WATERBORNE DISEASES

CONTINUING EDUCATION
PROFESSIONAL DEVELOPMENT COURSE



Technical Learning College

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CEU COURSE INTRODUCTION

WATERBORNE DISEASES CEU TRAINING COURSE

Review of commonly found water and wastewater diseases, symptoms, and identification techniques. This course will cover the federal rules concerning water and wastewater sampling techniques, waterborne disease control, general water quality operations and definitions; disease symptoms; disease diagnosis; history; susceptibility; and disease sources of contamination. This course will apply to all categories of water treatment/distribution and wastewater treatment/collection. It is our responsibility to identify, stop and control all waterborne diseases.

This course was designed for the enhancement of laboratory technical abilities. This course was designed for Water Laboratory Analysts, but can be utilized by Wastewater Treatment, Collections, Water Distribution, Well Drillers, Pump Installers, and Water Treatment Operators. The target audience for this course is any person that has at least 2 years of college lecture and laboratory course work in microbiology or a closely related field. This person should have at least 6 months of continuous bench experience with environmental protozoa detection techniques and must have successfully analyzed at least 50 water and/or wastewater samples for *Cryptosporidium* and *Giardia*. Six months of additional experience in the above areas may be substituted for two years of college. This course is also an excellent introduction for a person interested in working in the water quality field, water/wastewater treatment or distribution or a collections facility and wishing to maintain CEUs for certification license or to learn how to do the job safely and effectively, and/or to meet education needs for promotion. Every operator or customer service person that has contact with the public should have this booklet accessible to help answer water quality and waterborne disease related questions.

Course Procedures for Registration and Support

All of Technical Learning College's distance learning courses have complete registration and support services offered. Delivery of services will include e-mail, website, telephone, fax and mail support. TLC will attempt immediate and prompt service.

When a student registers for a distance or correspondence course, he/she is assigned a start date and an end date. It is the student's responsibility to note dates for assignments and keep up with the course work. If a student falls behind, he/she must contact TLC and request an end date extension in order to complete the course. It is the prerogative of TLC to decide whether to grant the request. All students will be tracked by their social security number or a unique number will be assigned to the student.

Course Assignment Material

Most of the EPA questions will come from the EPA summary, waterborne disease section identification, MCL listing, water sampling and laboratory procedures. Other detailed information will come from the Center of Disease Control. The EPA rules and laboratory procedures are also available on line at the EPA Web site: **www.epa.gov**. You are expected to read and understand all these rules and laboratory procedures.

Instructions for Written Assignments

The Waterborne Diseases distance learning course uses a multiple-choice style answer key. You can find the answer key in the rear of the assignment. You may have the option of completion of the laboratory assignment, either the heterotrophic plate count,

Method 1623 - Cryptosporidium and Giardia or most EPA's data gathering and monitoring programs under the Unregulated Contaminant Monitoring Rule. To receive alternate credit for the course, please contact TLC to receive permission from your Instructor.

Feedback Mechanism (examination procedures)
Each student will receive a feedback form as part of his or her study packet. You will be able to find this form in the rear of the course or lesson.

Security and Integrity
All students are required to do their own work. All lesson sheets and final exams are not returned to the student to discourage sharing of answers. Any fraud or deceit and the student will forfeit all fees and the appropriate agency will be notified. A random test generator will be implemented to protect the integrity of the assignment.

Grading Criteria
TLC will offer the student either pass/fail or a standard letter grading assignment. If TLC is not notified, you will only receive a pass/fail notice. In order to pass your final assignment, you are required to obtain a minimum score of 70% on your assignment.

Required Texts
The Waterborne Disease course comes complete with a short summary of the EPA's Rules and Regulations and related drinking water standards. If you need more information or a complete set of Rules, you can download them off the EPA's web page, www.epa.gov or contact your local state environmental agency. You may need to contact a laboratory or state agency to obtain-up-to date or certain sampling information.

Recordkeeping and Reporting Practices
TLC will keep all student records for a minimum of seven years. It is the student's responsibility to give the completion certificate to the appropriate agencies. TLC will not release any records to any party, except to the student. We will send the required information to Texas, Indiana and Pennsylvania for your certificate renewals.

ADA Compliance
TLC will make reasonable accommodations for persons with documented disabilities. Students should notify TLC and their instructors of any special needs. Course content may vary from this outline to meet the needs of this particular group. There is an option course assignment available, please contact an Instructor for further assistance.

Mission Statement
Our only product is educational service. Our goal is to provide you with the best possible education service possible. TLC will attempt to make your learning experience an enjoyable opportunity.

Educational Mission

The educational mission of TLC is:
To provide TLC students with comprehensive and ongoing training in the theory and skills needed for the environmental education field,

To provide TLC students opportunities to apply and understand the theory and skills needed for operator certification,

To provide opportunities for TLC students to learn and practice environmental educational skills with members of the community for the purpose of sharing diverse perspectives and experience,

To provide a forum in which students can exchange experiences and ideas related to environmental education,

To provide a forum for the collection and dissemination of current information related to environmental education, and to maintain an environment that nurtures academic and personal growth.

Professor Melissa Durbin Author and Dean of Instruction

30 years experience in water quality, water treatment and college instruction. I have taught this course to hundreds of students and still learn more each day about microorganisms. Call me or any of the other Instructors for course assistance. I welcome your input and comments and hope you enjoy this course.

Waterborne Diseases

Name	Causative organism	Source of organism	Disease
Viral gastroenteritis	**Rotavirus** (mostly in young children)	Human feces	Diarrhea or vomiting
Norwalk Agent	**Noroviruses** (genus *Norovirus*, family *Caliciviridae*) *1	Human feces; also, shellfish; lives in polluted waters	Diarrhea and vomiting
Salmonellosis	***Salmonella*** (bacterium)	Animal or human feces	Diarrhea or vomiting
Gastroenteritis Escherichia *coli*	-- ***E. coli O1* 57:H7** (bacterium): Other *E. coli* organisms:	Human feces	Symptoms vary with type caused
Typhoid	***Salmonella typhi*** (bacterium)	Human feces, urine	Inflamed intestine, enlarged spleen, high temperature-sometimes fatal
Shigellosis	***Shigella*** (bacterium)	Human feces	Diarrhea
Cholera	***Vibrio choleras*** (bacterium)	Human feces; also, shellfish; lives in many coastal waters	Vomiting, severe diarrhea, rapid dehydration, mineral loss-high mortality
Hepatitis A	**Hepatitis A** virus	Human feces; shellfish grown in polluted waters	Yellowed skin, enlarged liver, fever, vomiting, weight loss, abdominal pain-low mortality, lasts up to four months
Amebiasis	***Entamoeba histolytica*** (protozoan)	Human feces	Mild diarrhea, dysentery, extra intestinal infection
Giardiasis	***Giardia lamblia*** (protozoan)	Animal or human feces	Diarrhea, cramps, nausea, and general weakness — lasts one week to months
Cryptosporidiosis	***Cryptosporidium parvum***	Animal or human feces	Diarrhea, stomach pain — lasts (protozoan) days to weeks

Notes:

*1 http://www.cdc.gov/ncidod/dvrd/revb/gastro/norovirus.htm
http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5009a1.htm

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Common EPA Definitions **Larger Glossary and detailed Laboratory Terms and Procedures in the rear.**

The following are terms that will be found in this course, especially in the waterborne disease area and laboratory/sampling chapters.

As used in 40 CFR 141, the term:

Best available technology* or *BAT means the best technology, treatment techniques, or other means which the Administrator finds, after examination for efficacy under field conditions and not solely under laboratory conditions, are available (taking cost into consideration). For the purposes of setting MCLs for synthetic organic chemicals, any BAT must be at least as effective as granular activated carbon.

Community water system is a public water system which serves at least 15 service connections used by year-round residents or regularly serves at least 25 year-round residents.

Compliance cycle is the nine-year calendar year cycle during which public water systems must monitor. Each compliance cycle consists of three three-year compliance periods. The first calendar year cycle began January 1, 1993 and ends December 31, 2001; the second begins January 1, 2002 and ends December 31, 2010; the third begins January 1, 2011 and ends December 31, 2019.

Compliance period is a three-year calendar year period within a compliance cycle. Each compliance cycle has three three-year compliance periods. Within the first compliance cycle, the first compliance period began January 1, 1993 to December 31, 1995; the second from January 1, 1996 to December 31, 1998; the third from January 1, 1999 to December 31, 2001.

Contaminant is any physical, chemical, biological, or radiological substance or matter in water.

Maximum contaminant level is the maximum permissible level of a contaminant in water which is delivered to any user of a public water system.

Maximum contaminant level goal* or *MCLG is the maximum level of a contaminant in drinking water at which no known or anticipated adverse effect on the health of persons would occur, and which allows an adequate margin of safety. Maximum contaminant level goals are non-enforceable health goals.

Non-transient non-community water system* or *NTNCWS is a public water system that is not a community water system and that regularly serves at least 25 of the same persons over 6 months per year.

Point-of-entry treatment device (POE) is a treatment device applied to the drinking water entering a house or building for the purpose of reducing contaminants in the drinking water distributed throughout the house or building.

Point-of-use treatment device (POU) is a treatment device applied to a single tap used for the purpose of reducing contaminants in drinking water at that one tap.

Public water system is a system for the provision to the public of water for human consumption through pipes or, after August 5, 1998, other constructed conveyances, if such system has at least fifteen service connections or regularly serves an average of at least twenty-five individuals daily at least 60 days out of the year. Such term includes: any collection, treatment, storage, and distribution facilities under control of the operator of such system and used primarily in connection with such system; and any collection or pretreatment storage facilities not under such control which are used primarily in connection with such system. Such term does not include any **"*special irrigation district*."**

A public water system is either a ***"community water system"*** or a ***"noncommunity water system."***

State means the agency of the State or Tribal government which has jurisdiction over public water systems. During any period when a State or Tribal government does not have primary enforcement responsibility pursuant to section 1413 of the Act, the term ***"State"*** means the Regional Administrator, of the U.S. Environmental Protection Agency.

Surface water means all water which is open to the atmosphere and subject to surface runoff.

Microbes **Basic definitions, more detailed information in the next section.**

Coliform bacteria are common in the environment and are generally not harmful. However, the presence of these bacteria in drinking water is usually a result of a problem with the treatment system or the pipes which distribute water, and indicate that the water may be contaminated with germs that can cause disease.

Fecal Coliform and E coli are bacteria whose presence indicate that the water may be contaminated with human or animal wastes. Microbes in these wastes can cause short-term effects, such as diarrhea, cramps, nausea, headaches, or other symptoms.

Turbidity has no health effects. However, turbidity can interfere with disinfection and provide a medium for microbial growth. Turbidity may indicate the presence of disease causing organisms. These organisms include bacteria, viruses, and parasites that can cause symptoms such as nausea, cramps, diarrhea, and associated headaches.

Cryptosporidium is a parasite that enters lakes and rivers through sewage and animal waste. It causes cryptosporidiosis, a mild gastrointestinal disease. However, the disease can be severe or fatal for people with severely weakened immune systems. The EPA and CDC have prepared advice for those with severely compromised immune systems who are concerned about *Cryptosporidium*.

Giardia lamblia is a parasite that enters lakes and rivers through sewage and animal waste. It causes gastrointestinal illness (e.g. diarrhea, vomiting, and cramps).

Waterborne Disease CEU Course Introduction

Bacteria, viruses, and protozoans that cause disease are known as pathogens. Most pathogens are generally associated with diseases that cause intestinal illness and affect people in a relatively short amount of time, generally a few days to two weeks. They can cause illness through exposure to small quantities of contaminated water or food, or from direct contact with infected people or animals. Pathogens that may cause waterborne outbreaks through drinking water have one thing in common: they are spread by the fecal-oral, or feces-to-mouth, route.

Pathogens may get into water and spread when infected humans or animals pass the bacteria, viruses and protozoa in their stool. For another person to become infected, he or she must take that pathogen in through the mouth.

Waterborne pathogens are different from other types of pathogens such as the viruses that cause influenza (the flu) or the bacteria that cause tuberculosis. Influenza virus and tuberculosis bacteria are spread by secretions that are coughed or sneezed into the air by an infected person.

Human or animal wastes in watersheds, failing septic systems, failing sewage treatment plants or cross-connections of water lines with sewage lines provide the potential for contaminating water with pathogens. The water may not appear to be contaminated because the feces has been broken up, dispersed, and diluted into microscopic particles. These particles, containing pathogens, may remain in the water and be passed to humans or animals unless adequately treated.

Only proper treatment will ensure eliminating the spread of disease. In addition to water, other methods exist for spreading pathogens by the fecal-oral route. The foodborne route is one of the more common methods. A frequent source is a food handler who does not wash his hands after a bowel movement and then handles food with ***"unclean"*** hands. The individual who eats feces-contaminated food may become infected and ill. It is interesting to note the majority of foodborne diseases occur in the home, not restaurants.

Day care centers are another common source for spreading pathogens by the fecal-oral route. Here, infected children in diapers may get feces on their fingers, then put their fingers in a friend's mouth or handle toys that other children put into their mouths. You will usually be asked to sample at these facilities for Giardia.

The general public and some of the medical community usually refer to diarrhea symptoms as "***stomach flu***." Technically, influenza is an upper respiratory illness and rarely has diarrhea associated with it; therefore, stomach flu is a misleading description for foodborne or waterborne illnesses, yet is accepted by the general public. So the next time you get the stomach flu, you may want to think twice about what you've digested within the past few days.

Chain of Transmission

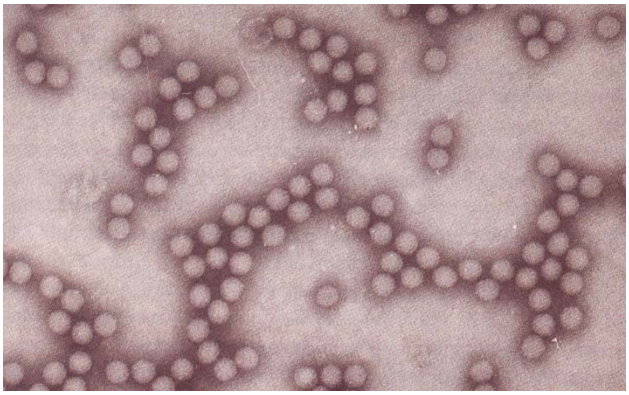
When water is contaminated with feces, this contamination may be of human or animal origin. If the human or animal source is not infected with a pathogen (disease-causing bacteria, viruses or protozoa), no disease will result.

The pathogens must survive in the water. This depends on the temperature of the water and the length of time the pathogens are in the water. Some pathogens will survive for only a short time in water, others, such as Giardia or Cryptosporidium, may survive for months. The pathogens in the water must enter the water system's intake and in numbers sufficient to infect people. The water is either not treated or inadequately treated for the pathogens present. A susceptible person must drink the water that contains the pathogen in order for illness (**disease**) to occur. This chain lists the events that must occur for the transmission of disease via drinking water. By breaking the chain at any point, the transmission of disease will be prevented.

Bacterial Diseases (More detailed information in the next chapters)
Campylobacteriosis is the most common diarrheal illness caused by bacteria. Symptoms include abdominal pain, malaise, fever, nausea and vomiting, and usually begin three to five days after exposure. The illness is frequently over within two to five days and usually lasts no more than 10 days. Campylobacteriosis outbreaks have most often been associated with food, especially chicken and unpasteurized milk as well as unchlorinated water. These organisms are also an important cause of "***travelers' diarrhea.***" Medical treatment generally is not prescribed for campylobacteriosis because recovery is usually rapid.

Cholera, Legionellosis, salmonellosis, shigellosis, yersiniosis, are other bacterial diseases that can be transmitted through water. All bacteria in water are readily killed or inactivated with chlorine or other disinfectants.

Viral Diseases or Viruses



Hepatitis A is a common example of a viral disease that may be transmitted through water. The onset is usually abrupt with fever, malaise, loss of appetite, nausea and abdominal discomfort, followed within a few days by jaundice. The disease varies in severity from a mild illness lasting one to two weeks, to a severely disabling disease lasting several months (rare). The incubation period is 15-50 days and averages 28-30 days. Hepatitis A outbreaks have been related to fecally contaminated water, food contaminated by infected food handlers, including sandwiches and salads that are not cooked or are handled after cooking, and raw or undercooked mollusks harvested from contaminated waters. Aseptic meningitis, polio, and viral gastroenteritis (Norwalk agent) are other viral diseases that can be transmitted through water. Most viruses in drinking water can be inactivated by chlorine or other disinfectants.

Terrorism
Recent investigations have shown proof the terrorist organizations have been able to reproduce most of these pathogens and have the technology and funding to attack our public water supply system. You will need to know these diseases and how to deal with these issues. Even diseases that we have not seen in years are easily and readily available for a terrorist to backflow into our distribution system, or pour into a wellhead or clearwell.

The Main Players- History and Biology Chapter 1

Before we define the major waterborne diseases, let's first examine the germs and other creatures that cause the diseases. Most of the following information may be simple or instruction that you already know. But to be safe, let's review the basics.

History of Research
By the last half of the 19th century, the microbial world was known to consist of protozoa, fungi, and bacteria, all visible with a light microscope. In the 1840s, the German scientist Jacob Henle suggested that there were infectious agents too small to be seen with a light microscope, but for the lack of direct proof, his hypothesis was not accepted. Although the French scientist Louis Pasteur was working to develop a vaccine for rabies in the 1880s, he did not understand the concept of a virus.



LOUIS PASTEUR

During the last half of the 19th century, several key discoveries were made that set the stage for the discovery of viruses. Pasteur is usually credited for dispelling the notion of spontaneous generation and proving that organisms reproduce new organisms. The German scientist Robert Koch, a student of Jacob Henle, and the British surgeon Joseph Lister developed techniques for growing cultures of single organisms that allowed the assignment of specific bacteria to specific diseases.

First Experiment
The first experimental transmission of a viral infection was accomplished in about 1880 by the German scientist Adolf Mayer, when he demonstrated that extracts from infected tobacco leaves could transfer tobacco mosaic disease to a new plant, causing spots on the leaves. Because Mayer was unable to isolate a bacterium or fungus from the tobacco leaf extracts, he considered the idea that tobacco mosaic disease might be caused by a soluble agent, but he concluded incorrectly that a new type of bacteria was likely to be the cause. The Russian scientist Dmitri Ivanofsky extended Mayer's observation and reported in 1892 that the tobacco mosaic agent was small enough to pass through a porcelain filter known to block the passage of bacteria. He too failed to isolate bacteria or fungi from the filtered material. But Ivanofsky, like Mayer, was bound by the dogma of his times and concluded in 1903 that the filter might be defective or that the disease agent was a toxin rather than a reproducing organism.

Unaware of Ivanofsky's results, the Dutch scientist Martinus Beijerinck, who collaborated with Mayer, repeated the filter experiment but extended this finding by demonstrating that the filtered material was not a toxin because it could grow and reproduce in the cells of the plant tissues. In his 1898 publication, Beijerinck referred to this new disease agent as a contagious living liquid—contagium vivum fluid—initiating a 20-year controversy over whether viruses were liquids or particles.

The conclusion that viruses are particles came from several important observations. In 1917 the French-Canadian scientist Félix H. d'Hérelle discovered that viruses of bacteria, which he named bacteriophage, could make holes in a culture of bacteria. Because each hole, or plaque, developed from a single bacteriophage, this experiment provided the first method for counting infectious viruses (the plaque assay). In 1935 the American biochemist Wendell Meredith Stanley crystallized tobacco mosaic virus to demonstrate that viruses had regular shapes, and in 1939 tobacco mosaic virus was first visualized using the electron microscope.

In 1898 the German bacteriologists Friedrich August Johannes Löffler and Paul F. Frosch (both trained by Robert Koch) described foot-and-mouth disease virus as the first filterable agent of animals, and in 1900, the American bacteriologist Walter Reed and colleagues recognized yellow fever virus as the first human filterable agent. For several decades viruses were referred to as filterable agents, and gradually the term virus (Latin for "slimy liquid" or "poison") was employed strictly for this new class of infectious agents. Through the 1940s and 1950s many critical discoveries were made about viruses through the study of bacteriophages because of the ease with which the bacteria they infect could be grown in the laboratory. Between 1948 and 1955, scientists at the National Institutes of Health (NIH) and at Johns Hopkins Medical Institutions revolutionized the study of animal viruses by developing cell culture systems that permitted the growth and study of many animal viruses in laboratory dishes.

Germ Theory of Disease History
Louis Pasteur along with Robert Koch developed the germ theory of disease which states that "a specific disease is caused by a specific type of microorganism." In 1876, Robert Koch established an experimental procedure to prove the germ theory of disease. This scientific procedure is known as Koch's postulates.

Koch's Postulates

• the causative agent must be present in every case of the disease and must not be present in healthy animals.
• the pathogen must be isolated from the diseased host animal and must be grown in pure culture.
• the same disease must be produced when microbes from the pure culture are inoculated into healthy susceptible animals.
• the same pathogen must be recoverable once again from this artificially infected animal and it must be able to be grown in pure culture.

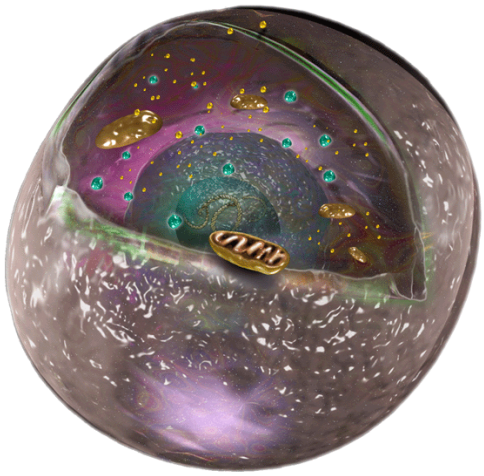
Koch's postulates not only proved the germ theory but also gave a tremendous boost to the development of microbiology by stressing a laboratory culture and identification of microorganisms.

Circumstances under which Koch's postulates do not easily apply

• Many healthy people carry pathogens but do not exhibit the symptoms of disease. These "carriers" may transmit the pathogens to others who then may become diseased. Example: epidemics of certain hospital acquired (nosocomial) infections, gonorrhea, typhoid, pneumonia, and AIDS.
• Some microbes are very difficult to grow under in-vitro (in the laboratory) conditions. Example: viruses, chlamydia, rickettsias, and bacteria that cause leprosy and syphilis. Some of the fastidious organisms can now be grown in cultures of human or animal cells or in small animals.
• Not all laboratory animals are susceptible to all pathogens. Many pathogens are species specific. Ethical considerations limit the use of laboratory animals and human volunteers.
• Certain diseases develop only when an opportunistic pathogen invades a susceptible host. These secondary invaders or opportunists cause disease only when a person is ill or recovering from another disease. For example, in the case of pneumonia and ear infections following influenza, isolation of bacteria causing pneumonia may mislead the isolation of influenza virus.
• Not all diseases are caused by microorganisms. Many diseases are caused by dietary deficiencies (scurvy, rickets). Some of the diseases are inherited or are caused by abnormality in chromosomes. Still others, such as cancer of the lungs and skin, are influenced by environmental factors.

Cells

Robert Hooke observed small empty chambers in the structure of cork with the help of his crude microscope. He called them cells. With the help of advanced microscopes it is now known that a cell is composed of many different substances and contains tiny particles called organelles that have important functions. Two German biologists Matthias Schleiden and Theodore Schwann proposed the "Cell theory' in 1838. According to this theory, all living things are composed of cells.



Rudolph Virchow completed the cell theory with the idea that all cells must arise from preexisting cells.

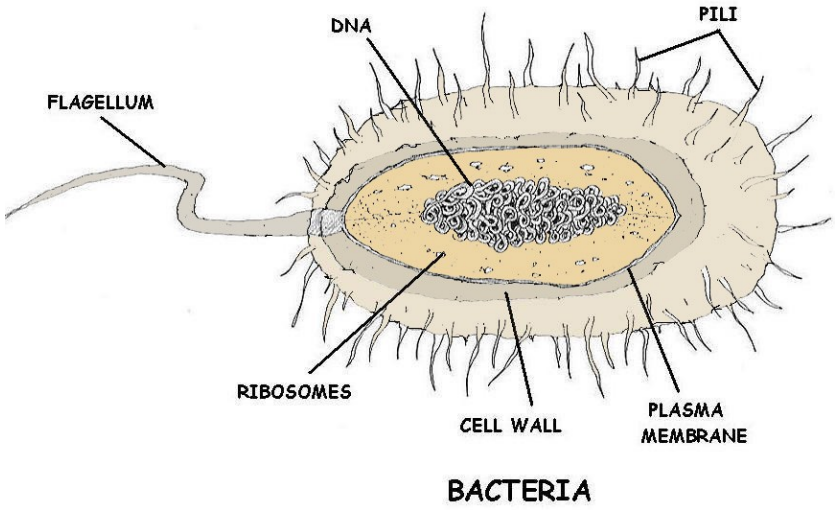
In biology, a cell is defined as the fundamental living unit of any organism and exhibits the basic characteristics of life. A cell obtains food from the environment to produce energy and nutrients for metabolism.

Metabolism
Metabolism is a term that describes all the chemical reactions by which food is transformed for use by the cells.

Through its metabolism, a cell can grow, reproduce and it can respond to changes in its environment. As a result of accidental changes in its environment, a cell can undergo changes in its genetic material. This is called mutation.

Bacteria

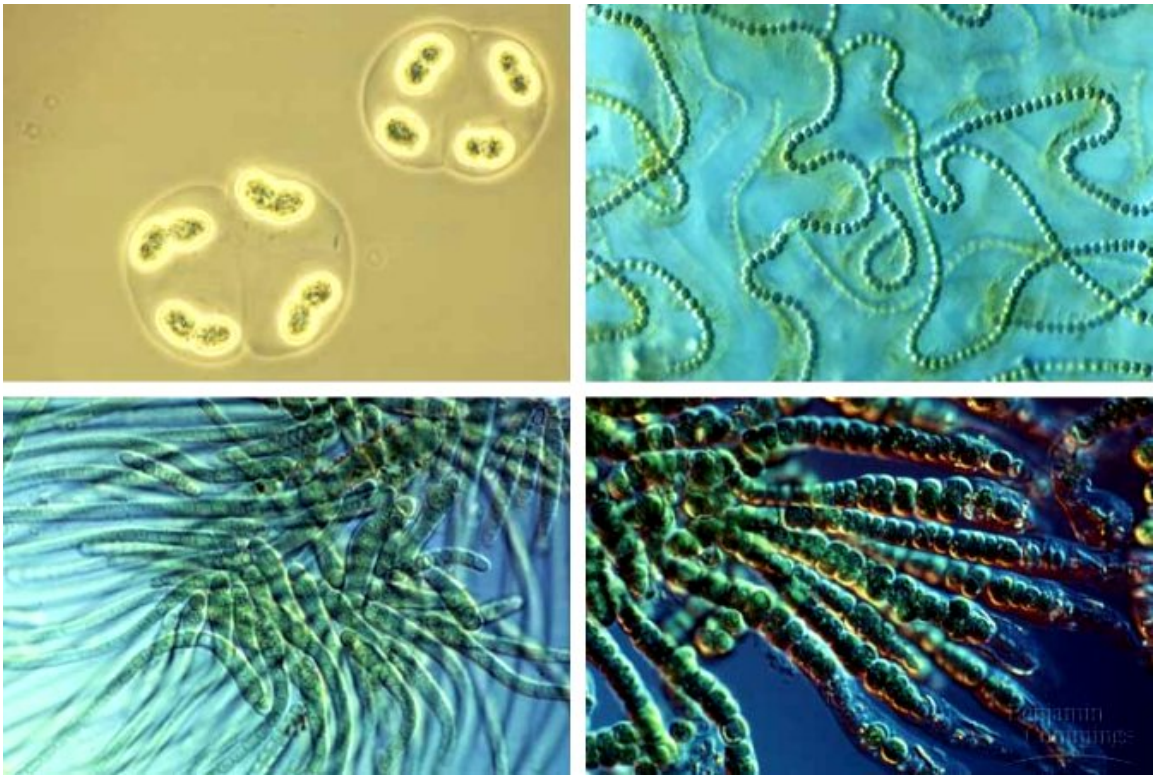
Bacteria consist of only a single cell, but don't let their small size and seeming simplicity fool you. They're an amazingly complex and fascinating group of creatures. Bacteria have been found that can live in temperatures above the boiling point and in cold that would freeze your blood. They "eat" everything from sugar and starch to sunlight, sulfur and iron. There's even a species of bacteria—*Deinococcus radiodurans*—that can withstand blasts of radiation 1,000 times greater than would kill a human being.



Schematic of a typical rod-type bacteria.

“Bacteria” is a plural word. The singular for this word is “bacterium” (**bacter** = rod, staff). Bacteria are prokaryotes (Kingdom Monera), which means that they have no true nucleus. They do have one chromosome of double-stranded DNA in a ring. They reproduce by binary fission. Most bacteria lack or have very few internal membranes, which means that they don’t have some kinds of organelles (like mitochondria or chloroplasts). Most bacteria are **benign** (**benign** = good, friendly, kind) or beneficial, and only a few are “bad guys” or **pathogens**.

Kingdom Monera is a very diverse group. There are some bacteria relatives that can do photosynthesis--they don't have chloroplasts, but their chlorophyll and other needed chemicals are built into their cell membranes.



These organisms are called **Cyanobacteria** (**cyano** = blue, dark blue) or bluegreen algae, although they're not really algae (real algae are in Kingdom Protista). Like us, some kinds of bacteria need and do best in O_2, while others are poisoned or killed by it.

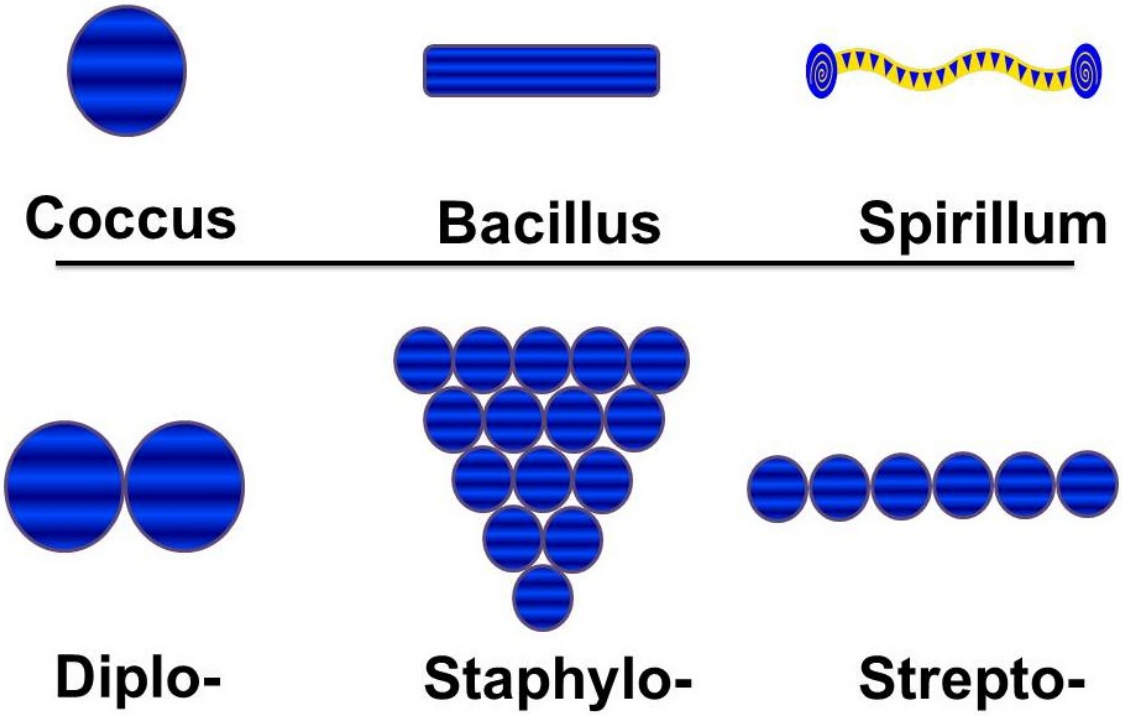
Prokaryotes
Bacteria and archaea are the only prokaryotes. All other life forms are Eukaryotes *(you-carry-oats)*, creatures whose cells have nuclei.

(Note: viruses are not considered true cells, so they don't fit into either of these categories; this will be covered in the next few pages.)

Early Origins
Bacteria are among the earliest forms of life that appeared on Earth thousands of years ago. Scientists think that bacteria helped shape and change the young planet's environment, eventually creating atmospheric oxygen that enabled other, more complex life forms to develop. Many believe that more complex cells developed as once free-living bacteria took up residence in other cells, eventually becoming the organelles in modern complex cells.

The mitochondria *(mite-oh-con-dree-uh)* that make energy for your body cells is one example of such an organelle.

There are thousands of species of bacteria, but all of them are basically one of three different shapes. Some are rod - or stick-shaped and called bacilli *(buh-sill-eye)*. Others are shaped like little balls and called cocci *(cox-eye)*.



Others still are helical or spiral in shape. Some bacterial cells exist as individuals while others cluster together to form pairs, chains, squares or other groupings. Bacteria live on or in just about every material and environment on Earth from soil to water to air, and from your house to arctic ice to volcanic vents. Each square centimeter of your skin averages about 100,000 bacteria. A single teaspoon of topsoil contains more than a billion (1,000,000,000) bacteria.

Peptidoglycan

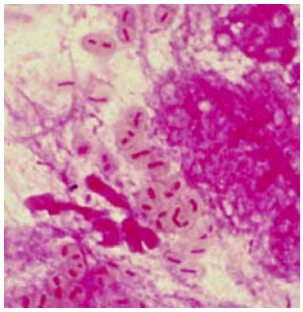
Most bacteria secrete a covering for themselves which we call a **cell wall**. However, bacterial cell walls are a totally different thing than the cell walls we talk about plants having. Bacterial cell walls do **NOT** contain cellulose like plant cell walls do. Bacterial cell walls are made mostly of a chemical called **peptidoglycan** (made of polypeptides bonded to modified sugars), but the amount and location of the peptidoglycan are different in the two possible types of cell walls, depending on the species of bacterium.

Some antibiotics, like penicillin, inhibit the formation of the chemical cross linkages needed to make peptidoglycan. These antibiotics don't kill the bacteria outright; just stop them from being able to make more cell wall so they can grow. That's why antibiotics must typically be taken for ten days until the bacteria, unable to grow, die of "old age". If a person stops taking the antibiotic sooner, any living bacteria could start making peptidoglycan, grow, and reproduce.

Gram Stain

However, because one of the two possible types of bacterial cell walls has more peptidoglycan than the other, antibiotics like penicillin are more effective against bacteria with that type of cell wall and less effective against bacteria with less peptidoglycan in their cell walls. Thus it is important, before beginning antibiotic treatment, to determine with which of the two types of bacteria one is dealing. **Dr. Hans Christian Gram**, a Danish physician, invented a staining process to tell these two types of bacteria apart, and in his honor, this process is called **Gram stain**. In this process, the amount of peptidoglycan in the cell walls of the bacteria under study will determine how those bacteria absorb the dyes with which they are stained; thus, bacterial cells can be $Gram^+$ or Gram $^-$. $Gram^+$ bacteria have simpler cell walls with lots of peptidoglycan, and stain a dark purple color. $Gram^-$ bacteria have more complex cell walls with less peptidoglycan, thus absorb less of the purple dye used and stain a pinkish color instead. Also, $Gram^-$ bacteria often incorporate toxic chemicals into their cell walls, and thus tend to cause worse reactions in our bodies. Because $Gram^-$ bacteria have less peptidoglycan, antibiotics like penicillin are less effective against them. As we have discussed before, taking antibiotics that don't work can be bad for you, thus a good doctor should always have a culture done before prescribing antibiotics to make sure the person is getting something that will help.

Pseudomonas aeruginosa is a strictly aerobic, oxidase positive, gram-negative non-fermentative bacterium. The Gram-stain appearance is not particularly characteristic although rods are somewhat thinner than those seen for the enteric-like bacteria. Mucoid strains that produce an extracellular polysaccharide are frequently isolated from patients with cystic fibrosis and this capsular material can be seen in the photo.



Two types of cells- Procaryotes and Eucaryotes
A Procaryotic cell exhibits all the characteristics of life but it lacks the complex system of membranes and organelles. ***Example***: Bacterial cells and cyanobacteria. A Eukaryotic cell has a complex structure. It contains a true nucleus and many membrane bound organelles. ***Example***: Protozoa, fungi, algae, all plants and animal cells.

Structure of a Eukaryotic Cell
Cell Membrane: The cell is enclosed and held intact by the cell membrane/plasma membrane/cytoplasmic membrane. It is composed of large molecules of proteins and phospholipids. These large molecules permit the passage of nutrients, waste products and secretions across the cellular membrane. The cell membrane is selectively permeable.

Nucleus
The Nucleus unifies, controls and integrates the function of the entire cell. The nucleus is enclosed in the nuclear membrane and contains chromosomes; the number and composition of chromosomes and the number of genes on each chromosome are characteristic of each species. Human cells have 46 (23 pairs) chromosomes.
Each chromosome consists of many genes. A gene is a coiled unit made up of DNA and proteins that code for or determine a particular characteristic of an individual organism.

Cytoplasm
Cytoplasm is the cellular material outside the nucleus. It is composed of a semifluid gelatinous nutrient matrix and cytoplasmic organelles including endoplasmic reticulum, ribosomes, Golgi complex, mitochondria, centrioles, microtubules, lysosomes and vacoules.

Cell Wall
A cell wall is found as an external structure of plant cells, algae, and fungi. It consists of cellulose, pectin, chitin, and some mineral salts. A rigid exterior cell wall defines the shape of bacterial cells. It is different from the simple cell wall of plant cells and is made up of macromolecular polymer-peptidoglycan (protein and polysaccharide chain). The thickness and its exact composition varies with the species of bacteria.

Cilia and Flagella
Some eukaryotic cells possess relatively long and thin structures called flagella. These are organs of locomotion. Cilia are also organs of locomotion but are shorter and more numerous

Structure of a Procaryotic Cell
All bacteria are procaryotes and are simple cells. They divide by binary fission.

Chromosome
The chromosome of a prokaryotic cell is not surrounded by a nuclear membrane, it has no definite shape and no protein material associated with it. It usually consists of a single circular DNA molecule and serves as the control center of the bacterial cell. A typical bacterial chromosome contains approximately 10,000 genes.

Cytoplasm
Cytoplasm is a semi-liquid that surrounds the chromosome and is contained within the plasma membrane. Located within the cytoplasm are several ribosomes, which are the sites of protein synthesis. Cytoplasmic granules occur in certain species of bacteria which can be specifically stained and used to identify the bacteria.

Cell Membrane
The Cell Membrane is similar to that of the eukaryotic cell membrane. It is selectively permeable and controls the substances entering or leaving the cell. It is very thin and can be seen by electron microscopes only.

Capsules
Some bacteria have a layer of material outside the cell wall. When highly organized and firmly attached to the cell wall, this layer is called a capsule or if it is not highly organized and not firmly attached, a slime layer. Capsules consist of complex sugars or polysaccharides combined with lipids and proteins. The composition of the capsule is useful in differentiating between different types of bacteria. Capsules are usually detected by negative staining, where the bacterial cell and the background become stained but the capsule remains unstained.

Encapsulated bacteria produce colonies on nutrient agar that are smooth, mucoid and glistening, whereas the noncapsulated bacteria produce rough and dry colonies. Capsules enable the bacterial species to attach to mucus membranes and protect the bacteria from phagocytosis.

Flagella
Flagella are thread-like proteins that enable the bacteria to move. Flagellated bacteria are said to be motile while non-flagellated bacteria are generally non-motile. The number and arrangement of flagella are species specific and can be used to classify bacteria.
Peritrichous bacteria- possess flagella over the entire surface.
Lophotrichous bacteria-possess a tuft of flagella at one or both ends.
Amphitrichous bacteria-bacteria with one flagellum at each end.
Monotrichous bacteria-bacteria with a single polar flagellum.

Pili or Fimbriae
Pili or Fimbriae are thin hair-like structures observed on gram negative bacteria. They are not associated with motility. They enable the bacteria to attach to other bacteria or to membrane surfaces such as intestinal linings or RBC. They are also used to transfer genetic material from one bacteria cell to another.

Spores
Some bacteria are capable of forming spores (also called endospore) as a means of survival under adverse conditions. During sporulation the genetic material is enclosed in several protein coats that are resistant to heat, drying and most chemicals. Spores have been shown to survive in soil or dust. When the dried spore lands on a nutrient rich surface, it forms a new vegetative cell. Spore formation is related to the survival of bacterial cells, not reproduction.

Bacterial Nutrition
All life has the same basic nutritional requirements which include:
Energy. This may be light (the sun or lamps) or inorganic substances like sulfur, carbon monoxide or ammonia, or preformed organic matter like sugar, protein, fats etc. Without energy life cannot exist and quickly dies or becomes inactive.
Nitrogen. This may be nitrogen gas, ammonia, nitrate/nitrite, or a nitrogenous organic compound like protein or nucleic acid.
Carbon. This can be carbon dioxide, methane, carbon monoxide, or a complex organic material.
Oxygen. All cells use oxygen in a bound form and many require gaseous oxygen (air), but oxygen is lethal to many microbes.
Phosphorous, Sulfur, Magnesium, Potassium, and Sodium.
Calcium. Most cells require calcium in significant quantities, but some seem to only need it in trace amounts.
Water. All life requires liquid water in order to grow and reproduce; which is why the Mars Mission is so interested in water on Mars. Some resting stages of cells, like bacterial spores, can exist for long periods without free water, but they do not grow or metabolize.
Iron, Zinc, Cobalt. These are called trace metals that are required by some enzymes to function.

The sources of these various requirements define an organism, so a description of every organism should include this information.

Fastidious

Many bacteria can synthesize every complex molecule they need from the basic minerals, but others, said to be fastidious, require preformed organic molecules like vitamins, amino acids, nucleic acids, carbohydrates; humans are ***fastidious***. In general bacterial pathogens need more preformed organic molecules than do non-pathogens, but that is not always true. For example, some bacteria that are found in milk hardly make any of their own basic organic molecules; that is, they let the cow (or more to the point the number of microbes that live in the cow's gut) make these things for them. A simple rule of thumb is "***if humans can use something for food, many microbes will also love it***". The reverse is not always true, as microbes can "digest" some very strange substances including cellulose, sulfur, some plastics, turkey feathers and asphalt, just to name a few.

Table of differences between Archaea, Bacteria and Eukaryotes

Characteristic	Archaea	Bacteria	Eukaryotes
Predominantly multicellular	No	No	Yes
Cell contains a nucleus and other membrane bound organelles	No	No	Yes
DNA occurs in a circular form*	Yes	Yes	No
Ribosome size	70s	70s	80s
Membrane lipids ester-linked**	No	Yes	Yes
Photosynthesis with chlorophyll	No	Yes	Yes
Capable of growth at temperatures greater than 80 C	Yes	Yes	No
Histone proteins present in cell	Yes	No	Yes
Methionine used as tRNA Initiator***	Yes	No	Yes
Operons present in DNA	Yes	Yes	No
Interon present in most genes	No	No	Yes
Capping and poly-A tailing of mRNA	No	No	Yes
Gas vesicles present	Yes	Yes	No
Capable of Methanogenesis	Yes	No	No
Sensitive to chloramphenicol, kanamycin and streptomycin	No	Yes	No
Transcription factors required	No	Yes	Yes
Capable of Nitrification	No	Yes	No
Capable of Denitrification	Yes	Yes	No
Capable of Nitrogen Fixation	Yes	Yes	No
Capable of Chemolithotrophy	Yes	Yes	No

* Eukaryote DNA is linear

** Archaea membrane lipids are ether-linked

*** Bacteria use Formylmethionine

What in the World is an Eukaryote?

Eukaryotes are organisms with complex cells, in which the genetic material is organized into membrane-bound nuclei. They include the animals, plants, and fungi, which are mostly multicellular, as well as various other groups called protists, many of which are unicellular. In contrast, other organisms such as bacteria lack nuclei and other complex cell structures, and are called prokaryotes. The eukaryotes share a common origin, and are often treated formally as a super kingdom, empire, or domain. The name comes from the Greek *eus* or true and *karyon* or nut, referring to the nucleus.

What are Protists?

- They are **eukaryotes** because they all have a **nucleus**.
- Most have **mitochondria** although some have later lost theirs. Mitochondria were derived from aerobic alpha-proteobacteria (prokaryotes) that once lived within their cells.
- Many have **chloroplasts** with which they carry on photosynthesis. Chloroplasts were derived from photosynthetic **cyanobacteria** (also prokaryotes) living within their cells.

Eukaryotic Cells

Eukaryotic cells are generally much larger than prokaryotes, typically with a thousand times their volumes. They have a variety of internal membranes and structures, called organelles, and a cytoskeleton composed of microtubules and microfilaments, which plays an important role in defining the cell's organization. Eukaryotic DNA is divided into several bundles called chromosomes, which are separated by a microtubular spindle during nuclear division. In addition to asexual cell division, most eukaryotes have some process of sexual reproduction via cell fusion, which is not found among prokaryotes.

Eukaryotic cells include a variety of membrane-bound structures, collectively referred to as the endomembrane system. Simple compartments, called vesicles or vacuoles, can form by budding off of other membranes. Many cells ingest food and other materials through a process of endocytosis, where the outer membrane invaginates and then pinches off to form a vesicle. It is probable that most other membrane-bound organelles are ultimately derived from such vesicles.

The nucleus is surrounded by a double membrane, with pores that allow material to move in and out. Various tube- and sheet-like extensions of the nuclear membrane form what is called the endoplasmic reticulum or ER, which is involved in protein transport. It includes rough sections where ribosomes are attached, and the proteins they synthesize enter the interior space or lumen. Subsequently, they generally enter vesicles, which bud off from the smooth section. In most eukaryotes, the proteins may be further modified in stacks of flattened vesicles, called Golgi bodies or dictyosomes.

Vesicles may be specialized for various purposes. For instance, lysosomes contain enzymes that break down the contents of food vacuoles, and peroxisomes are used to break down peroxide which is toxic otherwise.

Contractile Vacuoles

Many protozoa have contractile vacuoles, which collect and expel excess water, and extrusomes, which expel material used to deflect predators or capture prey. In multicellular organisms, hormones are often produced in vesicles. In higher plants, most of a cell's volume is taken up by a central vacuole or tonoplast, which maintains its osmotic pressure. Many eukaryotes have slender motile projections, usually called flagella when long and cilia when short. These are variously involved in movement, feeding, and sensation. These are entirely distinct from prokaryotic flagella. They are supported by a bundle of microtu-bules arising from a basal body, also called a kinetosome or centriole, characteristically arranged as nine doublets surrounding two singlets. Flagella also may have hairs or mastigonemes, scales, connecting membranes, and internal rods. Their interior is continuous with the cell's cytoplasm.

Centrioles

Centrioles are often present even in cells and groups that do not have flagella. They generally occur in groups of one or two, called kinetids that give rise to various microtubular roots. These form a primary component of the cytoskeletal structure, and are often assembled over the course of several cell divisions, with one flagellum retained from the parent and the other derived from it. Centrioles may also be associated in the formation of a spindle during nuclear division. Some protists have various other microtubule-supported organelles. These include the radiolaria and heliozoa, which produce axopodia used in flotation or to capture prey, and the haptophytes, which have a peculiar flagellum-like organelle called the haptonema.

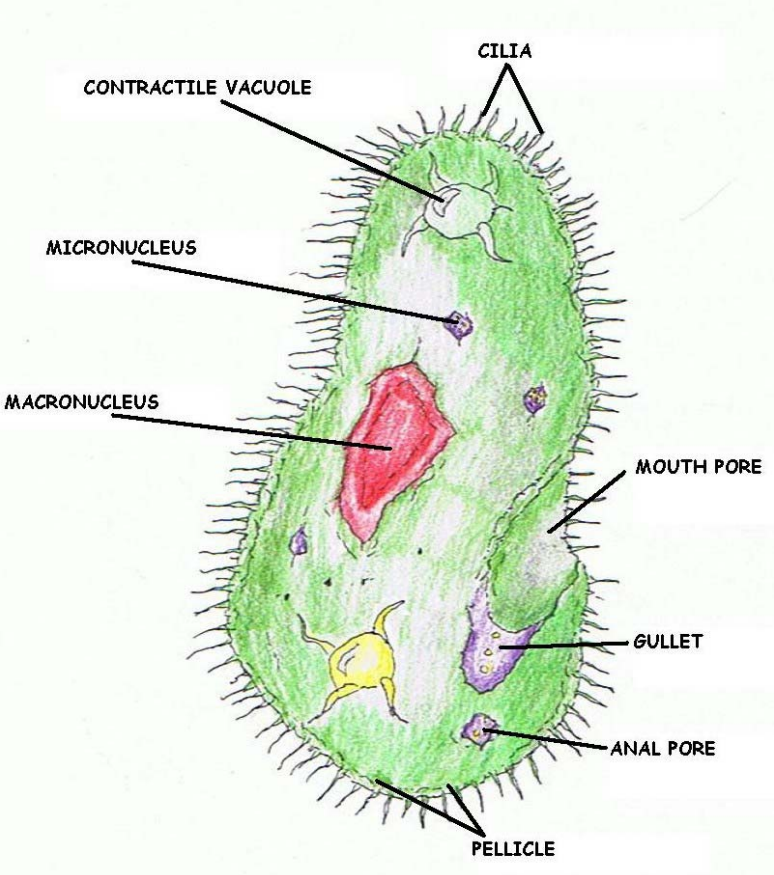
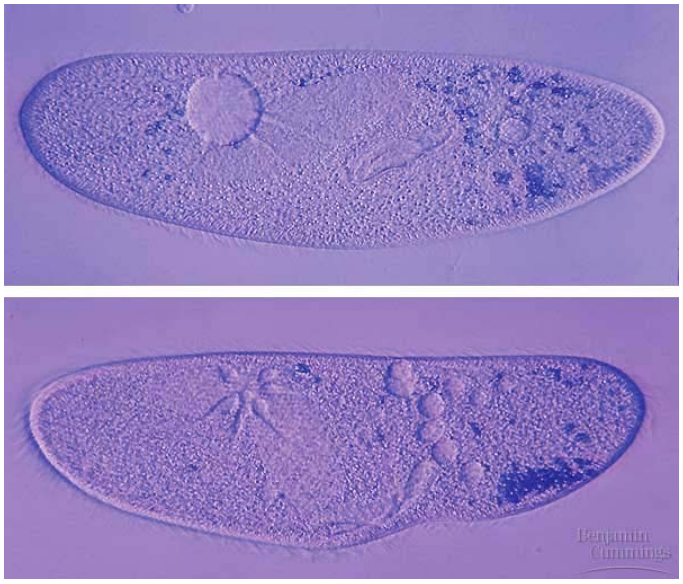


Figure 1. A diagram of *Paramecium* sp. with major organelles indicated.

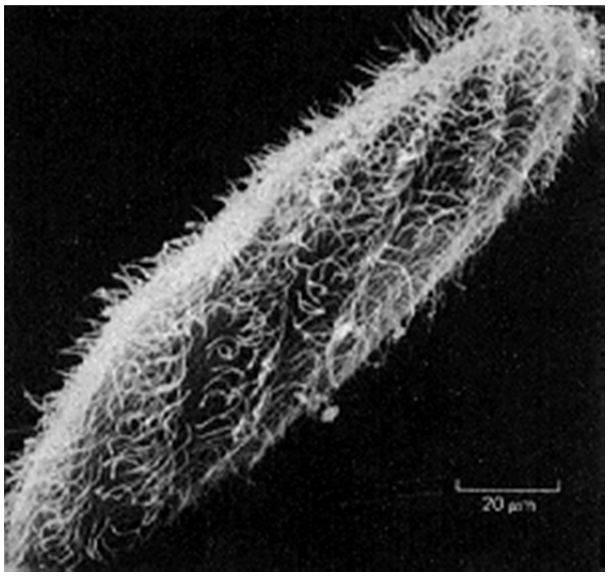


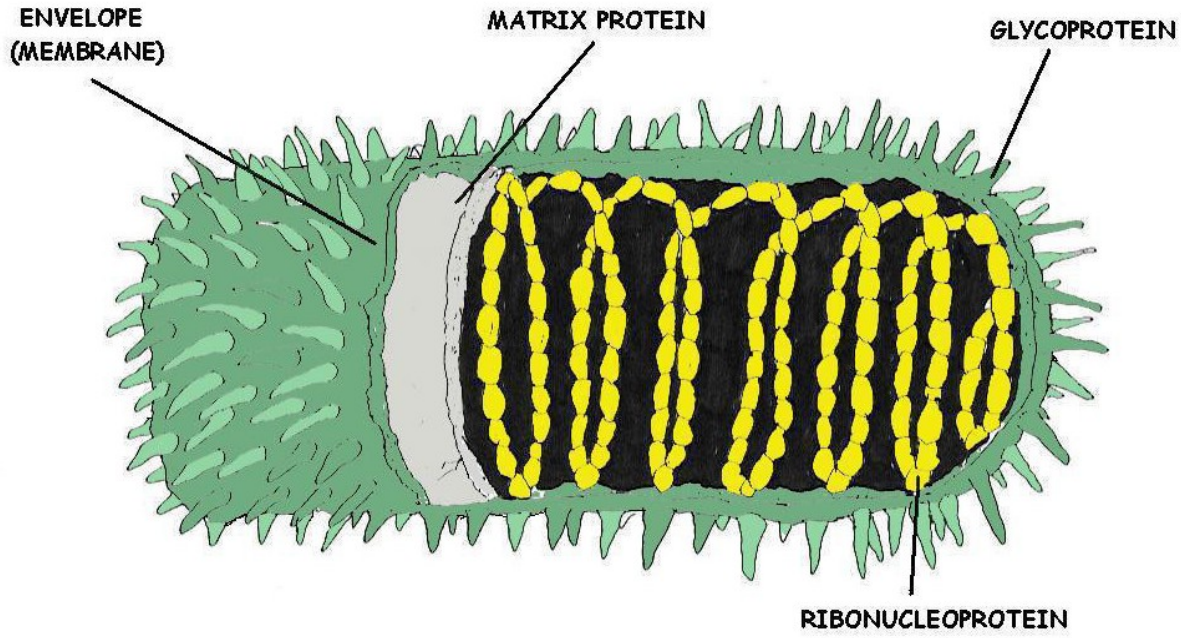
Contractile Vacuoles

Figure 2. The contractile vacuole when full (top) and after contraction (bottom).

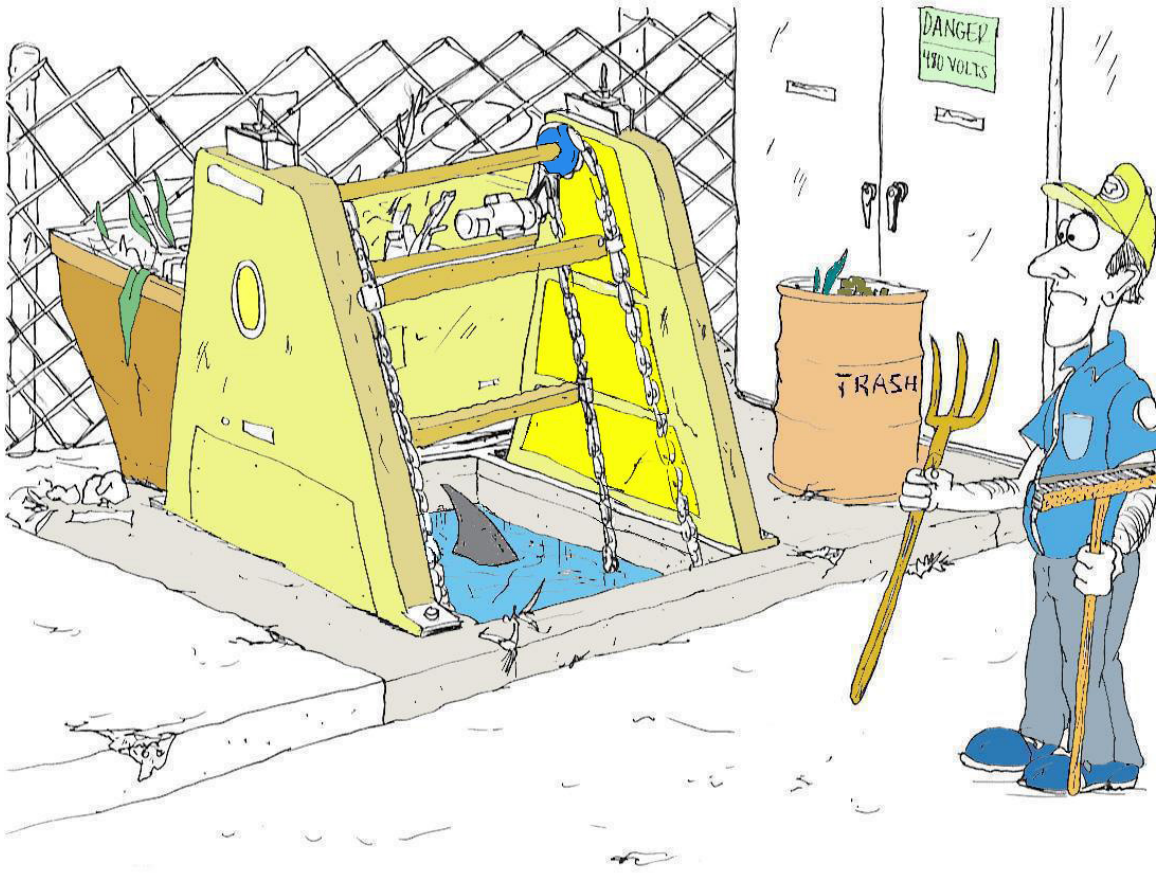
Paramecium

Members of the genus *Paramecium* are single-celled, freshwater organisms in the kingdom Protista. They exist in an environment in which the osmotic concentration in their external environment is much lower than that in their cytoplasm. More specifically, the habitat in which they live is **hypotonic** to their cytoplasm. As a result of this, *Paramecium* is subjected to a continuous influx of water, as water diffuses inward to a region of higher osmotic concentration. If *Paramecium* is to maintain homeostasis, water must be continually pumped out of the cell (against the osmotic gradient) at the same rate at which it moves in. This process, known as **osmoregulation**, is carried out by two organelles in *Paramecium* known as **contractile vacuoles** (Figures 1 and 2).



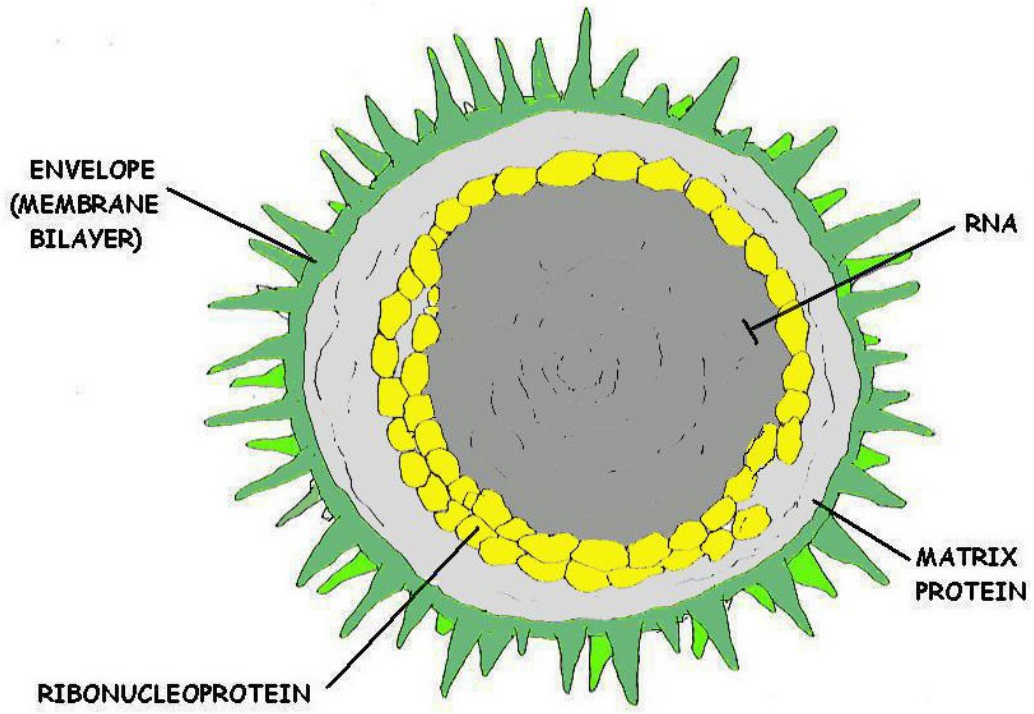

ENVELOPE
(MEMBRANE)
MATRIX PROTEIN
GLYCOPROTEIN
RIBONUCLEOPROTEIN

VIRUS


DANGER
480 VOLTS
TRASH

Viruses

Viruses are acellular microorganisms. They are made up of only genetic material and a protein coat. Viruses depend on the energy and metabolic machinery of the host cell to reproduce. A virus is an infectious agent found in virtually all life forms, including humans, animals, plants, fungi, and bacteria. Viruses consist of genetic material—either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA)—surrounded by a protective coating of protein, called a capsid, with or without an outer lipid envelope. Viruses are between 20 and 100 times smaller than bacteria and hence are too small to be seen by light microscopy.



CROSS SECTIONAL VIEW

Viruses vary in size from the largest poxviruses of about 450 nanometers (about 0.000014 in) in length to the smallest polioviruses of about 30 nanometers (about 0.000001 in). Viruses are not considered free-living, since they cannot reproduce outside of a living cell; they have evolved to transmit their genetic information from one cell to another for the purpose of replication. Viruses often damage or kill the cells that they infect, causing disease in infected organisms. A few viruses stimulate cells to grow uncontrollably and produce cancers. Although many infectious diseases, such as the common cold, are caused by viruses, there are no cures for these illnesses. The difficulty in developing antiviral therapies stems from the large number of variant viruses that can cause the same disease, as well as the inability of drugs to disable a virus without disabling healthy cells. However, the development of antiviral agents is a major focus of current research, and the study of viruses has led to many discoveries important to human health.

Virions
Individual viruses, or virus particles, also called virions, contain genetic material, or genomes, in one of several forms. Unlike cellular organisms, in which the genes always are made up of DNA, viral genes may consist of either DNA or RNA. Like cell DNA, almost all viral DNA is double-stranded, and it can have either a circular or a linear arrangement. Almost all viral RNA is single-stranded; it is usually linear, and it may be either segmented (with different genes on different RNA molecules) or nonsegmented (with all genes on a single piece of RNA).

Capsids
The viral protective shell, or capsid, can be either helical (spiral-shaped) or icosahedral (having 20 triangular sides). Capsids are composed of repeating units of one or a few different proteins. These units are called protomers or capsomers. The proteins that make up the virus particle are called structural proteins. Viruses also carry genes for making proteins that are never incorporated into the virus particle and are found only in infected cells. These viral proteins are called nonstructural proteins; they include factors required for the replication of the viral genome and the production of the virus particle.

Capsids and the genetic material (DNA or RNA) they contain are together referred to as nucleocapsids. Some virus particles consist only of nucleocapsids, while others contain additional structures.

Some icosahedral and helical animal viruses are enclosed in a lipid envelope acquired when the virus buds through host-cell membranes. Inserted into this envelope are glycoproteins that the viral genome directs the cell to make; these molecules bind virus particles to susceptible host cells.

Bacteriophages
The most elaborate viruses are the bacteriophages, which use bacteria as their hosts. Some bacteriophages resemble an insect with an icosahedral head attached to a tubular sheath. From the base of the sheath extend several long tail fibers that help the virus attach to the bacterium and inject its DNA to be replicated, direct capsid production, and virus particle assembly inside the cell.

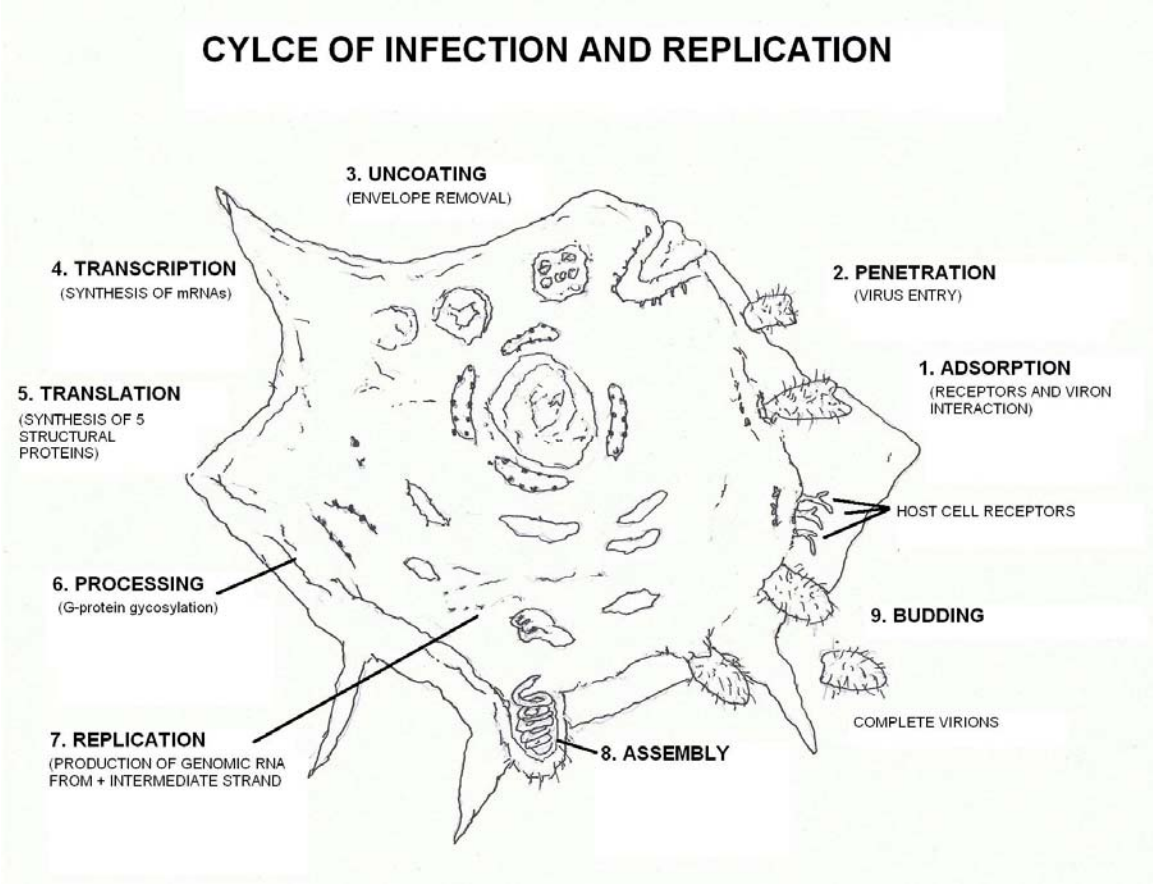
Viroids and Prions
Viroids and prions are smaller than viruses, but they are similarly associated with disease. Viroids are plant pathogens that consist only of a circular, independently replicating RNA molecule. The single-stranded RNA circle collapses on itself to form a rodlike structure. The only known mammalian pathogen that resembles plant viroids is the deltavirus (hepatitis D), which requires hepatitis B virus proteins to package its RNA into virus particles. Co-infection with hepatitis B and D can produce more severe disease than can infection with hepatitis B alone. Prions are mutated forms of a normal protein found on the surface of certain animal cells.

Virus Classification
Viruses are classified according to their type of genetic material, their strategy of replication, and their structure. The International Committee on Nomenclature of Viruses (ICNV), established in 1966, devised a scheme to group viruses into families, subfamilies, genera, and species. The ICNV report published in 1995 assigned more than 4000 viruses into 71 virus families. Hundreds of other viruses remain unclassified because of the lack of sufficient information.

Replication

The first contact between a virus particle and its host cell occurs when an outer viral structure docks with a specific molecule on the cell surface. For example, a glycoprotein called gp120 on the surface of the human immunodeficiency virus (HIV, the cause of acquired immunodeficiency syndrome, or AIDS) virion specifically binds to the CD4 molecule found on certain human T lymphocytes (a type of white blood cell). Most cells that do not have surface CD4 molecules generally cannot be infected by HIV.

After binding to an appropriate cell, a virus must cross the cell membrane. Some viruses accomplish this goal by fusing their lipid envelope to the cell membrane, thus releasing the nucleocapsid into the cytoplasm of the cell.



Other viruses must first be endocytosed (enveloped by a small section of the cell's plasma membrane that pokes into the cell and pinches off to form a bubble-like vesicle called an endosome) before they can cross the cell membrane. Conditions in the endosome allow many viruses to change the shape of one or more of their proteins.

These changes permit the virus either to fuse with the endosomal membrane or to lyse the endosome (cause it to break apart), allowing the nucleocapsid to enter the cell cytoplasm.

Once inside the cell, the virus replicates itself through a series of events. Viral genes direct the production of proteins by the host cellular machinery. The first viral proteins synthesized by some viruses are the enzymes required to copy the viral genome. Using a combination of viral and cellular components, the viral genome can be replicated

thousands of times. Late in the replication cycle for many viruses, proteins that make up the capsid are synthesized. These proteins package the viral genetic material to make newly formed nucleocapsids.
To complete the virus replication cycle, viruses must exit the cell. Some viruses bud out of the cell's plasma membrane by a process resembling reverse endocytosis. Other viruses cause the cell to lyse, thereby releasing newly formed virus particles ready to infect other cells. Still other viruses pass directly from one cell into an adjacent cell without being exposed to the extracellular environment. The virus replication cycle can be as short as a couple of hours for certain small viruses or as long as several days for some large viruses.

Virus Battle
Some viruses kill cells by inflicting severe damage resulting in cell lysis; other viruses cause the cell to kill itself in response to virus infection. This programmed cell suicide is thought to be a host defense mechanism to eliminate infected cells before the virus can complete its replication cycle and spread to other cells. Alternatively, cells may survive virus infection, and the virus can persist for the life of its host. Virtually all people harbor harmless viruses.

Retroviruses
Retroviruses, such as HIV, have RNA that is transcribed into DNA by the viral enzyme reverse transcriptase upon entry into the cell. (The ability of retroviruses to copy RNA into DNA earned them their name because this process is the reverse of the usual transfer of genetic information, from DNA to RNA.) The DNA form of the retrovirus genome is then integrated into the cellular DNA and is referred to as the provirus. The viral genome is replicated every time the host cell replicates its DNA and is thus passed on to daughter cells.

Hepatitis B Virus
Hepatitis B virus can also transcribe RNA to DNA, but this virus packages the DNA version of its genome into virus particles. Unlike retroviruses, hepatitis B virus does not integrate into the host cell DNA.

Viral Infections
Most viral infections cause no symptoms and do not result in disease. For example, only a small percentage of individuals who become infected with Epstein-Barr virus or western equine encephalomyelitis virus ever develop disease symptoms. In contrast, most people who are infected with measles, rabies, or influenza viruses develop the disease. A wide variety of viral and host factors determine the outcome of virus infections. A small genetic variation can produce a virus with increased capacity to cause disease. Such a virus is said to have increased virulence.

Infection
Viruses can enter the body by several routes. Herpes simplex virus and poxviruses enter through the skin by direct contact with virus-containing skin lesions on infected individuals. Ebola, hepatitis B, and HIV can be contracted from infected blood products. Hypodermic needles and animal and insect bites can transmit a variety of viruses through the skin. Viruses that infect through the respiratory tract are usually transmitted by airborne droplets of mucus or saliva from infected individuals who cough or sneeze.

Viruses that enter through the respiratory tract include orthomyxovirus (influenza), rhinovirus and adenovirus (common cold), and varicella-zoster virus (chicken pox).
Viruses such as rotavirus, coronavirus, poliovirus, hepatitis A, and some adenoviruses enter the host through the gastrointestinal tract. Sexually transmitted viruses, such as herpes simplex, HIV, and human papilloma viruses (HPV), gain entry through the genitourinary route. Other viruses, including some adenoviruses, echoviruses, Coxsackie viruses, and herpes viruses, can infect through the eye.

Localized or Systemic Infections
Virus infections can be either localized or systemic. The path of virus spread through the body in systemic infections differs among different viruses. Following replication at the initial site of entry, many viruses are spread to their target organs by the bloodstream or the nervous system.

The particular cell type can influence the outcome of virus infection. For example, herpes simplex virus undergoes lytic replication in skin cells around the lips but can establish a latent or dormant state in neuron cell bodies (located in ganglia) for extended periods of time. During latency, the viral genome is largely dormant in the cell nucleus until a stimulus such as a sunburn causes the reactivation of latent herpes virus, leading to the lytic replication cycle. Once reactivated, the virus travels from the ganglia back down the nerve to cause a cold sore on the lip near the original site of infection. The herpesvirus genome does not integrate into the host cell genome.

Virus-induced Illnesses
Virus-induced illnesses can be either acute, in which the patient recovers promptly, or chronic, in which the virus remains with the host or the damage caused by the virus is irreparable. For most acute viruses, the time between infection and the onset of disease can vary from three days to three weeks. In contrast, onset of AIDS following infection with HIV takes an average of 7 to 11 years.

Several human viruses are likely to be agents of cancer, which can take decades to develop. The precise role of these viruses in human cancers is not well understood, and genetic and environmental factors are likely to contribute to these diseases. But because a number of viruses have been shown to cause tumors in animal models, it is probable that many viruses have a key role in human cancers.

Alphaviruses and Flaviviruses
Some viruses—alphaviruses and flaviviruses, for example—must be able to infect more than one species to complete their life cycles. Eastern equine encephalomyelitis virus, an alphavirus, replicates in mosquitoes and is transmitted to wild birds when the mosquitoes feed. Thus, wild birds and perhaps mammals and reptiles serve as the virus reservoir, and mosquitoes serve as vectors essential to the virus life cycle by ensuring transmission of the virus from one host to another. Horses and people are accidental hosts when they are bitten by an infected mosquito, and they do not play an important role in virus transmission.

Defense
Although viruses cannot be treated with antibiotics, which are effective only against bacteria, the body's immune system has many natural defenses against virus infections. Infected cells produce interferons and other cytokines (soluble components that are largely responsible for regulating the immune response), which can signal adjacent

uninfected cells to mount their defenses, enabling uninfected cells to impair virus replication.

Cytokines

Some cytokines can cause a fever in response to viral infection; elevated body temperature retards the growth of some types of viruses. B lymphocytes produce specific antibodies that can bind and inactivate viruses. Cytotoxic T cells recognize virus-infected cells and target them for destruction. However, many viruses have evolved ways to circumvent some of these host defense mechanisms.

The development of antiviral therapies has been thwarted by the difficulty of generating drugs that can distinguish viral processes from cellular processes. Therefore, most treatments for viral diseases simply alleviate symptoms, such as fever, dehydration, and achiness. Nevertheless, antiviral drugs for influenza virus, herpesviruses, and HIV are available, and many others are in the experimental and developmental stages.

Prevention has been a more effective method of controlling virus infections. Viruses that are transmitted by insects or rodent excretions can be controlled with pesticides. Successful vaccines are currently available for poliovirus, influenza, rabies, adenovirus, rubella, yellow fever, measles, mumps, and chicken pox.

Vaccines are prepared from killed (inactivated) virus, live (attenuated or weakened) virus, or isolated viral proteins (subunits). Each of these types of vaccines elicits an immune response while causing little or no disease, and there are advantages and disadvantages to each. (For a more complete discussion of vaccines, see the Immunization article.)

Vaccination

The principle of vaccination was discovered by British physician Edward Jenner. In 1796 Jenner observed that milkmaids in England who contracted the mild cowpox virus infection from their cows were protected from smallpox, a frequently fatal disease. In 1798 Jenner formally demonstrated that prior infection with cowpox virus protected those that he inoculated with smallpox virus (an experiment that would not meet today's protocol standards because of its use of human subjects).

Mutation

Viruses undergo very high rates of mutation (genetic alteration) largely because they lack the repair systems that cells have to safeguard against mutations. A high mutation rate enables the virus to continually adapt to new intracellular environments and to escape from the host immune response.

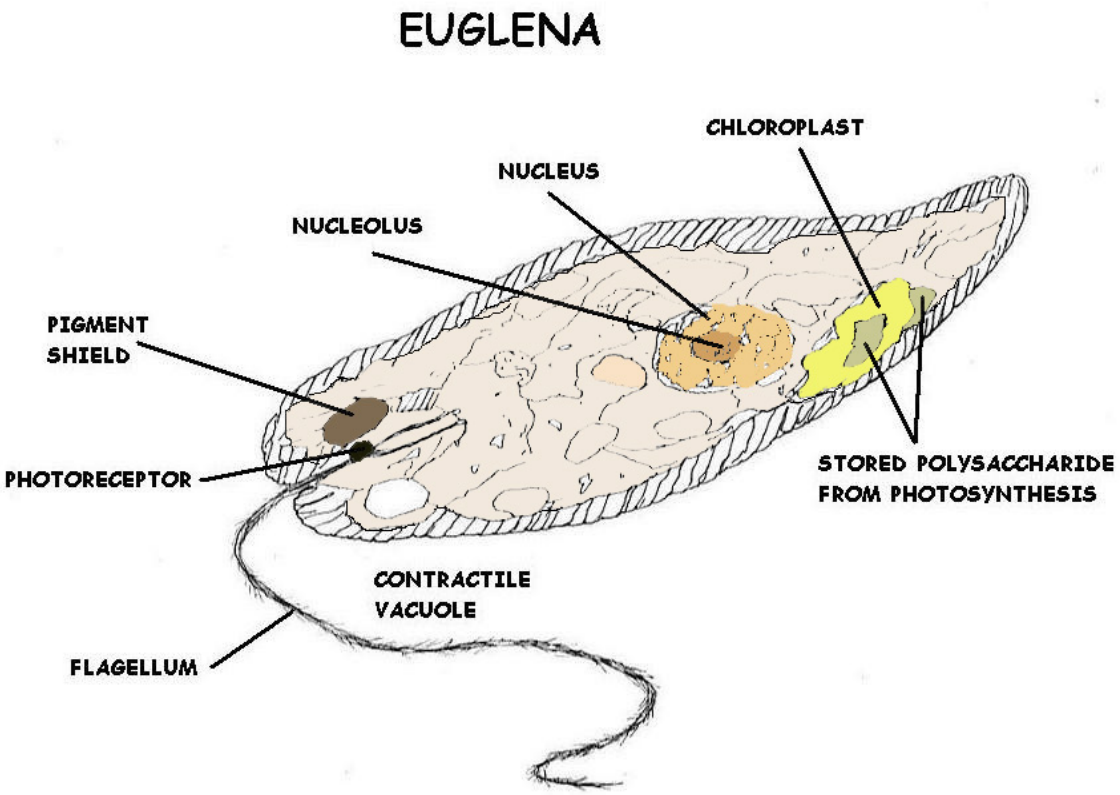
Co-infection of the same cell with different related viruses allows for genetic re-assortment (exchange of genome segments) and intramolecular recombination. Genetic alterations can alter virulence or allow viruses to gain access to new cell types or new animal hosts.

Many scientists believe that HIV is derived from a closely related monkey virus, SIV (simian immunodeficiency virus), that acquired the ability to infect humans. Many of today's emerging viruses may have similar histories.

Protozoa Section

The diverse assemblage of organisms that carry out all of their life functions within the confines of a single, complex eukaryotic cell are called protozoa.

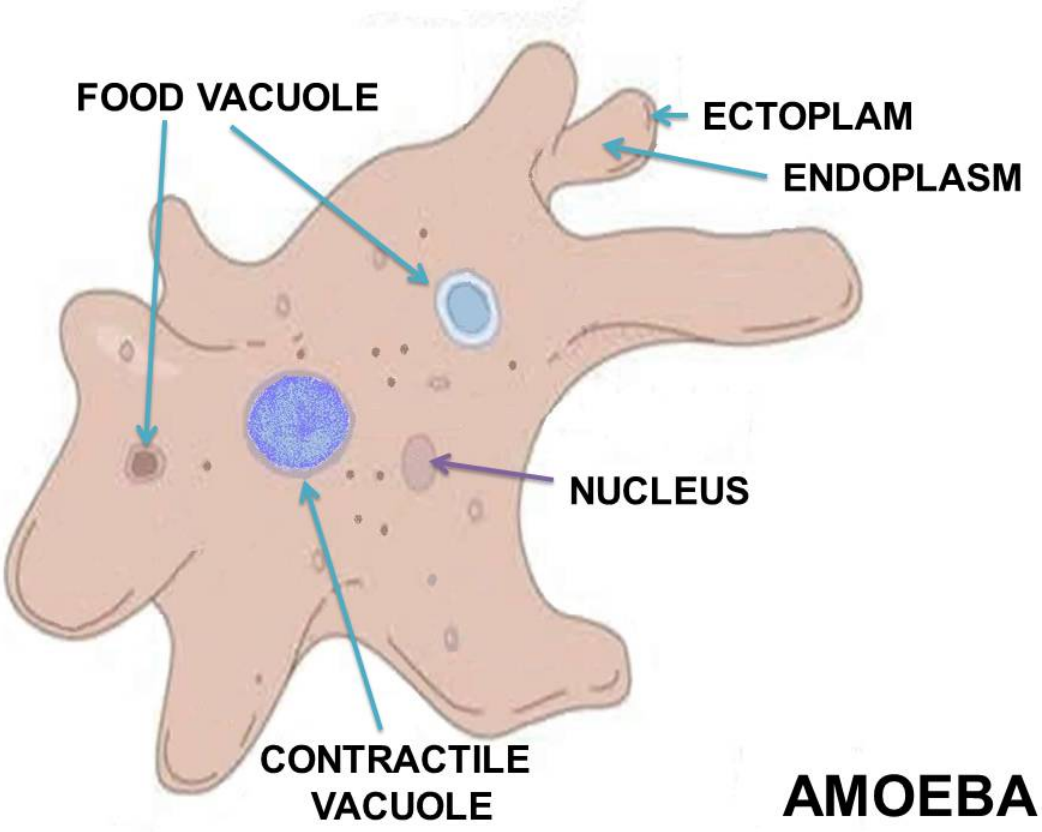
Paramecium, Euglena, and Amoeba are well-known examples of these major groups of organisms. Some protozoa are more closely related to animals, others to plants, and still others are relatively unique. Although it is not appropriate to group them together into a single taxonomic category, the research tools used to study any unicellular organism are usually the same, and the field of protozoology has been created to carry out this research. The unicellular photosynthetic protozoa are sometimes also called algae and are addressed elsewhere. This report considers the status of our knowledge of heterotrophic protozoa (protozoa that cannot produce their own food).

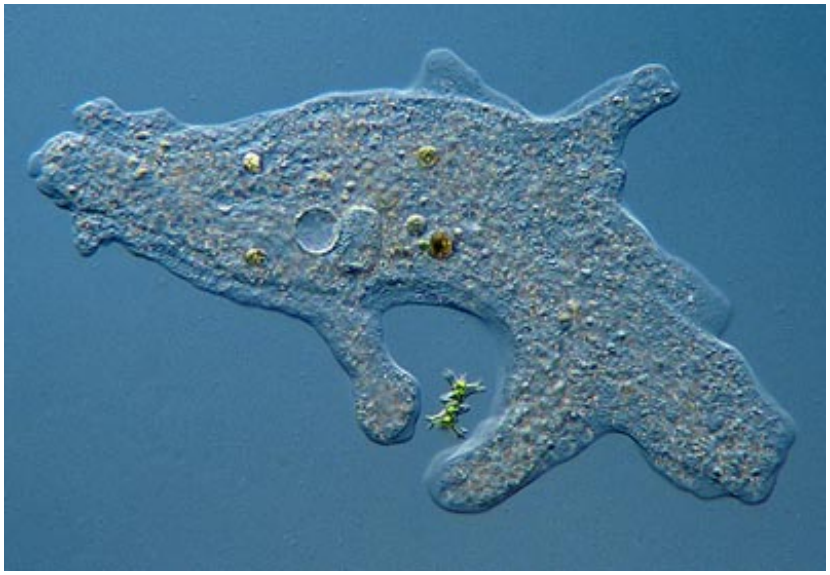


Free-living Protozoa

Protozoans are found in all moist habitats within the United States, but we know little about their specific geographic distribution. Because of their small size, production of resistant cysts, and ease of distribution from one place to another, many species appear to be cosmopolitan and may be collected in similar microhabitats worldwide (Cairns and Ruthven 1972). Other species may have relatively narrow limits to their distribution.

Marine ciliates inhabit interstices of sediment and beach sands, surfaces, deep sea and cold Antarctic environments, planktonic habitats, and the algal mats and detritus of estuaries and wetlands.





Amoeba proteus, pseudopods slowly engulf the small desmid *Staurastrum.*

Amoebas

Amoebas (Phylum Rhizopoda) are unicellular protists that are able to change their shape constantly. Each species has its own distinct repertoire of shapes.

How does an amoeba locomote?
Amoebas locomote by way of cytoplasmic movement. (cytoplasm is the cell content around the nucleus of the cell) The amoeba forms pseudopods (false feet) with which they 'flow' over a surface. The cytoplasma not only flows, it also changes from a fluid into a solid state.

These pseudopods are also used to capture prey, they simply engulf the food. They can detect the kind of prey and use different 'engulfing tactics'.

The image from the last page shows several cell organelles. Left from the center we can see aspherical water expelling vesicle and just right of it, the single nucleus of this species can be seen. Other species may have many nuclei. The cell is full of brown food vacuoles and also contains small crystals.

Protozoa Information
Our actual knowledge of salinity, temperature, and oxygen requirements of marine protozoa is poor (although some groups, such as the foraminifera, are better studied than others), and even the broadest outlines of their biogeographic ranges are usually a mystery. In general, freshwater protozoan communities are similar to marine communities except the specialized interstitial fauna of the sand is largely missing. In freshwater habitats, the foraminifera and radiolaria common in marine environments are absent or low in numbers while testate amoebae exist in greater numbers. Relative abundance of species in the marine versus freshwater habitat is unknown.

Soil-dwelling protozoa have been documented from almost every type of soil and in every kind of environment, from the peat-rich soil of bogs to the dry sands of deserts. In general, protozoa are found in greatest abundance near the soil surface, especially in the upper 15 cm (6 in), but occasional isolates can be obtained at depths of a meter (yard) or more.

Protozoa do not constitute a major part of soil biomass, but in some highly productive regions such as forest litter, the protozoa are a significant food source for the microinvertebrates, with a biomass that may reach 20 g/m2 of soil surface area there.

Environmental Quality Indicators
Polluted waters often have a rich and characteristic protozoan fauna. The relative abundance and diversity of protozoa are used as indicators of organic and toxic pollution (Cairns et al. 1972; Foissner 1987; Niederlehner et al. 1990; Curds 1992). Bick (1972), for example, provided a guide to ciliates that are useful as indicators of environmental quality of European freshwater systems, along with their ecological distribution with respect to parameters such as amount of organic material and oxygen levels.

Foissner (1988) clarified the taxonomy of European ciliates as part of a system for classifying the state of aquatic habitats according to their faunas.

Symbiotic Protozoa
Parasites
Protozoa are infamous for their role in causing disease, and parasitic species are among the best-known protozoa. Nevertheless, our knowledge has large gaps, especially of normally free-living protozoa that may become pathogenic in immunocompromised individuals. For example, microsporidia comprise a unique group of obligate, intracellular parasitic protozoa. Microsporidia are amazingly diverse organisms with more than 700 species and 80 genera that are capable of infecting a variety of plant, animal, and even other protist hosts.

They are found worldwide and have the ability to thrive in many ecological conditions. Until the past few years, their ubiquity did not cause a threat to human health, and few systematists worked to describe and classify the species. Since 1985, however, physicians have documented an unusual rise in worldwide infections in AIDS patients caused by four different genera of microsporidia (Encephalitozoon, Nosema, Pleistophora, and Enterocytozoon). According to the Centers for Disease Control in the United States, difficulties in identifying microsporidian species are impeding diagnosis and effective treatment of AIDS patients.

Protozoan Reservoirs of Disease
The presence of bacteria in the cytoplasm of protozoa is well known, whereas that of viruses is less frequently reported. Most of these reports simply record the presence of bacteria or viruses and assume some sort of symbiotic relationship between them and the protozoa. Recently, however, certain human pathogens were shown to not only survive but also to multiply in the cytoplasm of free-living, nonpathogenic protozoa. Indeed, it is now believed that protozoa are the natural habitat for certain pathogenic bacteria. To date, the main focus of attention has been on the bacterium Legionella pneumophila, the causative organism of Legionnaires' disease; these bacteria live and reproduce in the cytoplasm of some free-living amoebae (Curds 1992). More on this subject in the following chapters.

Symbionts
Some protozoa are harmless or even beneficial symbionts. A bewildering array of ciliates, for example, inhabit the rumen and reticulum of ruminates and the cecum and colon of equids. Little is known about the relationship of the ciliates to their host, but a few may aid the animal in digesting cellulose.

Data on Protozoa
While our knowledge of recent and fossil foraminifera in the U.S. coastal waterways is systematically growing, other free-living protozoa are poorly known. There are some regional guides and, while some are excellent, many are limited in scope, vague on specifics, or difficult to use. Largely because of these problems, most ecologists who include protozoa in their studies of aquatic habitats do not identify them, even if they do count and measure them for biomass estimates (Taylor and Sanders 1991).

Parasitic protozoa of humans, domestic animals, and wildlife are better known although no attempt has been made to compile this information into a single source. Large gaps in our knowledge exist, especially for haemogregarines, microsporidians, and myxosporidians (see Kreier and Baker 1987).

Museum Specimens
For many plant and animal taxa, museums represent a massive information resource. This is not true for protozoa. In the United States, only the National Natural History Museum (Smithsonian Institution) has a reference collection preserved on microscope slides, but it does not have a protozoologist curator and cannot provide species' identification or verification services. The American Type Culture Collection has some protozoa in culture, but its collection includes relatively few kinds of protozoa.

Ecological Role of Protozoa
Although protozoa are frequently overlooked, they play an important role in many communities where they occupy a range of trophic levels. As predators upon unicellular or filamentous algae, bacteria, and microfungi, protozoa play a role both as herbivores and as consumers in the decomposer link of the food chain. As components of the micro- and meiofauna, protozoa are an important food source for microinvertebrates. Thus, the ecological role of protozoa in the transfer of bacterial and algal production to successive trophic levels is important.

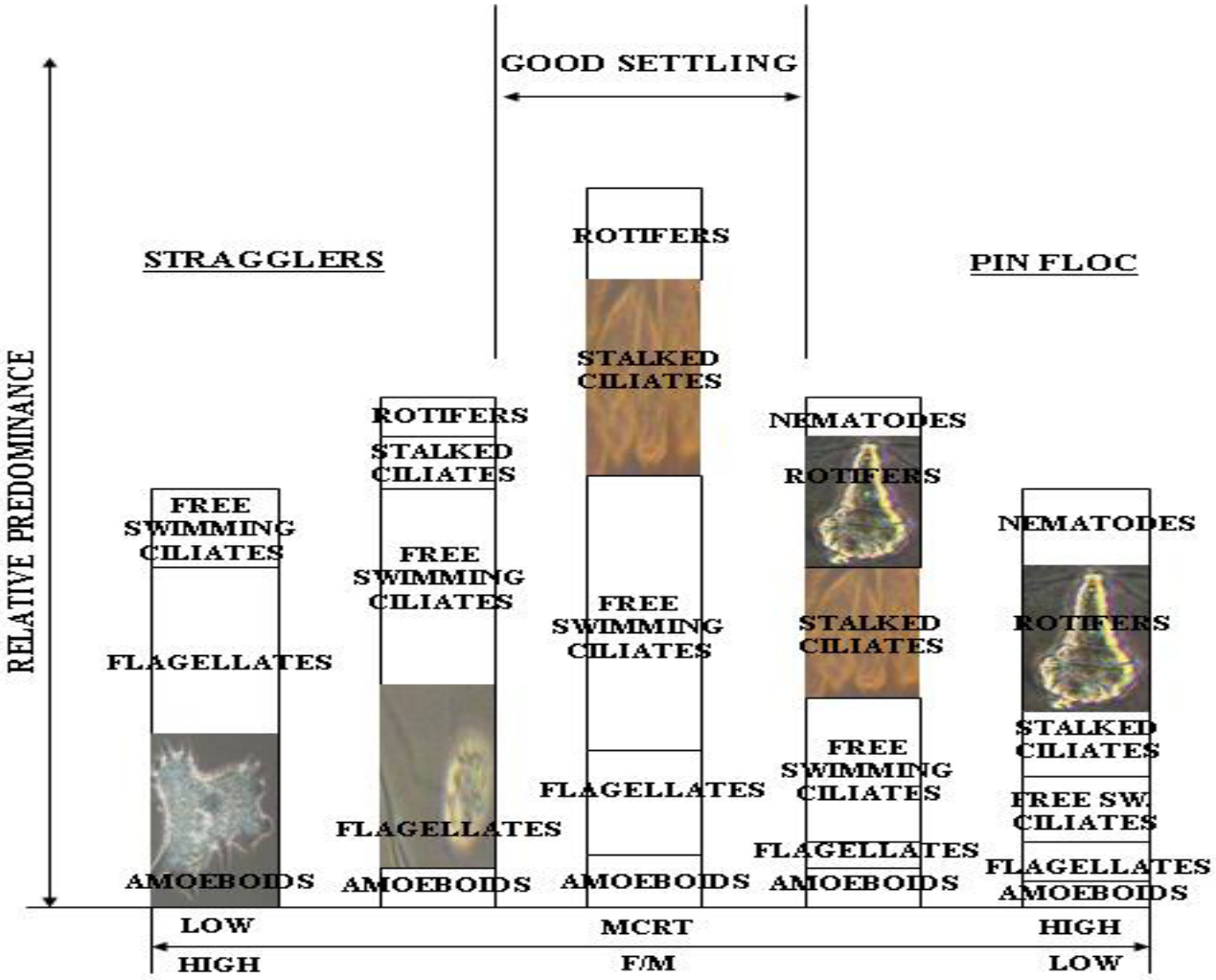
Factors Affecting Growth and Distribution
Most free-living protozoa reproduce by cell division (exchange of genetic material is a separate process and is not involved in reproduction in protozoa). The relative importance for population growth of biotic versus chemical-physical components of the environment is difficult to ascertain from the existing survey data. Protozoa are found living actively in nutrient-poor to organically rich waters and in fresh water varying between 0°C (32°F) and 50°C (122°F). Nonetheless, it appears that rates of population growth increase when food is not constrained and temperature is increased (Lee and Fenchel 1972; Fenchel 1974; Montagnes et al. 1988).

Comparisons of oxygen consumption in various taxonomic groups show wide variation (Laybourn and Finlay 1976), with some aerobic forms able to function at extremely low oxygen tensions and to thereby avoid competition and predation.

Many parasitic and a few free-living species are obligatory anaerobes (grow without atmospheric oxygen). Of the free-living forms, the best known are the plagiopylid ciliates that live in the anaerobic sulfide-rich sediments of marine wetlands (Fenchel et al. 1977). The importance of plagiopylids in recycling nutrients to aerobic zones of wetlands is potentially great.

Because of the small size of protozoa, their short generation time, and (for some species) ease of maintaining them in the laboratory, ecologists have used protozoan populations and communities to investigate competition and predation.

The result has been an extensive literature on a few species studied primarily under laboratory conditions. Few studies have been extended to natural habitats with the result that we know relatively little about most protozoa and their roles in natural communities. Intraspecific competition for common resources often results in cannibalism, sometimes with dramatic changes in morphology of the cannibals (Giese 1973). Field studies of interspecific competition are few and most evidence for such species interactions is indirect (Cairns and Yongue 1977).



Commonly found organisms that are present in wastewater treatment.

Wastewater Treatment Biology

Four (4) groups of bugs do most of the ***"eating"*** in the activated sludge process. The first group is the bacteria which eat the dissolved organic compounds. The second and third groups of bugs are microorganisms known as the free-swimming and stalked ciliates. These larger bugs eat the bacteria and are heavy enough to settle by gravity. The fourth group is a microorganism, known as Suctoria, which feed on the larger bugs and assist with settling.

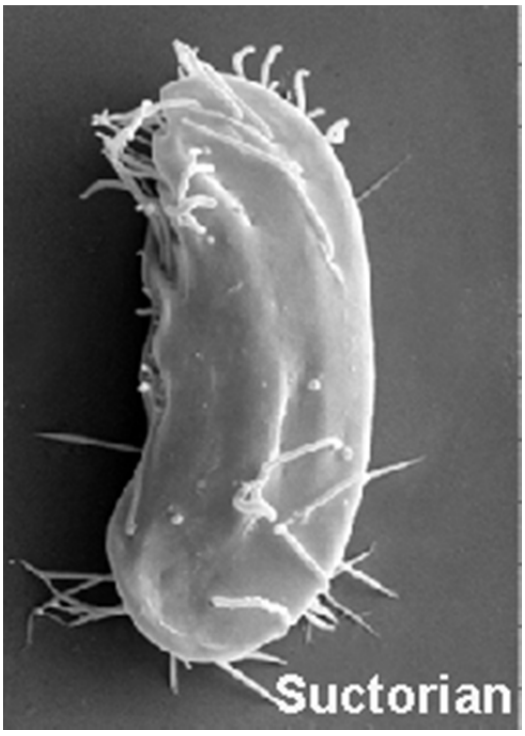
The interesting thing about the bacteria that eat the dissolved organics is that they have no mouth. The bacteria have an interesting property; their "fat reserve" is stored on the outside of their body. This fat layer is sticky and is what the organics adhere to.

Once the bacteria have "**contacted**" their food, they start the digestion process. A chemical enzyme is sent out through the cell wall to break up the organic compounds. This enzyme, known as hydrolytic enzyme, breaks the organic molecules into small units which are able to pass through the cell wall of the bacteria.

In wastewater treatment, this process of using bacteria-eating-bugs in the presence of oxygen to reduce the organics in water is called activated sludge. The first step in the process, the contact of the bacteria with the organic compounds, takes about 20 minutes. The second step is the breaking up, ingestion and digestion processes, which takes four (4) to 24 hours.

The fat storage property of the bacteria is also an asset in settling. As the bugs "bump" into each other, the fat on each of them sticks together and causes flocculation of the non-organic solids and biomass.

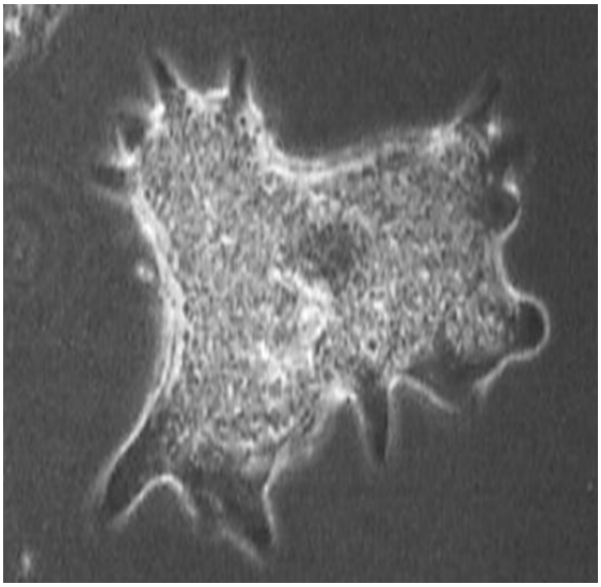
From the aeration tank, the wastewater, now called mixed liquor, flows to a secondary clarification basin to allow the flocculated biomass of solids to settle out of the water. The solids biomass, which is the activated sludge, contains millions of bacteria and other microorganisms, is used again by returning it to the influent of the aeration tank for mixing with the primary effluent and ample amounts of air.



Urostyla or Euplotes

Wastewater Treatment Microlife

Euglypha sp.

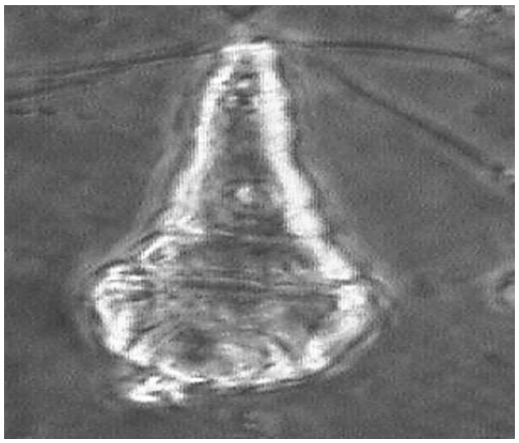


Euglypha (70-100 æm) is a shelled (testate) amoeba. Amoebas have jelly-like bodies. Motion occurs by extending a portion of the body (pseudopodia) outward. Shelled amoebas have a rigid covering which is either secreted or built from sand grains or other extraneous materials. The secreted shell of this Euglypha sp. consists of about 150 oval plates. Its spines project backward from the lower half of the shell. Euglypha spines may be single or in groups of two or three. The shell has an opening surrounded by 8-11 plates that resemble shark teeth under very high magnification.

The shell of Euglypha is often transparent, allowing the hyaline (watery) body to be seen inside the shell. The pseudopodia extend outward in long, thin, rays when feeding or moving. Euglypha primarily eats bacteria. Indicator: Shelled amoebas are common in soil, treatment plants, and stream bottoms where decaying organic matter is present. They adapt to a wide range of conditions and therefore are not good indicator organisms.

Euchlanis sp.

This microscopic animal is a typical rotifer. Euchlanis is a swimmer, using its foot and cilia for locomotion. In common with other rotifers, it has a head rimmed with cilia, a transparent body, and a foot with two strong swimming toes.



The head area, called the "corona," has cilia that beat rhythmically, producing a strong current for feeding or swimming. Euchlanis is an omnivore, meaning that its varied diet includes detritus, bacteria, and small protozoa. Euchlanis has a glassy shell secreted by its outer skin. The transparent body reveals the brain, stomach, intestines, bladder, and reproductive organs.

A characteristic of rotifers is their mastax, which is a jaw-like device that grinds food as it enters the stomach. At times the action of the mastax resembles the pulsing action of a heart. Rotifers, however, have no circulatory system. Indicator: Euchlanis is commonly found in activated sludge when effluent quality is good. It requires a continual supply of dissolved oxygen, evidence that aerobic conditions have been sustained.

Protozoan Diseases

Protozoan pathogens are larger than bacteria and viruses but still microscopic. They invade and inhabit the gastrointestinal tract. Some parasites enter the environment in a dormant form, with a protective cell wall, called a "***cyst***." The cyst can survive in the environment for long periods of time and be extremely resistant to conventional disinfectants such as chlorine. Effective filtration treatment is therefore critical to removing these organisms from water sources.

Giardiasis
Giardiasis is a commonly reported protozoan-caused disease. It has also been referred to as "***backpacker's disease***" and "***beaver fever***" because of the many cases reported among hikers and others who consume untreated surface water. Symptoms include chronic diarrhea, abdominal cramps, bloating, frequent loose and pale greasy stools, fatigue and weight loss. The incubation period is 5-25 days or longer, with an average of 7-10 days. Many infections are asymptomatic (no symptoms). Giardiasis occurs worldwide. Waterborne outbreaks in the United States occur most often in communities receiving their drinking water from streams or rivers without adequate disinfection or a filtration system. The organism, ***Giardia lamblia,*** has been responsible for more community-wide outbreaks of disease in the U.S. than any other pathogen. Drugs are available for treatment but are not 100% effective.

Cryptosporidiosis
Cryptosporidiosis is an example of a protozoan disease that is common worldwide, but was only recently recognized as causing human disease. The major symptom in humans is diarrhea, which may be profuse and watery. The diarrhea is associated with cramping abdominal pain. General malaise, fever, anorexia, nausea, and vomiting occur less often. Symptoms usually come and go, and end in fewer than 30 days in most cases. The incubation period is 1-12 days, with an average of about seven days. ***Cryptosporidium*** organisms have been identified in human fecal specimens from more than 50 countries on six continents. The mode of transmission is fecal-oral, either by person-to-person or animal-to-person. There is no specific treatment for ***Cryptosporidium*** infections.

All of these diseases, with the exception of hepatitis A, have one symptom in common: diarrhea. They also have the same mode of transmission, fecal-oral, whether through person-to-person or animal-to-person contact, and the same routes of transmission, being either foodborne or waterborne. Although most pathogens cause mild, self-limiting disease, on occasion, they can cause serious, even life threatening illness. Particularly vulnerable are persons with weak immune systems, such as those with HIV infections or cancer. By understanding the nature of waterborne diseases, the importance of properly constructed, operated and maintained public water systems becomes obvious. While water treatment cannot achieve sterile water (no microorganisms), the goal of treatment must clearly be to produce drinking water that is as pathogen-free as possible at all times. For those who operate water systems with inadequate source protection or treatment facilities, the potential risk of a waterborne disease outbreak is real. For those operating systems that currently provide adequate source protection and treatment, operating and maintaining the system at a high level on a continuing basis is critical to prevent disease.

Summary of Common Waterborne Diseases

Name Causative organism Source of organism Disease

Viral gastroenteritis ***Rotavirus*** mostly in young children; Human feces; Diarrhea or vomiting.

Norwalk-like viruses Human feces; also, shellfish; lives in polluted waters; Diarrhea and vomiting.

Salmonellosis ***Salmonella*** (bacterium) Animal or human feces; Diarrhea or vomiting.

Escherichia coli-- E. coli O157:H7 (bacterium) Other *E. coli* organisms. Human feces; Symptoms vary with type caused; gastroenteritis

Typhoid ***Salmonella typhi*** (bacterium) Human feces, urine Inflamed intestine, enlarged spleen, high temperature— sometimes fatal.

Shigellosis ***Shigella*** (bacterium) Human feces Diarrhea.

Cholera ***Vibrio cholerae*** (bacterium) Human feces; also, shellfish; lives in many coastal waters; Vomiting, severe diarrhea, rapid dehydration, mineral loss —high mortality.

Hepatitis A virus Human feces; shellfish grown in polluted waters; Yellowed skin, enlarged liver, fever, vomiting, weight loss, abdominal pain — low mortality, lasts up to four months.

Amebiasis ***Entamoeba histolytica*** Human feces; Mild diarrhea, dysentery, (protozoan) extra intestinal infection.

Giardiasis ***Giardia lamblia*** (protozoan) Animal or human feces; Diarrhea, cramps, nausea, and general weakness — lasts one week to months.

Cryptosporidiosis ***Cryptosporidium parvum*** (protozoan) Animal or human feces Diarrhea, stomach pain — lasts days to weeks.

Source: Adapted from American Water Works Association, *Introduction to Water Treatment: Principles and Practices of Water Supply Operation*s, Denver CO, 1984.

The Best Method to kill most of these Bugs

Disinfection is usually synonymous with chlorination. That is because chlorine addition is by far the most common form of disinfection used today. In this section, the main emphasis will be on chlorination: how it works, safety, types of chlorine, basic chemistry of chlorine and an introduction to **CT** values. Disinfection is the process of killing microorganisms in water that might cause disease (pathogens). Disinfection, however, should not be confused with sterilization, which is the destruction of all microorganisms. Disinfection is concerned only with killing pathogens. Cryptosporidium parvum and Giardia lamblia will require proper water treatment techniques.

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"YOUR MOM DOES NOT WORK HERE"
MLSS
CHOC MILK
UH, WHERE DID YOU SAY YOU GOT THAT CHOCOLATE MILK?

Chapter 1 Review

Example Question, fill-in-the blank with one correct answer.
_____ is usually credited for dispelling the notion of spontaneous generation and proving that organisms reproduce new organisms.
A. Louis Pasteur
B. Martinus Beijerinck
C. Dimitri Ivanofsky
D. Adolf Mayer
E. Robert Koch

Review, you can check your answers at the end of this section.
1. The German scientist _____, a student of Jacob Henle, and the British surgeon Joseph Lister developed techniques for growing cultures of single organisms that allowed the assignment of specific bacteria to specific diseases.
A. Louis Pasteur
B. Martinus Beijerinck
C. Dimitri Ivanofsky
D. Adolf Mayer
E. Robert Koch

2. The first experimental transmission of a viral infection was accomplished in about 1880 by the German scientist _____, when he demonstrated that extracts from infected tobacco leaves could transfer tobacco mosaic disease to a new plant, causing spots on the leaves.
A. Louis Pasteur
B. Martinus Beijerinck
C. Dimitri Ivanofsky
D. Adolf Mayer
E. Robert Koch

3. Because _____ was unable to isolate a bacterium or fungus from the tobacco leaf extracts, he considered the idea that tobacco mosaic disease might be caused by a soluble agent, but he concluded incorrectly that a new type of bacteria was likely to be the cause.
A. Louis Pasteur
B. Martinus Beijerinck
C. Dimitri Ivanofsky
D. Adolf Mayer
E. Robert Koch

4. The Russian scientist _____ extended Mayer's observation and reported in 1892 that the tobacco mosaic agent was small enough to pass through a porcelain filter known to block the passage of bacteria.
A. Louis Pasteur
B. Martinus Beijerinck
C. Dimitri Ivanofsky
D. Adolf Mayer
E. Robert Koch

5. In 1917 the French-Canadian scientist _____ discovered that viruses of bacteria, which he named bacteriophage, could make holes in a culture of bacteria.
A. Louis Pasteur
B. Robert Koch
C. Félix H. d'Hérelle
D. Wendell Meredith Stanley
E. Walter Reed

6. In 1935 the American biochemist _____ crystallized tobacco mosaic virus to demonstrate that viruses had regular shapes, and in 1939 tobacco mosaic virus was first visualized using the electron microscope.
A. Louis Pasteur
B. Robert Koch
C. Félix H. d'Hérelle
D. Wendell Meredith Stanley
E. Walter Reed

7. In 1898 the German bacteriologists Friedrich August Johannes Löffler and Paul F. Frosch (both trained by _____) described foot-and-mouth disease virus as the first filterable agent of animals.
A. Louis Pasteur
B. Robert Koch
C. Félix H. d'Hérelle
D. Wendell Meredith Stanley
E. Walter Reed

8. In 1900, the American bacteriologist _____ and colleagues recognized yellow fever virus as the first human filterable agent.
A. Louis Pasteur
B. Robert Koch
C. Félix H. d'Hérelle
D. Wendell Meredith Stanley
E. Walter Reed

9. For several decades viruses were referred to as filterable agents, and gradually the term virus (Latin for "_____" or "poison") was employed strictly for this new class of infectious agents.
A. Slimy liquid
B. Bacteriophages
C. Cell culture systems
D. Microorganism
E. Germ theory

10. Through the 1940s and 1950s many critical discoveries were made about viruses through the study of _____ because of the ease with which the bacteria they infect could be grown in the laboratory.
A. Slimy liquid
B. Bacteriophages
C. Cell culture systems
D. Microorganism
E. Germ theory

11. Between 1948 and 1955, scientists at the National Institutes of Health (NIH) and at Johns Hopkins Medical Institutions revolutionized the study of animal viruses by developing _____ that permitted the growth and study of many animal viruses in laboratory dishes.
A. Slimy liquid
B. Bacteriophages
C. Cell culture systems
D. Microorganism
E. Germ theory

12. Louis Pasteur along with _____ developed the germ theory of disease which states that "a specific disease is caused by a specific type of microorganism."
A. Robert Koch
B. Matthias Schleiden
C. Rudolph Virchow
D. Thedore Schwann
E. Robert Hooke

13. In 1876, _____ established an experimental procedure to prove the germ theory of disease. This scientific procedure is known as Koch's postulates.
A. Robert Koch
B. Matthias Schleiden
C. Rudolph Virchow
D. Thedore Schwann
E. Robert Hooke

14. _____ postulates not only proved the germ theory but also gave a tremendous boost to the development of microbiology by stressing a laboratory culture and identification of microorganisms.
A. Robert Koch
B. Matthias Schleiden
C. Rudolph Virchow
D. Thedore Schwann
E. Robert Hooke

15. _____ observed small empty chambers in the structure of cork with the help of his crude microscope. He called them cells.
A. Robert Koch
B. Matthias Schleiden
C. Rudolph Virchow
D. Thedore Schwann
E. Robert Hooke

16. Two German biologists _____ and Thedore Schwann proposed the "Cell theory' in 1838. According to this theory, all living things are composed of cells.
A. Robert Koch
B. Matthias Schleiden
C. Rudolph Virchow
D. Thedore Schwann
E. Robert Hooke

17. _____ completed the cell theory with the idea that all cells must arise from preexisting cells.
A. Robert Koch
B. Matthias Schleiden
C. Rudolph Virchow
D. Thedore Schwann
E. Robert Hooke

18. There's even a species of _____—Deinococcus radiodurans—that can withstand blasts of radiation 1,000 times greater than would kill a human being.
A. Metabolism
B. Cell
C. Mutation
D. Bacteria
E. None of the above

19. "Bacteria" is a singular word. The plural for this word is "bacterium" (**bacter** = rod, staff).
A. True
B. False

20. Bacteria are prokaryotes (Kingdom Monera), which means that they have a large nucleus.
A. True
B. False

21. Bacteria do have one chromosome of single-stranded DNA in a ring. They reproduce by binary fission.
A. True
B. False

22. Most bacteria lack or have very few internal membranes, which means that they don't have some kinds of organelles (like mitochondria or chloroplasts). Most bacteria are **benign** (**benign** = good, friendly, kind) or beneficial, and only a few are "bad guys" or **pathogens**.
A. True
B. False

23. Kingdom Monera is a very diverse group. All bacteria relatives can do photosynthesis—because they have chloroplasts, because chlorophyll and other needed chemicals are built into their cell membranes.
A. True
B. False

24. These organisms are called **Cyanobacteria** (**cyano** = blue, dark blue) or bluegreen algae, although they're not really algae (real algae are in Kingdom Protista). Like us, some kinds of bacteria need and do best in O_3, while others are poisoned/killed by it.
A. True
B. False

25. Bacteria fall into a category of life called the Prokaryotes (pro-carry-oats). Prokaryotes' genetic material, or DNA, is not enclosed in a cellular compartment called the nucleus.
A. True
B. False

26. Bacteria and archaea are the only prokaryotes. All other life forms are Eukaryotes (you-carry-oats), creatures whose cells have nuclei.
A. True
B. False

27. The mitochondria (mite-oh-con-dree-uh) that make energy for your body cells is one example of such an organelle.
A. True
B. False

28. There are less than 200 hundred of species of bacteria, but all of them are basically one of five different shapes.
A. True
B. False

29. Some bacteria are rod - or stick-shaped and are called _____.
A. Borrelia
B. Bacilli
C. Cocci
D. Peptidoglycan
E. None of the above

30. Some bacterial cells exist as individuals while others cluster together to form pairs, chains, squares or other _____.
A. Borrelia
B. Bacilli
C. Cocci
D. Peptidoglycan
E. None of the above

31. Most bacteria secrete a covering for themselves which we call a **cell wall**.
A. True
B. False

32. Bacterial cell walls do contain cellulose like plant cell walls do.
A. True
B. False

33. Bacterial cell walls are made mostly of a chemical called **peptidoglycan** (made of polypeptides bonded to modified sugars), but the amount and location of the peptidoglycan are different in the two possible types of cell walls, depending on the species of bacterium.
A. True
B. False

34. All antibiotics, like penicillin, prohibit the formation of the chemical cross linkages needed to make peptidoglycan.
A. True
B. False

35. That's why antibiotics must typically be taken for ten days until the bacteria, unable to grow, die of "old age". If a person stops taking the antibiotic sooner, any living bacteria could start making peptidoglycan, grow, and reproduce.
A. True
B. False

36. However, because one of the two possible types of bacterial cell walls has more peptidoglycan than the other, antibiotics like penicillin are more effective against bacteria with that type of cell wall and less effective against bacteria with less peptidoglycan in their cell walls.
A. True
B. False

37. Thus it is important, before beginning antibiotic treatment, to determine with which of the two types of bacteria one is dealing. Dr. Hans Christian Gram, a Danish physician, invented a staining process to tell these two types of bacteria apart, and in his honor, this process is called **Hetertropic Gram Count**.
A. True
B. False

38. In this process, the amount of cellulose in the cell walls of the bacteria under study will determine how those bacteria absorb the dyes with which they are stained, thus bacterial cells can be $Gram^{+}$ or $Gram^{-}$.
A. True
B. False

39. $Gram^{+}$ bacteria have simpler cell walls with lots of peptidoglycan, and stain a dark purple color.
A. True
B. False

40. Human cells have 46 (23 pairs) chromosomes. Each _____ consists of many genes.
A. Golgi complex
B. Nucleus
C. Chromosome
D. DNA
E. Genes

41. A gene is a coiled unit made up of _____ and proteins that codes for, or determines, a particular characteristic of an individual organism.
A. Golgi complex
B. Nucleus
C. Chromosome
D. DNA
E. Genes

42. The cellular material outside the _____; composed of a semifluid gelatinous nutrient matrix and cytoplasmic organelles including endoplasmic reticulum, ribosomes, Golgi complex, mitochondria, centrioles, microtubules, lysosomes and vacoules.
A. Golgi complex
B. Nucleus
C. Chromosome
D. DNA
E. Genes

43. Some eukaryotic cells possess relatively long and thin structures called _____. These are organs of locomotion.
A. Flagella
B. Cilia
C. Prokaryotes
D. Cytoplasm
E. Chromosome

44. _____ are also organs of locomotion but are shorter and more numerous.
A. Flagella
B. Cilia
C. Prokaryotes
D. Cytoplasm
E. Chromosome

45. All bacteria are _____.
A. Flagella
B. Cilia
C. Prokaryotes
D. Cytoplasm
E. Chromosome

46. The _____ of a procaryotic cell is not surrounded by a nuclear membrane, it has no definite shape and no protein material associated with it. It usually consists of a single circular DNA molecule and serves as the control center of the bacterial cell.
A. Flagella
B. Cilia
C. Prokaryotes
D. Cytoplasm
E. Chromosome

47. A typical bacterial _____ contains approximately 10,000 genes.
A. Flagella
B. Cilia
C. Prokaryotes
D. Cytoplasm
E. Chromosome

48. Semi-liquid, surrounds the chromosome and is contained within the plasma membrane. Within the _____ are located several ribosomes-which are the sites of protein synthesis.
A. Flagella
B. Cilia
C. Prokaryotes
D. Cytoplasm
E. Chromosome

49. Eukaryotes are organisms with complex cells, in which the genetic material is organized into membrane-bound nuclei.
A. True
B. False

50. The eukaryotes share a common origin, and are often treated formally as a superkingdom, empire, or domain.
A. True
B. False

51. The name "eukaryotes" comes from the Greek ***eus*** or true and ***karyon*** or nut, referring to the nucleus.
A. True
B. False

52. Protists are eukaryotes because they all have a nucleus.
A. True
B. False

53. Protists have mitochondria, although some have later lost theirs. _____ were derived from aerobic alpha-proteobacteria (prokaryotes) that once lived within their cells.
A. Vacuoles
B. Mitochondria
C. Prokaryotes
D. Microtubules
E. Chloroplasts

54. Many Protists have chloroplasts with which they carry on photosynthesis. _____ were derived from photosynthetic cyanobacteria (also prokaryotes) living within their cells.
A. Vacuoles
B. Mitochondria
C. Prokaryotes
D. Microtubules
E. Chloroplasts

55. Eukaryotic cells are generally much larger than _____, typically with a thousand times their volumes.
A. Vacuoles
B. Mitochondria
C. Prokaryotes
D. Microtubules
E. Chloroplasts

56. Eukaryotic cells have a variety of internal membranes and structures, called organelles, and a cytoskeleton composed of _____ and microfilaments, which plays an important role in defining the cell's organization.
A. Vacuoles
B. Mitochondria
C. Prokaryotes
D. Microtubules
E. Chloroplasts

57. Eukaryotic DNA is divided into several bundles called chromosomes, which are separated by a microtubular spindle during nuclear division. In addition to asexual cell division, most eukaryotes have some process of sexual reproduction via cell fusion, which is not found among _____.
A. Vacuoles
B. Mitochondria
C. Prokaryotes
D. Microtubules
E. Chloroplasts

58. Eukaryotic cells include a variety of membrane-bound structures, collectively referred to as the endomembrane system. Simple compartments, called vesicles or _____, can form by budding off of other membranes.
A. Vacuoles
B. Mitochondria
C. Prokaryotes
D. Microtubules
E. Chloroplasts

1.E, 2.D, 3.D, 4.C, 5.C, 6.D, 7.B, 8.E, 9.A, 10.B, 11.C, 12.A, 13.A, 14.A, 15.E, 16.B, 17.C, 18.D, 19.B, 20.B, 21.B, 22.A, 23.B, 24.B, 25.A, 26.A, 27.A, 28.B, 29.B, 30. E, 31.A, 32.B, 33.A, 34.B, 35.A, 36.A, 37.B, 38.B, 39.A, 40.C, 41.D, 42.B, 43.A, 44.B, 45.C, 46.E, 47.E, 48.D, 49.A, 50.A, 51.A, 52.A, 53.B, 54.E, 55.C, 56.D, 57.C, 58.A

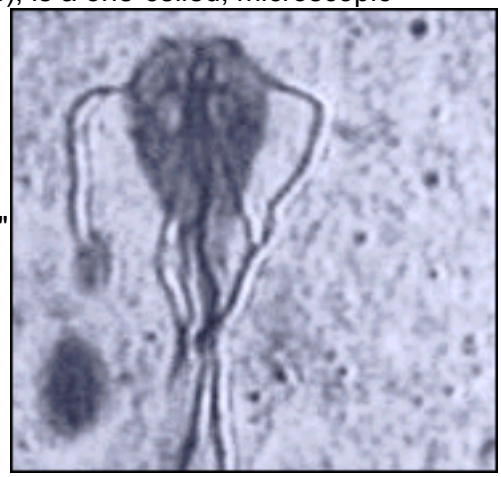
Microorganisms

Contaminant	MCLG[1] (mg/L)[2]	MCL or TT[1] (mg/L)[2]	Potential Health Effects from Ingestion of Water	Sources of Contaminant in Drinking Water
Cryptosporidium	zero	TT [3]	Gastrointestinal illness (e.g., diarrhea, vomiting, cramps)	Human and animal fecal waste
Giardia lamblia	zero	TT[3]	Gastrointestinal illness (e.g., diarrhea, vomiting, cramps)	Human and animal fecal waste
Heterotrophic plate count	n/a	TT[3]	HPC has no health effects; it is an analytic method used to measure the variety of bacteria that are common in water. The lower the concentration of bacteria in drinking water, the better maintained the water system is.	HPC measures a range of bacteria that are naturally present in the environment
Legionella	zero	TT[3]	Legionnaire's Disease, a type of pneumonia	Found naturally in water; multiplies in heating systems
Total Coliforms (including fecal coliform and *E. Coli*)	zero	5.0%[4]	Not a health threat in itself; it is used to indicate whether other potentially harmful bacteria may be present[5]	Coliforms are naturally present in the environment; as well as feces; fecal coliforms and *E. coli* only come from human and animal fecal waste.
Turbidity	n/a	TT[3]	Turbidity is a measure of the cloudiness of water. It is used to indicate water quality and filtration effectiveness (e.g., whether disease-causing organisms are present). Higher turbidity levels are often associated with higher levels of disease-causing microorganisms such as viruses, parasites and some bacteria. These organisms can cause symptoms such as nausea, cramps, diarrhea, and associated headaches.	Soil runoff
Viruses (enteric)	zero	TT[3]	Gastrointestinal illness (e.g., diarrhea, vomiting, cramps)	Human and animal fecal waste

Giardiasis *Giardia lamblia* Chapter 2

Giardia lamblia (intestinalis) is a single celled animal, i.e., a protozoa, that moves with the aid of five flagella. In Europe, it is sometimes referred to as Lamblia intestinalis.

Giardiasis is the most frequent cause of non-bacterial diarrhea in North America. *Giardia duodenalis*, cause of giardiasis (GEE-are-DYE-uh-sis), is a one-celled, microscopic parasite that can live in the intestines of animals and people. It is found in every region throughout the world and has become recognized as one of the most common causes of waterborne (and occasionally foodborne) illness often referred to as "Beaver Fever." It is commonly known as "traveler's diarrhea", and referred to as "Montezuma's Revenge" by those who travel to third world countries in the Western Hemisphere.

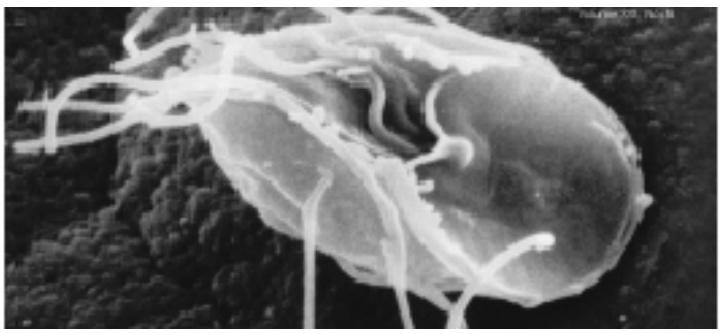


Approximately one week after ingestion of the *Giardia* cysts, prolonged, greasy diarrhea, gas, stomach cramps, fatigue, and weight loss begin.

It is possible to experience some, not all, of the symptoms, yet still shed cysts and pass the parasite onto others. Typically, the disease runs its course in a week or two, although in some cases, the disease may linger for months, causing severe illness and weight loss. Nonetheless, the basic biology of this parasite--including how it ravages the digestive tract--is poorly understood.

The organism exists in two different forms--a hardy, dormant cyst that contaminates water or food and an active, disease-causing form that emerges after the parasite is ingested. National Institute of General Medical Sciences grantee Dr. Frances Gillin of the University of California, San Diego and her colleagues cultivated the entire life cycle of this parasite in the lab and identified biochemical cues in the host's digestive system that trigger Giardia's life cycle transformations. They also uncovered several tricks the parasite uses to evade the defenses of the infected organism. One of Giardia's techniques is to alter the proteins on its surface, which confounds the ability of the infected animal's immune system to detect and combat the parasite. This work reveals why Giardia infections are extremely persistent and prone to recur. In addition, these insights into Giardia's biology and survival techniques may enable scientists to develop better strategies to understand, prevent, and treat Giardia infections.

Recently, Giardia has been found to possess mitochondrial remnants known as 'mitosomes', one assumption is that the condition of amitochondrialism is not primitive to eukaryotes but instead is a result of reductive evolution.



The microaerophilic Giardia uses these mitosomes in the maturation of iron-sulfur proteins rather than in ATP synthesis as is the case in mitochondria-possessing eukaryotes.

Nature of Disease

Organisms that appear identical to those that cause human illness have been isolated from domestic animals (dogs and cats) and wild animals (beavers and bears). A related but morphologically distinct organism infects rodents, although rodents may be infected with human isolates in the laboratory. Human giardiasis may involve diarrhea within 1 week of ingestion of the cyst, which is the environmental survival form and infective stage of the organism.

Normally illness lasts for 1 to 2 weeks, but there are cases of chronic infections lasting months to years. Chronic cases, both those with defined immune deficiencies and those without, are difficult to treat.

The disease mechanism is unknown, with some investigators reporting that the organism produces a toxin while others are unable to confirm its existence. The organism has been demonstrated inside host cells in the duodenum, but most investigators think this is such an infrequent occurrence that it is not responsible for disease symptoms. Mechanical obstruction of the absorptive surface of the intestine has been proposed as a possible pathogenic mechanism, as has a synergistic relationship with some of the intestinal flora.

Giardia can be excysted, cultured and encysted in vitro; new isolates have bacterial, fungal, and viral symbionts. Classically, the disease was diagnosed by demonstration of the organism in stained fecal smears.

Several strains of *G. lamblia* have been isolated and described through analysis of their proteins and DNA; type of strain, however, is not consistently associated with disease severity. Different individuals show various degrees of symptoms when infected with the same strain, and the symptoms of an individual may vary during the course of the disease.

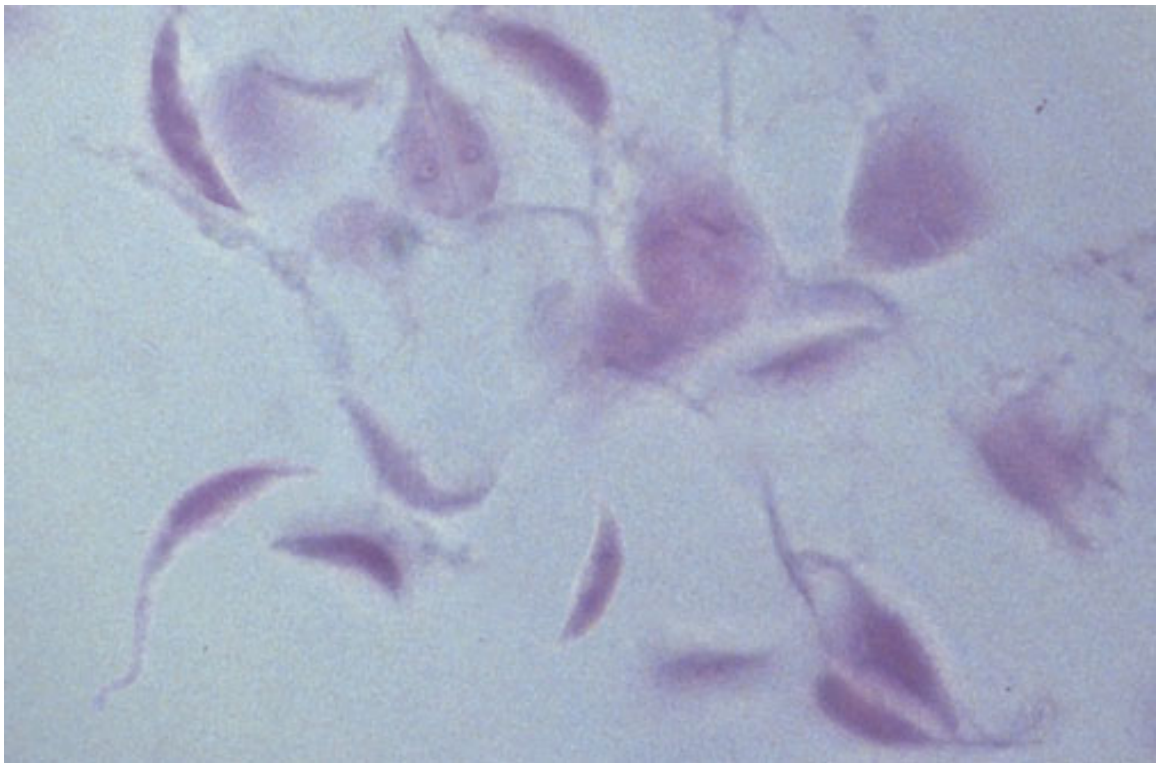
Diagnosis of Human Illness

Giardia lamblia is frequently diagnosed by visualizing the organism, either the trophozoite (active reproducing form) or the cyst (the resting stage that is resistant to adverse environmental conditions) in stained preparations or unstained wet mounts with the aid of a microscope. A commercial fluorescent antibody kit is available to stain the organism. Organisms may be concentrated by sedimentation or flotation; however, these procedures reduce the number of recognizable organisms in the sample. An enzyme linked immunosorbant assay (ELISA) that detects excretory secretory products of the organism is also available. So far, the increased sensitivity of indirect serological detection has not been consistently demonstrated.

Giardiasis is most frequently associated with the consumption of contaminated water. Five outbreaks have been traced to food contamination by infected or infested food handlers, and the possibility of infections from contaminated vegetables that are eaten raw cannot be excluded. Cool moist conditions favor the survival of the organism.

Relative Frequency of Disease
Giardiasis is more prevalent in children than in adults, possibly because many individuals seem to have a lasting immunity after infection. This organism is implicated in 25% of the cases of gastrointestinal disease and may be present asymptomatically. The overall incidence of infection in the United States is estimated at 2% of the population. This disease afflicts many homosexual men, both HIV-positive and HIV-negative individuals. This is presumed to be due to sexual transmission. The disease is also common in child day care centers, especially those in which diapering is done.

Acute outbreaks appear to be common with infants and is not usually associated with water but is related to child care and diaper changing hygiene procedures. When I worked for a major water provider, I would receive 2-3 calls a week about infants diagnosed with Giardiasis. The problem lies with the water provider in that we are obligated to investigate and analyze all water customer complaints and make sure that our water is safe.



This is an example of infectious diarrhea due to Giardia lamblia infection of the small intestine. The small pear-shaped trophozoites live in the duodenum and become infective cysts that are excreted. They produce a watery diarrhea. A useful test for diagnosis of infectious diarrheas is stool examination for ova and parasites.

Course of Disease and Complications
About 40% of those who are diagnosed with giardiasis demonstrate disaccharide intolerance during detectable infection and up to 6 months after the infection can no longer be detected. Lactose (i.e., milk sugar) intolerance is most frequently observed. Some individuals (less than 4%) remain symptomatic more than 2 weeks; chronic infections lead to a malabsorption syndrome and severe weight loss.

Chronic cases of giardiasis in immunodeficient and normal individuals are frequently refractile to drug treatment. Flagyl is normally quite effective in terminating infections. In some immune deficient individuals, giardiasis may contribute to a shortening of the life span.

Target Populations
Giardiasis occurs throughout the population, although the prevalence is higher in children than adults. Chronic symptomatic giardiasis is more common in adults than children.

Major Outbreaks
Major outbreaks are associated with contaminated water systems that do not use sand filtration or have a defect in the filtration system.

In April 1988, the Albuquerque Environmental Health Department and the New Mexico Health and Environment Department investigated reports of giardiasis among members of a church youth group in Albuquerque. The first two members to be affected had onset of diarrhea on March 3 and 4, respectively; stool specimens from both were positive for Giardia lamblia cysts. These two persons had only church youth group activities in common.

On August 8, 1983, the Utah Department of Health was notified by the Tooele County Health Department (TCHD) of an outbreak of diarrheal illness in Tooele, Utah, possibly associated with a contaminated public water supply that resulted from flooding during Utah's spring thaw.

References

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- Svard SG, Meng TC, Hetsko ML, McCaffery JM, Gillin FD. Differentiation-driven surface antigen variation in the ancient eukaryote. Molec. Microbiol. 1998;30:979-89.

- Tovar J, Levila G, Shez LB, Sutak R, Tachezy J, Van Der Giezen Mitochondrial remnant organelles of Giardia function in iron-sulphur protein maturation. Nature 2003;426:172-176

Giardia Images

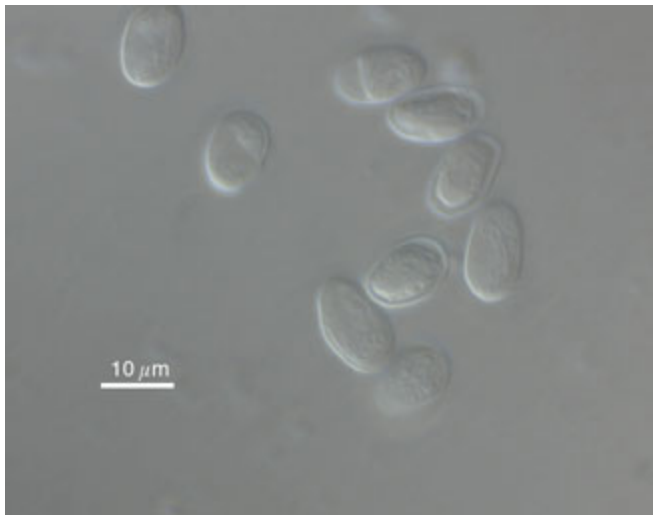


Photo Credit: H.D.A Lindquist, U.S. EPA

Above: Differential interference contrast (DIC) image of *Giardia lamblia* cysts, purified from Mongolian gerbil fecal material. Cysts are ovoidal or elipsoidal objects, usually 11-14 microns in length. Cysts may contain as many as 4 nuclei, and residual structures from their trophozoite or vegetative form. These residua include central axonemes, remnants of the striated disk, and remnant median bodies. In some cysts these structures will be indistinct. Scale bar is 10 microns.

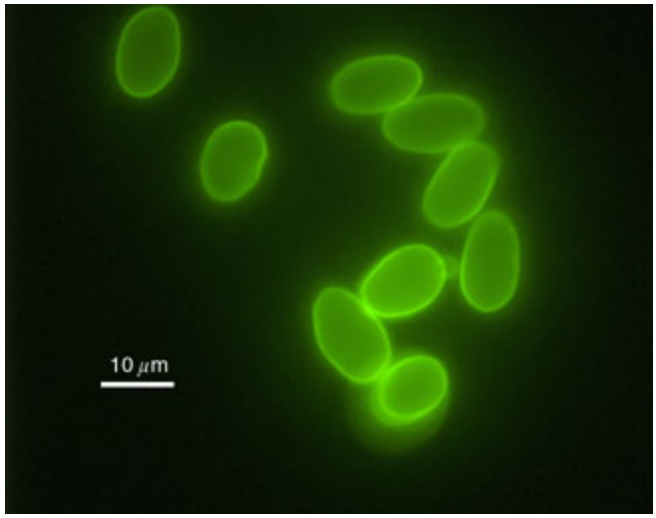


Photo Credit: H.D.A Lindquist, U.S. EPA

Above: Immunofluorescence image of *Giardia lamblia* cysts, purified from Mongolian gerbil fecal material. (Same field of view) Cysts were stained with commercially available immunofluorescent antibodies. Cysts should have an intense apple green fluorescence on the periphery of their cyst wall, and measure 11-14 microns in length. Scale bar is 10 microns.

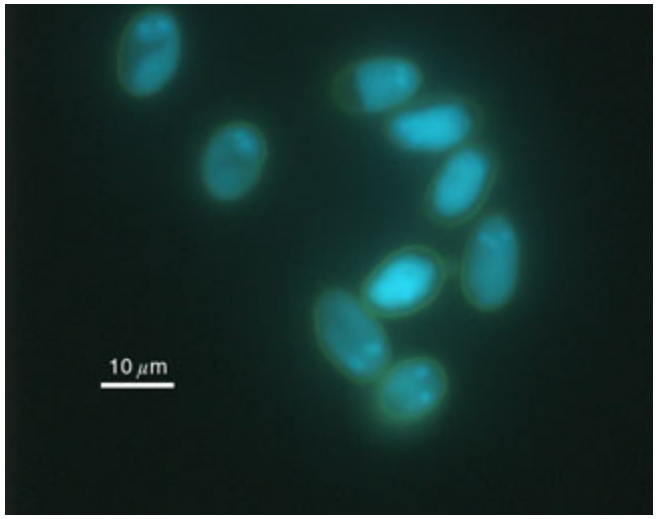


Photo Credit: H.D.A Lindquist, U.S. EPA

Above: Fluorescence image of *Giardia lamblia* cysts, purified from Mongolian gerbil fecal material. (Same field of view) Cysts were stained with 4,6-diamidino 2-phenyl-indole dihydrochloride (DAPI). DAPI interacts with nucleic acids and stains the nuclei within the cyst. There should be 4 nuclei in each cyst. Cysts that appear to have fewer than 4 stained nuclei may have 4 nuclei with the others not visible in this plane of focus.

Cysts with no nuclei visible, may be dead, may be resistant to DAPI staining, or may be organisms other than *G. lamblia*.

Giardiasis *Giardia lamblia* Chapter 2 Review

1. Giardia duodenalis, cause of giardiasis, is a one-celled, microscopic parasite that can live in the intestines of animals and people. It is found in every region throughout the world and has become recognized as one of the most common causes of waterborne (and occasionally foodborne) illness often referred to as "Beaver Fever." It is commonly known as "traveler's diarrhea", and referred to as "Montezuma's Revenge" by those who travel to third world countries in the Western Hemisphere.
A. True
B. False

2. Giardia lamblia (intestinalis) is a single celled animal, i.e., a protozoa, that moves with the aid of five flagella.
A. True
B. False

3. In Europe, Giardia lamblia is sometimes referred to as Lamblia intestinalis.
A. True
B. False

4. Giardiasis is the least frequent cause of non-bacterial diarrhea in North America.
A. True
B. False

5. Approximately one week after ingestion of the Giardia _____, prolonged, greasy diarrhea, gas, stomach cramps, fatigue, and weight loss begin.
A. Cysts
B. Immune system
C. Parasite
D. Amitochondrialism
E. None of the above

6. It is possible to experience some, not all, of the symptoms, yet still shed _____ and pass the parasite onto others. Typically, the disease runs its course in a week or two, although in some cases, the disease may linger for months, causing severe illness and weight loss. Nonetheless, the basic biology of this parasite--including how it ravages the digestive tract--is poorly understood.
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7. The organism exists in two different forms--a hardy, dormant _____that contaminates water or food and an active, disease-causing form that emerges after the parasite is ingested. National Institute of General Medical Sciences grantee Dr. Frances Gillin of the University of California, San Diego and her colleagues cultivated the entire life cycle of this parasite in the lab and identified biochemical cues in the host's digestive system that trigger Giardia's life cycle transformations. They also uncovered several tricks the parasite uses to evade the defenses of the infected organism.
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E. None of the above

8. One of Giardia's techniques is to alter the proteins on its surface, which confounds the ability of the infected animal's immune system to detect and combat the _____. This work reveals why Giardia infections are extremely persistent and prone to recur. In addition, these insights into Giardia's biology and survival techniques may enable scientists to develop better strategies to understand, prevent, and treat Giardia infections.
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C. Parasite
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E. None of the above

9. Recently, Giardia has been found to possess mitochondrial remnants known as 'mitosomes', which suggest that the condition of _____ is not primitive to eukaryotes but instead is a result of reductive evolution.
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B. Immune system
C. Parasite
D. Amitochondrialism
E. None of the above

10. The microaerophilic Giardia uses these _____ in the maturation of iron-sulfur proteins rather than in ATP synthesis as is the case in mitochondria-possessing eukaryotes.
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C. Parasite
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E. None of the above

11. Several strains of G. lamblia have been isolated and described through analysis of their _____ and DNA; type of strain, however, is not consistently associated with disease severity. Different individuals show various degrees of symptoms when infected with the same strain, and the symptoms of an individual may vary during the course of the disease.
A. Survival
B. Trophozoite
C. Excysted
D. Proteins
E. Enzyme

12. Giardia lamblia is frequently diagnosed by visualizing the organism, either the _____ (active reproducing form) or the cyst (the resting stage that is resistant to adverse environmental conditions) in stained preparations or unstained wet mounts with the aid of a microscope. A commercial fluorescent antibody kit is available to stain the organism.
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13. Organisms may be concentrated by sedimentation or flotation; however, these procedures reduce the number of recognizable organisms in the sample. An _____linked immunosorbant assay (ELISA) that detects excretory secretory products of the organism is also available. So far, the increased sensitivity of indirect serological detection has not been consistently demonstrated.
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B. Trophozoite
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E. Enzyme

14. Giardiasis is most frequently associated with the consumption of contaminated water. Five outbreaks have been traced to food contamination by infected or infested food handlers, and the possibility of infections from contaminated vegetables that are eaten raw cannot be excluded. Cool moist conditions favor the _____ of the organism.
A. Survival
B. Trophozoite
C. Excysted
D. Proteins
E. Enzyme

15. Giardiasis is more prevalent in children than in adults, possibly because many individuals seem to have a lasting immunity after infection. This organism is implicated in 25% of the cases of gastrointestinal disease and may be present asymptomatically.
A. True
B. False

16. The disease is also common in child day care centers, especially those in which diapering is done.
A. True
B. False

17. Acute outbreaks appear to be common with infants and are not usually associated with water but are related to child care and diaper changing hygiene procedures.
A. True
B. False

18. About 40% of those who are diagnosed with giardiasis demonstrate disaccharide intolerance during detectable infection and up to 6 months after the infection can no longer be detected. Lactose (i.e., milk sugar) intolerance is most frequently observed. Some individuals (less than 4%) remain symptomatic more than 2 weeks; chronic infections lead to a malabsorption syndrome and severe weight loss.
A. True
B. False

19. Chronic cases of giardiasis in immunodeficient and normal individuals are frequently refractile to drug treatment.
A. True
B. False

20. Flagyl is normally quite effective in terminating infections. In some immune deficient individuals, giardiasis may contribute to a shortening of the life span.
A. True
B. False

21. Giardiasis occurs throughout the population, although the prevalence is higher in children than adults. Chronic symptomatic giardiasis is more common in adults than children.
A. True
B. False

1.B, 2.A, 3.A, 4.B,5.A, 6.A, 7.A, 8.C, 9.D, 10.E, 11.D,12.B, 13.E, 14.A, 15.A, 16.A, 17.A, 18.A, 19.A, 20.A, 21.A

Cryptosporidiosis *Cryptosporidium* Chapter 3

Introduction

Until 1993, when over 400,000 people in Milwaukee became ill with diarrhea after drinking water contaminated with the parasite, few people had heard of *Cryptosporidium parvum*, or the disease it causes, cryptosporidiosis. Today, however, public health and water utility officials are increasingly called on to provide information and make decisions about the control of this protozoan found in public water supplies, recreational water and other areas.

Cryptosporidiosis is most particularly a danger for the immunocompromised, especially HIV-positive persons and persons with AIDS. Individuals with CD4 cell counts below 200 are more likely to experience severe complications, including prolonged diarrhea, dehydration, and possible death. Those with CD4 counts above 200 may recover from the symptoms of cryptosporidiosis yet maintain the infection asymptomatically, with symptoms potentially returning if their CD4 count later drops.

Other diseases besides AIDS can cause immunosuppression severe enough to lead to chronic cryptosporidiosis. Persons with these diseases should also be concerned about becoming infected. These diseases include congenital agammaglobulinemia, congenital IgA deficiency and cancer. Persons taking corticosteroids, for cancer and bone marrow or organ transplants, also need to be concerned about becoming infected. Even though persons who are taking immunosuppressive drugs may develop chronic and/or severe cryptosporidiosis, the infection usually resolves when these drugs are decreased or stopped. Persons taking immunosuppressive drugs need to consult with their healthcare provider if they believe they have cryptosporidiosis.

Persons at increased risk for contracting cryptosporidiosis include child care workers; diaper-aged children who attend child care centers; persons exposed to human feces by sexual contact; and caregivers who might come in direct contact with feces while caring for a person infected with cryptosporidiosis.

Transmission is by an oral-fecal route, including hand contact with the stool of infected humans or animals or with objects contaminated with stool. Transmission is also common from ingestion of food or water contaminated with stool, including water in the recreational water park and swimming pool settings.

Symptoms of cryptosporidiosis include, most commonly, watery diarrhea and cramps, sometimes severe. Weight loss, nausea, vomiting, and fever are also possible. The severity of symptoms varies with the degree of underlying immunosuppression, with immunocompetent patients commonly experiencing watery diarrhea for a few days to 4 or more weeks and occasionally having a recurrence of diarrhea after a brief period of recovery.

AIDS

Patients with AIDS can have a large number of stools per day for months or even years. There is currently no cure for cryptosporidiosis, though drug research is continuing. Patients who suspect they may have cryptosporidiosis should drink extra fluids and may wish to drink oral rehydration therapy liquid, to avoid dehydration.

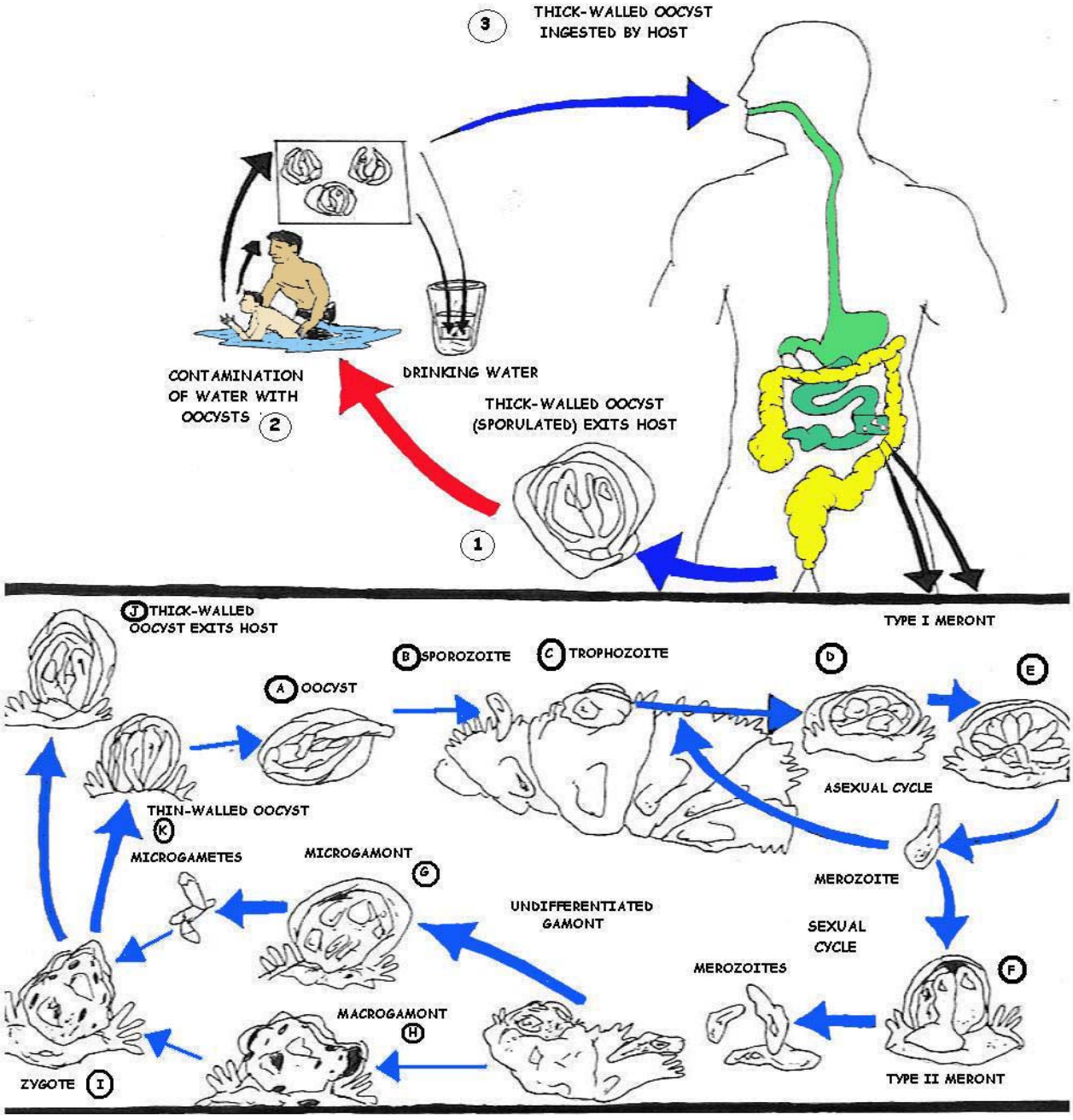
HIV-positive Individuals
HIV-positive individuals who suspect they have cryptosporidiosis should contact their healthcare provider. Infected individuals should be advised to wash their hands frequently, especially before preparing food and after going to the toilet.

They should also avoid close contact with anyone who has a weakened immune system. Individuals with diarrhea should not swim in public bathing areas while they have diarrhea and for at least 2 weeks after each attack of diarrhea.

Prevention
Washing hands is the most effective means of preventing cryptosporidiosis transmission.

For the immunocompromised, sex, including oral sex, that involves possible contact with stool should be avoided. Immunocompromised individuals should also avoid the stool of all animals and wash their hands thoroughly after any contact with animals or the living areas of animals. Immunocompromised persons may also wish to wash, peel, or cook all vegetables and to take extra measures, such as boiling or filtering their drinking water, to ensure its safety.





Life cycle of *Cryptosporidium parvum* and *C. hominis*.

Life cycle of *Cryptosporidium parvum* and *C. hominis*.

Cryptosporidium stages were reproduced from Juranek DD. Cryptosporidiosis. In: Strickland GT, editor. Hunter's Tropical Medicine and Emerging Infectious Diseases, 8th ed. Philadelphia: WB Saunders; 2000. Originally adapted from the life cycle that appears in Current WL, Garcia LS. Cryptosporidiosis. Clinc Microbiol Rev 1991;4:325-58.

Sporulated oocysts, containing 4 sporozoites, are excreted by the infected host through feces and possibly other routes such as respiratory secretions 1.

Transmission of *Cryptosporidium parvum* and *C. hominis* occurs mainly through contact with contaminated water (e.g., drinking or recreational water).

Occasionally food sources, such as chicken salad, may serve as vehicles for transmission. Many outbreaks in the United States have occurred in waterparks, community swimming pools, and day care centers. Zoonotic and anthroponotic transmission of *C. parvum* and anthroponotic transmission of *C. hominis* occur through exposure to infected animals or exposure to water contaminated by feces of infected animals 2.

Following ingestion (and possibly inhalation) by a suitable host 3, excystation a occurs. The sporozoites are released and parasitize epithelial cells (b, c) of the gastrointestinal tract or other tissues such as the respiratory tract. In these cells, the parasites undergo asexual multiplication (schizogony or merogony) (d, e, f) and then sexual multiplic-ation (gametogony) producing microgamonts (male) g and macrogamonts (female) h.

Upon fertilization of the macrogamonts by the microgametes (i), oocysts (j, k) develop that sporulate in the infected host. Two different types of oocysts are produced, the thick-walled, which is commonly excreted from the host j, and the thin-walled oocyst k, which is primarily involved in autoinfection.

Oocysts are infective upon excretion, thus permitting direct and immediate fecal-oral transmission.

Note that oocysts of *Cyclospora cayetanensis*, another important coccidian parasite, are unsporulated at the time of excretion and do not become infective until sporulation is completed.

Refer to the life cycle of *Cyclospora cayentanensis* for further details.

Cryptosporidiosis Oocysts



Cryptosporidium oocysts

Genus *Cryptosporidium*

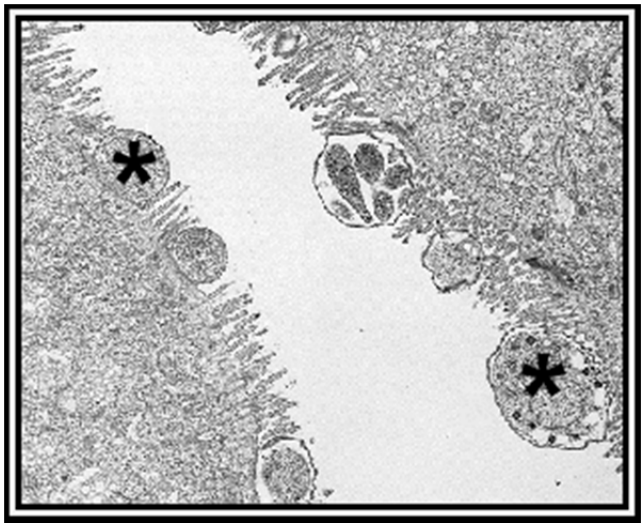
Members of the genus *Cryptosporidium* are parasites of the intestinal tracts of fishes, reptiles, birds, and mammals. It seems that members of this genus do not display a high degree of host specificity, so the number of species in this genus remains a matter of some discussion. *Cryptosporidium* isolated from humans is now referred to as *C. parvum*. *Cryptosporidium* infections have been reported from a variety of wild and domesticated animals, and in the last six or seven years literally hundreds of human infections have been reported, including epidemics in several major urban areas in the United States. Cryptosporidiosis is now recognized as an important opportunistic infection, especially in immunocompromised hosts.

Cryptosporidium is a small parasite, measuring about 3-5 µm. It lives on (or just under) the surface of the cells lining the small intestine, reproduces asexually, and oocysts are passed in the feces. Transmission of the infection occurs via the oocysts. Many human infections have been traced to the contamination of drinking water with oocysts from agricultural "run-off" (i.e., drainage from pastures), so it is considered a zoonosis.

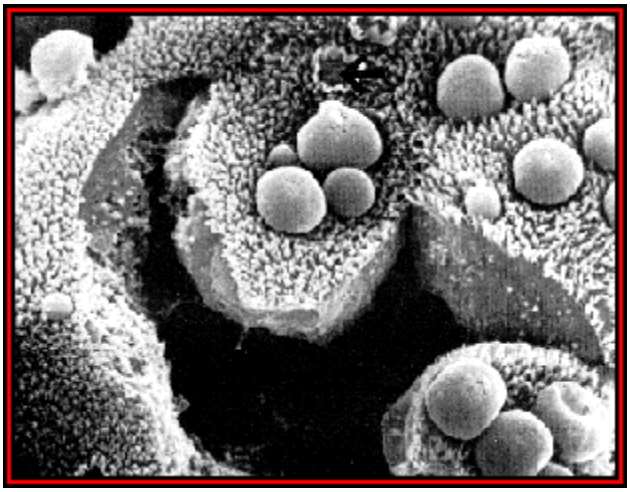
In most patients infected with cryptosporidiosis the infection causes a short term, mild diarrhea. Since such symptoms are associated with a number of ailments, infected individuals may not seek medical treatment, and the infection may subside on its own. Thus, it is difficult to say how many people are infected. On the other hand, in persons with compromised immune systems, this parasite can cause a pronounced, chronic diarrhea; in severe cases the infected individual may produce up to 15 liters/day of stools, and this may go on for weeks or months. Needless to say, such an infection, if not fatal unto itself, can exacerbate other opportunitistic infections common in immunocompromised hosts.



A scanning electron micrograph of a broken meront of *Cryptosporidium* showing the merozoites within. (From: Gardiner *et al*., 1988, An Atlas of Protozoan Parasites in Animal Tissues, USDA Agriculture Handbook No. 651.)



An electron micrograph showing several stages of *Cryptosporidium* (two are marked with asterisks) on the intestinal epithelium of a sheep. (From: Gardiner *et al*., 1988, An Atlas of Protozoan Parasites in Animal Tissues, USDA Agriculture Handbook No. 651.)



A scanning electron micrograph of *Cryptosporidium* lining the intestinal tract. (From: Gardiner *et al*., 1988, An Atlas of Protozoan Parasites in Animal Tissues, USDA Agriculture Handbook No. 651.)

How is cryptosporidiosis spread?
Cryptosporidium lives in the intestine of infected humans or animals. Millions of crypto germs can be released in a bowel movement from an infected human or animal.

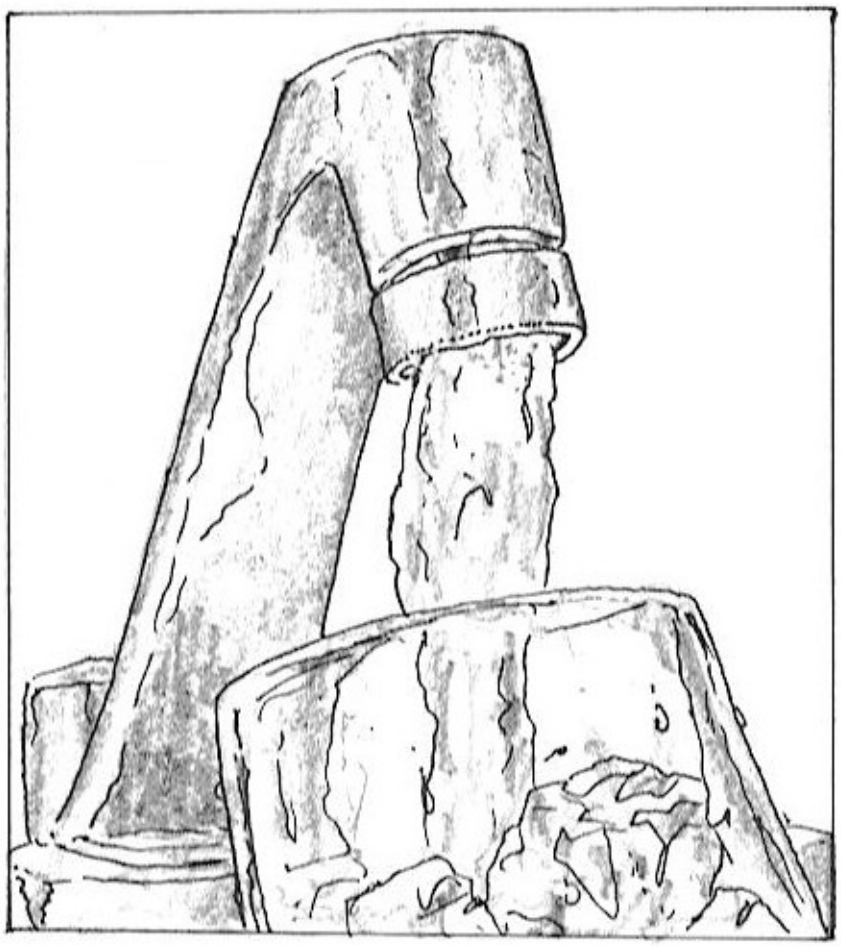
Consequently, *Cryptosporidium* is found in soil, food, water, or surfaces that have been contaminated with infected human or animal feces. If a person swallows the parasite they become infected. You **cannot** become infected through contact with blood. The parasite can be spread by:

- Accidentally putting something into your mouth or swallowing something that has come into contact with feces of a person or animal infected with *Cryptosporidium*.

- Swallowing recreational water contaminated with *Cryptosporidium* (Recreational water includes water in swimming pools, hot tubs, jacuzzis, fountains, lakes, rivers, springs, ponds, or streams that can be contaminated with sewage or feces from humans or animals.) **Note:** *Cryptosporidium* can survive for days in swimming pools with adequate chlorine levels.

- Eating uncooked food contaminated with *Cryptosporidium*. Thoroughly wash with clean, safe water all vegetables and fruits you plan to eat raw.

- Accidentally swallowing *Cryptosporidium* picked up from surfaces (such as bathroom fixtures, changing tables, diaper pails, or toys) contaminated with feces from an infected person.



Key Words

DISINFECT: The application of a chemical to kill most, but not all, microorganisms that may be present. Chlorine is added to public water drinking systems drinking water for disinfection. Depending on your state rule, drinking water must contain a minimum of 0.2 mg/L free chlorine. Disinfection makes drinking water safe to consume from the standpoint of killing pathogenic microorganisms including bacteria and viruses. Disinfection does not remove all bacteria from drinking water, but the bacteria that can survive disinfection with chlorine are not pathogenic bacteria that can cause disease in normal healthy humans.

DISINFECTION: The treatment of water to inactivate, destroy, and/or remove pathogenic bacteria, viruses, protozoa, and other parasites.

DISSOLVED OXYGEN: Can be added to zones within a lake or reservoir that would normally become anaerobic during periods of thermal stratification.

DISSOLVED SOLIDS: Solids in solution that cannot be removed by filtration with a 0.45 micron filter.

What are the Symptoms of Cryptosporidiosis?

The most common symptom of cryptosporidiosis is watery diarrhea. Other symptoms include:

- Dehydration
- Weight loss
- Stomach cramps or pain
- Fever
- Nausea
- Vomiting

Some people with crypto will have no symptoms at all. While the small intestine is the site most commonly affected, *Cryptosporidium* infections could possibly affect other areas of the digestive or the respiratory tract.

How long after infection do symptoms appear?
Symptoms of cryptosporidiosis generally begin 2 to 10 days (average 7 days) after becoming infected with the parasite.

How long will symptoms last?
In persons with healthy immune systems, symptoms usually last about 1 to 2 weeks. The symptoms may go in cycles in which you may seem to get better for a few days, then feel worse again before the illness ends.

If I have been diagnosed with *Cryptosporidium* , should I worry about spreading the infection to others?
Yes, *Cryptosporidium* can be very contagious. Follow these guidelines to avoid spreading the disease to others:

1. Wash your hands with soap and water after using the toilet, changing diapers, and before eating or preparing food.
2. Do not swim in recreational water (pools, hot tubs, lakes or rivers, the ocean, etc.) if you have cryptosporidiosis and for at least 2 weeks after diarrhea stops. You can pass *Cryptosporidium* in your stool and contaminate water for several weeks after your symptoms have ended. This has resulted in outbreaks of cryptosporidiosis among recreational water users.
   Note: Cryptosporidium can be spread in a chlorinated pool because it is resistant to chlorine and, therefore, can live for days in chlorine-treated swimming pools.
3. Avoid fecal exposure during sexual activity.

Who is most at risk for cryptosporidiosis?
People who are most likely to become infected with *Cryptosporidium* include:

- Children who attend day care centers, including diaper-aged children
- Child care workers
- Parents of infected children
- International travelers
- Backpackers, hikers, and campers who drink unfiltered, untreated water
- Swimmers who swallow water while swimming in swimming pools, lakes, rivers, ponds, and streams
- People who drink from shallow, unprotected wells
- People who swallow water from contaminated sources

Contaminated water includes water that has not been boiled or filtered. Several community-wide outbreaks of cryptosporidiosis have been linked to drinking municipal water or recreational water contaminated with *Cryptosporidium.*

Who is most at risk for getting seriously ill with cryptosporidiosis?
Although Crypto can infect all people, some groups are more likely to develop more serious illness.

- Young children and pregnant women may be more susceptible to the dehydration resulting from diarrhea and should drink plenty of fluids while ill.
- If you have a severely weakened immune system, you are at risk for more serious disease. Your symptoms may be more severe and could lead to serious or life-threatening illness. Examples of persons with weakened immune systems include those with HIV/AIDS; cancer and transplant patients who are taking certain immunosuppressive drugs; and those with inherited diseases that affect the immune system.

If you have a severely weakened immune system, talk to your health care provider for additional guidance. You can also call the CDC AIDS HOTLINE toll-free at 1-800-342-2437. Ask for more information on cryptosporidiosis, or go to the CDC fact sheet *Preventing Cryptosporidiosis: A Guide for People with Compromised Immune Systems* available by visiting http://www.cdc.gov/ncidod/dpd/parasites/cryptosporidiosis/factsht_crypto_prevent_ci.htm

What should I do if I think I may have cryptosporidiosis?
If you suspect that you have cryptosporidiosis, see your health care provider.

How is cryptosporidiosis diagnosed?
Your health care provider will ask you to submit stool samples to see if you are infected. Because testing for Crypto can be difficult, you may be asked to submit several stool specimens over several days. Tests for Crypto are not routinely done in most laboratories; therefore, your health care provider should specifically request testing for the parasite.

What is the treatment for cryptosporidiosis?
Although there is no standard treatment for cryptosporidiosis, the symptoms can be treated. Most people who have a healthy immune system will recover without treatment. If you have diarrhea, drink plenty of fluids to prevent dehydration. Rapid loss of fluids from diarrhea may be especially life threatening to babies; therefore, parents should talk to their health care provider about fluid replacement therapy options for infants. Antidiarrheal medicine may help slow down diarrhea, but talk to your health care provider before taking it. A new drug, nitazoxanide, has been approved for treatment of diarrhea caused by *Cryptosporidium* in healthy children less than 12 years old. Consult with your health care provider for more information. People who are in poor health or who have a weakened immune system are at higher risk for more severe and more prolonged illness. For persons with AIDS, anti-retroviral therapy that improves immune status will also decrease or eliminate symptoms of Crypto. However, even if symptoms disappear, cryptosporidiosis is usually not curable and the symptoms may return if the immune status worsens. See your health care provider to discuss anti-retroviral therapy used to improve your immune status.

How Can I Prevent Cryptosporidiosis?

Practice good hygiene.

1. Wash hands thoroughly with soap and water.
    a. Wash hands after using the toilet and before handling or eating food (especially for persons with diarrhea).
    b. Wash hands after every diaper change, especially if you work with diaper-aged children, even if you are wearing gloves.
2. Protect others by not swimming if you are experiencing diarrhea (essential for children in diapers).

Avoid water that might be contaminated.

1. Do not swallow recreational water
2. Do not drink untreated water from shallow wells, lakes, rivers, springs, ponds, and streams.
3. Do not drink untreated water during community-wide outbreaks of disease caused by contaminated drinking water.
4. Do not use untreated ice or drinking water when traveling in countries where the water supply might be unsafe.

For information on recreational water-related illnesses, visit CDC's Healthy Swimming website at http://www.cdc.gov/healthyswimming.

In the United States, nationally distributed brands of bottled or canned carbonated soft drinks are safe to drink. Commercially packaged non-carbonated soft drinks and fruit juices that do not require refrigeration until after they are opened (those that are stored un-refrigerated on grocery shelves) also are safe.

For information on choosing safe bottled water, see the CDC fact sheet entitled “Preventing Cryptosporidiosis: A Guide to Water Filters and Bottled Water,” available by visiting http://www.cdc.gov/ncidod/dpd/parasites/cryptosporidiosis/factsht_crypto_prevent_water.htm.

If you are unable to avoid using or drinking water that might be contaminated, then you can make the water safe to drink by doing one of the following:

- Heat the water to a rolling boil for at least 1 minute.
  OR
- Use a filter that has an absolute pore size of at least 1 micron or one that has been NSF rated for "cyst removal."

Do not rely on chemicals to disinfect water and kill *Cryptosporidium*. Because it has a thick outer shell, this particular parasite is highly resistant to disinfectants such as chlorine and iodine.

For information on choosing a water filter, see the CDC fact sheet entitled "Preventing Cryptosporidiosis: A Guide to Water Filters and Bottled Water," available by visiting http://www.cdc.gov/ncidod/dpd/parasites/cryptosporidiosis/factsht_crypto_prevent_water.htm.

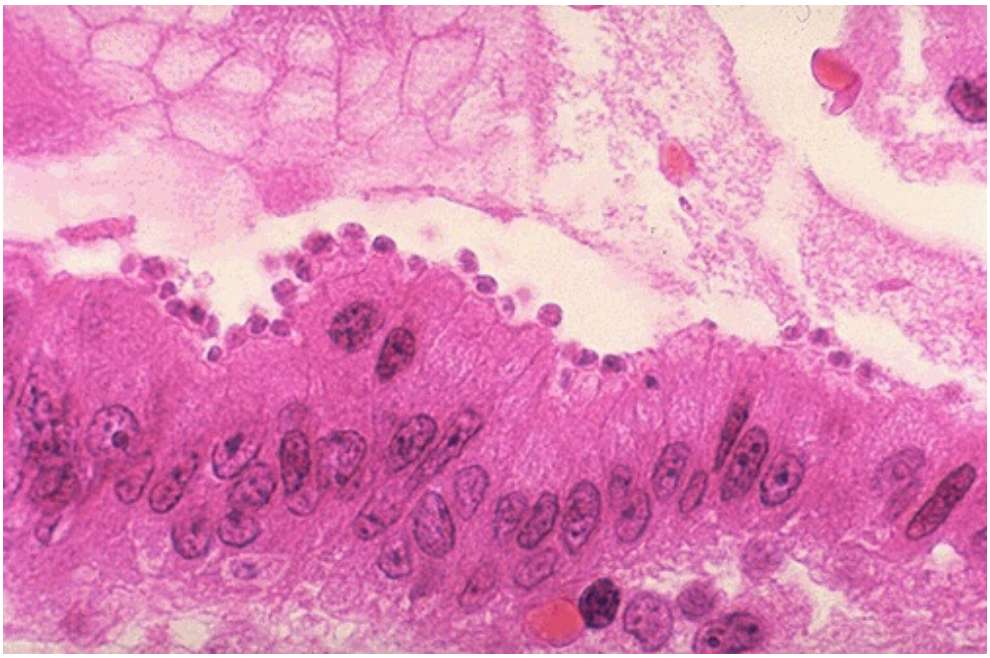
Avoid food that might be contaminated.

1. Wash and/or peel all raw vegetables and fruits before eating.
2. Use safe, uncontaminated water to wash all food that is to be eaten raw.
3. Avoid eating uncooked foods when traveling in countries with minimal water treatment and sanitation systems.

Take extra care when traveling.

If you travel to developing nations, you may be at a greater risk for *Cryptosporidium* infection because of poorer water treatment and food sanitation. Warnings about food, drinks, and swimming are even more important when visiting developing countries. Avoid foods and drinks, in particular raw fruits and vegetables, tap water, or ice made from tap water, unpasteurized milk or dairy products, and items purchased from street vendors. These items may be contaminated with *Cryptosporidium*. Steaming-hot foods, fruits you peel yourself, bottled and canned processed drinks, and hot coffee or hot tea are probably safe. Talk with your health care provider about other guidelines for travel abroad.

Avoid fecal exposure during sexual activity.



This is another infectious agent that is becoming more frequent in immunocompromised patients, particularly those with AIDS. The small round blue organisms at the lumenal border are cryptosporidia. Cryptosporidiosis produces a copious watery diarrhea.

A Guide to Water Filters and Bottled Water

Filtering tap water
Many, but not all, available home water filters remove Cryptosporidium. Some filter designs are more suitable for removal of *Cryptosporidium* than others. Filters that have the words "reverse osmosis" on the label protect against *Cryptosporidium*. Many other types of filters that work by micro-straining also work. Look for a filter that will remove particles that are less than or equal to 1 micron in diameter.

There are two types of these - "absolute 1 micron" filters and "nominal 1 micron" filters. The absolute 1 micron filter will more consistently remove *Cryptosporidium* than a nominal filter. Some nominal 1 micron filters will allow 20% to 30% of 1 micron particles to pass through.



NSF-International
NSF-International (NSF) does independent testing of filters to determine if they remove *Cryptosporidium*. To find out if a particular filter is certified to remove *Cryptosporidium*, you can look for the NSF trademark plus the words "cyst reduction" or "cyst removal" on the product label information. You can also contact the NSF at 789 N. Dixboro Road, Ann Arbor, MI 48105 USA, toll free1-877-867-3435, fax 313-769-0109, email info@nsf.org, or visit their Web site at www.nsf.org/certified/DWTU/.

At their Web site, you can enter the model number of the unit you intend to buy to see if it is on their certified list, or you can look under the section entitled "Reduction claims for drinking water treatment units - Health Effects" and check the box in front of the words "Cyst Reduction." This will display a list of filters tested for their ability to remove *Cryptosporidium*.

Because NSF testing is expensive and voluntary, some filters that may work against *Cryptosporidium* have not been NSF-tested. If you choose to use a product not NSF-certified, select those technologies more likely to reduce *Cryptosporidium*; this includes filters with reverse osmosis and those that have an absolute pore size of 1 micron or smaller.

Package and Label information for purchasing water filters:

Filters designed to remove crypto ***(any of the four messages below on a package label indicate that the filter should be able to remove crypto***	**Filters labeled only with these words may NOT be designed to remove crypto**
Reverse osmosis (with or without NSF testing)	*Nominal* pore size of 1 micron or smaller
Absolute pore size of 1 micron or smaller (with or without NSF testing)	One micron filter
Tested and certified by NSF Standard 53 or NSF Standard 58 for cyst removal	Effective against *Giardia*
Tested and certified by NSF Standard 53 or NSF Standard 58 for cyst reduction	Effective against parasites
	Carbon filter
	Water purifier
	EPA approved — Caution: EPA does not approve or test filters
	EPA registered — Caution: EPA does not register filters based on their ability to remove Cryptosporidium
	Activated carbon
	Removes chlorine
	Ultraviolet light
	Pentiodide resins
	Water softener

Note: Filters collect germs from water, so someone who is not HIV infected or immune impaired should change the filter cartridges. Anyone changing the cartridges should wear gloves and wash hands afterwards. Filters may not remove *Cryptosporidium* as

well as boiling does because even good brands of filters may sometimes have manufacturing flaws that allow small numbers of *Cryptosporidium* to get in past the filter. Selection of NSF-Certified filters provides additional assurance against such flaws. Also, poor filter maintenance or failure to replace the filter cartridges as recommended by the manufacturer can cause a filter to fail.

If you drink bottled water, read the label and look for this information:

Water so labeled has been processed by method effective against crypto	Water so labeled may not have been processed by method effective against crypto
Reverse osmosis treated	Filtered
Distilled	Micro-filtered
Filtered through an *absolute* 1 micron or smaller filter	Carbon-filtered
"One micron absolute"	Particle-filtered
	Multimedia-filtered
	Ozonated
	Ozone-treated
	Ultraviolet light-treated
	Activated carbon-treated
	Carbon dioxide-treated
	Ion exchange-treated
	Deionized
	Purified
	Chlorinated

Bottled water labels reading "well water," "artesian well water," "spring water," or "mineral water" do not guarantee that the water does not contain crypto. However, water that comes from protected well or protected spring water sources is less likely to contain crypto than bottled water or tap water from less protected sources, such as rivers and lakes.

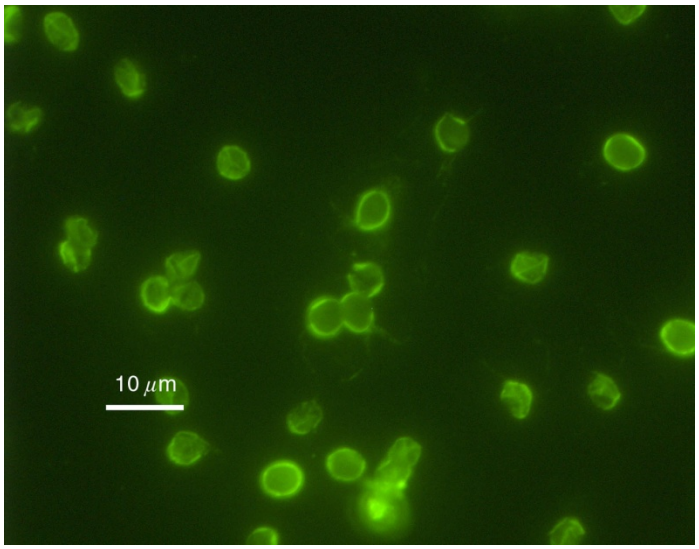
Home distillers: You can remove crypto and other germs from your water with a home distiller. If you use one, you need to carefully store your water as recommended for storing purified water.

Other drinks: Soft drinks and other beverages may or may not contain crypto. You need to know how they were prepared to know if they might contain crypto.

If you consume prepared beverages, look for drinks from which crypto has been removed:

Crypto killed or removed in preparation	Crypto may not be killed or removed in preparation
Canned or bottled soda, seltzer, and fruit drinks	Fountain drinks
Steaming hot (175 degrees F or hotter) tea or coffee	Fruit drinks you mix with tap water from frozen concentrate
Pasteurized drinks	Iced tea or coffee

Juices made from fresh fruit can also be contaminated with crypto. Several people became ill after drinking apple cider made from apples contaminated with crypto. You may wish to avoid unpasteurized juices or fresh juices if you do not know how they were prepared.



Immunofluorescence image of *Cryptosporidium parvum* oocysts, purified from murine fecal material. (Same field of view) Oocysts were stained with commercially available immunofluorescent antibodies. Oocysts should have an intense apple green fluorescence on the periphery of their oocyst wall, and measure 4 to 6 microns in diameter. Scale bar is 10 microns. Photo Credit: H.D.A Lindquist, U.S. EPA.

Cryptosporidiosis Chapter 3 Review

1. Until 1993, when over 400,000 people in Milwaukee became ill with diarrhea after drinking water contaminated with the parasite, few people had heard of Cryptosporidium parvum, or the disease it causes, cryptosporidiosis.
A. True
B. False

2. Transmission is also common from ingestion of food or water contaminated with stool, including water in the recreational water park and swimming pool settings.
A. True
B. False

3. Symptoms of cryptosporidiosis include, most commonly, watery diarrhea and cramps, sometimes severe. Weight loss, nausea, vomiting, and fever are also possible.
A. True
B. False

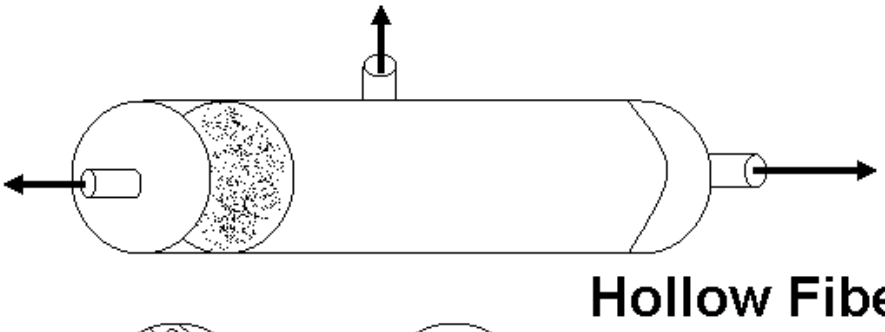
4. The severity of symptoms varies with the degree of underlying immunosuppression, with immunocompetent patients commonly experiencing watery diarrhea for a few days to 4 or more weeks and occasionally having a recurrence of diarrhea after a brief period of recovery.
A. True
B. False

5. Cryptosporidiosis is most particularly a danger for the immunocompromised, especially HIV-positive persons and persons with AIDS. Individuals with CD4 cell counts below 200 are more likely to experience severe complications, including prolonged diarrhea, dehydration, and possible death.
A. True
B. False

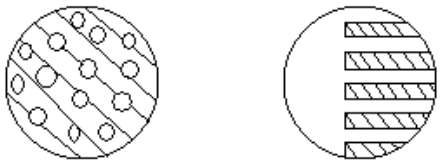
6. Persons at increased risk for contracting cryptosporidiosis include child care workers; diaper-aged children who attend child care centers; persons exposed to human feces by sexual contact; and caregivers who might come in direct contact with feces while caring for a person infected with cryptosporidiosis.
A. True
B. False

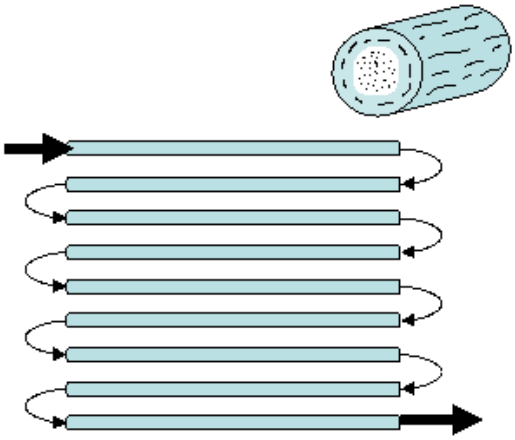
7. Transmission is by an oral-fecal route, including hand contact with the stool of infected humans or animals or with objects contaminated with stool.
A. True
B. False

1.A, 2.A, 3.A, 4.A, 5.A, 6.A, 7.A



Hollow Fiber





Tubular

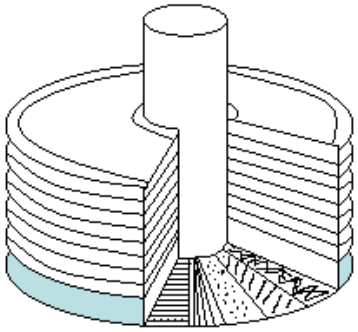
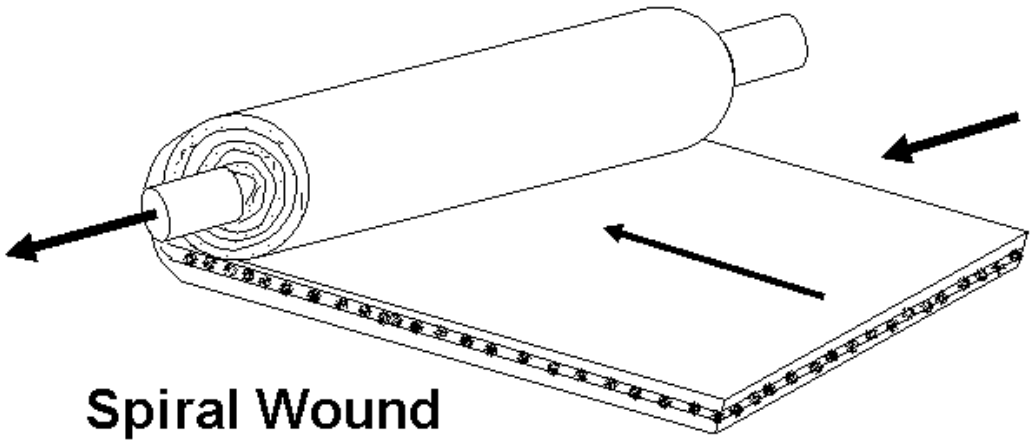


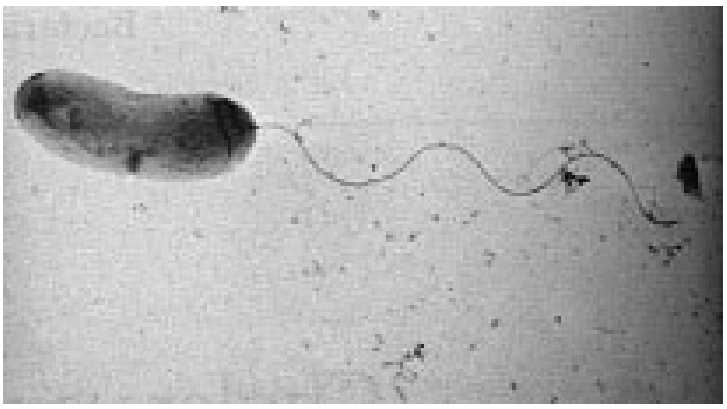
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Spiral Wound

Cholera *Vibrio cholerae* Chapter 4

Cholera has been very rare in industrialized nations for the last 100 years; however, the disease is still common today in other parts of the world, including the Indian subcontinent and sub-Saharan Africa. Although cholera can be life-threatening, it is easily prevented and treated. In the United States, because of advanced water and sanitation systems, cholera is not a major threat; however, everyone, especially travelers, should be aware of how the disease is transmitted and what can be done to prevent it.



Vibrio cholerae

Cholera, which is derived from a Greek term meaning "flow of bile," is caused by *Vibrio cholerae* and is the most feared epidemic diarrheal disease because of its severity. Dehydration and death can occur within a matter of hours of infection. In 1883, Robert Koch discovered *V cholerae* during a cholera outbreak in Egypt. The organism is a comma-shaped, gram-negative aerobic bacillus whose size varies from 1-3 mm in length by 0.5-0.8 mm in diameter. Its antigenic structure consists of a flagellar H antigen and a somatic O antigen. The differentiation of the latter allows for separation into pathogenic and nonpathogenic strains. *V cholerae* O1 or O139 are associated with epidemic cholera. *V cholerae* O1 has 2 major biotypes: classic and El Tor.

Currently, El Tor is the predominant cholera pathogen. Organisms in both biotypes are subdivided into serotypes according to the structure of the O antigen, as follows:

- Serotype Inaba - O antigens A and C
- Serotype Ogawa - O antigens A and B
- Serotype Hikojima - O antigens A, B, and C

How does a person get cholera?

A person may get cholera by drinking water or eating food contaminated with the cholera bacterium. In an epidemic, the source of the contamination is usually the feces of an infected person. The disease can spread rapidly in areas with inadequate treatment of sewage and drinking water. The cholera bacterium may also live in the environment in brackish rivers and coastal waters. Shellfish eaten raw have been a source of cholera, and a few persons in the United States have contracted cholera after eating raw or undercooked shellfish from the Gulf of Mexico. The disease is not likely to spread directly from one person to another; therefore, casual contact with an infected person is not a risk for becoming ill.

Vibrio Cholerae Bacterium
Cholera (also called Asiatic cholera) is a disease of the gastrointestinal tract caused by the Vibrio cholerae bacterium. These bacteria are typically ingested by drinking water contaminated by improper sanitation or by eating improperly cooked fish, especially shellfish. Symptoms include diarrhea, abdominal cramps, nausea, vomiting, and dehydration. Death is generally due to the dehydration caused by the illness. When left untreated, Cholera generally has a high mortality rate. Treatment is typically an aggressive rehydration regimen usually delivered intravenously, which continues until the diarrhea ceases.

* About one million Vibrio cholerae bacteria must be ingested to cause cholera in normally healthy adults, although increased susceptibility may be observed in those with a weakened immune system, individuals with decreased gastric acidity (as from the use of antacids), or those who are malnourished.

* 1,099,882 cases and 10,453 deaths were reported in the Western Hemisphere between January 1991 and July 1995.

* On average, one case of cholera is reported in the United States every week.
Vibrio cholerae causes disease by producing a toxin that disables the GTPase function of G proteins which are part of G protein-coupled receptors in intestinal cells. This has the effect that the G proteins are locked in the "on position" binding GTP (normally, the G proteins quickly return to "off" by hydrolyzing GTP to GDP). The G proteins then cause adenylate cyclases to produce large amounts of cyclic AMP (cAMP) which results in the loss of fluid and salts across the lining of the gut.

The resulting diarrhea allows the bacterium to spread to other people under unsanitary conditions.

What is the risk for cholera in the United States?
In the United States, cholera was prevalent in the 1800s but has been virtually eliminated by modern sewage and water treatment systems. However, as a result of improved transportation, more persons from the United States travel to parts of Latin America, Africa, or Asia where epidemic cholera is occurring. U.S. travelers to areas with epidemic cholera may be exposed to the cholera bacterium. In addition, travelers may bring contaminated seafood back to the United States; foodborne outbreaks have been caused by contaminated seafood brought into this country by travelers.

Although cholera can be life-threatening, it is easily prevented and treated. In the United States, because of advanced water and sanitation systems, cholera is not a major threat. The last major outbreak of cholera in the United States was in 1911. However, everyone, especially travelers, should be aware of how the disease is transmitted and what can be done to prevent it.

What should travelers do to avoid getting cholera?
The risk for cholera is very low for U.S. travelers visiting areas with epidemic cholera. When simple precautions are observed, contracting the disease is unlikely.

All travelers to areas where cholera has occurred should observe the following recommendations:

- Drink only water that you have boiled or treated with chlorine or iodine. Other safe beverages include tea and coffee made with boiled water and carbonated, bottled beverages with no ice.
- Eat only foods that have been thoroughly cooked and are still hot, or fruit that you have peeled yourself.
- Avoid undercooked or raw fish or shellfish, including ceviche.
- Make sure all vegetables are cooked, avoid salads.
- Avoid foods and beverages from street vendors.
- Do not bring perishable seafood back to the United States.

A simple rule of thumb is "***Boil it, cook it, peel it, or forget it***."

Treatment

The objective of treatment is to replace fluid and electrolytes lost through diarrhea. Depending on the condition of the person, oral or intravenous fluid will be given. Tetracycline and other antibiotics may shorten the duration of the symptoms.
Note: Tetracycline is usually not prescribed for children until after all the permanent teeth have come in, because it can permanently discolor teeth that are still forming.

The World Health Organization (WHO) has developed an oral rehydration solution that is cheaper and easier to use than the typical intravenous fluid. This solution of sugar and electrolytes is now being used internationally.

Expectations (prognosis)

Severe dehydration can cause death. Given adequate fluids, most people will make a full recovery.

Complications

- Severe dehydration.

Calling your health care provider

Call your health care provider if profuse watery diarrhea develops.

Call your health care provider if signs of dehydration occur, including rapid pulse (heart rate), dry skin, dry mouth, thirst, "glassy" eyes, lethargy, sunken eyes, no tears, reduced or no urine, and unusual sleepiness or tiredness.

Susceptibility

Recent genetic research has determined that a person's susceptibility to cholera and other diarrheas) is affected by their blood type. Those with type O blood are the most susceptible. Those with type AB are the most resistant, virtually immune. Between these two extremes are the A and B blood types, with type A being more resistant than type B.

Carriers of the cystic fibrosis gene are protected from the severe effects of cholera because they don't lose water as quickly. This explains the high incidence of cystic fibrosis among populations which were formerly exposed to cholera.

Epidemic control and preventive measures
When cholera appears in a community it is essential to ensure three things: hygienic disposal of human feces, an adequate supply of safe drinking water, and good food hygiene. Effective food hygiene measures include cooking food thoroughly and eating it while still hot; preventing cooked foods from being contaminated by contact with raw foods, including water and ice, contaminated surfaces or flies; and avoiding raw fruits or vegetables unless they are first peeled. Washing hands after defecation, and particularly before contact with food or drinking water, is equally important.

Routine treatment of a community with antibiotics, or "mass chemoprophylaxis", has no effect on the spread of cholera, nor does restricting travel and trade between countries or between different regions of a country. Setting up a *cordon sanitaire* at frontiers uses personnel and resources that should be devoted to effective control measures, and hampers collaboration between institutions and countries that should unite their efforts to combat cholera.

Limited stocks of two oral cholera vaccines that provide high-level protection for several months against cholera caused by *V. cholerae* O1 have recently become available in a few countries. Both are suitable for use by travelers but they have not yet been used on a large scale for public health purposes. Use of this vaccine to prevent or control cholera outbreaks is not recommended because it may give a false sense of security to vaccinated subjects and to health authorities, who may then neglect more effective measures.

Is a vaccine available to prevent cholera?
At the present time, the manufacture and sale of the only licensed cholera vaccine in the United States (Wyeth-Ayerst) has been discontinued. It has not been recommended for travelers because of the brief and incomplete immunity it offers. No cholera vaccination requirements exist for entry or exit in any country.

Two recently developed vaccines for cholera are licensed and available in other countries (Dukoral®, Biotec AB and Mutacol®, Berna). Both vaccines appear to provide a somewhat better immunity and fewer side-effects than the previously available vaccine. However, neither of these two vaccines are recommended for travelers nor are they available in the United States.

History and spread of epidemic cholera
Cholera has smoldered in an endemic fashion on the Indian subcontinent for centuries. There are references to deaths due to dehydrating diarrhea dating back to Hippocrates and Sanskrit writings. Epidemic cholera was described in 1563 by Garcia del Huerto, a Portuguese physician at Goa, India. The mode of transmission of cholera by water was proven in 1849 by John Snow, a London physician. In 1883, Robert Koch successfully isolated the cholera vibrio from the intestinal discharges of cholera patients and proved conclusively that it was the agent of the disease.

The first long-distance spread of cholera to Europe and the Americas began in 1817 and by the early 20th century, six waves of cholera had spread across the world in devastating epidemic fashion. Since then, until the 1960s, the disease contracted, remaining present only in southern Asia.

El Tor

In 1961, the "**El Tor**" biotype (distinguished from classic biotypes by the production of hemolysins) reemerged to produce a major epidemic in the Philippines and to initiate a seventh global pandemic. Since then this biotype has spread across Asia, the Middle East, Africa, and more recently, parts of Europe.

There are several characteristics of the El Tor strain that confer upon it a high degree of "epidemic virulence," allowing it to spread across the world as previous strains have done. First, the ratio of cases to carriers is much less than in cholera due to classic biotypes (1: 30-100 for El Tor vs. 1: 2 - 4 for "classic" biotypes). Second, the duration of carriage after infection is longer for the El Tor strain than the classic strains. Third, the El Tor strain survives for longer periods in the extraintestinal environment. Between 1969 and 1974, El Tor replaced the classic strains in the heartland of endemic cholera, the Ganges River Delta of India.

El Tor broke out explosively in Peru in 1991 (after an absence of cholera there for 100 years), and spread rapidly in Central and South America, with recurrent epidemics in 1992 and 1993. From the onset of the epidemic in January 1991 through September 1, 1994, a total of 1,041,422 cases and 9,642 deaths (overall case-fatality rate: 0.9%) were reported from countries in the Western Hemisphere to the Pan American Health Organization. In 1993, the numbers of reported cases and deaths were 204,543 and 2362, respectively.

So far, the United States has been spared except for imported cases, or clusters of infections from imported food. In the United States during 1993 and 1994, 22 and 47 cholera cases were reported to CDC, respectively. Of these, 65 (94%) were associated with foreign travel. In 1982, in Bangladesh, a classic biotype resurfaced with a new capacity to produce more severe illness, and it rapidly replaced the El Tor strain which was thought to be well-entrenched. This classic strain has not yet produced a major outbreak in any other country.

In December, 1992, a large epidemic of cholera began in Bangladesh, and large numbers of people have been involved. The organism has been characterized as *V. cholerae* **O139 "Bengal"**. It is derived genetically from the El Tor pandemic strain but it has changed its antigenic structure such that there is no existing immunity and all ages, even in endemic areas, are susceptible. The epidemic has continued to spread. and *V. cholerae* **O139** has affected at least 11 countries in southern Asia. Specific totals for numbers of *V. cholerae* O139 cases are unknown because affected countries do not report infections caused by O1 and O139 separately.

Antigenic Variation and LPS Structure in *Vibrio cholerae*

Antigenic variation plays an important role in the epidemiology and virulence of cholera. The emergence of the Bengal strain, mentioned above, is an example. The flagellar antigens of *V. cholerae* are shared with many water vibrios and therefore are of no use in distinguishing strains causing epidemic cholera. O antigens, however, do distinguish strains of *V. cholerae* into 139 known serotypes. Almost all of these strains of *V. cholerae* are nonvirulent. Until the emergence of the Bengal strain (which is "non-O1") a single serotype, designated O1, has been responsible for epidemic cholera.

However, there are three distinct **O1 biotypes**, named Ogawa, Inaba and Hikojima, and each biotype may display the "classical" or El Tor phenotype. The Bengal strain is a new serological strain with a unique O-antigen which partly explains the lack of residual immunity.

Antigenic Determinants of *Vibrio cholerae*

Serotype	O Antigens
Ogawa	A, B
Inaba	A, C
Hikojima	A, B, C

Endotoxin is present in *Vibrio cholerae* as in other Gram-negative bacteria. Fewer details of the chemical structure of *Vibrio cholerae* LPS are known than in the case of *E. coli* and *Salmonella typhimurium*, but some unique properties have been described. Most importantly, variations in LPS occur in vivo and in vitro, which may be correlated with reversion in nature of nonepidemic strains to classic epidemic strains and vice versa.

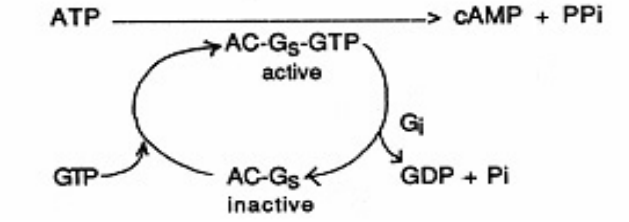
Cholera Toxin

Cholera toxin **activates the adenylate cyclase enzyme in cells of the intestinal mucosa** leading to increased levels of intracellular cAMP, and the secretion of H_20, Na^+, K^+, Cl^-, and HCO_3^- into the lumen of the small intestine. The effect is dependent on a specific receptor, monosialosyl ganglioside (GM1 ganglioside) present on the surface of intestinal mucosal cells. The bacterium produces an invasin, neuraminidase, during the colonization stage which has the interesting property of degrading gangliosides to the monosialosyl form, which is the specific receptor for the toxin.

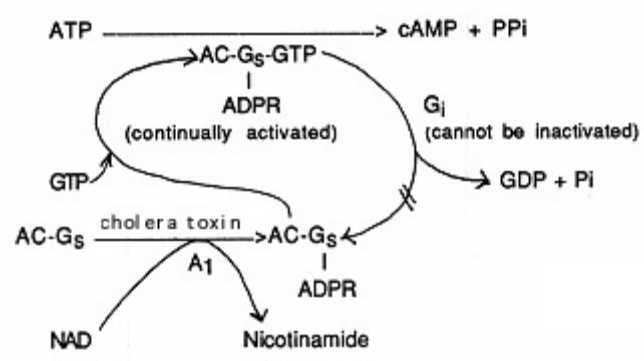
The toxin has been characterized and contains **5 binding (B) subunits** of 11,500 daltons, an active **(A1) subunit** of 23,500 daltons, and a **bridging piece (A2)** of 5,500 daltons that links A1 to the 5B subunits. Once it has entered the cell, the A1 subunit enzymatically transfers ADP ribose from NAD to a protein (called Gs or Ns), that regulates the adenylate cyclase system which is located on the inside of the plasma membrane of mammalian cells.

Enzymatically, fragment A1 catalyzes the transfer of the ADP-ribosyl moiety of NAD to a component of the adenylate cyclase system. The process is complex. Adenylate cyclase (AC) is activated normally by a regulatory protein (GS) and GTP; however activation is normally brief because another regulatory protein (Gi), hydrolyzes GTP.

The normal situation is described as follows.



The A1 fragment catalyzes the attachment of ADP-Ribose (ADPR) to the regulatory protein forming Gs-ADPR from which GTP cannot be hydrolyzed. Since GTP hydrolysis is the event that inactivates the adenylate cyclase, the enzyme remains continually activated. This situation can be illustrated as follows.



Thus, the net effect of the toxin is to cause cAMP to be produced at an abnormally high rate which stimulates mucosal cells to pump large amounts of Cl^- into the intestinal contents. H_2O, Na^+ and other electrolytes follow due to the osmotic and electrical gradients caused by the loss of Cl^-.

The lost H_2O and electrolytes in mucosal cells are replaced from the blood. Thus, the toxin-damaged cells become pumps for water and electrolytes, causing the diarrhea, loss of electrolytes, and dehydration that are characteristic of cholera.

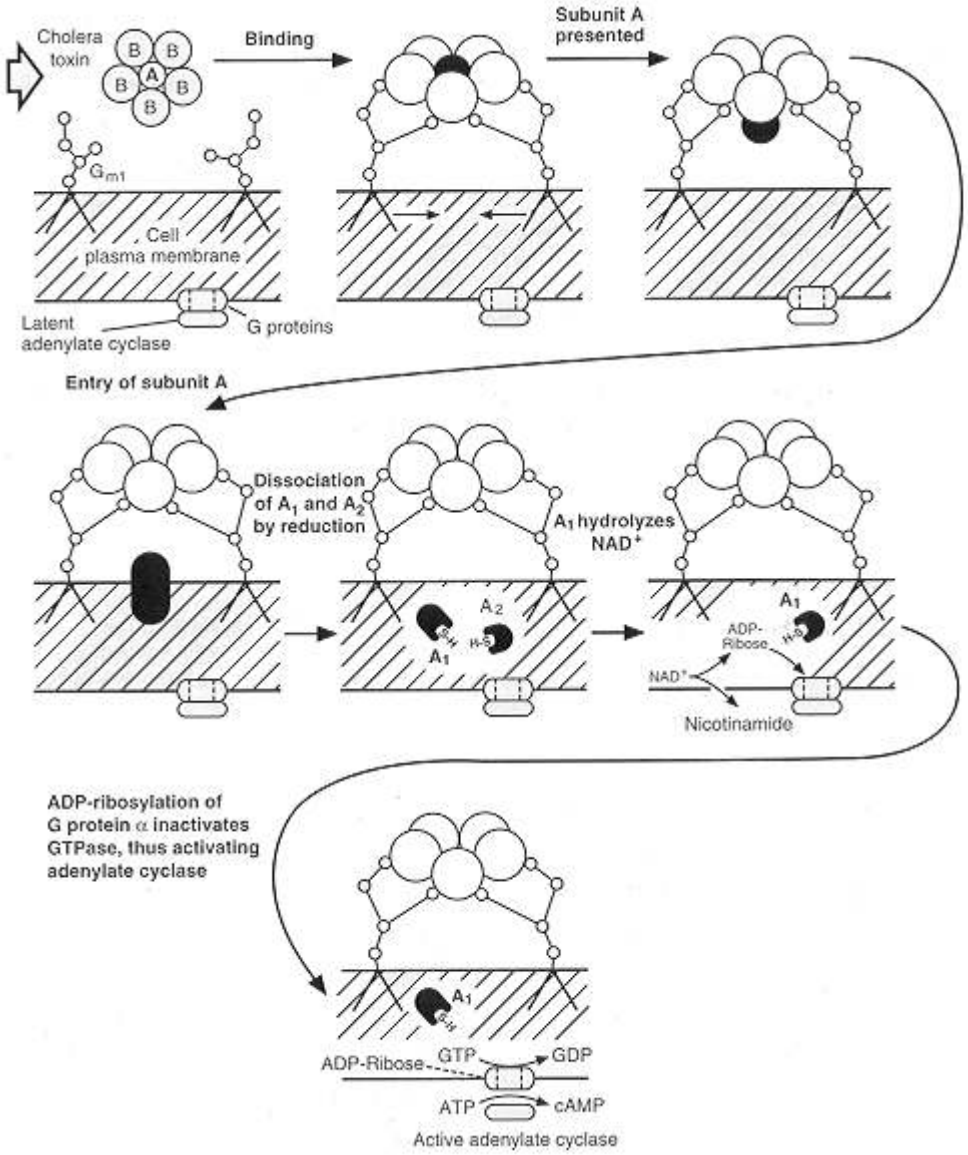
Last Word

E. coli produces a toxin, heat labile toxin (LT) that is very similar to the cholera toxin in structure and mode of action. The DNA that encodes the LT toxin is on a plasmid that can be transferred to other *E. coli* strains and probably to other enteric bacteria, as well. Close relationships between the genetic code for LT toxin and the cholera toxin undoubtedly exist but have not been documented as yet.

The genetic information for the toxin in *V. cholerae* is located on the bacterial chromosome. Other bacterial enterotoxins related to cholera toxin have been reported in non-group O *Vibrio* strains and a strain of *Salmonella*.

Enterotoxins, toxins which act in the GI tract, are produced by a wide variety of bacteria.

The family of heat-stable (ST) enterotoxins of *E. coli*, which activate guanylate cyclase, are unrelated to LT toxin or cholera toxin. Other enterotoxins, which elicit cytotoxic effects on intestinal epithelial cells, have been described from *Escherichia*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Aeromonas*, *Pseudomonas*, *Shigella*, *V. parahaemolyticus*, *Campylobacter*, *Yersinia enterocolitica*, *Bacillus cereus*, *Clostridium perfringens*, *C. difficile*, and *Staphylococcus aureus*.



Mechanism of action of cholera enterotoxin according to Finkelstein in Baron, Chapter 24. Cholera toxin approaches target cell surface. B subunits bind to oligosaccharide of GM1 ganglioside. Conformational alteration of holotoxin occurs, allowing the presentation of the A subunit to cell surface. The A subunit enters the cell. The disulfide bond of the A subunit is reduced by intracellular glutathione, freeing A1 and A2. NAD is hydrolyzed by A1, yielding ADP-ribose and nicotinamide. One of the G proteins of adenylate cyclase is ADP-ribosylated, inhibiting the action of GTPase and locking adenylate cyclase in the "on" mode.

Cholera Chapter 4 Review

1. Cholera, which is derived from a Greek term meaning “Running to the bathroom,” is caused by Vibrio cholerae and is the most feared epidemic diarrheal disease because of its severity. Dehydration and death can occur within a matter of minutes of infection.
A. True
B. False

2. In 1883, Louis Pasteur discovered V cholerae during a cholera outbreak in Egypt.
A. True
B. False

3. Cholera has been very common in industrialized nations for the last 100 years.
A. True
B. False

4. Cholera is always life-threatening, it is easily prevented and treated with choromines.
A. True
B. False

5. In the United States, because of advanced water and sanitation systems, cholera is not a major threat; however, everyone, especially travelers, should be aware of how the disease is transmitted and what can be done to prevent it.
A. True
B. False

6. The V cholerae organism is a comma-shaped, gram-negative aerobic bacillus whose size varies from 1-3 mm in length by 0.5-0.8 mm in diameter. Its antigenic structure consists of a flagellar H antigen and a somatic O antigen.
A. True
B. False

7. The differentiation of the latter allows for separation into pathogenic and nonpathogenic strains. V cholerae O1 or O139 are associated with epidemic cholera. V cholerae O1 has 2 major biotypes: classic and El Tor.
A. True
B. False

8. Currently, El Leche is the predominant cholera pathogen.
A. True
B. False

9. A person may get cholera by drinking water or eating food contaminated with the cholera bacterium. In an epidemic, the source of the contamination is usually the feces of an infected person. The disease can spread rapidly in areas with inadequate treatment of sewage and drinking water.
A. True
B. False

10. The cholera bacterium may also live in the environment in brackish rivers and coastal waters. Shellfish eaten raw have been a source of cholera, and a few persons in the United States have contracted cholera after eating raw or undercooked shellfish from the Gulf of Mexico. The disease is not likely to spread directly from one person to another; therefore, casual contact with an infected person is not a risk for becoming ill.
A. True
B. False

11. Cholera (also called Asiatic flu) is a disease of the respiratory tract caused by the Vibrio cholerae bacterium. These bacteria are typically ingested by drinking water contaminated by improper sanitation or by eating improperly cooked fish, especially shellfish.
A. True
B. False

12. About one hundred Vibrio cholerae bacteria must be ingested to cause cholera in normally healthy adults, although increased susceptibility may be observed in those with a strong immune system, individuals with increased gastric acidity, or those who are malnourished.
A. True
B. False

13. Vibrio cholerae causes disease by producing a toxin that disables the _____ of G proteins which are part of G protein-coupled receptors in intestinal cells. This has the effect that the G proteins are locked in the "on position" binding GTP (normally, the G proteins quickly return to "off" by hydrolyzing GTP to GDP).
A. GTPase function
B. G proteins
C. Bacterium
D. Antigenic
E. Flagellar antigens

14. The _____ then cause adenylate cyclases to produce large amounts of cyclic AMP (cAMP) which results in the loss of fluid and salts across the lining of the gut.
A. GTPase function
B. G proteins
C. Bacterium
D. Antigenic
E. Flagellar antigens

15. The resulting diarrhea allows the _____ to spread to other people under unsanitary conditions.
A. GTPase function
B. G proteins
C. Bacterium
D. Antigenic
E. Flagellar antigens

16. When cholera appears in a community it is essential to ensure three things: hygienic disposal of human feces, an _____ supply of safe drinking water, and good food hygiene.
A. GTPase function
B. G proteins
C. Bacterium
D. Antigenic
E. None of the above

17. _____ variation plays an important role in the epidemiology and virulence of cholera. The emergence of the Bengal strain is an example.
A. GTPase function
B. G proteins
C. Bacterium
D. Antigenic
E. Flagellar antigens

18. The _____ of V. cholerae are shared with many water vibrios and therefore are of no use in distinguishing strains causing epidemic cholera.
A. GTPase function
B. G proteins
C. Bacterium
D. Antigenic
E. Flagellar antigens

19. O antigens, however, do distinguish strains of V. cholerae into 139 known _____.
A. Serological strain
B. Nonvirulent
C. Serotypes
D. Phenotype
E. None of the above

20. Almost all strains of V. cholerae are _____.
A. Serological strain
B. Nonvirulent
C. Serotypes
D. Phenotype
E. None of the above

21. Until the emergence of the Bengal strain (which is "non-O1") a single serotype, designated O1, has been responsible for epidemic cholera. However, there are three distinct O1 biotypes, named Ogawa, Inaba and Hikojima, and each biotype may display the "classical" or El Tor _____.
A. Serological strain
B. Nonvirulent
C. Serotypes
D. Phenotype
E. None of the above

22. E. coli produces a toxin, heat labile toxin (LT) that is very similar to the cholera toxin in structure and mode of action. The DNA that encodes the LT _____ is on a plasmid that can be transferred to other E. coli strains and probably to other enteric bacteria, as well.
A. Toxin
B. In vitro
C. Adenylate cyclase enzyme
D. Enterotoxins
E. None of the above

23. Close relationships between the genetic code for LT _____ and the cholera toxin un-doubtedly exist but have not been documented as yet.
A. Toxin
B. In vitro
C. Adenylate cyclase enzyme
D. Enterotoxins
E. None of the above

24. The genetic information for the toxin in V. cholerae is located on the bacterial chromo-some. Other bacterial _____ related to cholera toxin have been reported in non-group O Vibrio strains and a strain of Salmonella.
A. Toxin
B. In vitro
C. Adenylate cyclase enzyme
D. Enterotoxins
E. None of the above

25. _____, toxins which act in the GI tract, are produced by a wide variety of bacteria. The family of heat-stable (ST) enterotoxins of E. coli, which activate guanylate cyclase, are unrelated to LT toxin or cholera toxin.
A. Toxin
B. In vitro
C. Adenylate cyclase enzyme
D. Enterotoxins
E. None of the above

26. Other _____, which elicit cytotoxic effects on intestinal epithelial cells, have been described from Escherichia, Klebsiella, Enterobacter, Citrobacter, Aeromonas, Pseudomonas, Shigella, V. parahaemolyticus, Campylobacter, Yersinia enterocolitica, Bacillus cereus, Clostridium perfringens, C. difficile, and Staphylococcus aureus.
A. Toxin
B. In vitro
C. Adenylate cyclase enzyme
D. Enterotoxins
E. None of the above

1.B, 2.B, 3.B, 4.B, 5.A, 6.A, 7.A, 8.B, 9.A,10.A, 11.B, 12.B, 13.A, 14.B, 15.C, 16.E, 17.D, 18.E, 19.C, 20.B, 21.D,22.A, 23.A, 24.D, 25.D, 26.D

Legionnaires' Disease *Legionella* Chapter 5

Introduction **Genus:** *Legionella* **Species:** *pneumophila*

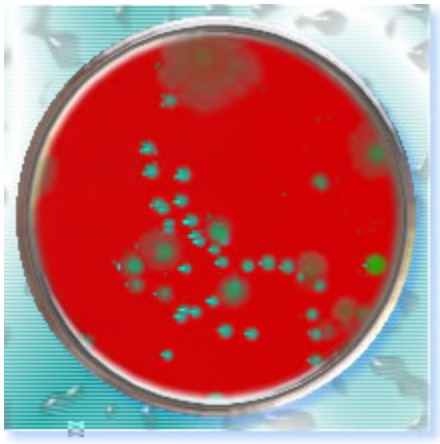
The first discovery of bacteria from genus Legionella came in 1976 when an outbreak of pneumonia at an American Legion convention led to 29 deaths. The causative agent, what would come to be known as Legionella pneumophila, was isolated and given its own genus. The organisms classified in this genus are Gram-negative bacteria that are considered intracellular parasites. The disease has two distinct forms:



- Legionnaires' disease, the more severe form of infection which includes pneumonia, and
- Pontiac fever, a milder illness.

What have been the water sources for Legionnaires' disease?
The major source is water distribution systems of large buildings, including hotels and hospitals. Cooling towers have long been thought to be a major source for *Legionella*, but new data suggest that this is an overemphasized mode of transmission. Other sources include mist machines, humidifiers, whirlpool spas, and hot springs. Air conditioners are not a source for Legionnaires' disease. They were suspected to be the source in the original American Legion outbreak in a Philadelphia hotel, but new data now suggests that the water in the hotel was the actual culprit.

Legionnaire's disease is caused most commonly by the inhalation of small droplets of water or fine aerosol containing Legionella bacteria. Legionella bacteria are naturally found in environmental water sources such as rivers, lakes and ponds and may colonize man-made water systems that include air conditioning systems, humidifiers, cooling tower waters, hot water systems, spas and pools.



How do people contract Legionella?
The most popular theory is that the organism is aerosolized in water and people inhale the droplets containing *Legionella*. However, new evidence suggests that another way of contracting *Legionella* is more common. "Aspiration" is the most common way that bacteria enter into the lungs to cause pneumonia. Aspiration means choking such that secretions in the mouth get past the choking reflexes and instead of going into the esophagus and stomach, mistakenly, enter the lung. The protective mechanisms to prevent aspiration is defective in patients who smoke or have lung disease. Aspiration now appears to be the most common mode of transmission.

Legionella may multiply to high numbers in cooling towers, evaporative condensers, air washers, humidifiers, hot water heaters, spas, fountains, and plumbing fixtures.

Within one month, Legionella can multiply, in warm water-containing systems, from less than 10 per milliliter to over 1,000 per milliliter of water.

Once high numbers of Legionella have been found, a relatively simple procedure for disinfecting water systems with chlorine and detergent is available. This procedure is not part of a routine maintenance program because equipment may become corroded.

Property owners have been sued for the spread of Legionella, resulting in expensive settlements. Regular monitoring with a battery of DFA monoclonal antibodies for several serogroups and species of Legionella morphologically intact bacteria provides a means for exercising 'reasonable care' to deter potential litigation.

Currently, there are no United States government regulations concerning permissible numbers of legionella in water systems and there are no federal or state certification programs for laboratories that perform legionella testing of environmental samples.

Epifluorescence Microscopy DFA Method
The epifluorescence microscopy DFA method that most labs use was published in the British Journal, Water Research 19:839-848, 1985 "Disinfection of circulating water systems by ultraviolet light and halogenation", R. Gilpin, et al. so we can count viable-but-nonculturable (VBNC) legionella.

Most labs will provide a quantitative epifluorescence microscopic analysis of your cooling tower and potable water samples for 14 serogroups of Legionella pneumophila and 15 other Legionella species (listed below).

Legionella anisa	*Legionella bozemanii* sg 1 & 2
Legionella dumoffi	*Legionella feeleii* sg 1 & 2
Legionella gormanii	*Legionella hackeliae* sg 1 & 2
Legionella jordanis	*Legionella longbeachae* sg 1& 2
Legionella maceachernii	*Legionella micdadei*
Legionella oakridgensis	*Legionella parisiensis*
Legionella pneumophila sg 1-14	*Legionella sainthelensi*
Legionella santicrucis	*Legionella wadsworthii*

Heterotrophic bacterial CFU are often inversely proportional to numbers of Legionella in cooling tower samples, in our experience. Routine biocide treatments will not eradicate Legionella bacteria in the environment, only in laboratory studies.

Culture methods are good during outbreaks for biotyping; but culture methods lack sensitivity for routine, quantitative monitoring. Many factors will inhibit growth or identification of legionella on BCYE with or without antimicrobial agents, heat or acid treatment.

Culture methods will not identify non-culturable legionella that can still cause outbreaks (non-culturable, viable legionella have been reported in several peer-reviewed journals). Only DFA tests performed by trained laboratory personnel can identify these legionella.

Direct fluorescent antibody (DFA) tests using a battery of monoclonal antibodies provide more useful routine monitoring information than culture methods.

Legionella species of bacteria cause Legionnaire's disease. They are gram negative (but stain poorly), strictly aerobic rods.

The U.S. Environmental Protection Agency and the U.S. Occupational Safety and Health Administration recommend routine maintenance of water-containing equipment. Most State health departments recommend monthly testing for Legionella as part of a routine maintenance program.

As far as we know, there are no federal or state certification programs for laboratories that perform Legionella testing of environmental samples. Therefore, care must be taken when selecting a testing laboratory.

More on Legionnaires' Disease Medical Aspects
Legionnaires' disease is caused by bacteria that belong to the family Legionellaceae. This family now includes 48 species and over 70 serogroups. Approximately half of these species have been implicated in human disease. Legionella pneumophila is responsible for approximately 90% of infections.

Most cases are caused by L. pneumophila, serogroup 1. Legionella species are small (0.3 to 0.9 μm in width and approximately 2 μm in length) faintly staining Gram-negative rods with polar flagella (except L. oakridgensis). They generally appear as small coccobacilli in infected tissue or secretions. They are distinguished from other saccharolytic bacteria by their requirement for L-cysteine and iron salts for primary isolation on solid media and by their unique cellular fatty acids and ubiquinones.

They grow well on buffered charcoal yeast extract agar, but it takes about five days to get sufficient growth. When grown on this medium, Legionella colonies appear off-white in color and circular in shape. Laboratory identification can also include microscopic examination in conjunction with a direct flourescent antibody (DFA) test. Since the initial discovery, many species have been added to the Legionella genus, but only two are within the scope of our discussion.

L. pneumophila and L. micdadei

L. pneumophila
L. pneumophila is the bacterium associated with Legionnaires' disease and Pontiac fever. Respiratory transmission of this organism can lead to infection, which is usually characterized by a gradual onset of flu-like symptoms.

Patients may experience fever, chills, and a dry cough as part of the early symptoms. Patients can develop severe pneumonia which is not responsive to penicillins or aminoglycosides. Legionnaires' disease also has the potential to spread into other organ-systems of the body such as the gastrointestinal tract and the central nervous system. Accordingly, patients with advanced infections may experience diarrhea, nausea, disorientation, and confusion.

The 1200 or so cases of Legionnaires' disease per year in the United States usually involve middle-aged or immunosuppressed individuals. Pontiac fever is also caused by *L. pneumophila* but does not produce the severity of the symptoms found in Legionnaires' disease.

The flu-like symptoms are still seen in Pontiac fever patients, but pneumonia does not develop and infection does not spread beyond the lungs. Most *L. pneomophila* infections are easily treated with erythromycin.

Laboratory Indications

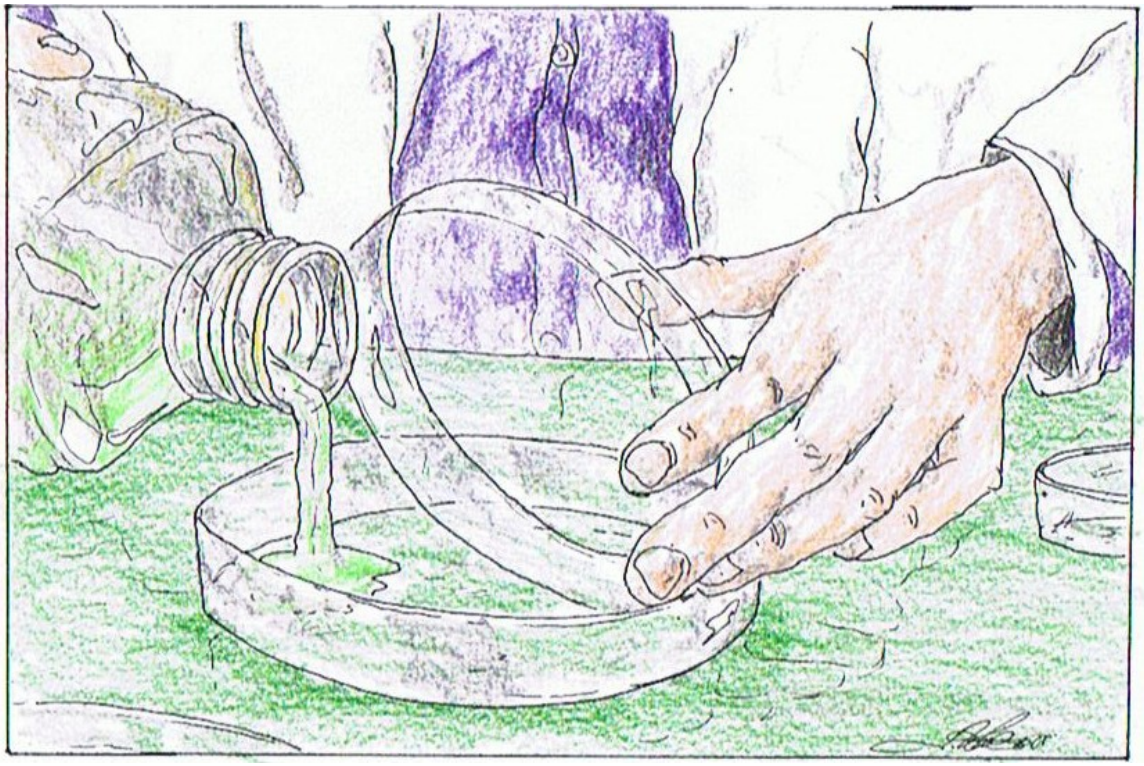
- Beta-lactamase +
- Hippurate hydrolysis +

L. micdadei

L. micdadei is the second most commonly isolated member of *Legionella*. This bacterium can cause the same flu-like symptoms and pneomonia which characterize an *L. pneumophila* infection. Unlike its relative, *L. micdadei* is sensitive to the penicillins because it does not produce beta-lactamase.

Laboratory Indications

- Beta-lactamase -
- Hippurate hydrolysis -
- Acid fast



Chlorine Dioxide

Prevention and Control
In the prevention and control of Legionnaires disease (legionella) causing microbes, chlorine dioxide has taken an eminent roll. The specific characteristics of the disinfectant make sure ClO^2 gets the job done where others fail.

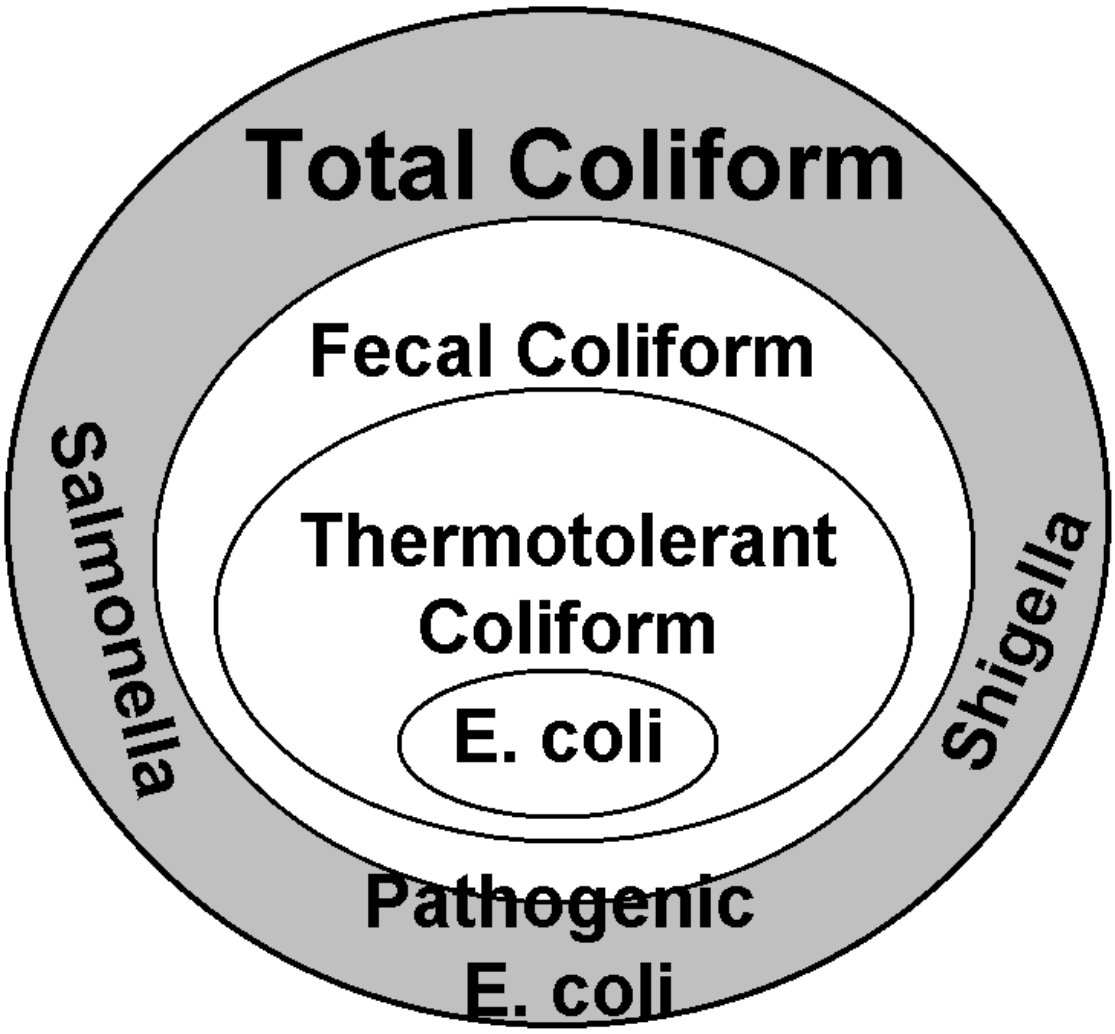
Biofilm in the piping can protect legionella from most of the disinfectants.

Chlorine dioxide however removes the biofilm and kills the bacteria, spores and viruses.
Other advantages are:
1. The bactericidal efficiency is relatively unaffected by pH values between 4 and 10;
2. The required contact time for ClO^2 is lower;
3. Chlorine dioxide has better solubility;
4. Chlorine dioxide does not react with NH3 or NH4+;
5. It destroys THM precursors and increases coagulation;
6. ClO^2 destroys phenols and has no distinct smell.

Hot and cold water systems
The advantages in using chlorine dioxide with hot and cold water systems have already been shown at the descriptions on legionella. There are however more advantages:

1. The bactericidal efficiency is relatively unaffected by pH values between 4 and 10;
2. Chlorine dioxide is clearly superior to chlorine in the destruction of spores, bacteria's, viruses and other pathogen organisms on an equal residual base (even cryptosporidium and giardia);
3. The required contact time for ClO^2 is lower;
4. Chlorine dioxide has better solubility;
5. No corrosion associated with high chlorine concentrations. Reduces long term maintenance costs;
6. Chlorine dioxide does not react with NH3 or NH4+;
7. It destroys THM precursors and increases coagulation;
8. ClO^2 destroys phenols and has no distinct smell;
9. It is better at removing iron and magnesia compounds than chlorine, especially complex bounds.

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Total Coliform
Fecal Coliform
Thermotolerant Coliform
E. coli
Salmonella
Shigella
Pathogenic E. coli

Legionella Chapter 5 Review

1. The first discovery of bacteria from genus Legionella came in 1976 when an outbreak of _____ at an American Legion convention led to 29 deaths.
A. Legionnaires' disease
B. Pneumonia
C. Pontiac fever
D. Legionella pneumophila
E. None of the above

2. The causative agent, what would come to be known as _____, was isolated and given its own genus.
A. Legionnaires' disease
B. Pneumonia
C. Pontiac fever
D. Legionella pneumophila
E. None of the above

3. The organisms classified in this genus are Gram-negative bacteria that are considered _____ .
A. Legionnaires' disease
B. Pneumonia
C. Pontiac fever
D. Intracellular parasites
E. None of the above

4. Legionnaires' disease is the more severe form of infection which includes pneumonia; _____is a milder illness.
A. Legionnaires' disease
B. Pneumonia
C. Pontiac fever
D. Legionella pneumophila
E. None of the above

5. The major source is water distribution systems of large buildings including hotels and hospitals. Cooling towers have long been thought to be a major source for _____, but new data suggest that this is an overemphasized mode of transmission.
A. Legionnaires' disease
B. Pneumonia
C. Pontiac fever
D. Legionella
E. None of the above

6. Other sources include mist machines, humidifiers, whirlpool spas, and hot springs. Air conditioners are not a source for _____. They were suspected to be the source in the original American Legion outbreak in a Philadelphia hotel, but new data now suggests that the water in the hotel was the actual culprit.
A. Legionnaires' disease
B. Pneumonia
C. Pontiac fever
D. Legionella pneumophila
E. None of the above

7. _____ is the most common way that bacteria enter into the lungs to cause pneumonia. It means choking such that secretions in the mouth get past the choking reflexes and instead of going into the esophagus and stomach, mistakenly, enter the lung. The protective mechanisms to prevent it is defective in patients who smoke or have lung disease.
A. Routine maintenance program
B. Aspiration
C. Aerosol
D. Naturally found
E. Multiply

8. Legionella may _____ to high numbers in cooling towers, evaporative condensers, air washers, humidifiers, hot water heaters, spas, fountains, and plumbing fixtures.
A. Routine maintenance program
B. Aspiration
C. Aerosol
D. Naturally found
E. Multiply

9. Legionnaire's disease is caused most commonly by the inhalation of small droplets of water or fine _____ containing Legionella bacteria.
A. Routine maintenance program
B. Aspiration
C. Aerosol
D. Naturally found
E. Multiply

10. Legionella bacteria are _____ in environmental water sources such as rivers, lakes and ponds and may colonise man made water systems that include air conditioning systems, humidifiers, cooling tower waters, hot water systems, spas and pools.
A. Routine maintenance program
B. Aspiration
C. Aerosol
D. Naturally found
E. Multiply

11. Within one month, Legionella can _____, in warm water-containing systems, from less than 10 per milliliter to over 1,000 per milliliter of water.
A. Routine maintenance program
B. Aspiration
C. Aerosol
D. Naturally found
E. Multiply

12. Once high numbers of Legionella have been found, a relatively simple procedure for disinfecting water systems with chlorine and detergent is available. This procedure is not part of a _____ because equipment may become corroded.
A. Routine maintenance program
B. Aspiration
C. Aerosol
D. Naturally found
E. Multiply

13. Currently, there are no United States government regulations concerning _____ numbers of legionella in water systems and there are no federal or state certification programs for laboratories that perform legionella testing of environmental samples.
A. Serogroups
B. Permissible
C. Ultraviolet light
D. Biocide
E. Quantitative monitoring

14. The epifluorescence microscopy DFA method that most labs use was published in the British Journal, Water Research 19:839-848, 1985 "Disinfection of circulating water systems by _____ and halogenation", R. Gilpin, et al. so we can count viable-but-nonculturable (VBNC) legionella.
A. Serogroups
B. Permissible
C. Ultraviolet light
D. Biocide
E. Quantitative monitoring

15. Most labs will provide a _____ microscopic analysis of your cooling tower and potable water samples for 14 serogroups of Legionella pneumophila and 15 other Legionella species.
A. Serogroups
B. Permissible
C. Ultraviolet light
D. Biocide
E. Quantitative epifluorescence

16. Culture methods will not identify _____ legionella that can still cause outbreaks (non-culturable, viable legionella have been reported in several peer-reviewed journals). Only DFA tests performed by trained laboratory personnel can identify these legionella.
A. Aerobic rods
B. Non-culturable
C. Ultraviolet light
D. Fluorescent
E. Quantitative monitoring

17. Direct _____ antibody (DFA) tests using a battery of monoclonal antibodies provide more useful routine monitoring information than culture methods.
A. Aerobic rods
B. Non-culturable
C. Ultraviolet light
D. Fluorescent
E. Quantitative monitoring

18. Legionella species of bacteria cause Legionnaire's disease. They are gram negative (but stain poorly), strictly _____.
A. Aerobic rods
B. Non-culturable
C. Ultraviolet light
D. Fluorescent
E. Quantitative monitoring

19. Legionnaires' disease is caused by bacteria that belong to the family _____.
A. Coccobacilli
B. Legionella genus
C. Legionellaceae
D. Serogroups
E. Legionella pneumophila

20. This family now includes 48 species and over 70 _____. Approximately half of these species have been implicated in human disease.
A. Coccobacilli
B. Legionella genus
C. Legionellaceae
D. Serogroups
E. Legionella pneumophila

21. _____ is responsible for approximately 90% of infections.
A. Coccobacilli
B. Legionella genus
C. Legionellaceae
D. Serogroups
E. Legionella pneumophila

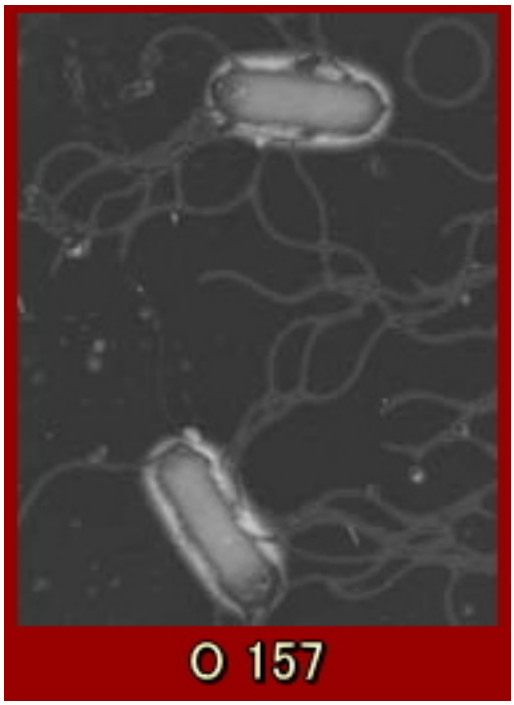
1.B, 2.D, 3.D, 4.C, 5.D, 6.A, 7.B, 8.E, 9.C, 10.D, 11.E, 12.A, 13.B, 14.C, 15.E, 16.B, 17.D, 18.A, 19.C, 20.D, 21.E

Escherichia Coli Chapter 6

Fecal Coliform Bacteria. More information in the Laboratory section.
Fecal coliform bacteria are microscopic organisms that live in the intestines of warm-blooded animals. They also live in the waste material, or feces, excreted from the intestinal tract. When fecal coliform bacteria are present in high numbers in a water sample, it means that the water has received fecal matter from one source or another. Although not necessarily agents of disease, fecal coliform bacteria may indicate the presence of disease-carrying organisms, which live in the same environment as the fecal coliform bacteria.

Reasons for Natural Variation
Unlike the other conventional water quality parameters, fecal coliform bacteria are living organisms. They do not simply mix with the water and float straight downstream. Instead they multiply quickly when conditions are favorable for growth, or die in large numbers when conditions are not. Because bacterial concentrations are dependent on specific conditions for growth, and these conditions change quickly, fecal coliform bacteria counts are not easy to predict. For example, although winter rains may wash more fecal matter from urban areas into a stream, cool water temperatures may cause a major die-off. Exposure to sunlight (with its ultraviolet disinfection properties) may have the same effect, even in the warmer water of summertime.

Expected Impact of Pollution
The primary sources of fecal coliform bacteria to fresh water are wastewater treatment plant discharges, failing septic systems, and animal waste. Bacteria levels do not necessarily decrease as a watershed develops from rural to urban. Instead, urbanization usually generates new sources of bacteria. Farm animal manure and septic systems are replaced by domestic pets and leaking sanitary sewers. In fact, stormwater runoff in urbanized areas has been found to be surprisingly high in fecal coliform bacteria concentrations.



The presence of old, disintegrating storm and sanitary sewers, misplaced sewer pipes, and good breeding conditions are common explanations for the high levels measured.

Coliform Standards (in colonies/100ml)
Drinking water.....1FC
Total body contact (swimming).....200FC
Partial body contact (boating).....1000FC
Threatened sewage effluent .....not to exceed 200 FC
*Total coliform (TC) includes bacteria from cold-blooded animals and various soil organisms. According to recent literature, total coliform counts are normally about 10 times higher than fecal coliform (FC) counts.

Indicator Connection Varies
General coliforms, E. Coli, and Enterococcus bacteria are the "indicator" organisms generally measured to assess microbiological quality of water. However, these aren't generally what get people sick. Other bacteria, viruses, and parasites are what we are actually worried about.

Because it is so much more expensive and tedious to do so, actual pathogens are virtually never tested for. Over the course of a professional lifetime pouring over indicator tests, in a context where all standards are based on indicators, water workers tend to forget that the indicators are not the things we actually care about.

What are these indicators? More information in the Laboratory section.

- **General coliforms** indicate that the water has come in contact with plant or animal life. General coliforms are universally present, including in pristine spring water. They are of little concern at low levels, except to indicate the effectiveness of disinfection. Chlorinated water and water from perfectly sealed tube wells is the only water I've tested which had zero general coliforms. At very high levels they indicate there is what amounts to a lot of compost in the water, which could easily include pathogens (Ten thousand general coliform bacteria will get you a beach closure, compared to two or four hundred fecal coliforms, or fifty enterococcus).
- **Fecal coliforms**, particularly E. coli, indicate that there are mammal or bird feces in the water.
- **Enterococcus bacteria** also indicate that there are feces from warm blooded animals in the water. Enterococcus are a type of fecal streptococci. They are another valuable indicator for determining the amount of fecal contamination of water.
  According to studies conducted by the EPA, enterococci have a greater correlation with swimming-associated gastrointestinal illness in both marine and fresh waters than other bacterial indicator organisms, and are less likely to "die off" in saltwater.

The more closely related the animal, the more likely pathogens excreted with their feces can infect us.

Human feces are the biggest concern, because anything which infects one human could infect another. There isn't currently a quantitative method for measuring specifically human fecal bacteria (expensive genetic studies can give a presence/absence result).

Ingesting a human stranger's feces via contaminated water supply is a classic means for infections to spread rapidly. The more pathogens an individual carries, the more hazardous their feces. Ingesting feces from someone who is not carrying any pathogens may gross you out, but it can't infect you. Infection rates are around 5% in the US, and approach 100% in areas with poor hygiene and contaminated water supplies.

Keep in the back of your mind that **the ratio of indicators to actual pathogens is not fixed**. It will always be different, sometimes very different. Whenever you are trying to form a mental map of reality based on water tests, you should include in the application of your water intuition an adjustment factor for your best guess of the ratio between indicators and actual pathogens.



This lab equipment is used for testing parameters in water such as Metals, Pesticides and Hydrocarbons. Often geotechnical water testing that is performed to support characterization regarding the fate and transport of contaminants in soils and sediments. Information such as the grain size distribution provides insight into the absorption, accumulation and movement of contaminants as it may relate to various particle sizes.

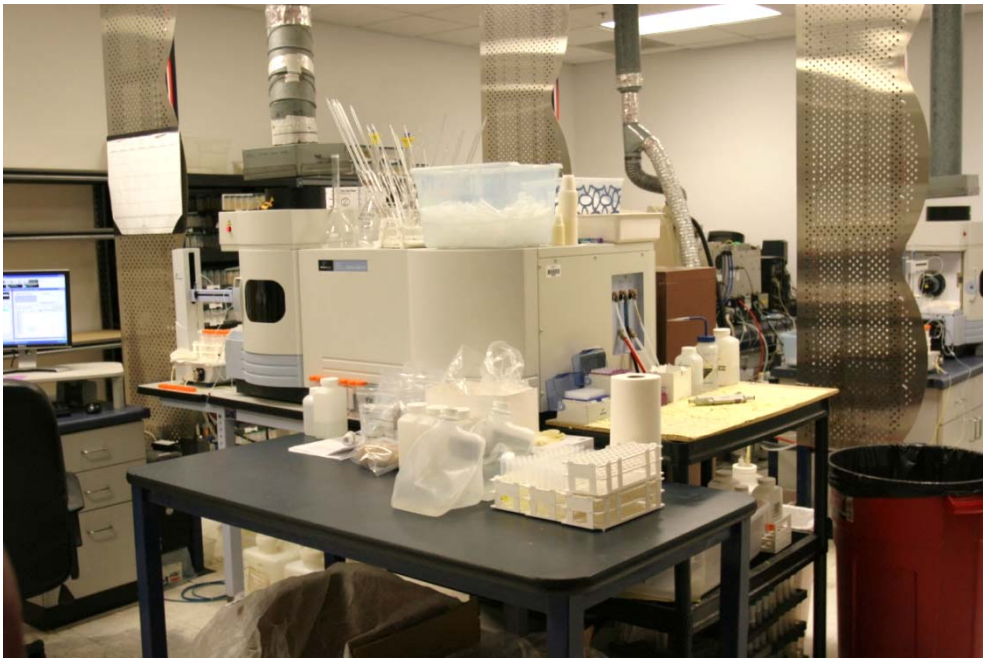




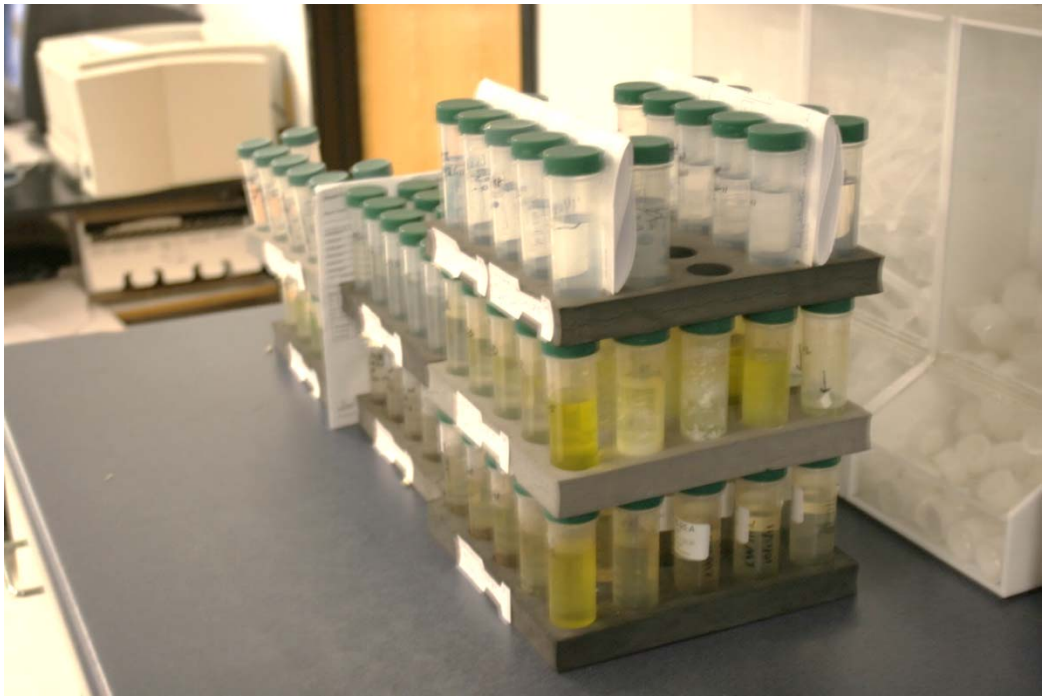
Top Photo: Extraction is a process used to concentrate trace levels of materials, in this case metals. Some metals are able to be complexed with an organic chelating agent. The organic chelating agent, Ammonium pyrrolidine dithiocarbamate (APDC), chemically binds the metals in the water. APDC is easily dissolved in the nonpolar organic solvent, methyl isobutyl ketone (MIBK). Because MIBK is nonpolar, it will not dissolve in water and will separate from the water like oil and water.

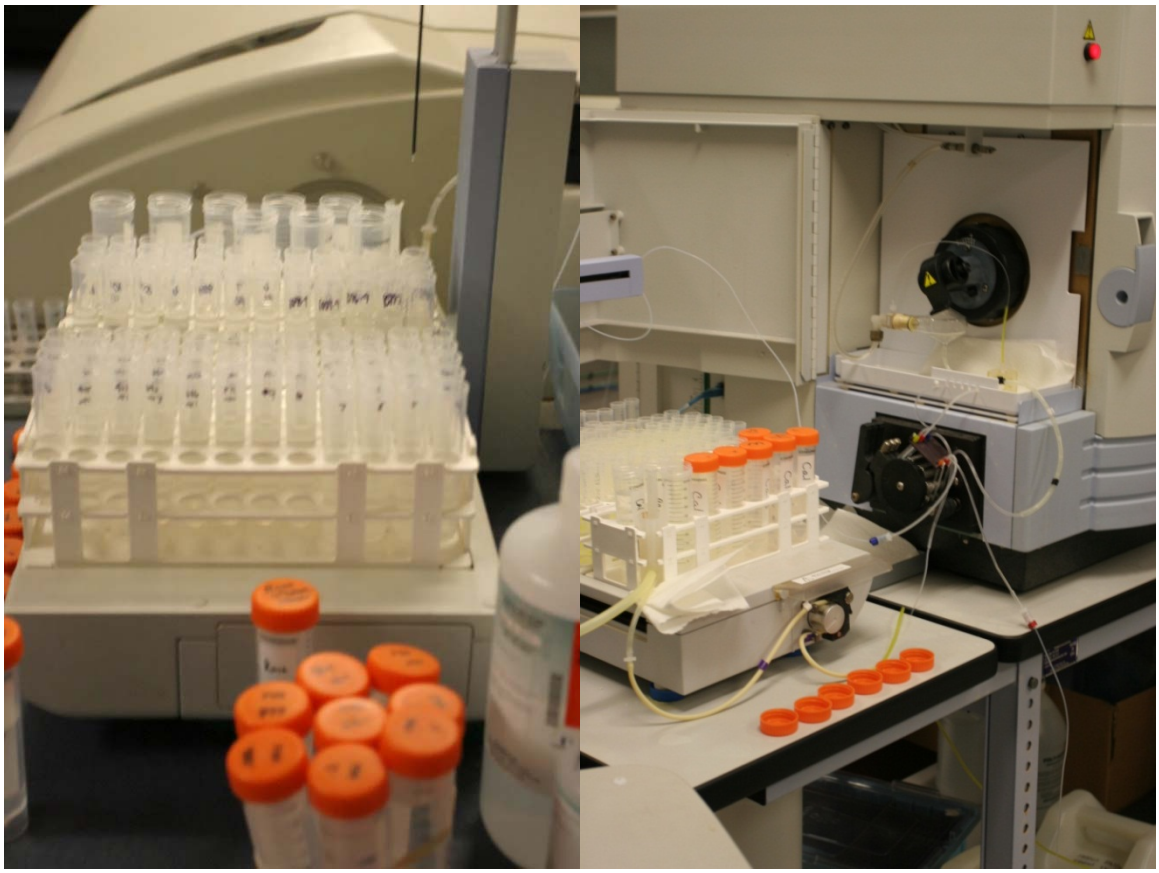
Bottom Photo: Atomic absorption requires the presence of ground state metal atoms (Me^{o}). The initial process which takes place in an atomic absorption spectrophotometer is to create a population of ground state atoms. This is accomplished in a variety of ways, usually classified as flame and non-flame. The simplest and most common method uses a flame to produce these atoms.





Vapor atomic fluorescence spectroscopy (CVAFS) analytical techniques were designed by the EPA for the analysis of Mercury at low and sub parts per trillion (ppt) concentrations. Low detection limits were developed to support risk assessment, Mercury bioaccumulation in fish is a heightened concern. Thirty-nine states have issued fish consumption advisories due to mercury contamination. Mercury is a toxic pollutant across most regulatory programs (air, water, hazardous waste & pollution prevention). It is persistent and harmful to human health and the environment at relatively low levels.





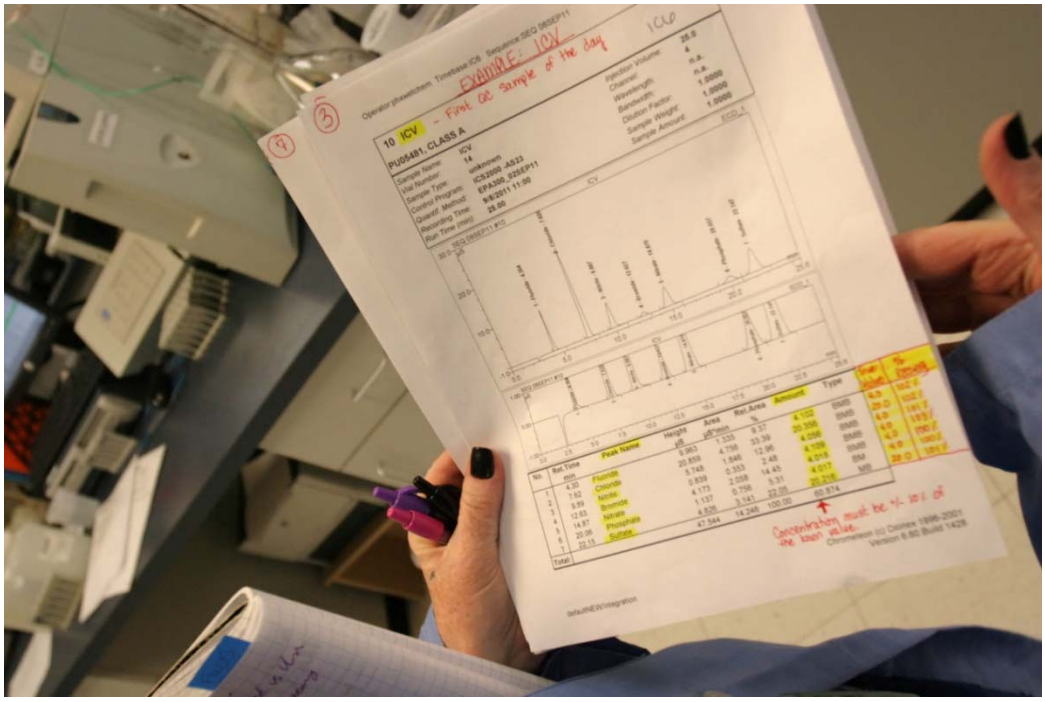
This lab's Quality Assurance Quality Control (QAQC) methods includes spikes, performance samples, replicates, detection limits and blanks.





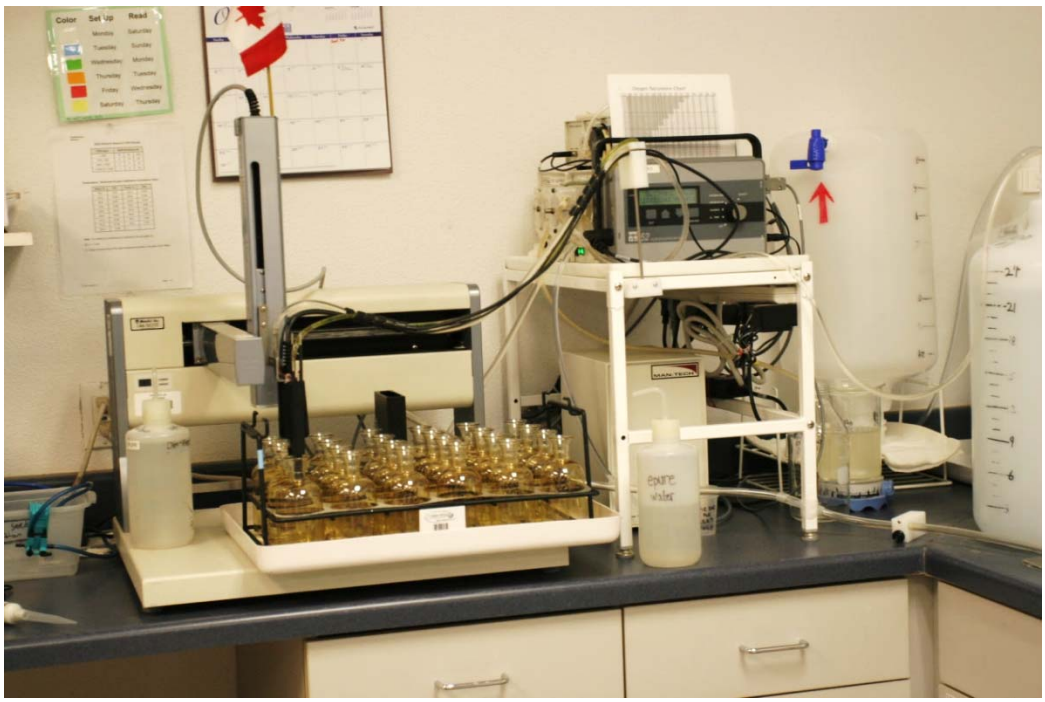
Prepared samples stored for metal analysis.

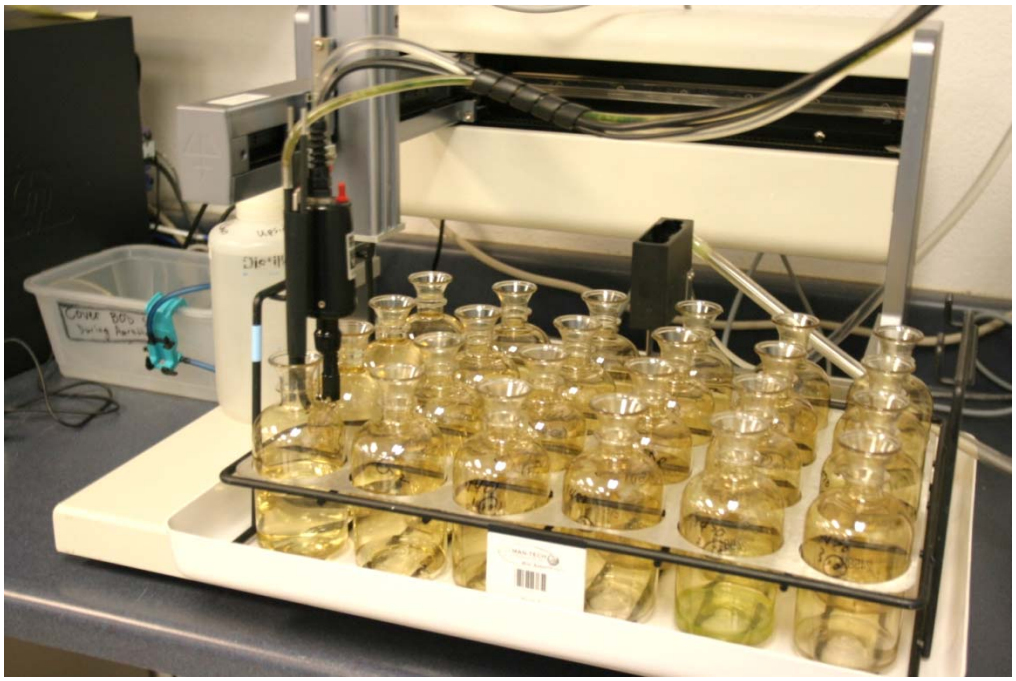




Top Photo: This form shows a typical ion chromatography run will have a standard curve consisting of 4 or 5 points for each ion of interest. A combined ion stock standard is used. The correlation coefficient of the standard curve for each ion should be >.998. The coefficient is calculated by plotting the peak area against the standard concentration using a linear fit.

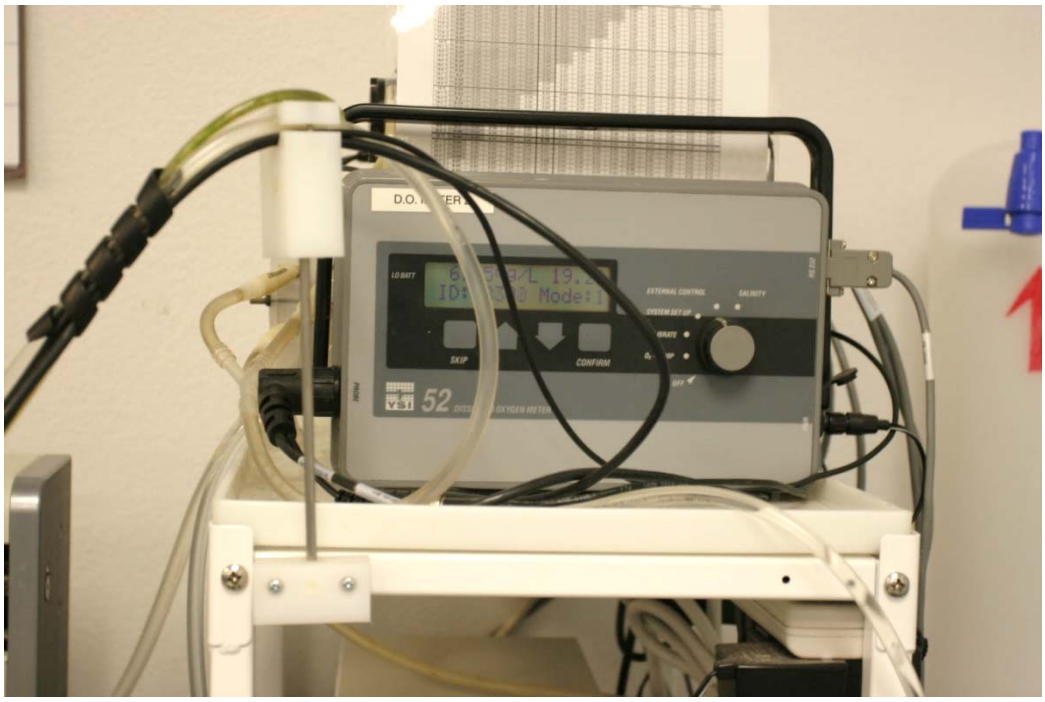
Bottom Photo: Some labs have the luxury of automatic filling dilution water for BOD bottles. If the BOD bottle is filled too rapidly, excessive agitation and bubbling may result in supersaturated water. This device also has the capability of measuring the oxygen level.

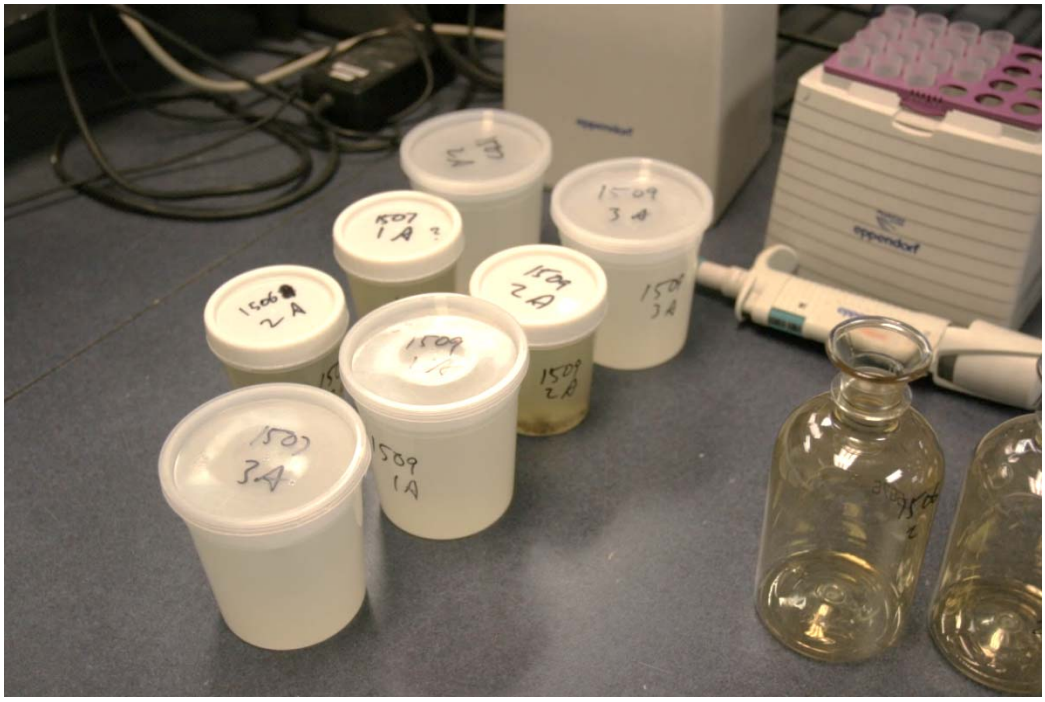




Top Photo: Running a BOD analysis on a sample consists of placing a portion of a sample (along with prepared dilution water) into an air-tight bottle (300 ml volume) and incubating the bottle at 20 +/- 1 deg C for (usually) 5 days.

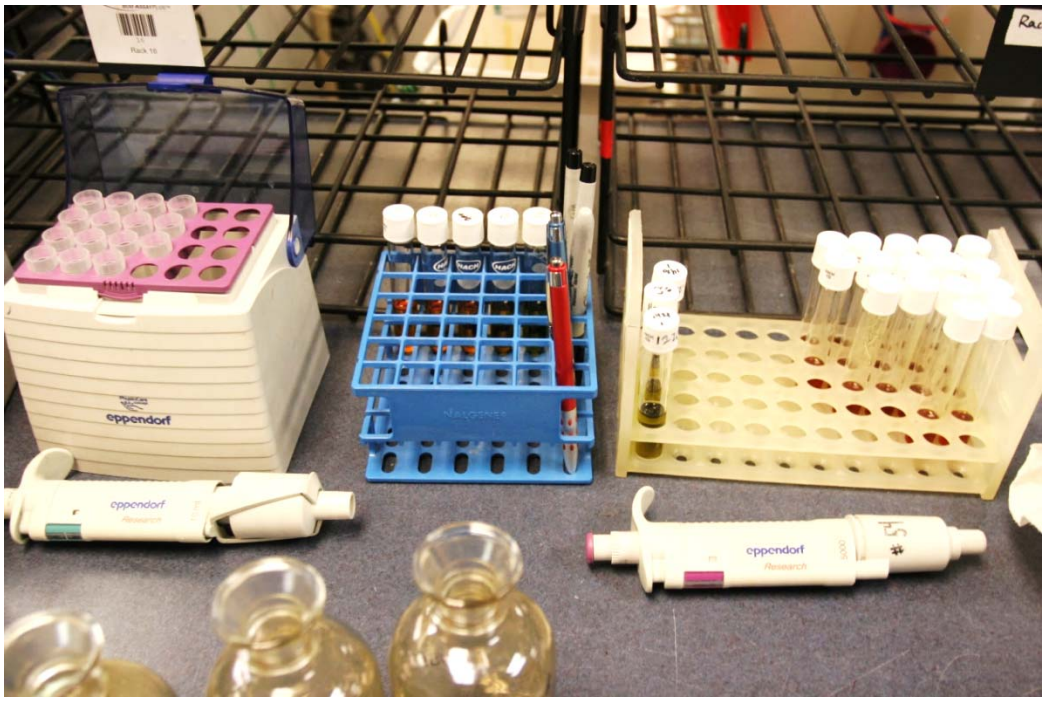
Bottom Photo: Dissolved oxygen is often measured to determine the oxygen level in receiving streams, in the aeration basin, and in the BOD test. The DO meter and electrode work in similar fashion to other electronic meters but unlike the others, this meter must be continually "on" to remain polarized.





Top Photo: Collecting the seed in a 500 ml bottle and let settle at least 1 hour and up to 36 hours. This will allow settleable solids to settle and help assure the seed is homogeneous.

Bottom Photo: In order to validate the BOD test conditions, reagents, and procedure, it is necessary to measure a solution with a known amount of organics. A solution that has a known value is called a standard.





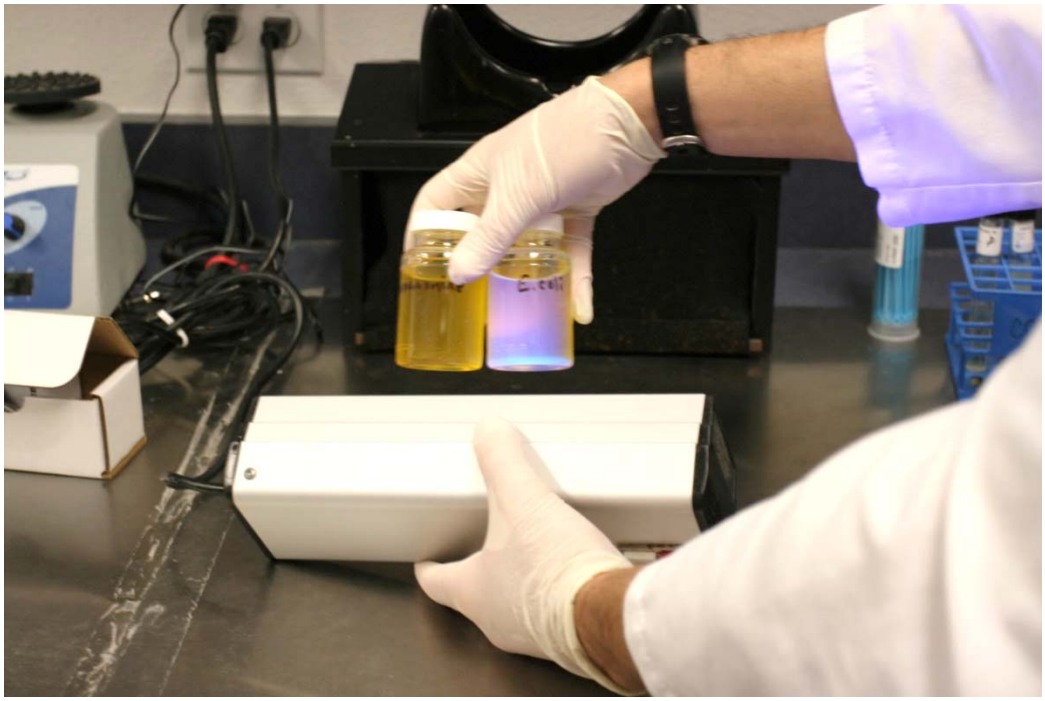
Top Photo: This area is used for Fecal coliform which the most common dilution is prepared by transferring 11 ml of sample to 99 ml of sterile phosphate dilution water using a sterile serological pipet.

Bottom Photo: Some labs perform a variation of coliform testing this technician is making sure his area follows QAQC standards.





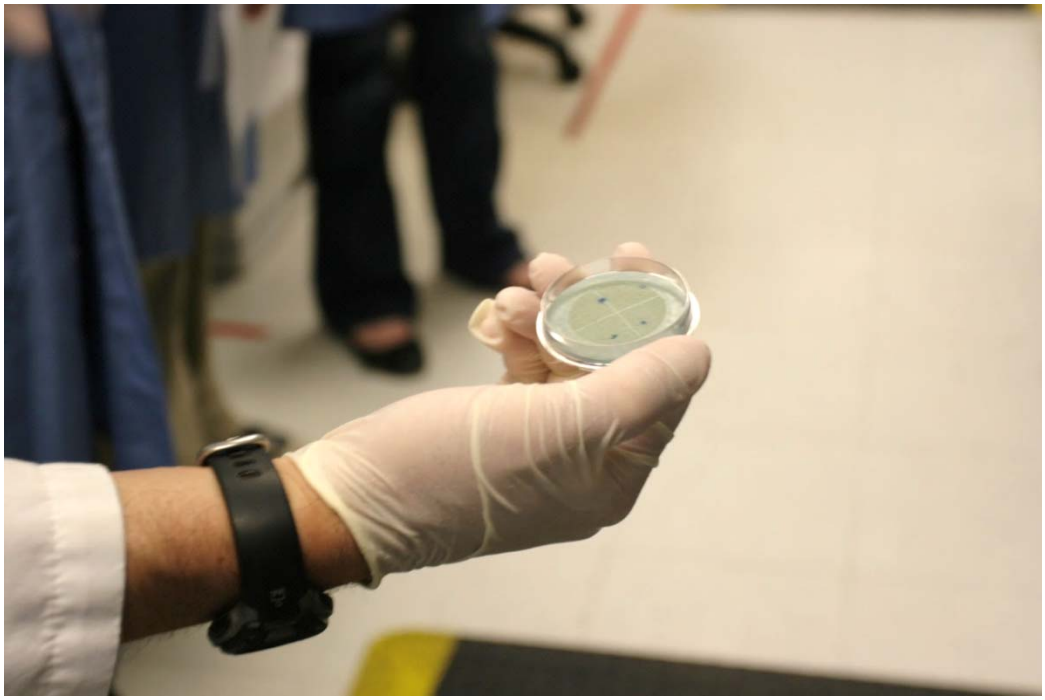
IDEXX bottles are clear plastic disposable containers that are supplied by the manufacturer with the colilert testing kits. Many labs that use the colilert method also issue the IDEXX bottles. Positive identification of total coliform is indicated by fluorescent under the UV light. E-coli are indicated by fluorescent blue colonies.





Top Photo: This technician is using Colilert which is a commercially available enzyme-substrate liquid-broth medium (IDEXX Laboratories, Inc.) that allows the simultaneous detection of total coliforms and *Escherichia coli* (*E. coli*). It is available in the most-probable number (MPN) or the presence/absence (PA) format. The MPN method is facilitated by use of a specially designed disposable incubation tray called the Quanti-Tray®.

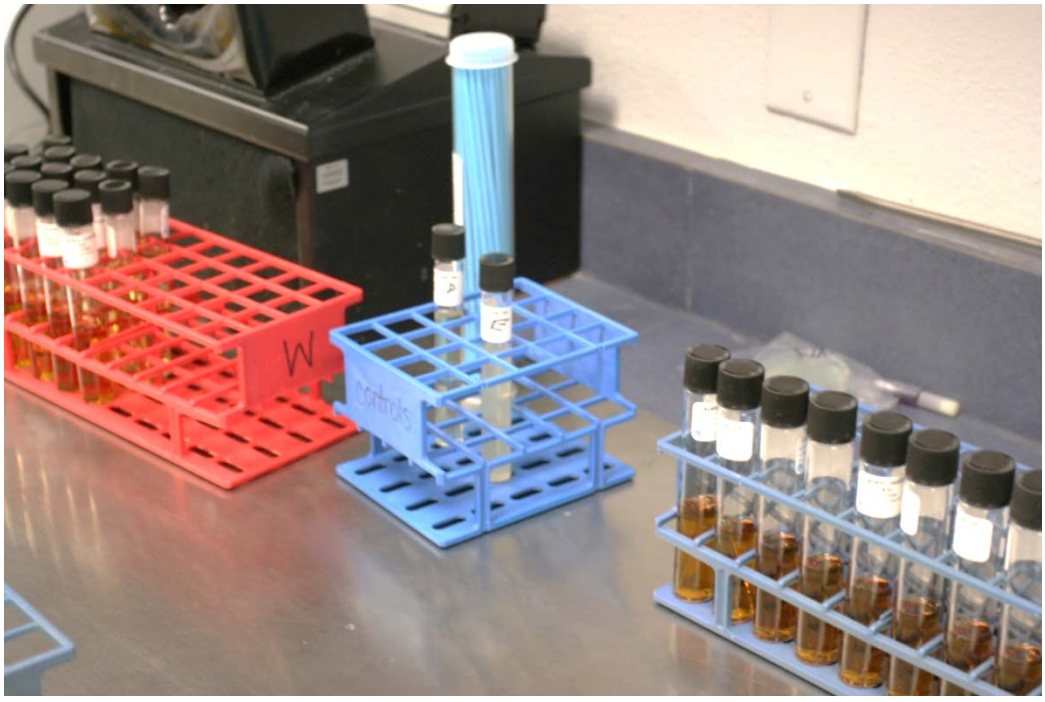
Bottom Photo: Another method is using a petri dish with a filter membrane. The broth and membrane used vary depending on the sample type for water or wastewater.

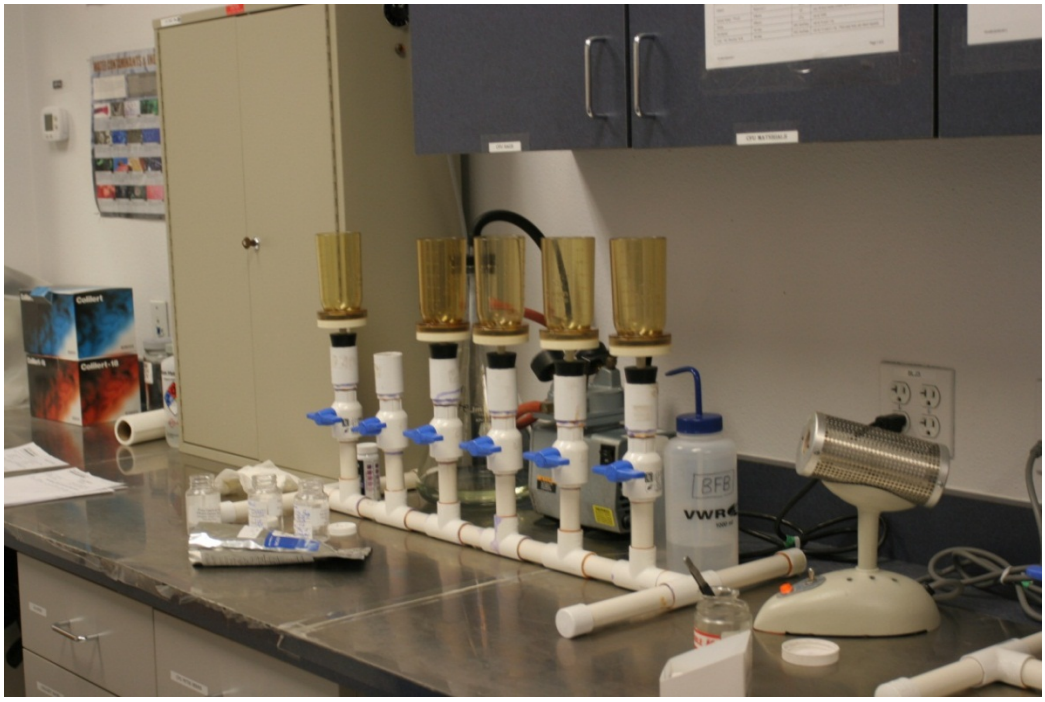




The MPN method (also called Multiple Tube Fermentation Technique) for fecal coliform detection is often used when the sample has excessive turbidity. Excessive turbidity in the sample will plug the membrane filter, causing poor bacteria recovery and slow filtration times.

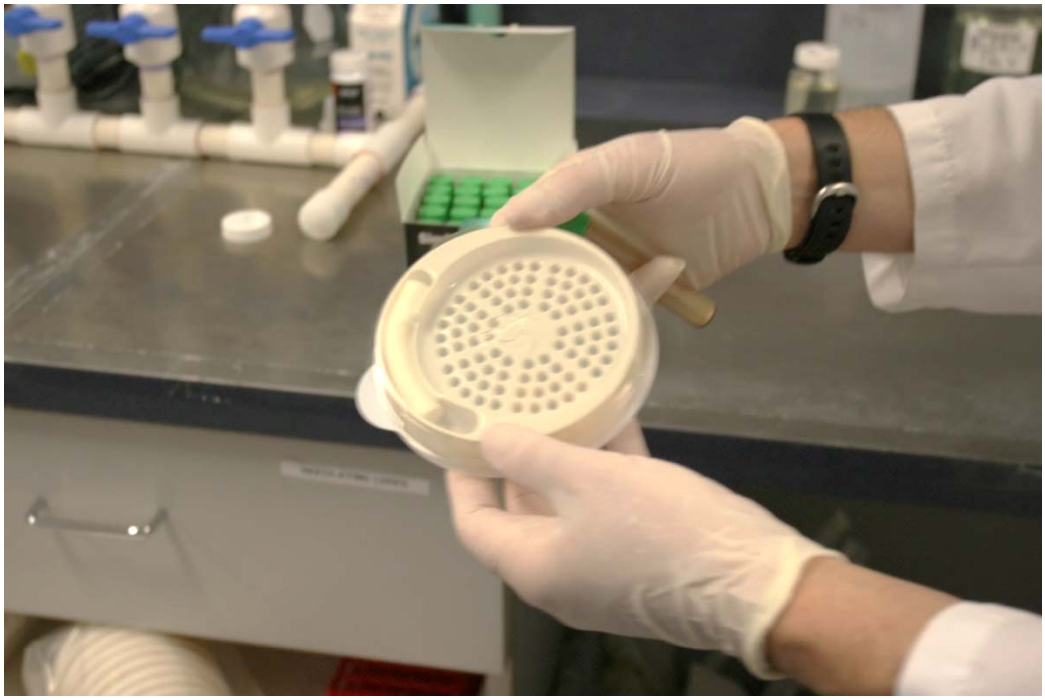
The MPN method involves adding the wastewater sample to a series of 5 sets of tubes, each of which contains either lactose broth or lauryl tryptose broth and an inverted tube. The tubes are then incubated at 35 + 0.5oC for 24 to 48 hours.





Top Photo: Analytical funnels are 100 ml filtration units that allow the membrane to be removed.

Bottom Photo: The heterotrophic plate count (HPC) is a procedure for estimating the number of live heterotrophic bacteria (requiring organic compounds of carbon and nitrogen for nourishment) in water. The technician will count the colored holes for the results.



Water Quality & River Sampling Photos



Top Photo: Technicians use several different devices to sample wells depending on the depth of the water table. Some use hand bailers or small gas powered submersible pumps.

Bottom Photo: Technicians are always concerned with Quality Assurance; here the containers have DI water for rinsing in the field. The containers that look like milk bottles are used for the equal width depth integrated sampler.





Top Photo: This multi probe device measures dissolved oxygen (DO), pH, conductivity and total dissolved solids (TDS), the device requires calibration for each parameter.

Bottom Photo: Collection of isokinetic, depth-integrated samples is done using either an equal-width-increment (EWI) or equal-discharge-increment (EDI) sampling method. The methods typically result in a composite sample that represents the streamflow-weighted concentrations of the stream cross section being sampled. That sample is poured into the bottles that resemble old fashion milk bottles.





The churn splitter was designed to facilitate the withdrawal of a representative subsample from a large composite sample of a water-sediment mixture. For example, samples from several verticals in a stream cross section, differing slightly from each other in chemical quality and sediment concentration, can be placed in the churn and be mixed into a relatively homogenous suspension, any subsample withdrawn from the churn should be equal in chemical quality and sediment concentration to any other subsample from the churn.





Ampullariidae, common name the apple snails, is a family of large freshwater snails, aquatic gastropod mollusks with a gill and an operculum. This family is in the superfamily Ampullarioidea and is the type family of that superfamily. The Ampullariidae are unusual because they have both a gill and a lung, the mantle cavity being divided in order to separate the two types of respiratory structures. This adaptation allows these snails to be amphibious. Bottom photo, snail eggs clusters on rocks.





Top Photo: When sampling in the river it is suggested that a minimum of two people participate. One person is holding the collection net while the other carefully disturbs the sediment for collection.

Bottom Photo: This river contained larvae of mayfly and stone flies along with leaches.





Sieving invertebrate samples reduces the volume of sediment that must be sorted through in the lab. A #60 sieve is recommended because the smaller invertebrates will be retained by the #60 sieve and should yield more complete invertebrate community data for a site. Any large debris should be cleaned (remove invertebrates and add them to the sample) and removed from the sample. The sample is then washed through the sieve over the side of the boat or in a tub with site water until no more fine sediment washes through the mesh.

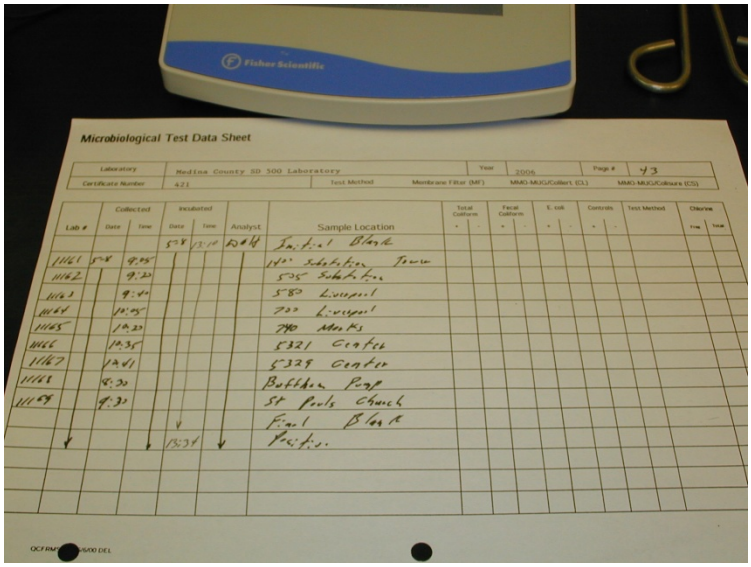


Membrane Filter Total Coliform Technique

The membrane filter total Coliform technique is used at Medina County for drinking water quality testing. The following is a summary of this test. A sampling procedure sheet is given to all sample takers by Medina County.

The samples are taken in sterile 100 mL containers. These containers, when used for chlorinated water samples, have a sodium thiosulfate pill or solution to dechlorinate the sample.

The sample is placed in cold storage after proper sample taking procedures are followed. (See sample procedures below)



The samples are taken to the laboratory with a chain of custody to assure no tampering of samples can occur.

These samples are logged in at the laboratory.

No longer than 30 hours can lapse between the time of sampling and time of test incubation. (8 hours for heterotrophic, nonpotable 6 hours, others not longer than 24 hours)

All equipment is sterilized by oven and autoclave.
Glassware in oven at 170°C ± 10°C with foil (or other suitable wrap) loosely fitting and secured immediately after sterilization.

Filtration units in autoclave at 121°C for 30 minutes.



Use sterile petri dishes, grid, and pads bought from a reliable company – certified, quality assured - test for satisfactory known positive amounts.

Incubators – 35°C ± .5°C (60% relative humidity)
M-endo medium is prepared and heated to near boiling removed from heat cooled to 45°C pH adjusted to 7.2 ± .2 and immediately dispensed 8ml to plates. Keep refrigerated and discard after 2 weeks.

Plates can be stored in a dated box with expiration date and discarded if not used.No denatured alcohol should be used. Everclear or 95% proof alcohol or absolute methyl may be used for sterilizing forceps by flame.

Procedure:
Counters are alcohol wiped.
Bench sheets are filled out.
Samples are removed from refrigeration.
Sterile wrapped utensils are placed on counters.
Filtration units are placed onto sterile membrane filters by aseptic technique using sterile forceps.
Sterile petri dishes are labeled.
The samples closures are clipped.
The sample is shaken 25 times 1 foot in length within 7 seconds.
100 mL is filtered and rinsed with sterile distilled water 3 times.
The membrane filter is aseptically removed from filter holder.
A sterile padded petri dish is used and the membrane filter is rolled onto the pad making sure no air bubbles form.
The sterile labeled lid is placed on the petri dish.
2 blanks and a known is run with each series of samples.
The samples are placed in the 35^{o}C $\pm$ .5^{o}C incubator stacked no higher than 3 for 22 – 24 hours (Humidity can be maintained by saturated paper towels placed under containers holding petri dishes.)
After 22- 24 hours view the petri dishes under a 10 –15 power magnification with cool white fluorescent light.
Count all colonies that appear pink to dark red with a metallic surface sheen – the sheen may vary in size from a pin head to complete coverage.
Report as Total Coliform per 100 mL.
If no colonies are present report as <1 coliform/100mL.

Anything greater than 1 is over the limit for drinking water for 2 samples taken 24 hours apart. Further investigation may be necessary – follow Standard Methods accordingly.



Photograph and Credits to Mary McPherson
Aran™ Aqua Analytical Laboratory Director.

Escherichia coli

Two types of pathogenic Escherichia coli, enteropathogenic E. coli (EPEC) and enterohemorrhagic E. coli (EHEC), cause diarrheal disease by disrupting the intestinal environment through the intimate attachment of the bacteria to the intestinal epithelium.

E. coli O157:H7
E. coli O157:H7 (bacterium) found in human feces. Symptoms vary with type caused gastroenteritis.

Escherichia coli O157:H7 is an emerging cause of foodborne illness. An estimated 73,000 cases of infection and 61 deaths occur in the United States each year. Infection often leads to bloody diarrhea, and occasionally to kidney failure. Most illnesses have been associated with eating undercooked, contaminated ground beef. Person-to-person contact in families and child care centers is also an important mode of transmission. Infection can also occur after drinking raw milk and after swimming in or drinking sewage-contaminated water.

Consumers can prevent *E. coli* O157:H7 infection by thoroughly cooking ground beef, avoiding unpasteurized milk, and washing hands carefully. Because the organism lives in the intestines of healthy cattle, preventive measures on cattle farms and during meat processing are being investigated.

What is *Escherichia coli* O157:H7?
E. coli O157:H7 is one of hundreds of strains of the bacterium Escherichia coli. Although most strains are harmless and live in the intestines of healthy humans and animals, this strain produces a powerful toxin and can cause severe illness.

E. coli O157:H7 was first recognized as a cause of illness in 1982 during an outbreak of severe bloody diarrhea; the outbreak was traced to contaminated hamburgers. Since then, most infections have come from eating undercooked ground beef.

The combination of letters and numbers in the name of the bacterium refers to the specific markers found on its surface and distinguishes it from other types of *E. coli.*

Currently, there are four recognized classes of enterovirulent *E. coli* (collectively referred to as the EEC group) that cause gastroenteritis in humans. Among these is the enterohemorrhagic (EHEC) strain designated *E. coli* O157:H7. *E. coli* is a normal inhabitant of the intestines of all animals, including humans. When aerobic culture methods are used, *E. coli* is the dominant species found in feces.

Normally *E. coli* serves a useful function in the body by suppressing the growth of harmful bacterial species and by synthesizing appreciable amounts of vitamins. A minority of *E. coli* strains are capable of causing human illness by several different mechanisms. *E. coli* serotype O157:H7 is a rare variety of *E. coli* that produces large quantities of one or more related, potent toxins that cause severe damage to the lining of the intestine. These toxins [verotoxin (VT), shiga-like toxin] are closely related or identical to the toxin produced by *Shigella dysenteriae*.

How does *E. coli* or other fecal coliforms get in the water?
E. coli comes from human and animal wastes. During rainfalls, snow melts, or other types of precipitation, *E. coli* may be washed into creeks, rivers, streams, lakes, or groundwater. When these waters are used as sources of drinking water and the water is not treated or inadequately treated, *E. coli* may end up in drinking water.

How is water treated to protect me from *E. coli*?
The water can be treated using chlorine, ultra-violet light, or ozone, all of which act to kill or inactivate *E. coli.* Systems using surface water sources are required to disinfect to ensure that all bacterial contamination such as *E. coli*. is inactivated. Systems using ground water sources are not required to disinfect, although many of them do.

How does the U.S. Environmental Protection Agency regulate *E. coli*?
According to EPA regulations, a system that operates at least 60 days per year, and serves 25 people or more or has 15 or more service connections, is regulated as a public water system under the Safe Drinking Water Act. If a system is not a public water system as defined by EPA regulations, it is not regulated under the Safe Drinking Water Act, although it may be regulated by state or local authorities.

Under the Safe Drinking Water Act, the EPA requires public water systems to monitor for coliform bacteria. Systems analyze first for total coliform, because this test is faster to produce results. Any time that a sample is positive for total coliform, the same sample must be analyzed for either fecal coliform or *E. coli*. Both are indicators of contamination with animal waste or human sewage.

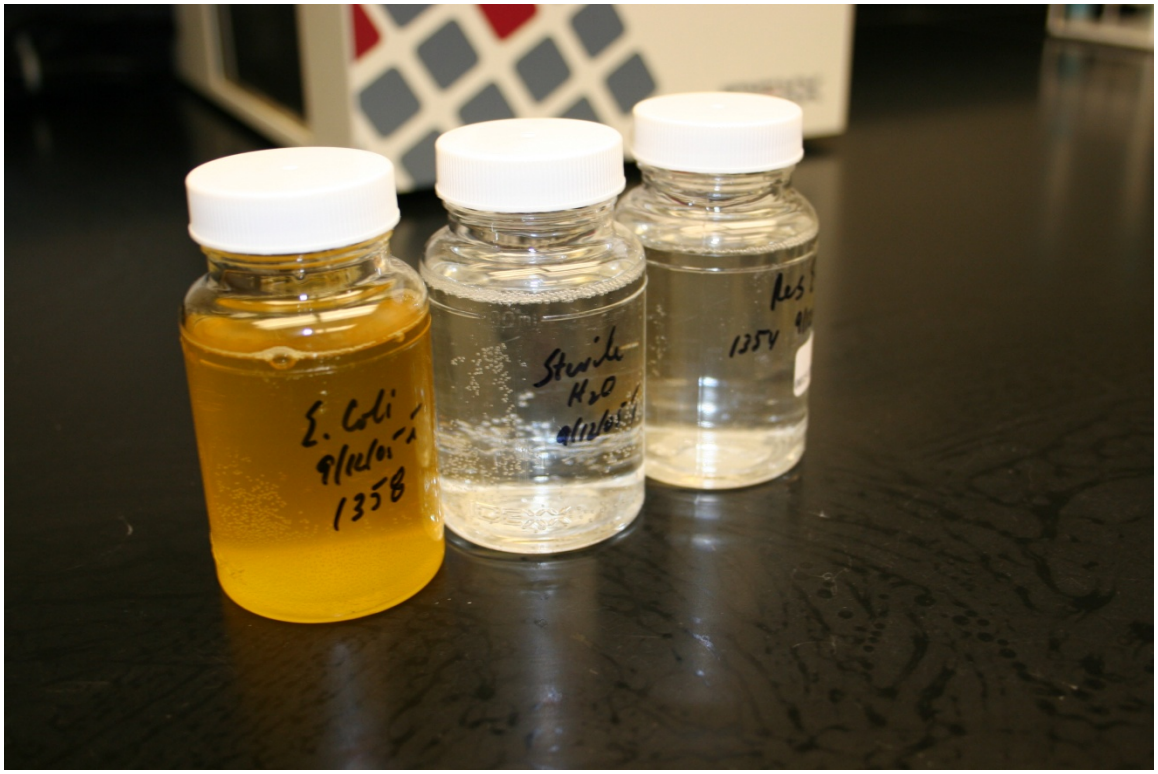
The largest public water systems (serving millions of people) must take at least 480 samples per month. Smaller systems must take at least five samples a month unless the state has conducted a sanitary survey – a survey in which a state inspector examines system components and ensures they will protect public health – at the system within the last five years.

Systems serving 25 to 1,000 people typically take one sample per month. Some states reduce this frequency to quarterly for ground water systems if a recent sanitary survey shows that the system is free of sanitary defects. Some types of systems can qualify for annual monitoring. Systems using surface water, rather than ground water, are required to take extra steps to protect against bacterial contamination because surface water sources are more vulnerable to such contamination. At a minimum, all systems using surface waters must disinfect. Disinfection will kill *E. coli* O157:H7.

What can I do to protect myself from *E. coli* O157:H7 in drinking water?
Approximately 89 percent of Americans are receiving water from community water systems that meet all health-based standards. Your public water system is required to notify you if, for any reason, your drinking water is not safe. If you wish to take extra precautions, you can boil your water for one minute at a rolling boil, longer at higher altitudes.

To find out more information about your water, see the Consumer Confidence Report from your local water supplier or contact your local water supplier directly. You can also obtain information about your local water system on the EPA's website at www.epa.gov/safewater/dwinfo.htm.

Positive Tests
If you draw water from a private well, you can contact your state health department to obtain information on how to have your well tested for total coliforms and *E. coli* contamination. If your well tests positive for *E. coli*, there are several steps that you should take: (1) begin boiling all water intended for consumption, (2) disinfect the well according to procedures recommended by your local health department, and (3) monitor your water quality to make certain that the problem does not recur. If the contamination is a recurring problem, you should investigate the feasibility of drilling a new well or install a point-of-entry disinfection unit, which can use chlorine, ultraviolet light, or ozone.

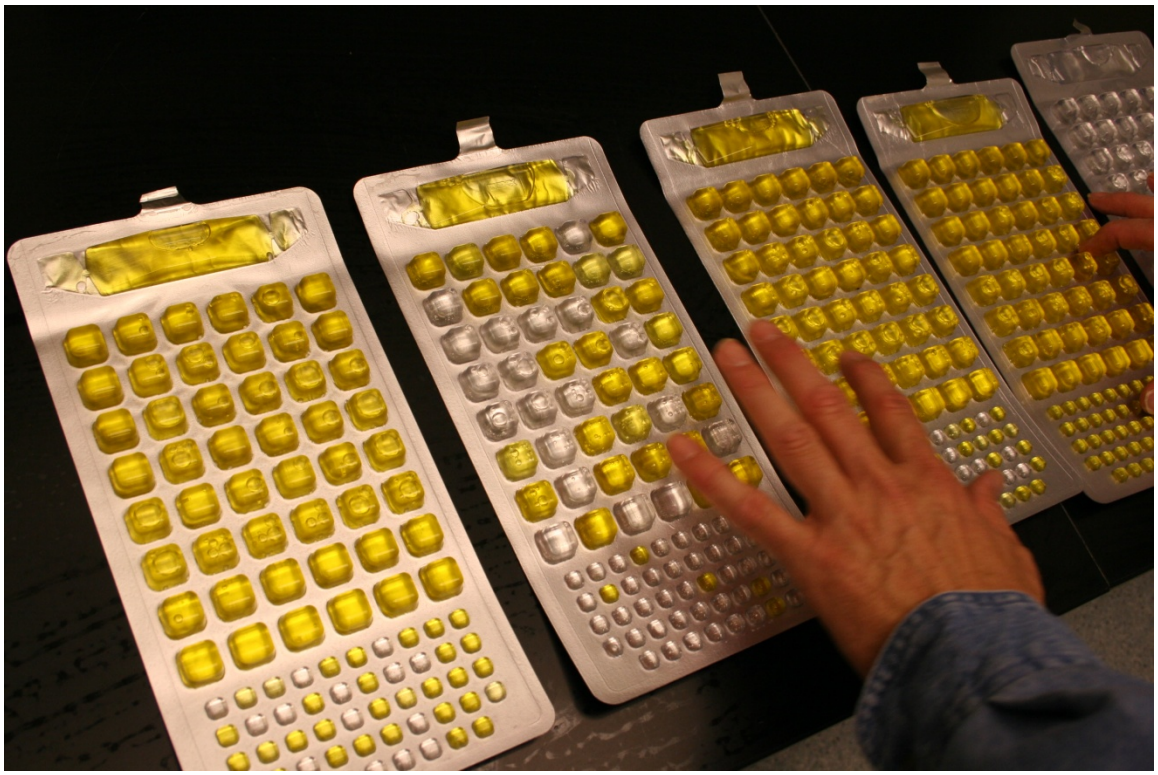


How is *E. coli* O157:H7 spread?
The organism can be found on a small number of cattle farms and can live in the intestines of healthy cattle. Meat can become contaminated during slaughter, and organisms can be thoroughly mixed into beef when it is ground. Bacteria present on a cow's udders or on equipment may get into raw milk. Eating meat, especially ground beef that has not been cooked sufficiently to kill *E. coli* O157:H7 can cause infection. Contaminated meat looks and smells normal. Although the number of organisms required to cause disease is not known, it is suspected to be very small.

Among other known sources of infection are consumption of sprouts, lettuce, salami, unpasteurized milk and juice, and swimming in or drinking sewage-contaminated water. Bacteria in diarrheal stools of infected persons can be passed from one person to another if hygiene or handwashing habits are inadequate. This is particularly likely among toddlers who are not toilet trained. Family members and playmates of these children are at high risk of becoming infected. Young children typically shed the organism in their feces for a week or two after their illness resolves. Older children rarely carry the organism without symptoms.

What illness does *E. coli* O157:H7 cause?
E. coli O157:H7 infection often causes severe bloody diarrhea and abdominal cramps; sometimes the infection causes nonbloody diarrhea or no symptoms. Usually little or no fever is present, and the illness resolves in 5 to 10 days. Hemorrhagic colitis is the name of the acute disease caused by *E. coli* O157:H7.

In some persons, particularly children under 5 years of age and the elderly, the infection can also cause a complication called hemolytic uremic syndrome, in which the red blood cells are destroyed and the kidneys fail. About 2%-7% of infections lead to this complication. In the United States, hemolytic uremic syndrome is the principal cause of acute kidney failure in children, and most cases of hemolytic uremic syndrome are caused by *E. coli* O157:H7.



How is *E. coli* O157:H7 infection diagnosed?
Infection with *E. coli* O157:H7 is diagnosed by detecting the bacterium in the stool. Most laboratories that culture stool do not test for *E. coli* O157:H7, so it is important to request that the stool specimen be tested on sorbitol-MacConkey (SMAC) agar for this organism. All persons who suddenly have diarrhea with blood should get their stool tested for *E. coli* O157:H7.

How is the illness treated?
Most persons recover without antibiotics or other specific treatment in 5-10 days. There is no evidence that antibiotics improve the course of disease, and it is thought that treatment with some antibiotics may precipitate kidney complications. Antidiarrheal agents, such as loperamide (Imodium), should also be avoided. Hemolytic uremic syndrome is a life-threatening condition usually treated in an intensive care unit. Blood

transfusions and kidney dialysis are often required. With intensive care, the death rate for hemolytic uremic syndrome is 3%-5%.

What are the long-term consequences of infection?
Persons who only have diarrhea usually recover completely. About one-third of persons with hemolytic uremic syndrome have abnormal kidney function many years later, and a few require long-term dialysis. Another 8% of persons with hemolytic uremic syndrome have other lifelong complications, such as high blood pressure, seizures, blindness, paralysis, and the effects of having part of their bowel removed.

What can be done to prevent the infection?
E. coli O157:H7 will continue to be an important public health concern as long as it contaminates meat. Preventive measures may reduce the number of cattle that carry it and the contamination of meat during slaughter and grinding. Research into such prevention measures is just beginning.

What can you do to prevent *E. coli* O157:H7 infection?
Cook all ground beef and hamburger thoroughly. Because ground beef can turn brown before disease-causing bacteria are killed, use a digital instant-read meat thermometer to ensure thorough cooking. Ground beef should be cooked until a thermometer inserted into several parts of the patty, including the thickest part, reads at least 160º F. Persons who cook ground beef without using a thermometer can decrease their risk of illness by not eating ground beef patties that are still pink in the middle.

If you are served an undercooked hamburger or other ground beef product in a restaurant, send it back for further cooking. You may want to ask for a new bun and a clean plate, too. Avoid spreading harmful bacteria in your kitchen. Keep raw meat separate from ready-to-eat foods. Wash hands, counters, and utensils with hot soapy water after they touch raw meat. Never place cooked hamburgers or ground beef on the unwashed plate that held raw patties. Wash meat thermometers in between tests of patties that require further cooking. Drink only pasteurized milk, juice, or cider.

Commercial juice with an extended shelf-life that is sold at room temperature (e.g. juice in cardboard boxes, vacuum sealed juice in glass containers) has been pasteurized, although this is generally not indicated on the label. Juice concentrates are also heated sufficiently to kill pathogens.

Wash fruits and vegetables thoroughly, especially those that will not be cooked. Children under 5 years of age, immunocompromised persons, and the elderly should avoid eating alfalfa sprouts until their safety can be assured. Methods to decontaminate alfalfa seeds and sprouts are being investigated.

Drink municipal water that has been treated with chlorine or other effective disinfectants. Avoid swallowing lake or pool water while swimming. Make sure that persons with diarrhea, especially children, wash their hands carefully with soap after bowel movements to reduce the risk of spreading infection, and that persons wash hands after changing soiled diapers. Anyone with a diarrheal illness should avoid swimming in public pools or lakes, sharing baths with others, and preparing food for others.

Chapter References

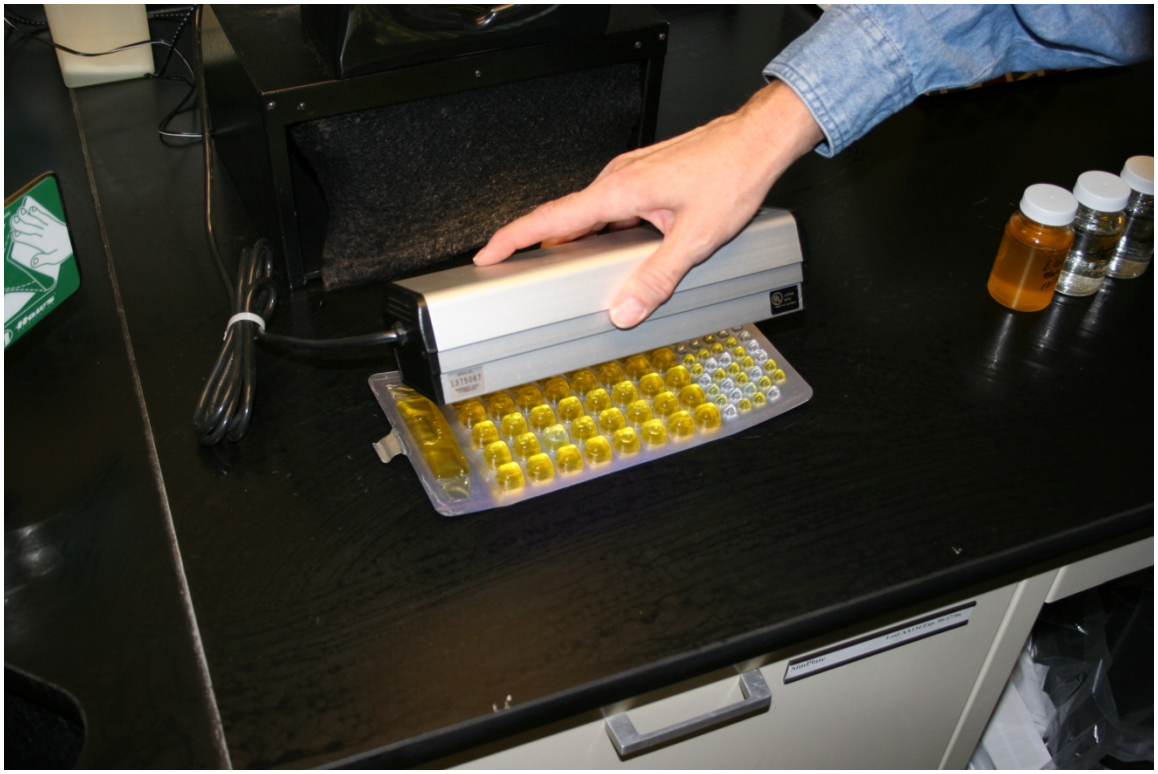
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Pathogenic *Escherichia coli* MLST Database – Clonal Group Definition

Clonal Group	Class	Reference Strain	Comments
0	N/A	TW08017	Undefined Clonal Group
1	atypical B13	TW08889	cluster within E. coli with Boydii 13 (B13) antigen
2	STEC 13	TW08045	includes STEC R:H18 strain
3	EPEC 3	TW06584	O86:H34 strains from infant diarrhea
4	H51	TW08260	cluster with H51 antigen
5	NT-1	TW08997	no common traits
6	EPEC 1	TW06375	Classical EPEC with H6
7	EPEC 4	TW03173	O119:H6 strains, basal to EPEC 1
8	NT-2	TW08983	no common traits
9	NT-3	TW08990	no common traits
10	Shigella 3	TW08837	Reeves Shigella Group 3 including serotypes F1a, F2a, F3a, F3b, F4, F5a FY
11	EHEC 1	TW08264	O157:H7 and relatives including atypical EPEC O55:H7
12	STEC 12	TW00964	STEC O145 strains
13	ETEC P	TW00601	ETEC from pigs including O157:H43 clone
14	EHEC 2	TW00970	O26:H11 and O111:H8 and relatives including RDEC
15	NT-4	TW09177	no common traits
16	EIEC 2	TW01095	Invasive strains with O type 29, 124, 152, 164
17	EPEC 2	TW01120	Classical EPEC with H2 antigen
18	STEC 8	TW04909	Serotype O104:H21, includes Montana outbreak strain G5506
19	STEC 9	TW08580	Serotype O174:H8
20	STEC 10	TW07618	Various serotypes
21	STEC 11	TW07613	Serotype O111:H28
22	EIEC 1	TW01116	Invasive strains with O types 29, 124, 152, 164
23	ECOR A	TW00073	Includes atypical EPEC O111:H12
24	STEC 3	TW08023	Serotype O121:H19
25	Shigella 1	TW07572	Reeves Shigella Group 1 including serotypes F6, D3, D6, D7, D9, D11, D12, B1, B2, B3, B4, B8, B10, B14, B15, B18
26	Shigella 2a	TW02615	Reeves Shigella Group 2 including serotypes D2, D4, B9, B15
27	Shigella 2b	TW01151	Reeves Shigella Group 2 including serotypes B5, B11, B17
28	EPEC 5	TW04892	Atypical EPEC with serotype O111:H9, includes Finland outbreak strain 921
29	Sonnei	TW01150	Shigella sonnei
30	STEC 2	TW01391	Includes serotype O113:H21 and ECOR 30
31	NT-5	TW00676	no common traits
32	NT-6	TW07795	no common traits

33	NT-13	TW09011	no common traits
34	STEC 1	TW01393	STEC with H21 antigen including B2F1
35	SMEC 1	TW02268	Extraintestinal strains including RS218
36	NT-7	TW07608	no common traits
37	Sand 2	TW09237	Environmental E. coli clone
38	UTI 1	TW08018	Uropathogens including CFT073
39	STEC 4	TW07995	Mix of serotypes O121, O116, also includes an EIEC 1758-70
40	NT-8	TW09214	no common traits
41	STEC 14	TW08574	Serotype O174:H2
42	NT-7	TW07612	no common traits
43	STEC	TW01670	serotype O8:H19
44	NT-10	TW10091	no comment traits
45	NT-11	TW08942	no comment traits
46	NT-12	TW09069	no comment traits



Quebec Colony Counter

Escherichia Coli Chapter 6 Review

1. _____ is one of hundreds of strains of the bacterium Escherichia coli. Although most strains are harmless and live in the intestines of healthy humans and animals, this strain produces a powerful toxin and can cause severe illness.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

2. _____ was first recognized as a cause of illness in 1982 during an outbreak of severe bloody diarrhea; the outbreak was traced to contaminated hamburgers. Since then, most infections have come from eating undercooked ground beef.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

3. Two types of pathogenic _____, enteropathogenic E. coli (EPEC) and enterohemorrhagic E. coli (EHEC), cause diarrheal disease by disrupting the intestinal environment through the intimate attachment of the bacteria to the intestinal epithelium.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

4. _____ (bacterium) found in human feces. Symptoms vary with type caused gastroenteritis.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

5. _____O157:H7 is an emerging cause of foodborne illness. An estimated 73,000 cases of infection and 61 deaths occur in the United States each year. Infection often leads to bloody diarrhea, and occasionally to kidney failure.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

6. The combination of letters and numbers in the name of the _____ refers to the specific markers found on its surface and distinguishes it from other types of E. coli.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

7. Currently, there are four recognized classes of enterovirulent E. coli (collectively referred to as the EEC group) that cause gastroenteritis in humans. Among these is the _____ (EHEC) strain designated E. coli O157:H7.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

8. E. coli is a normal inhabitant of the intestines of all animals, including humans. When _____ are used, E. coli is the dominant species found in feces.
A. Enterohemorrhagic
B. Escherichia coli
C. E. coli O157:H7
D. Bacterium
E. None of the above

9. _____ are bacteria that are associated with human or animal wastes. They usually live in human or animal intestinal tracts, and their presence in drinking water is a strong indication of recent sewage or animal waste contamination.
A. Safe Drinking Water Act
B. Vitamins
C. Shigella dysenteriae
D. Fecal coliforms
E. Chlorine

10. The water can be treated using _____, ultra-violet light, or ozone, all of which act to kill or inactivate E. coli. Systems using surface water sources are required to disinfect to ensure that all bacterial contamination is inactivated, such as E. coli. Systems using ground water sources are not required to disinfect, although many of them do.
A. Safe Drinking Water Act
B. Vitamins
C. Shigella dysenteriae
D. Fecal coliforms
E. Chlorine

11. According to EPA regulations, a system that operates at least 60 days per year, and serves 25 people or more or has 15 or more service connections, is regulated as a public water system under the _____.
A. Safe Drinking Water Act
B. Vitamins
C. Shigella dysenteriae
D. Fecal coliforms
E. Chlorine

12. If a system is not a public water system as defined by EPA regulations, it is not regulated under the _____, although it may be regulated by state or local authorities.
A. Safe Drinking Water Act
B. Vitamins
C. Shigella dysenteriae
D. Fecal coliforms
E. Chlorine

13. Under the _____, the EPA requires public water systems to monitor for coliform bacteria. Systems analyze first for total coliform, because this test is faster to produce results. Any time that a sample is positive for total coliform, the same sample must be analyzed for either fecal coliform or E. coli. Both are indicators of contamination with animal waste or human sewage.
A. Safe Drinking Water Act
B. Vitamins
C. Shigella dysenteriae
D. Fecal coliforms
E. Chlorine

14. The largest public water systems (serving millions of people) must take at least 50 samples per month. Smaller systems must take at least 20 samples a month unless the state has conducted a sanitary survey – a survey in which a state inspector examines system components and ensures they will protect public health – at the system within the last year.
A. True
B. False

15. Systems serving 25 to 1,000 people typically take one sample per month. Some states reduce this frequency to quarterly for ground water systems if a recent sanitary survey shows that the system is free of sanitary defects. Some types of systems can qualify for annual monitoring. Systems using surface water, rather than ground water, are required to take extra steps to protect against bacterial contamination because surface water sources are more vulnerable to such contamination. At a minimum, all systems using surface waters must disinfect.
A. True
B. False

16. Disinfection will kill E. coli O157:H7.
A. True
B. False

1.C, 2.C, 3.B, 4.C, 5.B, 6.D, 7.A, 8.E, 9.D, 10.E, 11.A, 12.A, 13.A, 14.B, 15.A, 16.A

Waterborne Diseases

Name	Causative organism	Source of organism	Disease
Viral gastroenteritis	**Rotavirus** (mostly in young children)	Human feces	Diarrhea or vomiting
Norwalk Agent	**Noroviruses** (genus *Norovirus*, family *Caliciviridae*) [*1]	Human feces; also, shellfish; lives in polluted waters	Diarrhea and vomiting
Salmonellosis	***Salmonella*** (bacterium)	Animal or human feces	Diarrhea or vomiting
Gastroenteritis Escherichia *coli*	-- ***E. coli O1* 57:H7** (bacterium): Other *E. coli* organisms:	Human feces	Symptoms vary with type caused
Typhoid	***Salmonella typhi*** (bacterium)	Human feces, urine	Inflamed intestine, enlarged spleen, high temperature-sometimes fatal
Shigellosis	***Shigella*** (bacterium)	Human feces	Diarrhea
Cholera	***Vibrio choleras*** (bacterium)	Human feces; also, shellfish; lives in many coastal waters	Vomiting, severe diarrhea, rapid dehydration, mineral loss-high mortality
Hepatitis A	**Hepatitis A** virus	Human feces; shellfish grown in polluted waters	Yellowed skin, enlarged liver, fever, vomiting, weight loss, abdominal pain-low mortality, lasts up to four months
Amebiasis	***Entamoeba histolytica*** (protozoan)	Human feces	Mild diarrhea, dysentery, extra intestinal infection
Giardiasis	***Giardia lamblia*** (protozoan)	Animal or human feces	Diarrhea, cramps, nausea, and general weakness — lasts one week to months
Cryptosporidiosis	***Cryptosporidium parvum***	Animal or human feces	Diarrhea, stomach pain — lasts (protozoan) days to weeks

Notes:
*1 **http://www.cdc.gov/**

Related Diseases and Associated Illnesses Chapter 7

Water providers should be alert to illness patterns and diagnostic clues that might indicate an unusual infectious disease outbreak associated with intentional release of a biologic agent and should report any clusters or findings to their local or state health department.

The covert release of a biologic agent may not have an immediate impact because of the delay between exposure and illness onset, and outbreaks associated with intentional releases might closely resemble naturally occurring outbreaks. Indications of intentional release of a biologic agent include 1) an unusual temporal or geographic clustering of illness (e.g., persons who attended the same public event or gathering) or patients presenting with clinical signs and symptoms that suggest an infectious disease outbreak (e.g., ≥2 patients presenting with an unexplained febrile illness associated with sepsis, pneumonia, respiratory failure, or rash or a botulism-like syndrome with flaccid muscle paralysis, especially if occurring in otherwise healthy persons); 2) an unusual age distribution for common diseases (e.g., an increase in what appears to be a chickenpox-like illness among adult patients, but which might be smallpox); and 3) a large number of cases of acute flaccid paralysis with prominent bulbar palsies, suggestive of a release of *botulinum* toxin.

CDC defines three categories of biologic agents with potential to be used as weapons, based on ease of dissemination or transmission, potential for major public health impact (e.g., high mortality), potential for public panic and social disruption, and requirements for public health preparedness.

Agents of highest concern are *Bacillus anthracis* (anthrax), *Yersinia pestis* (plague), variola major (smallpox), *Clostridium botulinum* toxin (botulism), *Francisella tularensis* (tularemia), filoviruses (Ebola hemorrhagic fever, Marburg hemorrhagic fever); and arenaviruses (Lassa [Lassa fever], Junin [Argentine hemorrhagic fever], and related viruses). The following summarizes the clinical features of these agents.

Anthrax
A nonspecific prodrome (i.e., fever, dyspnea, cough, and chest discomfort) follows inhalation of infectious spores. Approximately 2--4 days after initial symptoms, sometimes after a brief period of improvement, respiratory failure and hemodynamic collapse ensue. Inhalational anthrax also might include thoracic edema and a widened mediastinum on chest radiograph. Gram-positive bacilli can grow on blood culture, usually 2--3 days after onset of illness. Cutaneous anthrax follows deposition of the organism onto the skin, occurring particularly on exposed areas of the hands, arms, or face. An area of local edema becomes a pruritic macule or papule, which enlarges and ulcerates after 1--2 days. Small, 1--3 mm vesicles may surround the ulcer. A painless, depressed, black eschar, usually with surrounding local edema, subsequently develops. The syndrome also may include lymphangitis and painful lymphadenopathy.

Plague
Clinical features of pneumonic plague include fever, cough with muco-purulent sputum (gram-negative rods may be seen on gram stain), hemoptysis, and chest pain. A chest radiograph will show evidence of bronchopneumonia.

Botulism
Clinical features include symmetric cranial neuropathies (i.e., drooping eyelids, weakened jaw clench, and difficulty swallowing or speaking), blurred vision or diplopia, symmetric descending weakness in a proximal to distal pattern, and respiratory dysfunction from respiratory muscle paralysis or upper airway obstruction without sensory deficits. Inhalational botulism would have a similar clinical presentation as foodborne botulism; however, the gastrointestinal symptoms that accompany foodborne botulism may be absent.

Smallpox (variola)
The acute clinical symptoms of smallpox resemble other acute viral illnesses, such as influenza, beginning with a 2--4 day nonspecific prodrome of fever and myalgias before rash onset. Several clinical features can help clinicians differentiate varicella (chickenpox) from smallpox. The rash of varicella is most prominent on the trunk and develops in successive groups of lesions over several days, resulting in lesions in various stages of development and resolution. In comparison, the vesicular/pustular rash of smallpox is typically most prominent on the face and extremities, and lesions develop at the same time.

Inhalational tularemia
Inhalation of *F. tularensis* causes an abrupt onset of an acute, nonspecific febrile illness beginning 3--5 days after exposure, with pleuropneumonitis developing in a substantial proportion of cases during subsequent days.

Hemorrhagic fever (such as would be caused by Ebola or Marburg viruses).
After an incubation period of usually 5--10 days (range: 2--19 days), illness is characterized by abrupt onset of fever, myalgia, and headache. Other signs and symptoms include nausea and vomiting, abdominal pain, diarrhea, chest pain, cough, and pharyngitis. A maculopapular rash, prominent on the trunk, develops in most patients approximately 5 days after onset of illness. Bleeding manifestations, such as petechiae, ecchymoses, and hemorrhages, occur as the disease progresses (*8*).

Laboratory Personnel
Although unidentified gram-positive bacilli growing on agar may be considered as contaminants and discarded, CDC recommends that these bacilli be treated as a "finding" when they occur in a suspicious clinical setting (e.g., febrile illness in a previously healthy person).

The laboratory should attempt to characterize the organism, such as motility testing, inhibition by penicillin, absence of hemolysis on sheep blood agar, and further biochemical testing or species determination.

An unusually high number of samples, particularly from the same biologic medium (e.g., blood and stool cultures), may alert laboratory personnel to an outbreak. In addition, central laboratories that receive clinical specimens from several sources should be alert to increases in demand or unusual requests for culturing (e.g., uncommon biologic specimens such as cerebrospinal fluid or pulmonary aspirates).

Collection of Samples

When collecting or handling specimens, laboratory personnel should:
1) use Biological Safety Level II (BSL-2) or Level III (BSL-3) facilities and practices when working with clinical samples considered potentially infectious;
2) handle all specimens in a BSL-2 laminar flow hood with protective eyewear (e.g., safety glasses or eye shields), use closed-front laboratory coats with cuffed sleeves, and stretch the gloves over the cuffed sleeves;
3) avoid any activity that places persons at risk for infectious exposure, especially activities that might create aerosols or droplet dispersal;
4) decontaminate laboratory benches after each use and dispose of supplies and equipment in proper receptacles;
5) avoid touching mucosal surfaces with their hands (gloved or ungloved), and never eat or drink in the laboratory; and 6) remove and reverse their gloves before leaving the laboratory and dispose of them in a biohazard container, and wash their hands and remove their laboratory coat.

When a laboratory is unable to identify an organism in a clinical specimen, it should be sent to a laboratory where the agent can be characterized, such as the state public health laboratory or, in some large metropolitan areas, the local health department laboratory. Any clinical specimens suspected to contain variola (smallpox) should be reported to local and state health authorities and then transported to CDC. All variola diagnostics should be conducted at CDC laboratories. Clinical laboratories should report any clusters or findings that could indicate intentional release of a biologic agent to their state and local health departments.

After the terrorist attacks of September 11, state and local health departments initiated various activities to improve surveillance and response, ranging from enhancing communications (between state and local health departments and between public health agencies and health-care providers) to conducting special surveillance projects.

These special projects have included active surveillance for changes in the number of hospital admissions, emergency department visits, and occurrence of specific syndromes. Activities in bioterrorism preparedness and emerging infections over the past few years have better positioned public health agencies to detect and respond to the intentional release of a biologic agent. Immediate review of these activities to identify the most useful and practical approaches will help refine syndrome surveillance efforts in various clinical situations.

Additional information about responding to bioterrorism is available from CDC at <http://www.bt.cdc.gov>; the U.S. Army Medical Research Institute of Infectious Diseases at <http://www.usamriid.army.mil/education/bluebook.html>; the Association for Infection Control Practitioners at <http://www.apic.org>; and the Johns Hopkins Center for Civilian Biodefense at <http://www.hopkins-biodefense.org>.

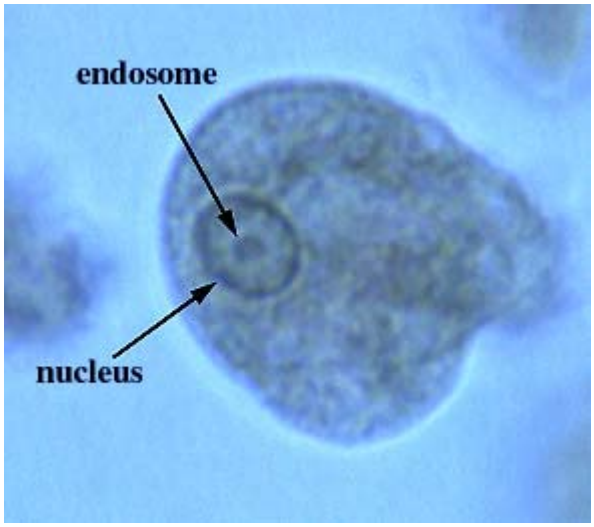
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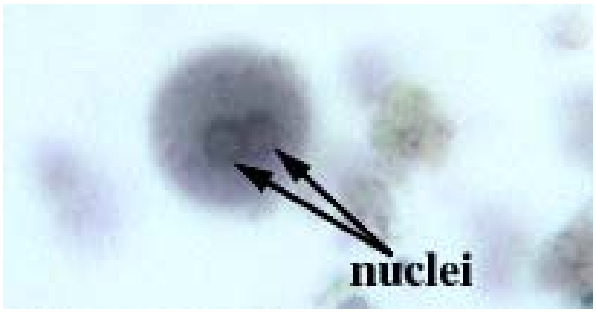


Amebiasis *Entamoeba histolytica* Section

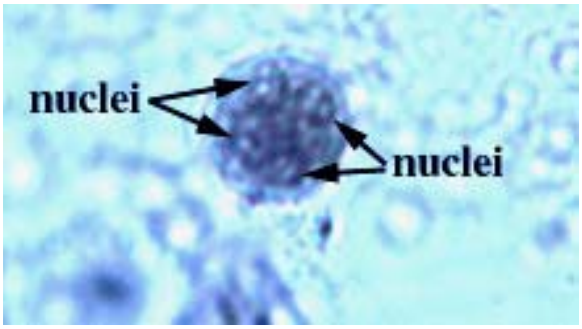
The life cycle of *Entamoeba histolytica* involves trophozoites (the feeding stage of the parasite) that live in the host's large intestine and cysts that are passed in the host's feces. Humans are infected by ingesting cysts, most often via food or water contaminated with human fecal material (view diagram of the life cycle). The trophozoites can destroy the tissues that line the host's large intestine, so of the amoebae infecting the human gastrointestinal tract, *E. histolytica* is potentially the most pathogenic.



Entamoeba histolytica *trophozoite*



Entamoeba histolytica *immature cyst*



Entamoeba histolytica *mature cyst*

Entamoeba histolytica is an amoeboid protozoan parasite of the intestinal tract and in some cases other visceral organs especially the liver. There are several species in this genus, distinguished by their number of nuclei in the cyst and position of the endosome, whether or not they form a cyst, and whether they invade tissues or remain in the intestinal lumen. *Entamoeba histolytica* has four nuclei in the cyst, a central endosome, forms a cyst, and can be a tissue invader. The amoeboid trophozoites can live in the intestinal crypts, feeding on intestinal contents and host tissue, and multiplying by fission.

Trophozoites
The trophozoites can be carried out in the feces. As the feces pass through the colon they dehydrate. The dehydration of the feces causes the trophozoites to begin the process of encystment. Undigested food is discharged, and the trophozoite condenses and forms a spherical shape to form what is called the pre-cyst, and the cyst wall is secreted. Within the cyst there are two nuclear divisions resulting in 2 nuclei in the immature cyst and 4 nuclei within the mature cyst. The cyst can resist desiccation for 1-2 weeks. When the cyst is ingested by another host the parasite excysts in the intestine and undergoes cytoplasmic division to produce 4 trophozoites. In some cases the trophozoites secrete proteolytic enzymes which destroy the intestinal epithelium allowing the trophozoiute to enter the host tissue.

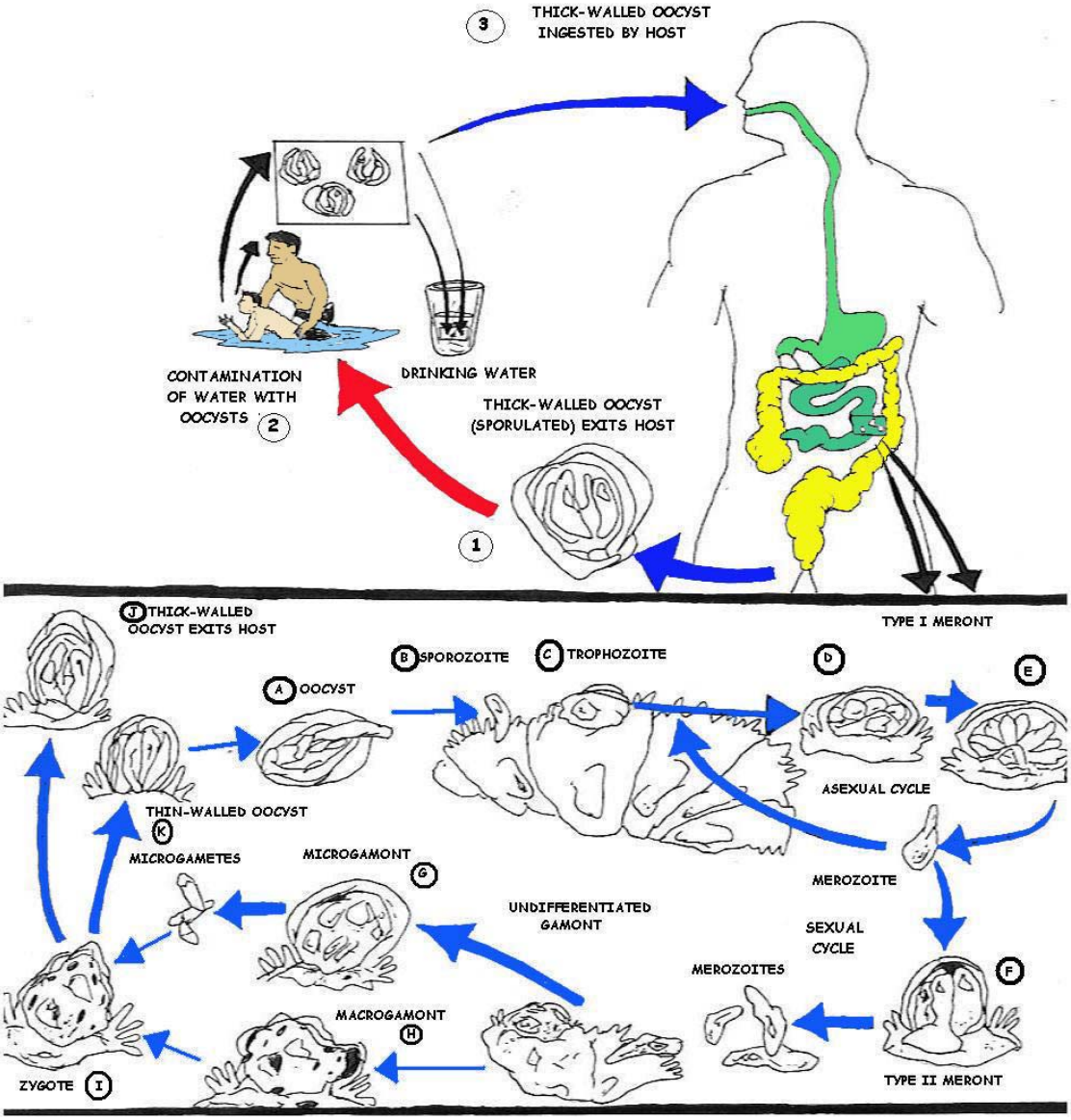
Extensive Tissue Destruction
These can form large abscesses that may allow the parasite to enter the blood stream and be carried to the liver and other organs. In these extra-intestinal sites the trophozoites also can cause extensive tissue destruction. If the intestinal tissue has been invaded the feces can be bloody and diarrheic.

Trophoziotes in diarrheic feces are not stimulated to encyst because the feces are not dehydrating. If they are not encysted they cannot long survive in the external environment. Secondary bacterial infection can complicate an already severe pathology.

Accurate diagnosis of this parasite is important to prevent unnecessary treatment of a non-pathogenic strain, and to ensure treating a pathogenic strain. Definitive diagnosis is based on morphological characteristics of the trophozoites and cysts, the presence of erythrocytes in the trophozoites, and clinical symptoms.

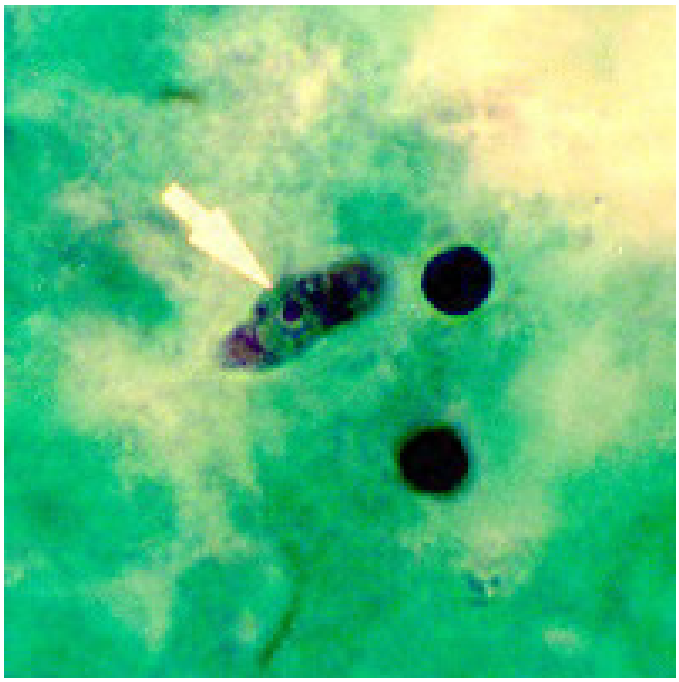
Symptoms of Amoebiasis
In most infected humans the symptoms of "amoebiasis" (or "amebiasis") are intermittent and mild (various gastrointestinal upsets, including colitis and diarrhea). In more severe cases the gastrointestinal tract hemorrhages, resulting in dysentery. In some cases the trophozoites will enter the circulatory system and infect other organs, most often the liver (hepatic amoebiasis), or they may penetrate the gastrointestinal tract resulting in acute peritonitis; such cases are often fatal.

As with most of the amoebae, infections of *E. histolytica* are often diagnosed by demonstrating cysts or trophozoites in a stool sample.


3
THICK-WALLED OOCYST
INGESTED BY HOST
CONTAMINATION
OF WATER WITH
OOCYSTS
2
DRINKING WATER
THICK-WALLED OOCYST
(SPORULATED) EXITS HOST
1
J THICK-WALLED
OOCYST EXITS HOST
A OOCYST
B SPOROZOITE
C TROPHOZOITE
D
E
TYPE I MERONT
ASEXUAL CYCLE
MEROZOITE
THIN-WALLED OOCYST
K
MICROGAMETES
MICROGAMONT
G
UNDIFFERENTIATED
GAMONT
SEXUAL
CYCLE
MEROZOITES
F
MACROGAMONT
H
ZYGOTE
I
TYPE II MERONT

Amebic Meningoencephalitis PAM *Naegleria fowleri*

What is primary amebic meningoencephalitis (PAM)?
Primary Amebic Meningoencephalitis (PAM) is a rare and usually deadly disease caused by infection with the ameba (a single-celled organism that constantly changes shape) *Naegleria fowleri*. *[Naegleria fowleri] [Acanthamoeba spp.] [Balamuthia mandrillaris]*



Naegleria fowleri trophozoite in spinal fluid. Trichrome stain. Note the typically large karyosome and the monopodial locomotion. Image contributed by Texas SHD.

What are the symptoms of PAM? What does PAM cause?
Following an incubation period of 2-15 days, there is a relatively sudden start of severe meningitis-like symptoms, which begin with fever and headache. These are rapidly followed by sensitivity to light, nausea, projectile vomiting, stiff neck, and, in many cases, disturbances to taste and smell. Changes in behavior and seizures may also be present. As conditions worsen the patient falls into a coma. Death usually occurs 3-7 days after the onset of symptoms.

How common is PAM?
The ameba that causes the infection lives in soil and in freshwater ponds, lakes, rivers, poorly or non-chlorinated pools, discharge or holding basins, and hot springs throughout the world. *Naegleria* thrives in warm, stagnant bodies of fresh water when temperatures are high, usually above 80 degrees.

Although the ameba is commonly found in the environment, PAM is very rare. In the last 30 years, only a few hundred cases have been reported worldwide.

Who should be especially careful about PAM?
Cases are usually reported in children and young adults who have had recent exposure to freshwater lakes or streams.

How is PAM spread? How do people get *Naegleria* infection?
The ameba is believed to enter the body through the nose and travel to the brain via the olfactory (smell) nerve. The disease is not spread from person to person.

How do I protect myself from PAM?
To protect yourself against *Naegleria* or any harmful organism that is present in the water:

- Never swim in stagnant or polluted water.
- Do not swim in areas posted as "No Swimming."
- Hold your nose or use nose plugs when jumping or diving into water.
- Avoid swallowing water from rivers, lakes, streams, or stock ponds.
- Use earplugs, swim goggles, or masks if you tend to get ear or eye infections.
- Swim only in properly maintained pools.
- Keep wading pools clean and change the water daily.
- Wash open skin cuts and scrapes with clean water and soap.

What do I do if I think I have PAM?
Seek immediate medical attention and mention any recent fresh water exposure.

How is PAM diagnosed?
The disease is initially suspected based on patient history. The diagnosis is made through the examination of the fluid in the patient's spinal chord or frequently after death through the examination of brain tissue.

How are *Naegleria* infections treated?
PAM is a severe illness that does not respond to routine treatments. Aggressive use of some antifungal medications has been successful only in a handful of cases. Intensive supportive care is necessary along with the medication.

Recent Outbreak Information

Health Stream Article - Issue 28 December 2002

Naegleria Deaths In Arizona
Residents of the Arizona towns of Peoria and Glendale have been shocked by the deaths of two five-year old boys from amoebic meningitis caused by Naegleria fowleri. The source of the infections has not been positively established but suspicion has fallen on a small unchlorinated ground water supply operated by a private company.

This supply was taken off-line on 3 November, a boil water notice was issued and 6,000 consumers were warned not to use unboiled tap water for drinking, cooking or bathing. Schools and restaurants in the suspect area were also closed, and residents were advised to drain and clean spas and hyperchlorinate swimming pools.

Supply to the affected area was switched to a chlorinated surface water source, and a flushing program with hyperchlorinated water was carried out to remove possible contamination from the water distribution system.

One of the victims lived in Peoria and the other in the neighboring town of Glendale, some four miles away. They attended separate schools; however the Glendale boy frequently visited his grandparents' home a few blocks from the other boy's residence in Peoria. Both boys became ill on 9 October and died a few days later on 12 and 13 October respectively. Health authorities then began investigating possible common sources of Naegleria exposure including drinking water, pools, bathtubs, spas and fountains.

About 100,000 of Peoria's 120,000 residents receive chlorinated drinking water from the municipal supply. This supply is predominantly drawn from surface water sources but is supplemented by groundwater in times of high demand. As Arizona state law prevents counties from supplying water to areas outside the incorporated municipal zones, the remaining 20,000 residents in the rapidly growing town are served by private water companies which mainly rely on groundwater sources. Some of these companies chlorinate their groundwater supplies and some do not.

The suspect water supply is drawn from a deep aquifer and is not routinely chlorinated, although periodic chlorination has been used after new connections, line breaks or incidents that might allow ingress of microbial contamination.

Tests by the Centers for Disease Control and Prevention have detected N. fowleri in three samples:

· one pre-chlorination water sample from a municipal well that was routinely chlorinated.
· one tank water sample from the suspect unchlorinated groundwater system.
· the refrigerator filter from the home of the grandparents of one of the boys.

The chlorinated well is believed unlikely to be the source of infection as chlorination is effective in killing *N. fowleri*.

Naegleria fowleri is a free living amoeba which is common in the environment and grows optimally at temperatures of 35 to 45 degrees C. Exposure to the organism is believed to be relatively common but infections resulting in illness are rare. The disease was first described in 1965 by Dr Malcolm Fowler, an Australian pathologist, who identified the amoeba in a patient who had died from meningitis.

Most reported cases of N. fowleri meningitis are associated with swimming in natural surface freshwater bodies, and infection occurs through introduction of the organism into the nasal cavities. Cases are often reported to be associated with jumping or falling into the water, providing conditions where water is forced into the nose at pressure. The amoeba may then penetrate the cribiform plate, a semiporous barrier, and spread to the meninges (the membrane surrounding the brain) and often to the brain tissue itself. The cribiform plate is more permeable in children, making them more susceptible to infection than adults. People with immune deficiencies may also be more prone to infection.

The incubation period is usually 2 to 5 days, and the infection cannot be transmitted from person to person. In early studies, transmission by contaminated dust was suspected as an infection route but this has since been discounted as the organism does not survive desiccation.

N. fowleri meningitis causes non-specific symptoms such as fever, drowsiness, confusion, vomiting, irritability, high pitched crying and convulsions. Similar symptoms also occur in viral and bacterial forms of meningitis which are much more common than the amoebic form. Most cases of *N. fowleri* meningitis are fatal, with only four survivors known among about 100 cases in the US since 1965.

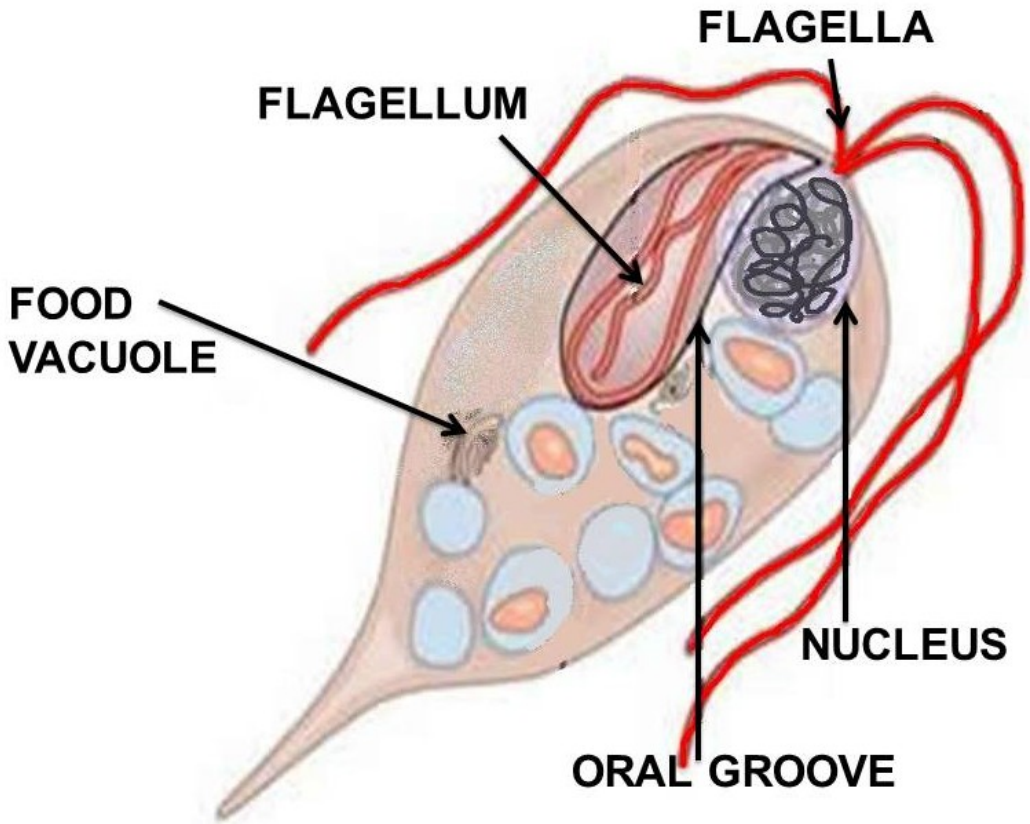
Cases of disease have also been associated with swimming pools where disinfection levels were inadequate, and inhalation of tap water from surface water supplies that have been subject to high temperatures.

The involvement of tap water supplies was first documented in South Australia, where a number of cases occurred in the 1960s and 70s in several towns served by unchlorinated surface water delivered through long above-ground pipelines. About half of the cases in the state did not have a recent history of freshwater swimming, but had intra-nasal exposure to tap water through inhaling or squirting water into the nose.

Investigators found *N. fowleri* in the water supply pipelines, and concluded that the high water temperatures reached in summer provided a suitable environment for growth of the organism. Tap water may also have been the primary source of infections attributed to swimming pools in these towns. The incidence of disease was greatly reduced by introduction of reliable chlorination facilities along the above-ground pipelines and introduction of chloramination in the 1980s led to virtual elimination of *N. fowleri* from the water supplies. Cases of disease have also been recorded in Western Australia, Queensland and New South Wales, and *N. fowleri* has been detected in water supplies in each of these states as well as the Northern Territory.

Prior to the incidents in Peoria, *N. fowleri* infections had not been reported to be associated with groundwater supplies. However as the organism may be found in moist soil, it is feasible that the amoeba may penetrate poorly constructed bores or be introduced by occasional contamination events.

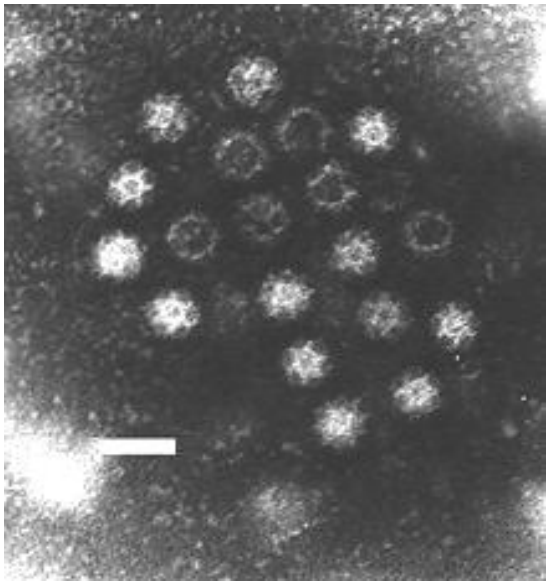
Warm water conditions and the absence of free chlorine may then allow it to proliferate in the system. Local health authorities in Arizona are continuing their investigation into the two deaths with assistance from CDC personnel. Plans are also underway to install a continuous chlorination plant on the groundwater supply, and some residents have called for the municipality to purchase the private water company and take over its operations.



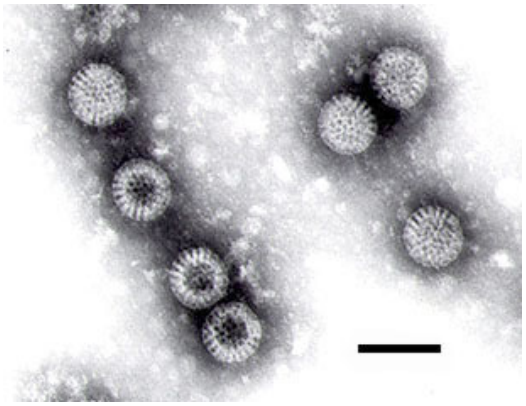
GIARDIA LAMBLIA

Calicivirus

See Gastroenteritis section, Norovirus Infection (aka Norwalk virus, calicivirus, viral gastroenteritis)



Note the 'Star of David' image exhibited by individual virus particles. This is distinct from the star-like images exhibited by astrovirus particles. Bar = 50 nanometers.
Source: Stool sample from an individual with gastroenteritis.
Method: Negative-stain Transmission Electron Microscopy



Rotovirus
Note the wheel-like appearance of some of the rotavirus particles. The observance of such particles gave the virus its name ('rota' being the Latin word meaning wheel). Bar = 100 nanometers.
Source: Cell culture.
Method: Negative-stain Transmission Electron Microscopy

Photographs and information courtesy from the U.S. EPA and F.P. Williams, U.S. EPA



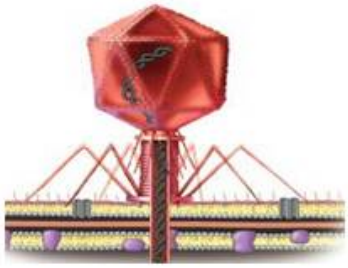
COCCI
(SPHERICAL)



SPIRILLI
(SPIRAL)

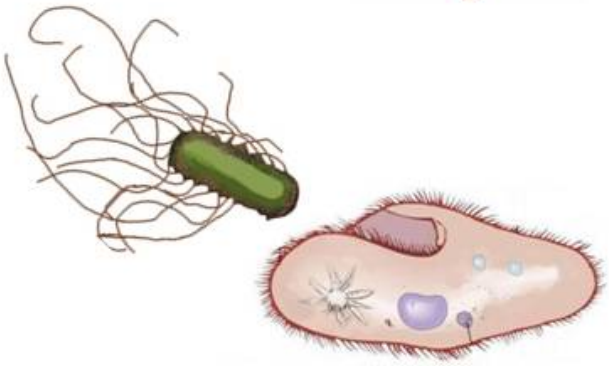


BACILLI
(ROD-SHAPED)

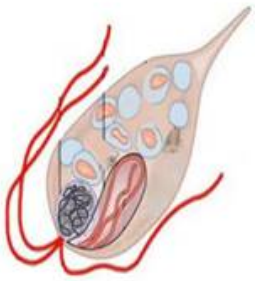




VIRUSES



BACTERIA



PROTOZOA

BACTERIA TYPES

Schistosomes and Other Trematodes

Schistosomiasis, also called snail fever or bilharziasis, is thought to cause more illness and disability than any other parasitic disease, except malaria. Almost unknown in industrialized countries, schistosomiasis infects 200 million people in 76 countries of the tropical developing world.

A Flatworm that spends part of its life in a freshwater snail host causes schistosomiasis. Multiplying in the snail, a microscopic infective larval stage is released that can penetrate human skin painlessly in 30 to 60 seconds. The larvae grow to adulthood and migrate to the veins around the intestines or bladder, where mating occurs. The eggs produced may lodge in these tissues and cause disease, or they are passed out in urine or feces, where they reach fresh water and hatch to infect snails.

Multiplication and Life Cycle
Free-swimming larvae (cercariae) are given off by infected snails. These either penetrate the skin of the human definitive host (schistosomes) or are ingested after encysting as metacercariae in or on various edible plants or animals (all other trematodes). After entering a human, the larvae develop into adult males and females (schistosomes) or hermaphrodites (other flukes), which produce eggs that pass out of the host in excreta. These eggs hatch in fresh water into miracidia which infect snails.



Cercariae

Pathogenesis
In schistosomiasis, eggs trapped in the tissues produce granulomatous inflammatory reactions, fibrosis, and obstruction. The hermaphroditic flukes of the liver, lungs, and intestines induce inflammatory and toxic reactions.

Host Defenses
Host defenses against schistosomiasis include antibody or complement-dependent cellular cytotoxicity and modulation of granulomatous hypersensitivity. The defenses against hermaphroditic flukes are unknown.

Epidemiology
Most infected individuals show no overt disease. In a relatively small proportion of individuals, heavy infections due to repeated exposure to parasitic larvae will lead to the development of clinical manifestations. The distribution of flukes is limited by the distribution of their snail intermediate host. Larvae from snails infect a human by penetrating the skin (schistosomes) or by being eaten (encysted larvae of other trematodes).

Diagnosis
Diagnosis is suggested by clinical manifestations, geographic history, and exposure to infective larvae. The diagnosis is confirmed by the presence of parasite eggs in excreta.

Control
As a control measure, exposure to parasite larvae in water and food should be prevented. Treatment with praziquantel is effective.

Clinical Manifestations
Signs and symptoms are related largely to the location of the adult worms. Infections with *Schistosoma mansoni* and *S japonicum* (mesenteric venules) result in eosinophilia, hepatomegaly, splenomegaly, and hematemesis. *Schistosoma haematobium* (vesical venules) causes dysuria, hema turia, and uremia. *Fasciola hepatica, Clonorchis sinensis,* and *Opisthorchis viverrini* (bile ducts) cause fever, hepatomegaly, abdominal pain, and jaundice. Infections with *Paragonimus westermani* (lungs, brain) result in cough, hemoptysis, chest pain, and epilepsy. *Fasciolopsis buski* (intestines) causes abdominal pain, diarrhea, and edema.

Structure
Trematodes are multicellular eukaryotic helminths.

Gastroenteritis

What is viral gastroenteritis?
Gastroenteritis means inflammation of the stomach and small and large intestines. Viral gastroenteritis is an infection caused by a variety of viruses that results in vomiting or diarrhea. It is often called the "stomach flu," although it is not caused by the influenza viruses.

What causes viral gastroenteritis?
Many different viruses can cause gastroenteritis, including rotaviruses, adenoviruses, caliciviruses, astroviruses, Norwalk virus, and a group of Noroviruses. Viral gastroenteritis is not caused by bacteria (such as *Salmonella* or *Escherichia coli*) or parasites (such as *Giardia*), or by medications or other medical conditions, although the symptoms may be similar. Your doctor can determine if the diarrhea is caused by a virus or by something else.

What are the symptoms of viral gastroenteritis?
The main symptoms of viral gastroenteritis are watery diarrhea and vomiting. The affected person may also have headache, fever, and abdominal cramps ("stomach ache"). In general, the symptoms begin 1 to 2 days following infection with a virus that causes gastroenteritis and may last for 1 to 10 days, depending on which virus causes the illness.

Is viral gastroenteritis a serious illness?
For most people, it is not. People who get viral gastroenteritis almost always recover completely without any long-term problems. Gastroenteritis is a serious illness, however, for persons who are unable to drink enough fluids to replace what they lose through vomiting or diarrhea. Infants, young children, and persons who are unable to care for themselves, such as the disabled or elderly, are at risk for dehydration from loss of fluids. Immune compromised persons are at risk for dehydration because they may get a more serious illness, with greater vomiting or diarrhea. They may need to be hospitalized for treatment to correct or prevent dehydration.

Is the illness contagious? How are these viruses spread?
Yes, viral gastroenteritis is contagious. The viruses that cause gastroenteritis are spread through close contact with infected persons (for example, by sharing food, water, or eating utensils). Individuals may also become infected by eating or drinking contaminated foods or beverages.

How does food get contaminated by gastroenteritis viruses?
Food may be contaminated by food preparers or handlers who have viral gastroenteritis, especially if they do not wash their hands regularly after using the bathroom. Shellfish may be contaminated by sewage, and persons who eat raw or undercooked shellfish harvested from contaminated waters may get diarrhea. Drinking water can also be contaminated by sewage and be a source of spread of these viruses.

Where and when does viral gastroenteritis occur?
Viral gastroenteritis affects people in all parts of the world. Each virus has its own seasonal activity. For example, in the United States, rotavirus and astrovirus infections occur during the cooler months of the year (October to April), whereas adenovirus

infections occur throughout the year. Viral gastroenteritis outbreaks can occur in institutional settings, such as schools, child care facilities, and nursing homes, and can occur in other group settings, such as banquet halls, cruise ships, dormitories, and campgrounds.

Who gets viral gastroenteritis?
Anyone can get it. Viral gastroenteritis occurs in people of all ages and backgrounds. However, some viruses tend to cause diarrheal disease primarily among people in specific age groups. Rotavirus infection is the most common cause of diarrhea in infants and young children under 5 years old. Adenoviruses and astroviruses cause diarrhea mostly in young children, but older children and adults can also be affected. Norwalk and Noroviruses are more likely to cause diarrhea in older children and adults.

How is viral gastroenteritis diagnosed?
Generally, viral gastroenteritis is diagnosed by a physician on the basis of the symptoms and medical examination of the patient. Rotavirus infection can be diagnosed by laboratory testing of a stool specimen. Tests to detect other viruses that cause gastroenteritis are not in routine use.

How is viral gastroenteritis treated?
The most important of treating viral gastroenteritis in children and adults is to prevent severe loss of fluids (dehydration). This treatment should begin at home. Your physician may give you specific instructions about what kinds of fluid to give. CDC recommends that families with infants and young children keep a supply of oral rehydration solution (ORS) at home at all times and use the solution when diarrhea first occurs in the child. ORS is available at pharmacies without a prescription.

Follow the written directions on the ORS package, and use clean or boiled water. Medications, including antibiotics (which have no effect on viruses) and other treatments, should be avoided unless specifically recommended by a physician.

Can viral gastroenteritis be prevented?
Yes, persons can reduce their chance of getting infected by frequent handwashing, prompt disinfection of contaminated surfaces with household chlorine bleach-based cleaners, and prompt washing of soiled articles of clothing. If food or water is thought to be contaminated, it should be avoided.

Is there a vaccine for viral gastroenteritis?
There is no vaccine or medicine currently available that prevents viral gastroenteritis. A vaccine is being developed, however, that protects against severe diarrhea from rotavirus infection in infants and young children.

Rotovirus Information

Clinical Features
Rotavirus is the most common cause of severe diarrhea among children, resulting in the hospitalization of approximately 55,000 children each year in the United States and the death of over 600,000 children annually worldwide. The incubation period for rotavirus disease is approximately 2 days. The disease is characterized by vomiting and watery diarrhea for 3 - 8 days, and fever and abdominal pain occur frequently. Immunity after infection is incomplete, but repeat infections tend to be less severe than the original infection.

The Virus
A rotavirus has a characteristic wheel-like appearance when viewed by electron microscopy (the name rotavirus is derived from the Latin rota, meaning "wheel"). Rotaviruses are nonenveloped, double-shelled viruses. The genome is composed of 11 segments of double-stranded RNA, which code for six structural and five nonstructural proteins. The virus is stable in the environment.

Epidemiologic Features
The primary mode of transmission is fecal-oral, although some have reported low titers of virus in respiratory tract secretions and other body fluids. Because the virus is stable in the environment, transmission can occur through ingestion of contaminated water or food and contact with contaminated surfaces. In the United States and other countries with a temperate climate, the disease has a winter seasonal pattern, with annual epidemics occurring from November to April. The highest rates of illness occur among infants and young children, and most children in the United States are infected by 2 years of age. Adults can also be infected, though disease tends to be mild.

Diagnosis
Diagnosis may be made by rapid antigen detection of rotavirus in stool specimens. Strains may be further characterized by enzyme immunoassay or reverse transcriptase polymerase chain reaction, but such testing is not commonly done.

Treatment
For persons with healthy immune systems, rotavirus gastroenteritis is a self-limited illness, lasting for only a few days. Treatment is nonspecific and consists of oral rehydration therapy to prevent dehydration. About one in 40 children with rotavirus gastroenteritis will require hospitalization for intravenous fluids.

Prevention
In 1998, the U.S. Food and Drug Administration approved a live virus vaccine (Rotashield) for use in children. However, the Advisory Committee on Immunization Practices (ACIP) recommended that Rotashield no longer be recommended for infants in the United States because of data that indicated a strong association between Rotashield and intussusception (bowel obstruction) among some infants during the first 1-2 weeks following vaccination.

More information about rotavirus vaccine is available from the National Immunization Program.

Recent Newspaper Article

Norwalk Virus
The Gila County Department of Health is currently investigating an outbreak of viral gastroenteritis in the Globe / Miami area. The outbreak has been laboratory confirmed by the Arizona State Laboratory as Norwalk virus. Please be aware of the following symptoms and recommendations.

Norwalk Symptoms
Usually a mild to moderate infection that often occurs in outbreaks with clinical symptoms of nausea, vomiting, diarrhea, abdominal pain, low grade fever, or any combination of these symptoms. Gastrointestinal symptoms will characteristically last 24 to 48 hours, resolving on their own.

Mode of Transmission and Communicability
Fecal to oral route is the most likely mode of transmission. This virus is easily transmitted. Patients are communicable during the acute phase of the illness and up to 48 hours after the symptoms resolve.

If you are experiencing symptoms consistent with this disease, please exclude yourself from school, work, or any group activity. Hand washing and disinfection are essential to stop the spread of this virus. Anyone experiencing severe complications from this ailment should seek medical attention.

Please report all suspected group outbreaks to the Gila County Department of Health by phone immediately. (928) 425-3189



Noroviruses

Noroviruses (genus *Norovirus*, family *Caliciviridae*) are a group of related, single-stranded RNA, nonenveloped viruses that cause acute gastroenteritis in humans. Norovirus was recently approved as the official genus name for the group of viruses provisionally described as "Norwalk-like viruses" (NLV).

What are the symptoms of illness caused by noroviruses?
The symptoms of norovirus illness usually include nausea, vomiting, diarrhea, and some stomach cramping. Sometimes people additionally have a low-grade fever, chills, headache, muscle aches, and a general sense of tiredness. The illness often begins suddenly, and the infected person may feel very sick. The illness is usually brief, with symptoms lasting only about 1 or 2 days. In general, children experience more vomiting than adults. Most people with norovirus illness have both of these symptoms.

What is the name of the illness caused by noroviruses?
Illness caused by norovirus infection has several names, including:

- Stomach flu – this "stomach flu" is ***not*** related to the flu (or influenza), which is a respiratory illness caused by influenza virus.
- Viral gastroenteritis – the most common name for illness caused by norovirus. Gastroenteritis refers to an inflammation of the stomach and intestines.
- Acute gastroenteritis.
- Non-bacterial gastroenteritis.
- Food poisoning (although there are other causes of food poisoning).
- Calicivirus infection.

How serious is norovirus disease?
Norovirus disease is usually not serious, although people may feel very sick and vomit many times a day. Most people get better within 1 or 2 days, and they have no long-term health effects related to their illness. However, sometimes people are unable to drink enough liquids to replace the liquids they lose because of vomiting and diarrhea. These persons can become dehydrated and may need special medical attention. This problem with dehydration is usually only seen among the very young, the elderly, and persons with weakened immune systems. There is no evidence to suggest that an infected person can become a long-term carrier of norovirus.

How do people become infected with noroviruses?
Noroviruses are found in the stool or vomit of infected people. People can become infected with the virus in several ways, including:

- eating food or drinking liquids that are contaminated with norovirus;
- touching surfaces or objects contaminated with norovirus, and then placing their hand in their mouth;
- having direct contact with another person who is infected and showing symptoms (for example, when caring for someone with illness, or sharing foods or eating utensils with someone who is ill).

Persons working in day-care centers or nursing homes should pay special attention to children or residents who have norovirus illness. This virus is very contagious and can spread rapidly throughout such environments.

When do symptoms appear?
Symptoms of norovirus illness usually begin about 24 to 48 hours after ingestion of the virus, but they can appear as early as 12 hours after exposure.

Are noroviruses contagious?
Noroviruses are very contagious and can spread easily from person to person. Both stool and vomit are infectious. Particular care should be taken with young children in diapers who may have diarrhea.

How long are people contagious?
People infected with norovirus are contagious from the moment they begin feeling ill to at least 3 days after recovery. Some people may be contagious for as long as 2 weeks after recovery. Therefore, it is particularly important for people to use good handwashing and other hygienic practices after they have recently recovered from norovirus illness.

Who gets norovirus infection?
Anyone can become infected with these viruses. There are many different strains of norovirus, which makes it difficult for a person's body to develop long-lasting immunity. Therefore, norovirus illness can recur throughout a person's lifetime. In addition, because of differences in genetic factors, some people are more likely to become infected and develop more severe illness than others.

What treatment is available for people with norovirus infection?
Currently, there is no antiviral medication that works against norovirus and there is no vaccine to prevent infection. Norovirus infection cannot be treated with antibiotics. This is because antibiotics work to fight bacteria and not viruses.

Norovirus illness is usually brief in healthy individuals. When people are ill with vomiting and diarrhea, they should drink plenty of fluids to prevent dehydration. Dehydration among young children, the elderly, and the sick can be common, and it is the most serious health effect that can result from norovirus infection. By drinking oral rehydration fluids (ORF), juice, or water, people can reduce their chance of becoming dehydrated. Sports drinks do not replace the nutrients and minerals lost during this illness.

Can norovirus infections be prevented?
Yes. You can decrease your chance of coming in contact with noroviruses by following these preventive steps:

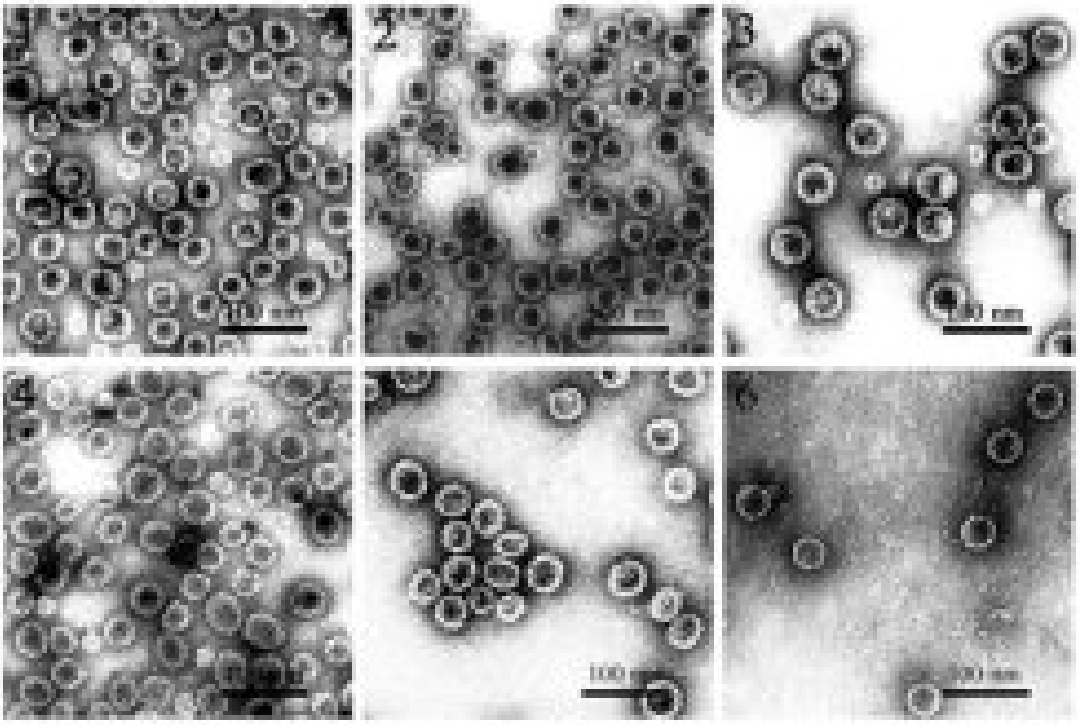
- Frequently wash your hands, especially after toilet visits and changing diapers and before eating or preparing food.
- Carefully wash fruits and vegetables, and steam oysters before eating them.
- Thoroughly clean and disinfect contaminated surfaces immediately after an episode of illness by using a bleach-based household cleaner.
- Immediately remove and wash clothing or linens that may be contaminated with virus after an episode of illness (use hot water and soap).
- Flush or discard any vomitus and/or stool in the toilet and make sure that the surrounding area is kept clean.

Persons who are infected with norovirus should not prepare food while they have symptoms and for 3 days after they recover from their illness. Food that may have been contaminated by an ill person should be disposed of properly.

Hepatitis Section

Viral hepatitis is a group of diseases of the liver that can be caused by consuming contaminated water or food, using dirty needles or syringes, or practicing unsafe sex.

Scientists have identified six hepatitis viruses, but three - known as A, B and C - cause about 90 percent of acute hepatitis cases in the United States. People infected with hepatitis can experience effects ranging from mild illness to serious liver damage. Many recover completely from an infection, while others become carriers of the disease and can spread it to others unknowingly. It is especially important for women who are pregnant or are trying to become pregnant to get tested for hepatitis.



Typical symptoms of acute hepatitis are:

- fever
- appetite loss
- nausea
- abdominal pain
- jaundice (yellowish color on the skin and eyeballs)

Hepatitis A virus found in human feces; shellfish grown in polluted waters. Yellowed skin, enlarged liver, fever, vomiting, weight loss, and abdominal pain — low mortality, lasts up to four months. Hepatitis A is a liver disease caused by the hepatitis A virus (HAV). Hepatitis A can affect anyone. In the United States, hepatitis A can occur in situations ranging from isolated cases of disease to widespread epidemics.

Each year, an estimated 100 persons die as a result of acute liver failure in the United States due to Hepatitis A. Approximately 30 - 50,000 cases occur yearly in the United States and the direct and indirect costs of these cases exceed $300 million.



The unfortunate aspect of these statistics is that with 21st century medicine, Hepatitis A is totally preventable, and isolated cases, especially outbreaks relegated to food consumption, need not occur.

Viral Hepatitis is a major public health concern in the United States, and a source of significant morbidity and mortality. The Hepatitis A virus or "HAV" is heat stable and will survive for up to a month at ambient temperatures in the environment.

Hepatitis A is a communicable (or contagious) disease that spreads from person to person. (It is not acquired from animals, insects, or other means.) It is transmitted by the "fecal – oral route." This does not mean, or course, that Hepatitis A transmission requires that fecal material from an infectious individual must come in contact directly with the mouth of a susceptible individual. It is almost always true that the virus infects a susceptible individual when he or she ingests it, but it gets to the mouth by an indirect route.

Where and how does hepatitis A virus get into drinking water?
Hepatitis A is found in every part of the United States and throughout the world. When water sources such as private wells are contaminated with feces from infected humans, the water will spread the hepatitis A virus. The virus can enter the water through various ways, including sewage overflows or broken sewage systems.

How do I remove hepatitis A from my drinking water?
Heating water at a full boil for 1 minute (3 minutes if you live in a high altitude) will kill or inactivate the hepatitis A virus. Water should then be stored in a clean container with a lid and refrigerated. Because of the small size of the virus, using a point-of-use filter will not remove it from water.

Most Common Method of Transmission
Food contaminated with the virus is the most common vehicle transmitting Hepatitis A.

The food preparer or cook is the individual most often contaminating the food. He or she is generally not ill: the peak time of infectivity (i.e., when the most virus is present in the stool of an infectious individual) is during the 2 weeks before illness begins. Hepatitis A is spread almost exclusively through fecal-oral contact, generally from person-to-person, or via contaminated food or water. Outbreaks associated with food have been increasingly implicated as a significant source of Hepatitis A infection.

Such "outbreaks are usually associated with contamination of food during preparation by an HAV-infected food handler."

Indeed, “[v]iral gastroenteritis was reported as the most common food-borne illness in Minnesota from 1984 to 1991, predominantly associated with poor personal hygiene of infected food handlers.”

Although ingestion of contaminated food is the most common means of spread for Hepatitis A, it may also commonly be spread by household contact among families or roommates, sexual contact, by the ingestion of contaminated water, by the ingestion of raw or undercooked fruits and vegetables or shellfish (like oysters), and by direct inoculation from persons sharing illicit drugs. Children often have asymptomatic or unrecognized infections and can pass the virus through ordinary play, unknown to their parents, who may later become infected from contact with their children.

Hepatitis A: is much more common in countries with under-developed sanitation systems. This includes most of the world: an increased transmission rate is seen in all countries other than the United States, Canada, Japan, Australia, New Zealand, and the countries of Western Europe. Within the United States, Native American reservations also experience a greatly increased rate of disease.

Hepatitis B: is a serious disease caused by a virus that attacks the liver. The virus, which is called hepatitis B virus (HBV), can cause lifelong infection, cirrhosis (scarring) of the liver, liver cancer, liver failure, and death.

Hepatitis C: is a liver disease caused by the hepatitis C virus (HCV), which is found in the blood of persons who have the disease. HCV is spread by contact with the blood of an infected person.

Hepatitis D: is a liver disease caused by the hepatitis D virus (HDV), a defective virus that needs the hepatitis B virus to exist. Hepatitis D virus (HDV) is found in the blood of persons infected with the virus.

Hepatitis E: is a liver disease caused by the hepatitis E virus (HEV) transmitted in much the same way as hepatitis A virus. Hepatitis E, however, does not occur often in the United States.

Medical Testing
Hepatitis virus tests require a blood sample. It is not necessary for the patient to withhold food or fluids before any of these tests, unless requested to do so by the physician.

Risks
Risks for these tests are minimal for the patient, but may include slight bleeding from the blood-drawing site, fainting or feeling lightheaded after venipuncture, or hematoma (blood accumulating under the puncture site).

Normal Medical Results
Reference ranges for the antigen/antibody tests are as follows:

- Hepatitis A antibody, IgM: Negative
- Hepatitis B core antibody: Negative
- Hepatitis B e antibody: Negative
- Hepatitis B e-antigen: Negative
- Hepatitis B surface antibody: Varies with clinical circumstance

- (Note: As the presence of anti-HBs indicates past infection with resolution of previous hepatitis B infection, or vaccination against hepatitis B, additional patient history may be necessary for diagnosis.)
- Hepatitis B surface antigen: Negative
- Hepatitis C serology: Negative
- Hepatitis D serology: Negative.

Abnormal Medical Results
Hepatitis A: A single positive anti-HAV test may indicate previous exposure to the virus, but due to the antibody persisting so long in the bloodstream, only evidence of a rising anti-HAV titer confirms hepatitis A. Determining recent infection rests on identifying the antibody as IgM (associated with recent infection). A negative anti-HAV test rules out hepatitis A.

Hepatitis B: High levels of HBsAg that continue for three or more months after onset of acute infection suggest development of chronic hepatitis or carrier status. Detection of anti-HBs signals late convalescence or recovery from infection. This antibody remains in the blood to provide immunity to re-infection.

Hepatitis C (non-A, non-B hepatitis): Anti-HBc develops after exposure to hepatitis B. As an early indicator of acute infection, antibody (IgM) to core antigen (anti-HBc IgM) is rarely detected in chronic infection, so it is useful in distinguishing acute from chronic infection, and hepatitis B from non-A, non-B.

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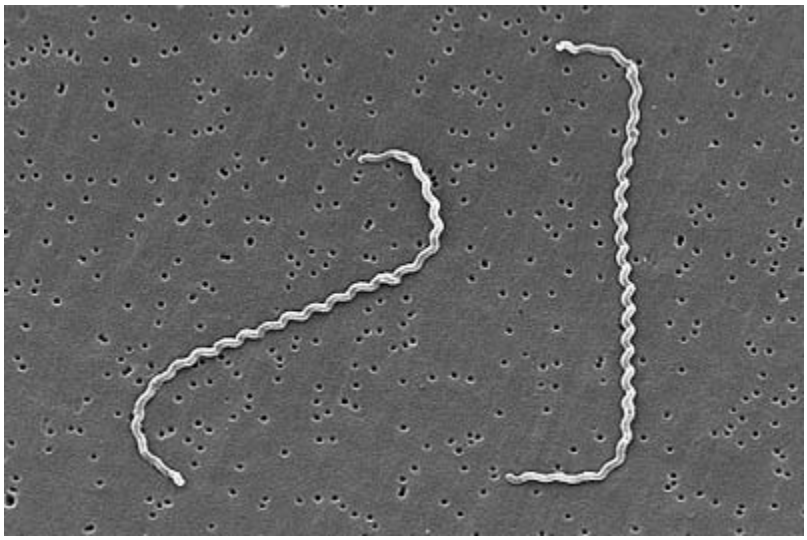
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Leptospirosis Leptospira Section

Leptospirosis is a bacterial disease that affects humans and animals. It is caused by bacteria of the genus Leptospira. In humans it causes a wide range of symptoms, and some infected persons may have no symptoms at all. Symptoms of leptospirosis include high fever, severe headache, chills, muscle aches, and vomiting, and may include jaundice (yellow skin and eyes), red eyes, abdominal pain, diarrhea, or a rash. If the disease is not treated, the patient could develop kidney damage, meningitis (inflammation of the membrane around the brain and spinal cord), liver failure, and respiratory distress. In rare cases death occurs. Leptospira interrogans causes leptospirosis, a usually mild febrile illness that may result in liver or kidney failure.



Structure, Classification, and Antigenic Types
Leptospira is a flexible, spiral-shaped, Gram-negative spirochete with internal flagella. Leptospira interrogans has many serovars based on cell surface antigens.

How do people get Leptospirosis?
Outbreaks of leptospirosis are usually caused by exposure to water contaminated with the urine of infected animals. Many different kinds of animals carry the bacterium; they may become sick but sometimes have no symptoms. Leptospira organisms have been found in cattle, pigs, horses, dogs, rodents, and wild animals. Humans become infected through contact with water, food, or soil containing urine from these infected animals. This may happen by swallowing contaminated food or water or through skin contact, especially with mucosal surfaces, such as the eyes or nose, or with broken skin.

The disease is not known to be spread from person to person.

Pathogenesis
Leptospira enters the host through mucosa and broken skin, resulting in bacteremia. The spirochetes multiply in organs, most commonly the central nervous system, kidneys, and liver. They are cleared by the immune response from the blood and most tissues but persist and multiply for some time in the kidney tubules. Infective bacteria are shed in the urine. The mechanism of tissue damage is not known.

Host Defenses
Serum antibodies are responsible for host resistance.

Epidemiology
Leptospirosis is a worldwide zoonosis affecting many wild and domestic animals. Humans acquire the infection by contact with the urine of infected animals. Human-to-human transmission is extremely rare.

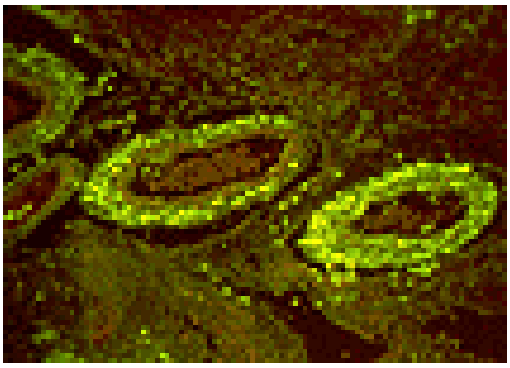
Diagnosis
Clinical diagnosis is usually confirmed by serology. Isolation of spirochetes is possible, but it is time-consuming and requires special media.

Control
Animal vaccination and eradication of rodents are important. Treatment with tetracycline and penicillin G is effective. No human vaccine is available.

Can Leptospirosis be prevented?
The risk of acquiring leptospirosis can be greatly reduced by not swimming or wading in water that might be contaminated with animal urine. Protective clothing or footwear should be worn by those exposed to contaminated water or soil because of their job or recreational activities.

Meningoencephalitis

Refer to amoebic meningoencephalitis (PAM), Naegleria fowleri and granulomatious amoebic encephalitis (GAE), acanthamoebic keratitis or acanthamoebic uveitis. These organisms are ubiquitous in the environment, in soil, water, and air. Infections in humans are rare and are acquired through water entering the nasal passages (usually during swimming) and by inhalation.



Granulomatous Amoebic Encephalitis due to Acanthamoeba castellanii

With immunofluorescent antibody techniques, the amoebae showed distinct fluorescence with anti-A. castellanii at 1:20 and 1:50 dilutions, but they were negative or weakly positive with anti-A. culbertsoni, A. polyphaga, A. rhysodes, and A. astronyxis sera. Protozoa were also seen in autopsy lung tissue, and identified as A. castellanii. There was also necrotizing amoebic panniculitis in subcutaneous, peripancreatic, mesenteric and peri-aortic tissue. There were occasional amoebae in the liver, but not enough to account for all of the patient's liver disease, so part of his liver disease was most likely due to sepsis due to his disseminated amoebiasis.

Pseudomonas aeruginosa Section

Pseudomonas aeruginosa *is the epitome of an opportunistic pathogen of humans. The bacterium almost never infects uncompromised tissues, yet there is hardly any tissue that it cannot infect if the tissue defenses are compromised in some manner.*

Pseudomonas aeruginosa is an opportunistic pathogen, meaning that it exploits some break in the host defenses to initiate an infection. It causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed. Pseudomonas aeruginosa infection is a serious problem in patients hospitalized with cancer, cystic fibrosis, and burns. The case fatality rate in these patients is 50 percent.

Pseudomonas aeruginosa is primarily a nosocomial pathogen. According to the CDC, the overall incidence of P. aeruginosa infections in US hospitals averages about 0.4 percent (4 per 1000 discharges), and the bacterium is the fourth most commonly-isolated nosocomial pathogen accounting for 10.1 percent of all hospital-acquired infections.

Pseudomonas aeruginosa is a Gram-negative bacterium that is noted for its environmental versatility, ability to cause disease in particular susceptible individuals, and its resistance to antibiotics. The most serious complication of cystic fibrosis is respiratory tract infection by the ubiquitous bacterium Pseudomonas aeruginosa. Cancer and burn patients also commonly suffer serious infections by this organism, as do certain other individuals with immune system deficiencies.

Unlike many environmental bacteria, P. aeruginosa has a remarkable capacity to cause disease in susceptible hosts. It has the ability to adapt to and thrive in many ecological niches, from water and soil to plant and animal tissues. The bacterium is capable of utilizing a wide range of organic compounds as food sources, thus giving it an exceptional ability to colonize ecological niches where nutrients are limited. P. aeruginosa can produce a number of toxic proteins which not only cause extensive tissue damage, but also interfere with the human immune system's defense mechanisms. These proteins range from potent toxins that enter and kill host cells at or near the site of colonization to degradative enzymes that permanently disrupt the cell membranes and connective tissues in various organs. This bacterium is also noted for its resistance to many antibiotics.

P. aeruginosa is widely studied by scientists who are interested in not only its ability to cause disease and resist antibiotics, but also its metabolic capability and environmental versatility. Analysis of its genome sequence has identified genes involved in locomotion, attachment, transport and utilization of nutrients, antibiotic efflux, and systems involved in sensing and responding to environmental changes.

The typical Pseudomonas bacterium in nature might be found in a biofilm, attached to some surface or substrate, or in a planktonic form, as a unicellular organism, actively swimming by means of its flagellum. Pseudomonas is one of the most vigorous, fast-swimming bacteria seen in hay infusions and pond water samples.

In its natural habitat Pseudomonas aeruginosa is not particularly distinctive as a pseudomonad, but it does have a combination of physiological traits that are noteworthy and may relate to its pathogenesis.

--Pseudomonas aeruginosa has very simple nutritional requirements. It is often observed "growing in distilled water" which is evidence of its minimal nutritional needs. In the laboratory, the simplest medium for growth of Pseudomonas aeruginosa consists of acetate for carbon and ammonium sulfate for nitrogen.

--P. aeruginosa possesses the metabolic versatility for which pseudomonads are so renowned. Organic growth factors are not required, and it can use more than seventy-five organic compounds for growth.

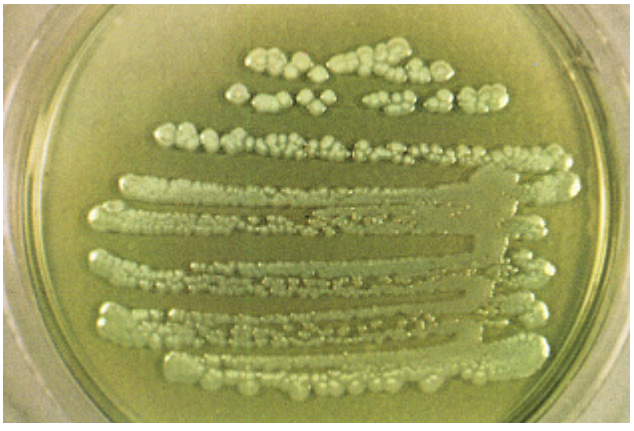
--Its optimum temperature for growth is 37°C, and it is able to grow at temperatures as high as 42°C.

--It is tolerant to a wide variety of physical conditions, including temperature. It is resistant to high concentrations of salts and dyes, weak antiseptics, and many commonly used antibiotics.

--Pseudomonas aeruginosa has a predilection for growth in moist environments, which is probably a reflection of its natural existence in soil and water.

These natural properties of the bacterium undoubtedly contribute to its ecological success as an opportunistic pathogen. They also help explain the ubiquitous nature of the organism and its prominance as a nosocomial pathogen.

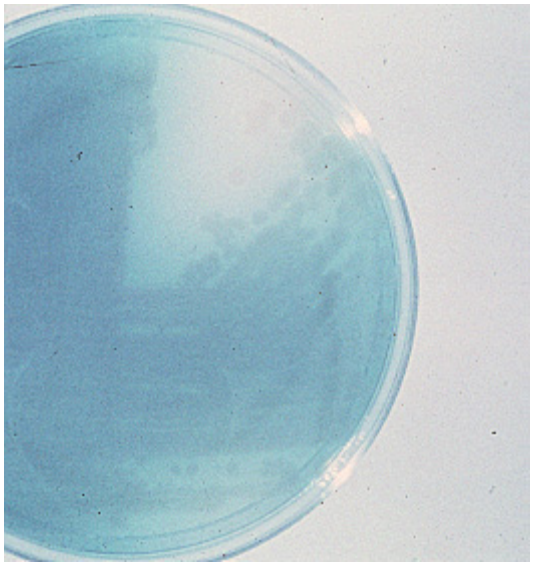
P. aeruginosa isolates may produce three colony types. Natural isolates from soil or water typically produce a small, rough colony. Clinical samples, in general, yield one or another of two smooth colony types. One type has a fried-egg appearance which is large and smooth, with flat edges and an elevated appearance. Another type, frequently obtained from respiratory and urinary tract secretions, has a mucoid appearance, which is attributed to the production of alginate slime. The smooth and mucoid colonies are presumed to play a role in colonization and virulence.



Pseudomonas aeruginosa colonies on agar.

Pyoverdin and the blue pigment Pyocyanin

P. aeruginosa strains produce two types of soluble pigments, the fluorescent pigment ***pyoverdin*** *and the blue pigment* ***pyocyanin****. The latter is produced abundantly in media of low-iron content and functions in iron metabolism in the bacterium. Pyocyanin (from "pyocyaneus") refers to "blue pus" which is a characteristic of suppurative infections caused by Pseudomonas aeruginosa.*



The soluble blue pigment pyocyanin is produced by many, but not all, strains of Pseudomonas aeruginosa.

Pseudomonas aeruginosa is notorious for its resistance to antibiotics and is, therefore, a particularly dangerous and dreaded pathogen. The bacterium is naturally resistant to many antibiotics due to the permeability barrier afforded by its outer membrane LPS. Also, its tendency to colonize surfaces in a biofilm form makes the cells impervious to therapeutic concentrations antibiotics. Since its natural habitat is the soil, living in association with the bacilli, actinomycetes and molds, it has developed resistance to a variety of their naturally-occurring antibiotics. Moreover, Pseudomonas maintains antibiotic resistance plasmids, both R-factors and RTFs, and it is able to transfer these genes by means of the bacterial processes of transduction and conjugation. Only a few antibiotics are effective against Pseudomonas, including fluoroquinolones, gentamicin and imipenem, and even these antibiotics are not effective against all strains. The futility of treating Pseudomonas infections with antibiotics is most dramatically illustrated in cystic fibrosis patients, virtually all of whom eventually become infected with a strain that is so resistant that it cannot be treated.

Diagnosis

Diagnosis of P. aeruginosa infection depends upon isolation and laboratory identification of the bacterium. It grows well on most laboratory media and commonly is isolated on blood agar or eosin-methylthionine blue agar. It is identified on the basis of its Gram morphology, inability to ferment lactose, a positive oxidase reaction, its fruity odor, and its ability to grow at 42° C. Fluorescence under ultraviolet light is helpful in early identification of P. aeruginosa colonies. Fluorescence is also used to suggest the presence of P. aeruginosa in wounds.

Pathogenesis

For an opportunistic pathogen such as Pseudomonas aeruginosa, the disease process begins with some alteration or circumvention of normal host defenses. The pathogenesis of Pseudomonas infections is multifactorial, as suggested by the number and wide array of virulence determinants possessed by the bacterium. Multiple and diverse determinants of virulence are expected in the wide range of diseases caused, which include septicemia, urinary tract infections, pneumonia, chronic lung infections, endocarditis, dermatitis, and osteochondritis.

Most Pseudomonas infections are both invasive and toxinogenic. The ultimate Pseudomonas infection may be seen as composed of three distinct stages: (1) bacterial attachment and colonization; (2) local invasion; (3) disseminated systemic disease. However, the disease process may stop at any stage. Particular bacterial determinants of virulence mediate each of these stages and are ultimately responsible for the characteristic syndromes that accompany the disease.

Colonization

Although colonization usually precedes infections by Pseudomonas aeruginosa, the exact source and mode of transmission of the pathogen are often unclear because of its ubiquitous presence in the environment. It is sometimes present as part of the normal flora of humans, although the prevalence of colonization of healthy individuals outside the hospital is relatively low (estimates range from 0 to 24 percent depending on the anatomical locale).

The fimbriae of Pseudomonas will adhere to the epithelial cells of the upper respiratory tract and, by inference, to other epithelial cells as well. These adhesions appear to bind to specific galactose, mannose or sialic acid receptors on epithelial cells. Colonization of the respiratory tract by Pseudomonas requires fimbrial adherence and may be aided by production of a protease enzyme that degrades fibronectin in order to expose the underlying fimbrial receptors on the epithelial cell surface. Tissue injury may also play a role in colonization of the respiratory tract since P. aeruginosa will adhere to tracheal epithelial cells of mice infected with Influenza virus but not to normal tracheal epithelium.

This has been called opportunistic adherence, and it may be an important step in Pseudomonas keratitis and urinary tract infections, as well as infections of the respiratory tract. The receptor on tracheal epithelial cells for Pseudomonas pili is probably sialic acid (N-acetylneuraminic acid). Mucoid strains, which produce an exopolysaccharide (alginate) have an additional or alternative adhesion which attaches to the tracheobronchial mucin (N-acetylglucosamine). Besides pili and the mucoid polysaccharide, there are possibly two other cell surface adhesions utilized by Pseudomonas to colonize the respiratory epithelium or mucin. Also, it is likely that surface-bound exoenzyme S could serve as an adhesion for glycolipids on respiratory cells. The mucoid exopolysaccharide produced by P. aeruginosa is a repeating polymer of mannuronic and glucuronic acid referred to as alginate. Alginate slime forms the matrix of the Pseudomonas biofilm which anchors the cells to their environment and, in medical situations, it protects the bacteria from the host defenses such as lymphocytes, phagocytes, the ciliary action of the respiratory tract, antibodies and complement. Biofilm mucoid strains of P. aeruginosa are also less susceptible to antibiotics than their planktonic counterparts. Mucoid strains of P. aeruginosa are most often isolated from patients with cystic fibrosis and they are usually found in post mortem lung tissues from such individuals.

Shigellosis Shigella Section

Shigella dysenteriae type 1(or bacillary dysentery) is the only cause of epidemic dysentery. This organism is generally found in the stool of infected individuals, as well as in contaminated water supplies. It is known to be able to survive on soiled linens for up to seven weeks, in water supplies for 5-11 days, and in kitchen waste for 1-4 days. Shigella can even survive in dust particles for six weeks at room temperature.

Infected humans act as host for this particular organism, as well as primates. The infections caused by this organism are generally seen in developing countries and areas of poor sanitation. Transmission occurs via direct or indirect contact with individuals who are infected by ingesting contaminated water, or food, as well as contact with fecal material.

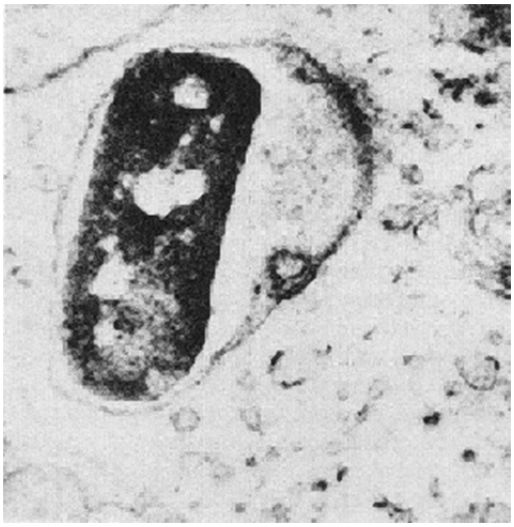
What sort of germ is Shigella?
The Shigella germ is actually a family of bacteria that can cause diarrhea in humans. They are microscopic living creatures that pass from person to person. Shigella were discovered over 100 years ago by a Japanese scientist named Shiga, for whom they are named. There are several different kinds of Shigella bacteria: Shigella sonnei, also known as "Group D" Shigella, accounts for over two-thirds of the shigellosis in the United States. A second type, Shigella flexneri, or "group B" Shigella, accounts for almost all of the rest. Other types of Shigella are rare in this country, though they continue to be important causes of disease in the developing world. One type found in the developing world, Shigella dysenteriae type 1, causes deadly epidemics there.

Microbial Characteristics
Shigella dysenteriae is a Gram (-), non-spore forming bacillus that survives as a facultative anaerobe. It is part of the family Enterobacteriaceae. When testing for it in the laboratory, you can help identify it by the fact that it is non-motile, and lactose and lysine (-). This organism, unlike some enterics, does not produce gas when breaking down carbohydrates.

Shigella dysenteriae is the organism responsible for bacillary dysentery. This disease is most often associated with areas of overcrowding and poor sanitation (developing countries). Illness does, however, tend to be seasonal, happening when it is hot and wet. Symptoms of dysentery due to this organism include mild to severe diarrhea, which is sometimes bloody or watery.

There is also fever and nausea that accompany the diarrhea. Some people, however, also suffer from vomiting and cramping, and some show no symptoms at all. The symptoms of the disease will generally show between 12-96 hours (1-3 days) after becoming infected.

During this incubation period, the organism will penetrate the mucosal epithelial cells of the intestine through use of an intestinal adherence factor. This penetration causes severe irritation which is responsible for the cramps and watery, bloody diarrhea. Dehydration can become a complication.



Micrograph of intra-epithelial membrane-enclosed Shigella
(from Microbiology: Fundamentals and Applications by R. M. Atlas, p. 609)

How can Shigella infections be diagnosed?
Many different kinds of diseases can cause diarrhea and bloody diarrhea, and the treatment depends on which germ is causing the diarrhea. Determining that Shigella is the cause of the illness depends on laboratory tests that identify Shigella in the stools of an infected person. These tests are sometimes not performed unless the laboratory is instructed specifically to look for the organism. The laboratory can also do special tests to tell which type of Shigella the person has and which antibiotics, if any, would be best to treat it.

How can Shigella infections be treated?
Shigellosis can usually be treated with antibiotics. The antibiotics commonly used for treatment are ampicillin, trimethoprim/sulfamethoxazole (also known as Bactrim or Septra*), nalidixic acid, or ciprofloxacin. Appropriate treatment kills the Shigella bacteria that might be present in the patient's stools, and shortens the illness. Unfortunately, some Shigella bacteria have become resistant to antibiotics and using antibiotics to treat shigellosis can actually make the germs more resistant in the future.*

Persons with mild infections will usually recover quickly without antibiotic treatment. Therefore, when many persons in a community are affected by shigellosis, antibiotics are sometimes used selectively to treat only the more severe cases. Antidiarrheal agents such as loperamide (Imodium) or diphenoxylate with atropine (Lomotil*) are likely to make the illness worse and should be avoided.*

Are there long term consequences to a Shigella infection?
Persons with diarrhea usually recover completely, although it may be several months before their bowel habits are entirely normal. About 3% of persons who are infected with one type of Shigella, Shigella flexneri, will later develop pains in their joints, irritation of the eyes, and painful urination. This is called Reiter's syndrome. It can last for months or years, and can lead to chronic arthritis which is difficult to treat. Reiter's syndrome is caused by a reaction to Shigella infection that happens only in people who are genetically predisposed to it.

Once someone has had shigellosis, they are not likely to get infected with that specific type again for at least several years. However, they can still get infected with other types of Shigella.

How do people catch Shigella?
The Shigella bacteria pass from one infected person to the next. Shigella are present in the diarrheal stools of infected persons while they are sick and for a week or two afterwards. Most Shigella infections are the result of the bacterium passing from stools or soiled fingers of one person to the mouth of another person.

This happens when basic hygiene and handwashing habits are inadequate. It is particularly likely to occur among toddlers who are not fully toilet-trained. Family members and playmates of such children are at high risk of becoming infected.

Shigella infections may be acquired from eating contaminated food. Contaminated food may look and smell normal. Food may become contaminated by infected food handlers who forget to wash their hands with soap after using the bathroom. Vegetables can become contaminated if they are harvested from a field with sewage in it. Flies can breed in infected feces and then contaminate food. Shigella infections can also be acquired by drinking or swimming in contaminated water. Water may become contaminated if sewage runs into it, or if someone with shigellosis swims in it.

What can a person do to prevent this illness?
There is no vaccine to prevent shigellosis. However, the spread of Shigella from an infected person to other persons can be stopped by frequent and careful handwashing with soap. Frequent and careful handwashing is important among all age groups. Frequent, supervised handwashing of all children should be followed in day care centers and in homes with children who are not completely toilet-trained (including children in diapers). When possible, young children with a Shigella infection who are still in diapers should not be in contact with uninfected children.

People who have shigellosis should not prepare food or pour water for others until they have been shown to no longer be carrying the Shigella bacterium.

If a child in diapers has shigellosis, everyone who changes the child's diapers should be sure the diapers are disposed of properly in a closed-lid garbage can, and should wash his or her hands carefully with soap and warm water immediately after changing the diapers. After use, the diaper changing area should be wiped down with a disinfectant such as household bleach, Lysol or bactericidal wipes.*

Basic food safety precautions and regular drinking water treatment prevents shigellosis. At swimming beaches, having enough bathrooms near the swimming area helps keep the water from becoming contaminated.

Simple precautions taken while traveling to the developing world can prevent getting shigellosis. Drink only treated or boiled water, and eat only cooked hot foods or fruits you peel yourself. The same precautions prevent traveler's diarrhea in general.

How common is shigellosis?
Every year, about 18,000 cases of shigellosis are reported in the United States. Because many milder cases are not diagnosed or reported, the actual number of infections may be twenty times greater. Shigellosis is particularly common and causes recurrent problems in settings where hygiene is poor and can sometimes sweep through entire communities. Shigellosis is more common in summer than winter. Children, especially toddlers aged 2 to 4, are the most likely to get shigellosis. Many cases are related to the spread of illness in child-care settings, and many more are the result of the spread of the illness in families with small children.

In the developing world, shigellosis is far more common and is present in most communities most of the time. Chinese scientists have sequenced the genome of a bacterium that is a leading cause of infant mortality in developing countries. About one million people die of Shigella infections every year, most of them children. The bacterium Shigella flexneri causes sudden and severe diarrhea in humans, known as shigellosis.

New treatments are needed for this highly infectious microbe because antibiotics are often inadequate and drug-resistant strains are on the rise. Currently, no vaccines exist and the World Health Organization considers the development of a vaccine a priority.

The publication of the genome sequence is an important step achieving this goal. The researchers identified regions of DNA linked to the virulence of the organism; these are promising targets for vaccines.

The sequenced S. flexneri strain was isolated from a patient with severe acute shigellosis in Beijing in 1984. The bacterium is commonly found in water polluted with human feces. It is transmitted in contaminated food or water and through contact between people. Upon infection, humans develop severe abdominal cramps, fever, and frequent passage of bloody stools.



Highly infectious microbe Shigella flexneri.
Courtesy P. Sansonetti, Institut Pasteur, Paris, France

The bacterium has about 4,700 genes. The S. flexneri genome consists of a chromosome and a smaller DNA structure called a virulence plasmid, which contains genes important in causing disease. The plasmid includes regions that are densely populated with genes called pathogenicity islands.

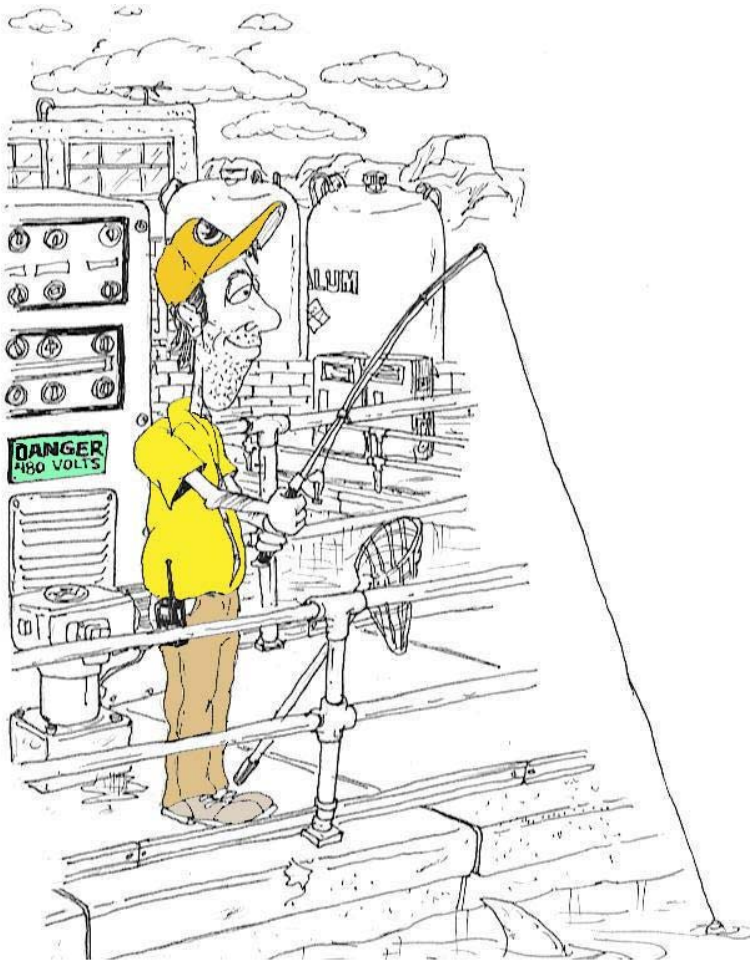
What else can be done to prevent shigellosis?
It is important for the public health department to know about cases of shigellosis. It is important for clinical laboratories to send isolates of Shigella to the City, County or State Public Health Laboratory so the specific type can be determined and compared to other Shigella. If many cases occur at the same time, it may mean that a restaurant, food or water supply has a problem which needs correction by the public health department. If a number of cases occur in a day-care center, the public health department may need to coordinate efforts to improve handwashing among the staff, children, and their families.

When a community-wide outbreak occurs, a community-wide approach to promote handwashing and basic hygiene among children can stop the outbreak. Improvements in hygiene for vegetables and fruit picking and packing may prevent shigellosis caused by contaminated produce.

Some prevention steps occur every day, without thinking about it. Making municipal water supplies safe and treating sewage are highly effective prevention measures that have been in place for many years.

What is the government doing about shigellosis?
The Centers for Disease Control and Prevention (CDC) monitors the frequency of Shigella infections in the country, and assists local and State health departments to investigate outbreaks, determine means of transmission and devise control measures. CDC also conducts research to better understand how to identify and treat shigellosis.

The Food and Drug Administration inspects imported foods, and promotes better food preparation techniques in restaurants and food processing plants. The Environmental Protection Agency regulates and monitors the safety of our drinking water supplies. The government has also maintained active research into the development of a Shigella vaccine.





Here is one of TLC's professors Marcos Aparecido Silva Bueno showing microscopic views of commonly found MO's in a classroom setting. Professor Marcos Aparecido Silva Bueno is a world renowned microbiological expert.



Typhoid Fever Salmonella typhi Section

Typhoid fever is a life-threatening illness caused by the bacterium Salmonella Typhi. In the United States about 400 cases occur each year, and 70% of these are acquired while traveling internationally. Typhoid fever is still common in the developing world, where it affects about 12.5 million persons each year.

Typhoid fever can be prevented and can usually be treated with antibiotics. If you are planning to travel outside the United States, you should know about typhoid fever and what steps you can take to protect yourself.



Salmonella typhi

Salmonella Typhi lives only in humans. Persons with typhoid fever carry the bacteria in their bloodstream and intestinal tract. In addition, a small number of persons, called carriers, recover from typhoid fever but continue to carry the bacteria. Both ill persons and carriers shed S. Typhi in their feces (stool).

You can get typhoid fever if you eat food or drink beverages that have been handled by a person who is shedding S. Typhi or if sewage contaminated with S. Typhi bacteria gets into the water you use for drinking or washing food. Therefore, typhoid fever is more common in areas of the world where handwashing is less frequent and water is likely to be contaminated with sewage. Once S. Typhi bacteria are eaten or drunk, they multiply and spread into the blood-stream. The body reacts with fever and other signs and symptoms.

In 1885, pioneering American veterinary scientist, Daniel E. Salmon, discovered the first strain of Salmonella from the intestine of a pig. This strain was called Salmonella choleraesuis, the designation that is still used to describe the genus and species of this common human pathogen. Salmonella is a type of bacteria that causes typhoid fever and many other infections of intestinal origin. Typhoid fever, rare in the U.S., is caused by a particular strain designated Salmonella typhi. But illness due to other Salmonella strains, just called "salmonellosis," is common in the U.S. Today, the number of known strains (technically termed "serotypes" or "serovars") of this bacteria total over 2300.

Serotypes
Salmonella serotypes typhimurium and enteritidis are the most common serotypes in the United States. In recent years, concerns have been raised because many strains of Salmonella have become resistant to several of the antibiotics traditionally used to treat it, in both animals and humans.

Getting vaccinated
If you are traveling to a country where typhoid is common, you should consider being vaccinated against typhoid. Visit a doctor or travel clinic to discuss your vaccination options.

Remember that you will need to complete your vaccination at least 1 week before you travel so that the vaccine has time to take effect. Typhoid vaccines lose effectiveness after several years; if you were vaccinated in the past, check with your doctor to see if it is time for a booster vaccination. Taking antibiotics will not prevent typhoid fever; they only help treat it.

The chart below provides basic information on typhoid vaccines that are available in the United States.

Vaccine Name	***How given***	***Number of doses necessary***	***Time between doses***	***Total time needed to set aside for vaccination***	***Minimum age for vaccination***	***Booster needed every...***
Ty21a (Vivotif Berna, Swiss Serum and Vaccine Institute)	*1 capsule by mouth*	*4*	*2 days*	*2 weeks*	*6 years*	*5 years*
ViCPS (Typhim Vi, Pasteur Merieux)	*Injection*	*1*	*N/A*	*1 week*	*2 years*	*2 years*

The parenteral heat-phenol-inactivated vaccine (manufactured by Wyeth-Ayerst) has been discontinued.

Tularemia Francisella tularensis Section

What is Tularemia?
Tularemia is a potentially serious illness that occurs naturally in the United States. It is caused by the bacterium *Francisella tularensis* found in animals (especially rodents, rabbits, and hares).

What are the Symptoms of Tularemia?
Symptoms of tularemia could include:

- *sudden fever*
- *chills*
- *headaches*
- *diarrhea*
- *muscle aches*
- *joint pain*
- *dry cough*
- *progressive weakness*

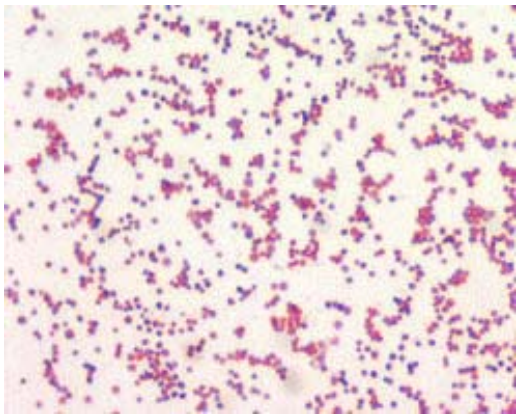
People can also catch pneumonia and develop chest pain, bloody sputum, and can have trouble breathing, even sometimes stop breathing.

Other symptoms of tularemia depend on how a person was exposed to the tularemia bacteria. These symptoms can include ulcers on the skin or mouth, swollen and painful lymph glands, swollen and painful eyes, and a sore throat.

How Does Tularemia Spread?
People can get tularemia many different ways:

- *being bitten by an infected tick, deerfly, or other insect*
- *handling infected animal carcasses*
- *eating or drinking contaminated food or water*
- breathing in the bacteria, *F. tularensis*

Tularemia is not known to be spread from person to person. People who have tularemia do not need to be isolated. People who have been exposed to the tularemia bacteria should be treated as soon as possible. The disease can be fatal if it is not treated with the right antibiotics.



F. tularensis
Gram stain

How Soon Do Infected People Get Sick?
Symptoms usually appear 3 to 5 days after exposure to the bacteria, but can take as long as 14 days.

What Should I Do if I Think I Have Tularemia?
Consult your doctor at the first sign of illness. Be sure to let the doctor know if you are pregnant or have a weakened immune system.

How Is Tularemia Treated?
Your doctor will most likely prescribe antibiotics, which must be taken according to the directions supplied with your prescription to ensure the best possible result. Let your doctor know if you have any allergy to antibiotics. A vaccine for tularemia is under review by the Food and Drug Administration and is not currently available in the United States.

What Can I Do To Prevent Becoming Infected with Tularemia?
Tularemia occurs naturally in many parts of the United States. Use insect repellent containing DEET on your skin, or treat clothing with repellent containing permethrin, to prevent insect bites. Wash your hands often, using soap and warm water, especially after handling animal carcasses. Be sure to cook your food thoroughly and that your water is from a safe source.

Note any change in the behavior of your pets (especially rodents, rabbits, and hares) or livestock, and consult a veterinarian if they develop unusual symptoms.

Can Tularemia Be Used As a Weapon?
Francisella tularensis is very infectious. A small number (10-50 or so organisms) can cause disease. If *F. tularensis* were used as a weapon, the bacteria would likely be made airborne for exposure by inhalation. People who inhale an infectious aerosol would generally experience severe respiratory illness, including life-threatening pneumonia and systemic infection, if they are not treated. The bacteria that cause tularemia occur widely in nature and could be isolated and grown in quantity in a laboratory, although manufacturing an effective aerosol weapon would require considerable sophistication.

F. tularensis is a small Gram-negative aerobic bacillus with two main serotypes: Jellison Type A and Type B. Type A is the more virulent form. The causative agent of the disease was named after Dr. Edward Francis and the location where the organism was discovered, Tulare County, California. Tularemia is frequently spread by direct contact with rabbits, leading to the term "rabbit fever." However, the disease can also be spread by other animals, typically rodents, and by arthropods. It is a primarily rural disease that is found in all 50 states, except Hawaii.

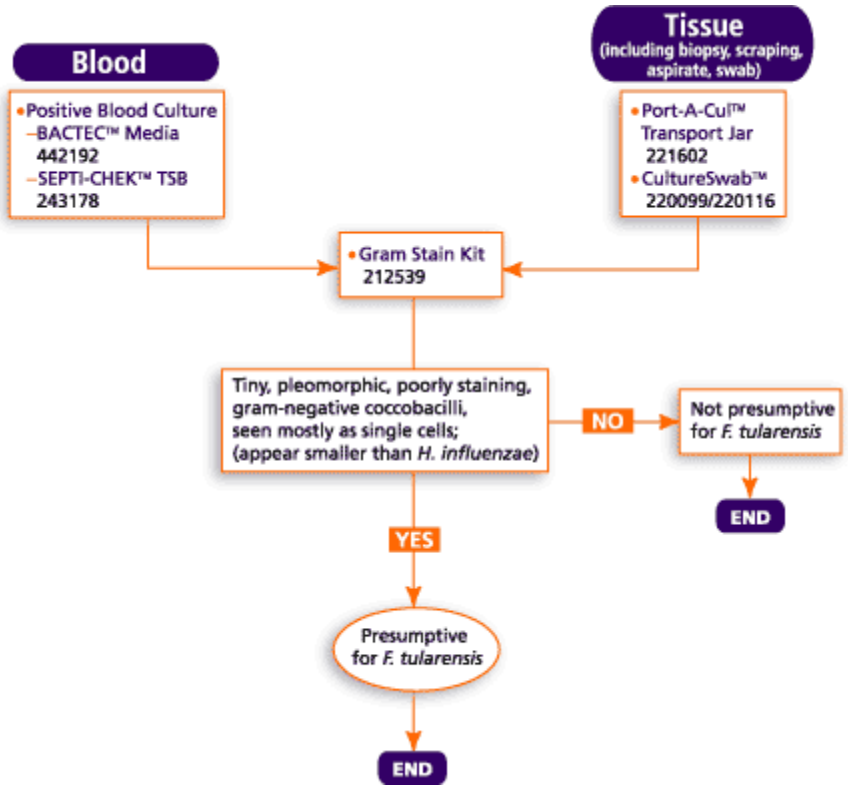
Pathogenesis
Historical commentaries reference the virulence of the disease, indicating that people have been aware of pathogenicity of Francisella for thousands of years. However, there is still much to be learned about this extremely virulent organism. The disease can be contracted by ingestion, inhalation, or by direct skin contact. Tularemia occurs in six different forms: typhoidal, pneumonic, oculoglandular, oropharyngeal, ulceroglandular, and glandular. Clinical diagnosis can be difficult since the disease mimics a slough of other illnesses. Pathogenesis varies greatly depending on mode of infection.

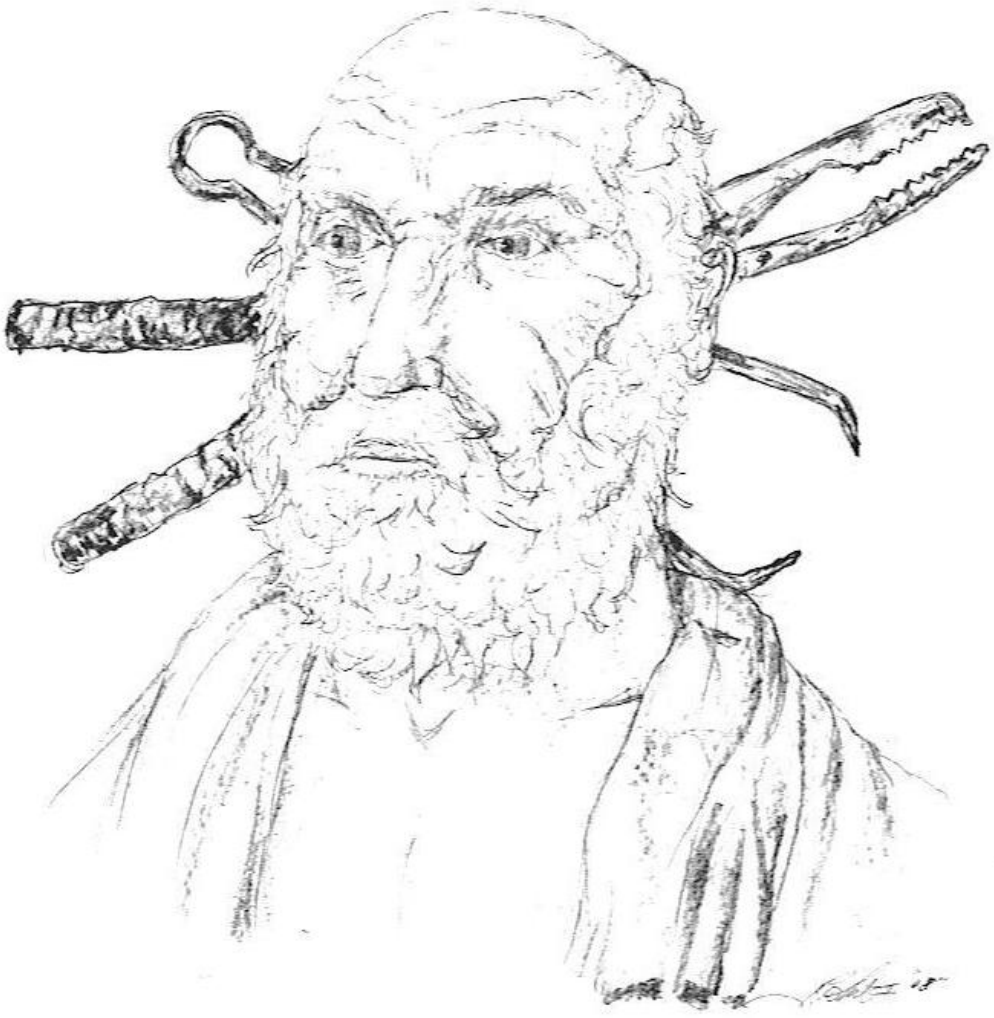
Manifestations
The incubation period is about 3-5 days but it can take as long as two weeks for symptoms to appear. Symptoms vary based on mode of infection, but generally include fever, chills, joint and muscle pain, headache, weakness, and sometimes pneumonia. People who develop pneumonic tularemia experience chest pain, bloody sputum, and difficultly breathing. The disease is easily cured by antibiotic treatment.

Treatment
If infection is suspected, diagnosis can be made based on serological assays since *F. tularensis* is difficult to culture on standard media. Agglutination titers can be performed following the first week of infection and reach a peak during the 4-8 weeks. Infected individuals are normally placed on a regimen of streptomycin or gentamycin for 10-14 days. Beta-lactams are generally ineffective due to beta-lactamase activity.

What is CDC Doing About Tularemia?
The CDC operates a national program for bioterrorism preparedness and response that incorporates a broad range of public health partnerships. Other things CDC is doing include:

- Stockpiling antibiotics to treat infected people.
- Coordinating a nation-wide program where states share information about tularemia.
- Creating new education tools and programs for health professionals, the public, and the media.





HIPPOCRATES

MIB and Geosmin Section

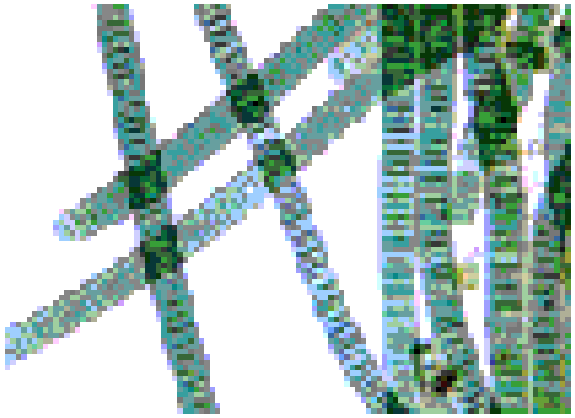
Aesthetics only and not a disease concern
While I was working in the water quality laboratory, we would be overwhelmed by customers calling in and worrying about tastes and odors. While this small section is not really about a waterborne disease, water customers will react to this as if was a disease.

Be prepared
Seasonal occurrences of musty/moldy or earthy tastes and odors may be detected in the system water. Research by laboratories dedicated to this subject, has determined the culprits are naturally occurring algal and fungal (microbiological) by-products. As algae in the canals die, compounds known as Methyl-Isoborneol (MIB) and Geosmin are released into the water.

These stable complex compounds present in parts per trillion are difficult to remove with current technology. The detection of these compounds is dependent upon an individual's olfactory sensitivity. Many people may never detect them, while others who are sensitive may detect the musty/moldy taste and smell at levels below instrument detection levels. Most water providers use activated carbon to adsorb the MIB and Geosmin, thus alleviating the taste and odor.

Earthy-musty tastes and odors are produced by certain cyanobacteria (blue-green algae), actinomycetes, and a few fungi. The substances are produced by actinomycetes and cyanobacteria that cause tastes and odors in drinking-water include geosmin, methyl-isoborneol (MIB), and cardin-4-ene-1-ol. Growing algae produce numerous volatile and nonvolatile organic substances, including aliphatic alcohols, aldehydes, ketones, esters, thioesters, and sulfides.

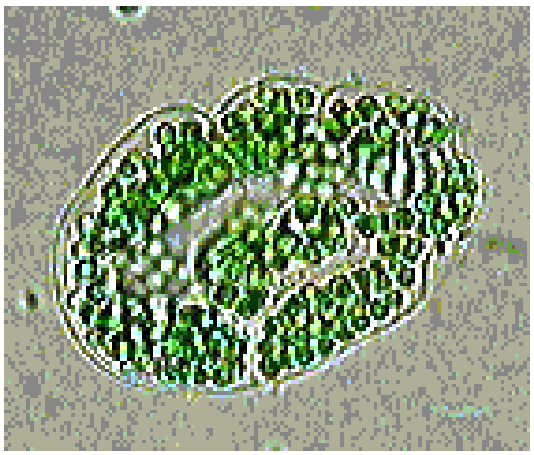
Occasionally, taste and odor problems in water are caused by other bacteria, fungi, zooplankton, and nemathelminthes. Ferrobacteria in water-distribution systems may produce tastes and odors, and some species of Pseudomonas can cause a swampy odor, whereas others can convert sulfur-containing amino acids into hydrogen sulfide, methylthiol, and dimethylpolysulfide.



Oscillatoria chalybea

Blooms

Blooms will produce the noxious substances 2-methyl isoborneol (MIB) and geosmin, compounds that are responsible for causing an off-flavor in catfish. MIB and geosmin are often described as smelling like "***sweaty socks***."



Microcystis aeruginosa

Commonly found in lakes and ponds. In the spring, large numbers floating on the water surface produce a blue-tinge. Blooms of this cyanobacterium are also notorious for producing a liver toxin that in large amount can kill fish and livestock.

Chemical Related Diseases

Arsenic

Arsenic is a naturally occurring element used since ancient times and has long been known to be toxic to humans. Arsenic in ground water is largely the result of minerals dissolving from weathered rocks and soils. Several types of cancer have been linked to arsenic in water. In 2001 the US Environmental Protection Agency lowered the maximum level of arsenic permitted in drinking water from 50 micrograms per liter (ug/L) to 10 ug/L.

Gastrointestinal and nervous system effects are common and the ingestion of relatively small amounts can result in death. A recent study indicates that arsenic disrupts the activity of glucocorticoids, compounds that have a variety of functions including the regulation of blood sugar.

Interestingly, this same study suggested that arsenic at high levels inhibits those mechanisms that normally suppress tumor production. This finding led to the suggestion that instead of causing cancer arsenic promotes the growth of tumors triggered by other carcinogens. And by the way, arsenic-induced effects appeared at concentrations as low as 2 micrograms per liter. Keep in mind, the new EPA regulation calls for 10 micrograms per liter and the average person ingests 10-15 micrograms per day. These numbers suggest we're getting our minimal daily requirement.



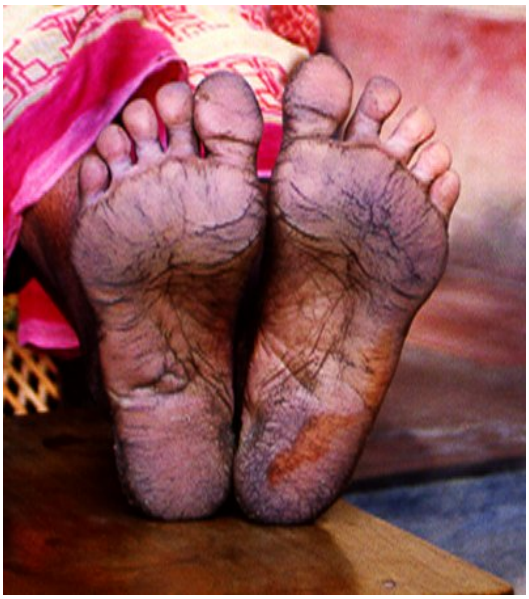
Other epidemiological studies suggest an association between drinking arsenic-tainted water and skin, lung, liver and bladder cancers. A 1999 report by the National Academy of Sciences estimated that daily ingestion of water containing 50 micrograms of arsenic per liter would add about 1 percent to a person's lifetime risk of dying from cancer.

Some studies also found that arsenic harms the central and peripheral nervous systems as well as heart and blood vessels. Arsenic has been associated with birth defects and reproductive problems.

Conservative estimates based on all these data suggest that more than 34 million Americans drink tap water supplied by systems containing average levels of arsenic that pose unacceptable cancer risks. In October 2001, the Environmental Protection Agency implemented new standards for arsenic in drinking water, lowering the maximum acceptable level in parts per billion from 50ppb to 10ppb.

Exposure to higher than average levels of arsenic occurs mostly in the workplace, near hazardous waste sites, or in areas with high natural levels. At high levels, inorganic arsenic can cause death. Exposure to lower levels for a long time can cause a discoloration of the skin and the appearance of small corns or warts.

Arsenic has been found at 1,014 of the 1,598 National Priority List sites identified by the Environmental Protection Agency (EPA).



Keratosis of the feet



Blackfoot disease

What happens to arsenic when it enters the environment?

- Arsenic cannot be destroyed in the environment. It can only change its form.
- Arsenic in air will settle to the ground or is washed out of the air by rain.
- Many arsenic compounds can dissolve in water.
- Fish and shellfish can accumulate arsenic, but the arsenic in fish is mostly in a form that is not harmful.

Is there a medical test to show whether I've been exposed to arsenic?

There are tests to measure the level of arsenic in blood, urine, hair, or fingernails. The urine test is the most reliable test for arsenic exposure within the last few days. Tests on hair and fingernails can measure exposure to high levels of arsenic over the past 6-12 months.

These tests can determine if you have been exposed to above-average levels of arsenic. They cannot predict how the arsenic levels in your body will affect your health.

Blue Baby Syndrome (Methemoglobinemia) Section

Methemoglobin is an abnormal form of hemoglobin which is unable to transport oxygen. Methemoglobinemia can be an inherited disorder, but it also can be acquired through exposure to chemicals such as nitrates (nitrate-contaminated water), aniline dyes, and potassium chlorate.

Causes, incidence, and risk factors
There are two forms of inheritable methemoglobinemia, Type I and Type II. Most hereditary cases are Type II, and result from a deficiency in the enzyme cytochrome b5 reductase. The other inheritable type, called hemoglobin M disease (Type I), is an autosomal dominant condition (you only need one affected parent to inherit it) characterized by an inability to convert methemoglobin back to hemoglobin. This usually causes few problems.

Acquired by Drinking Water and Other Causes
Exposure to certain chemicals may also cause an increase in the production of methemoglobin. These chemicals include nitrites (used commonly to prevent spoilage of meat), xylocaine, and benzene.

Nitrates and nitrites are nitrogen-oxygen chemical units which combine with various organic and inorganic compounds. The greatest use of nitrates is as a fertilizer. Most nitrogenous materials in natural waters tend to be converted to nitrate, so all sources of combined nitrogen, particularly organic nitrogen and ammonia, should be considered as potential nitrate sources. Primary sources of organic nitrates include human sewage and livestock manure, especially from feedlots.

What happens to nitrates/nitrites when they are released to the environment?
Since they are very soluble and do not bind to soils, nitrates have a high potential to migrate to ground water. Because they do not evaporate, nitrates/nitrites are likely to remain in water until consumed by plants or other organisms.

Short-term
Excessive levels of nitrate in drinking water have caused serious illness and sometimes death. The serious illness in infants is due to the conversion of nitrate to nitrite by the body, which can interfere with the oxygen-carrying capacity of the child's blood. This can be an acute condition in which health deteriorates rapidly over a period of days. Symptoms include shortness of breath and blueness of the skin. Long-term: Nitrates and nitrites have the potential to cause the following effects from a lifetime exposure at levels above the MCL: diuresis, increased starchy deposits and hemorrhaging of the spleen.

Follow-up
Retest regularly. Nitrate levels greater than 5 mg/L indicate the possibility that agricultural chemicals may be reaching the water source, and pesticide testing is recommended.

Point of Use Filtration
Nitrates cannot be removed from water by such treatments as UV lights, chlorinators, carbon filters, water softeners, iron filters, neutralizers. Nitrates can be removed by properly designed distillers, RO systems, and anion exchange systems.

Whereas distillers and RO systems are only suitable for point of use (i.e., one or two faucets in the home), anion exchange systems remove nitrates from the whole house.

Nitrate specific resin should be used with anion exchange systems to prevent the possibility of a maladjusted or malfunctioning anion exchange system from increasing the nitrate level due to sulfate exchange. We recommend that persons shopping for nitrate removal systems shop carefully and purchase only from a dealer experienced in nitrate removal.



Related Diseases and Associated Illnesses Chapter 7 Review

Amebiasis Section Entamoeba histolytica

1. The life cycle of Entamoeba histolytica involves trophozoites (the feeding stage of the parasite) that live in the host's large intestine and _____ that are passed in the host's feces.
A. Cysts
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

2. Humans are infected by ingesting _____, most often via food or water contaminated with human fecal material (view diagram of the life cycle).
A. Cysts
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

3. Entamoeba histolytica has four nuclei in the cyst, a central endosome, forms a cyst, and can be a tissue invader. The amoeboid trophozoites can live in the intestinal _____, feeding on intestinal contents and host tissue, and multiplying by fission.
A. Crypts
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

4. The _____ can destroy the tissues that line the host's large intestine, so of the amoebae infecting the human gastrointestinal tract, E. histolytica is potentially the most pathogenic.
A. Cyst
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

5. Entamoeba histolytica is an amoeboid _____ of the intestinal tract and in some cases other visceral organs, especially the liver.
A. Cyst
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

6. There are several species in this genus, distinguished by their number of _____ in the cyst and position of the endosome, whether or not they form a cyst, and whether they invade tissues or remain in the intestinal lumen.
A. Cyst
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

7. Within the _____ there are two nuclear divisions resulting in 2 nuclei in the immature cyst and 4 nuclei within the mature cyst. The cyst can resist desiccation for 1-2 weeks.
A. Cyst
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

8. When the cyst is ingested by another host the parasite excysts in the intestine and undergoes cytoplasmic division to produce 4 _____.
A. Cyst
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

9. In some cases the _____ secrete proteolytic enzymes which destroy the intestinal epithelium allowing the trophozoiute to enter the host tissue.
A. Cyst
B. Trophozoites
C. Protozoan parasite
D. Nuclei
E. Erythrocytes

10. In some cases the trophozoites will enter the circulatory system and infect other organs, most often the liver (hepatic amoebiasis), or they may penetrate the gastrointestinal tract resulting in acute peritonitis; such cases are often fatal. As with most of the amoebae, infections of E. histolytica are often diagnosed by demonstrating cysts or trophozoites in a stool sample.
A. True
B. False

Amebic Meningoencephalitis PAM Section Naegleria fowleri
11. Primary Amebic Meningoencephalitis (PAM) is a common and usually deadly disease caused by infection with the ameba (a multi-celled organism that maintains the original shape).
A. True
B. False

12. Following an incubation period of 2-15 days, there is a relatively sudden start of severe meningitis-like symptoms, which begin with fever and headache. These are rapidly followed by sensitivity to light, nausea, projectile vomiting, stiff neck, and, in many cases, disturbances to taste and smell. Changes in behavior and seizures may also be present. As conditions worsen the patient falls into a coma. Death usually occurs 3-7 days after the onset of symptoms.
A. True
B. False

13. The ameba that causes the infection lives in soil and in freshwater ponds, lakes, rivers, poorly or non-chlorinated pools, discharge or holding basins, and hot springs throughout the world. Naegleria thrives in warm, stagnant bodies of fresh water when temperatures are high, usually above 80 degrees.
A. True
B. False

14. Although the ameba is commonly found in the environment, PAM is very rare. In the last 30 years, only a few hundred cases have been reported worldwide.
A. True
B. False

15. The ameba is believed to enter the body through the mouth and travel to the stomach. The disease is easily spread from person to person.
A. True
B. False

16. The disease is initially suspected based on patient history. The diagnosis is made through the examination of the fluid in the digestive tract or frequently before death through the examination of digestive lining.
A. True
B. False

17. PAM is a mild illness that responds to routine treatments. Aggressive use of some antifungal medications have always been successful. Intensive supportive care is rarely necessary along with the medication.
A. True
B. False

Schistosomes and Other Trematodes Section

18. Schistosomiasis, also called snail fever or bilharziasis, is thought to cause more illness and disability than any other parasitic disease, except _____.
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

19. Almost unknown in industrialized countries, _____ infects 200 million people in 76 countries of the tropical developing world.
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

20. A Flatworm that spends part of its life in a freshwater snail host causes _____. Multiplying in the snail, a microscopic infective larval stage is released that can penetrate human skin painlessly in 30 to 60 seconds. The larvae grow to adulthood and migrate to the veins around the intestines or bladder, where mating occurs. The eggs produced may lodge in these tissues and cause disease, or they are passed out in urine or feces, where they reach fresh water and hatch to infect snails.
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

21. Free-swimming larvae (_____) are given off by infected snails. These either penetrate the skin of the human definitive host (schistosomes) or are ingested after encysting as metacercariae in or on various edible plants or animals (all other trematodes).
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

22. After entering a human the larvae develop into adult males and females (schistosomes) or _____ (other flukes), which produce eggs that pass out of the host in excreta. These eggs hatch in fresh water into miracidia which infect snails.
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

23. In _____, eggs trapped in the tissues produce granulomatous inflammatory reactions, fibrosis, and obstruction. The hermaphroditic flukes of the liver, lungs, and intestines induce inflammatory and toxic reactions.
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

24. The distribution of flukes is limited by the distribution of their snail intermediate host. Larvae from snails infect a human by penetrating the skin (schistosomes) or by being eaten (encysted larvae of other _____).
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. Trematodes

25. _____ is suggested by clinical manifestations, geographic history, and exposure to infective larvae. The diagnosis is confirmed by the presence of parasite eggs in excreta.
A. Cercariae
B. Hermaphrodites
C. Malaria
D. Schistosomiasis
E. None of the above

26. Signs and symptoms are related largely to the location of the adult worms. Infections with Schistosoma mansoni and S japonicum (mesenteric venules) result in eosinophilia, hepatomegaly, splenomegaly, and hematemesis. Schistosoma haematobium (vesical venules) causes dysuria, hema turia, and uremia. Fasciola hepatica, Clonorchis sinensis, and Opisthorchis viverrini (bile ducts) cause fever, hepatomegaly, abdominal pain, and jaundice. Infections with Paragonimus westermani (lungs, brain) result in cough, hemoptysis, chest pain, and epilepsy. Fasciolopsis buski (intestines) causes abdominal pain, diarrhea, and edema.
A. True
B. False

27. Trematodes are single cellular eukaryotic helminths.
A. True
B. False

Gastroenteritis Section
28. _____ means inflammation of the stomach and small and large intestines.
A. Contagious
B. Gastroenteritis
C. Virus(es)
D. Caliciviruses
E. None of the above

29. Viral gastroenteritis is an infection caused by a variety of _____ that results in vomiting or diarrhea. It is often called the "stomach flu," although it is not caused by the influenza viruses.
A. Contagious
B. Gastroenteritis
C. Virus(es)
D. Caliciviruses
E. None of the above

30. Many different viruses can cause gastroenteritis, including rotaviruses, adenoviruses, _____, astroviruses, Norwalk virus, and a group of Noroviruses. Viral gastroenteritis is not caused by bacteria (such as Salmonella or Escherichia coli) or parasites (such as Giardia), or by medications or other medical conditions, although the symptoms may be similar.
A. Contagious
B. Gastroenteritis
C. Virus(es)
D. Caliciviruses
E. None of the above

31. The main symptoms of viral _____ are watery diarrhea and vomiting.
A. Contagious
B. Gastroenteritis
C. Virus(es)
D. Caliciviruses
E. None of the above

32. The affected person may also have headache, fever, and abdominal cramps ("stomach ache"). In general, the symptoms begin 1 to 2 days following infection with a virus that causes gastroenteritis and may last for 1 to 10 days, depending on which _____ causes the illness.
A. Contagious
B. Gastroenteritis
C. Virus(es)
D. Caliciviruses
E. None of the above

33. Viral gastroenteritis is _____. The viruses that cause gastroenteritis are spread through close contact with infected persons (for example, by sharing food, water, or eating utensils). Individuals may also become infected by eating or drinking contaminated foods or beverages.
A. Contagious
B. Gastroenteritis
C. Virus(es)
D. Caliciviruses
E. None of the above

Rotovirus Information Section
34. The incubation period for _____ disease is approximately 2 days. The disease is characterized by vomiting and watery diarrhea for 3 - 8 days, and fever and abdominal pain occur frequently. Immunity after infection is incomplete, but repeat infections tend to be less severe than the original infection.
A. Rotavirus
B. Antigen
C. Genome
D. Gastroenteritis
E. None of the above

35. A _____ has a characteristic wheel-like appearance when viewed by electron microscopy (the name is derived from the Latin rota, meaning "wheel").
A. Rotavirus
B. Antigen
C. Genome
D. Gastroenteritis
E. None of the above

36. Rotaviruses are nonenveloped, double-shelled viruses. The _____ is composed of 11 segments of double-stranded RNA, which code for six structural and five nonstructural proteins. The virus is stable in the environment.
A. Rotavirus
B. Antigen
C. Genome
D. Gastroenteritis
E. None of the above

37. The primary mode of transmission is fecal-oral, although some have reported low titers of virus in respiratory tract secretions and other body fluids. Because the virus is stable in the _____, transmission can occur through ingestion of contaminated water or food and contact with contaminated surfaces.
A. Rotavirus
B. Antigen
C. Genome
D. Gastroenteritis
E. None of the above

38. Diagnosis may be made by rapid antigen detection of rotavirus in stool specimens. Strains may be further characterized by _____ or reverse transcriptase polymerase chain reaction, but such testing is not commonly done.
A. Rotavirus
B. Antigen
C. Genome
D. Gastroenteritis
E. None of the above

39. For persons with healthy immune systems, rotavirus _____ is a self-limited illness, lasting for only a few days. Treatment is nonspecific and consists of oral rehydration therapy to prevent dehydration. About one in 40 children will require hospitalization for intravenous fluids.
A. Rotavirus
B. Antigen
C. Genome
D. Gastroenteritis
E. None of the above

Noroviruses Section

40. Noroviruses (genus Norovirus, family Caliciviridae) are a group of related, single-stranded RNA, nonenveloped viruses that cause acute gastroenteritis in humans. Norovirus was recently approved as the official genus name for the group of viruses provisionally described as "Norwalk-like viruses" (NLV).
A. True
B. False

41. The symptoms of norovirus illness usually include nausea, vomiting, diarrhea, and some stomach cramping. Sometimes people additionally have a low-grade fever, chills, headache, muscle aches, and a general sense of tiredness. The illness often begins suddenly, and the infected person may feel very sick. The illness is usually brief, with symptoms lasting only about 1 or 2 days. In general, children experience more vomiting than adults. Most people with norovirus illness have both of these symptoms.
A. True
B. False

42. Persons who are infected with norovirus should not prepare food while they have symptoms and for 3 weeks after they recover from their illness. Food that may have been contaminated by an ill person can be eaten.
A. True
B. False

43. Illness caused by norovirus infection has several names, including stomach flu – this "stomach flu" is **not** related to the flu (or influenza), which is a respiratory illness caused by influenza virus.
A. True
B. False

44. Noroviruses are found in the stool or vomit of infected people. People can become infected with the virus in several ways, including eating food or drinking liquids that are contaminated with norovirus; touching surfaces or objects contaminated with norovirus, and then placing their hand in their mouth; having direct contact with another person who is infected and showing symptoms (for example, when caring for someone with illness, or sharing foods or eating utensils with someone who is ill).
A. True
B. False

45. Persons working in day-care centers or nursing homes should pay special attention to children or residents who have norovirus illness. This virus is very contagious and can spread rapidly throughout such environments.
A. True
B. False

Hepatitis Section

46. Hepatitis A is a liver disease caused by the hepatitis A virus (HAV). Hepatitis A can affect anyone. In the United States, hepatitis A can occur in situations ranging from isolated cases of disease to widespread _____.

A. Epidemics
B. Preventable
C. Acute liver failure
D. Communicable
E. None of the above

47. Each year, an estimated 100 persons die as a result of _____ in the United States due to Hepatitis A. Approximately 30 - 50,000 cases occur yearly in the United States and the direct and indirect costs of these cases exceed $300 million.

A. Epidemics
B. Preventable
C. Acute liver failure
D. Communicable
E. None of the above

48. The unfortunate aspect of these statistics is that with 21st century medicine, Hepatitis A is totally _____, and isolated cases, especially outbreaks relegated to food consumption, need not occur.

A. Epidemics
B. Preventable
C. Acute liver failure
D. Communicable
E. None of the above

49. Viral Hepatitis is a major public health concern in the United States, and a source of significant morbidity and mortality. The Hepatitis A virus or "HAV" is heat stable and will survive for up to a month at _____ in the environment.

A. Epidemics
B. Preventable
C. Acute liver failure
D. Communicable
E. None of the above

50. Hepatitis A is a _____ (or contagious) disease that spreads from person to person. (It is not acquired from animals, insects, or other means.) It is transmitted by the "fecal – oral route." This does not mean, or course, that Hepatitis A transmission requires that fecal material from an infectious individual must come in contact directly with the mouth of a susceptible individual. It is almost always true that the virus infects a susceptible individual when he or she ingests it, but it gets to the mouth by an indirect route.

A. Epidemics
B. Preventable
C. Acute liver failure
D. Communicable
E. None of the above

51. Hepatitis A is found in every part of the United States and throughout the world. When water sources such as private wells are contaminated with feces from infected humans, the water will spread the hepatitis A virus. The _____ can enter the water through various ways, including sewage overflows or broken sewage systems.
A. Epidemics
B. Preventable
C. Acute liver failure
D. Communicable
E. None of the above

52. Hepatitis ____ is a liver disease caused by the hepatitis____ virus, a defective virus that needs the hepatitis B virus to exist. Hepatitis ____ virus is found in the blood of persons infected with the virus.
A. Hepatitis A
B. Hepatitis B
C. Hepatitis C
D. Hepatitis D
E. Hepatitis E

53. Hepatitis ____ is a liver disease caused by the hepatitis ____ virus transmitted in much the same way as hepatitis A virus. Hepatitis ____, however, does not occur often in the United States.
A. Hepatitis A
B. Hepatitis B
C. Hepatitis C
D. Hepatitis D
E. Hepatitis E

54. Hepatitis ____is a serious disease caused by a virus that attacks the liver. The virus, which is called hepatitis ____ virus, can cause lifelong infection, cirrhosis (scarring) of the liver, liver cancer, liver failure, and death.
A. Hepatitis A
B. Hepatitis B
C. Hepatitis C
D. Hepatitis D
E. Hepatitis E

55. Hepatitis ____ is a liver disease caused by the hepatitis ____ virus, which is found in the blood of persons who have the disease. ____ is spread by contact with the blood of an infected person.
A. Hepatitis A
B. Hepatitis B
C. Hepatitis C
D. Hepatitis D
E. Hepatitis E

Leptospirosis Section Leptospira

56. _____ is a bacterial disease that affects humans and animals. It is caused by bacteria of the genus Leptospira. In humans it causes a wide range of symptoms, and some infected persons may have no symptoms at all.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

57. Symptoms of leptospirosis include high fever, severe headache, chills, muscle aches, and vomiting, and may include jaundice (yellow skin and eyes), red eyes, abdominal pain, diarrhea, or a rash. If the disease is not treated, the patient could develop kidney damage, meningitis (inflammation of the membrane around the brain and spinal cord), liver failure, and respiratory distress. In rare cases death occurs. Leptospira interrogans causes leptospirosis, a usually mild _____ that may result in liver or kidney failure.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

58. Leptospira is a flexible, spiral-shaped, _____ with internal flagella. Leptospira interrogans has many serovars based on cell surface antigens.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

59. Outbreaks of _____ are usually caused by exposure to water contaminated with the urine of infected animals. Many different kinds of animals carry the bacterium; they may become sick but sometimes have no symptoms.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

60. Leptospira organisms have been found in cattle, pigs, horses, dogs, rodents, and wild animals. Humans become infected through contact with water, food, or soil containing urine from these infected animals. This may happen by swallowing contaminated food or water or through skin contact, especially with mucosal surfaces, such as the eyes or nose, or with _____. The disease is not known to be spread from person to person.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

61. Leptospira enters the host through mucosa and broken skin, resulting in _____. The spirochetes multiply in organs, most commonly the central nervous system, kidneys, and liver. They are cleared by the immune response from the blood and most tissues but persist and multiply for some time in the kidney tubules. Infective bacteria are shed in the urine. The mechanism of tissue damage is not known.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

62. _____ are responsible for host resistance.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

63. Leptospirosis is a worldwide _____ affecting many wild and domestic animals. Humans acquire the infection by contact with the urine of infected animals. Human-to-human transmission is extremely rare.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

64. Clinical diagnosis is usually confirmed by _____. Isolation of spirochetes is possible, but it is time-consuming and requires special media.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

65. Animal vaccination and eradication of rodents are important. Treatment with _____ and penicillin G is effective. No human vaccine is available.
A. Serum antibodies
B. Zoonosis
C. Leptospirosis
D. Tetracycline
E. None of the above

Pseudomonas aeruginosa Section

66. Pseudomonas aeruginosa is the _____ of an opportunistic pathogen of humans.
A. Gram-negative bacterium
B. Cystic fibrosis
C. Epitome
D. Uncompromised
E. Gastrointestinal infections

67. The bacterium almost never infects _____ tissues, yet there is hardly any tissue that it cannot infect if the tissue defenses are compromised in some manner.
A. Gram-negative bacterium
B. Cystic fibrosis
C. Epitome
D. Uncompromised
E. Gastrointestinal infections

68. Pseudomonas aeruginosa is an opportunistic pathogen, meaning that it exploits some break in the host defenses to initiate an infection. It causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, _____ and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed.
A. Gram-negative bacterium
B. Cystic fibrosis
C. Epitome
D. Uncompromised
E. Gastrointestinal infections

69. Pseudomonas aeruginosa infection is a serious problem in patients hospitalized with cancer, _____, and burns. The case fatality rate in these patients is 50 percent.
A. Gram-negative bacterium
B. Cystic fibrosis
C. Epitome
D. Uncompromised
E. Gastrointestinal infections

70. Pseudomonas aeruginosa is primarily a _____. According to the CDC, the overall incidence of P. aeruginosa infections in US hospitals averages about 0.4 percent (4 per 1000 discharges), and the bacterium is the fourth most commonly-isolated nosocomial pathogen accounting for 10.1 percent of all hospital-acquired infections.
A. Gram-negative bacterium
B. Cystic fibrosis
C. Nosocomial pathogen
D. Uncompromised
E. Gastrointestinal infections

71. Unlike many environmental bacteria, P. aeruginosa has a remarkable capacity to cause disease in susceptible hosts. It has the ability to adapt to and thrive in many _____, from water and soil to plant and animal tissues.
A. Genome sequence
B. Metabolic capability
C. Permanently disrupt
D. Ecological niches
E. None of the above

72. The bacterium is capable of utilizing a wide range of organic compounds as food sources, thus giving it an exceptional ability to _____ where nutrients are limited.
A. Genome sequence
B. Metabolic capability
C. Permanently disrupt
D. Colonize ecological niches
E. None of the above

73. P. aeruginosa can produce a number of toxic proteins which not only cause extensive tissue damage, but also interfere with the human immune system's defense mechanisms. These proteins range from potent toxins that enter and kill host cells at or near the site of colonization to degradative enzymes that _____ the cell membranes and connective tissues in various organs.
A. Genome sequence
B. Metabolic capability
C. Permanently disrupt
D. Colonize ecological niches
E. None of the above

74. This bacterium is also noted for _____to many antibiotics.
A. Genome sequence
B. Metabolic capability
C. Permanently disrupt
D. Colonize ecological niches
E. Its resistance

75. P. aeruginosa is widely studied by scientists who are interested in not only its ability to cause disease and resist antibiotics, but also its _____ and environmental versatility.
A. Genome sequence
B. Metabolic capability
C. Permanently disrupt
D. Colonize ecological niches
E. None of the above

76. Analysis of its genome sequence has identified genes involved in locomotion, attachment, transport and utilization of nutrients, antibiotic efflux, _____ and systems involved in sensing and responding to environmental changes.
A. Genome sequence
B. Metabolic capability
C. Permanently disrupt
D. Colonize ecological niches
E. None of the above

Shigellosis Section Shigella

77. _____ type 1(or bacillary dysentery) is the only cause of epidemic dysentery.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella dysenteriae
E. None of the above

78. This organism is generally found in the stool of _____, as well as in contaminated water supplies. It is known to be able to survive on soiled linens for up to seven weeks, in water supplies for 5-11 days, and in kitchen waste for 1-4 days.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri
E. None of the above

79. _____ can even survive in dust particles for six weeks at room temperature.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri
E. None of the above

80. Infected humans act as host for this particular organism, as well as _____.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri
E. None of the above

81. The infections caused by this organism are generally seen in developing countries and areas of poor sanitation. _____ occurs via direct or indirect contact with individuals who are infected by ingesting contaminated water, or food, as well as contact with fecal material.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri
E. None of the above

82. The _____ germ is actually a family of bacteria that can cause diarrhea in humans. They are microscopic living creatures that pass from person to person.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri

83. A second type, _____, or "group B" Shigella, accounts for almost all of the rest.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri
E. None of the above

84. Other types of _____ are rare in this country, though they continue to be important causes of disease in the developing world.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella flexneri
E. None of the above

85. One type found in the developing world, _____ type 1, causes deadly epidemics there.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella dysenteriae
E. None of the above

86. _____ is a Gram (-), non-spore forming bacillus that survives as a facultative anaerobe. It is part of the family Enterobacteriaceae.
A. Shiga
B. Shigella
C. Shigella sonnei
D. Shigella dysenteriae
E. None of the above

87. When testing for it in the laboratory, you can help identify it by the fact that it is non-motile, and _____. This organism, unlike some enterics, does not produce gas when breaking down carbohydrates.
Incubation period
A. Lactose and lysine (-)
B. Bacillary dysentery
C. Bacterium passing
D. Shigellosis swims
E. None of above

88. Shigella dysenteriae is the organism responsible for _____.
A. Lactose and lysine (-)
B. Bacillary dysentery
C. Bacterium passing
D. Shigellosis swims
E. None of above

89. During this _____, the organism will penetrate the mucosal epithelial cells of the intestine through use of an intestinal adherence factor. This penetration causes severe irritation which is responsible for the cramps and watery, bloody diarrhea. Dehydration can become a complication.
A. Lactose and lysine (-)
B. Bacillary dysentery
C. Bacterium passing
D. Shigellosis swims
E. None of above

90. Most Shigella infections are the result of the _____ from stools or soiled fingers of one person to the mouth of another person. This happens when basic hygiene and handwashing habits are inadequate. It is particularly likely to occur among toddlers who are not fully toilet-trained. Family members and playmates of such children are at high risk of becoming infected.
A. Lactose and lysine (-)
B. Bacillary dysentery
C. Bacterium passing
D. Shigellosis swims
E. None of above

91. _____ may be acquired from eating contaminated food. Contaminated food may look and smell normal. Food may become contaminated by infected food handlers who forget to wash their hands with soap after using the bathroom.
A. Lactose and lysine (-)
B. Bacillary dysentery
C. Bacterium passing
D. Shigellosis swims
E. None of above

92. Vegetables can become contaminated if they are harvested from a field with sewage in it. Flies can breed in infected feces and then contaminate food. Shigella infections can also be acquired by drinking or swimming in contaminated water. Water may become contaminated if sewage runs into it, or if someone with _____ in it.
A. Lactose and lysine (-)
B. Bacillary dysentery
C. Bacterium passing
D. Shigellosis swims
E. None of above

Salmonella typhi Section
93. _____ is a life-threatening illness caused by the bacterium Salmonella Typhi.
A. Salmonella Typhi
B. Typhoid fever
C. Bacterium
D. Carriers
E. None of the above

94. _____is still common in the developing world, where it affects about 12.5 million persons each year.
A. Salmonella Typhi
B. Typhoid fever
C. Bacterium
D. Carriers
E. None of the above

95. Typhoid fever can be prevented and can usually be treated with _____.
A. Salmonella Typhi
B. Typhoid fever
C. Bacterium
D. Carriers
E. None of the above

96. Salmonella Typhi lives only in humans. Persons with typhoid fever carry the bacteria in their bloodstream and intestinal tract. In addition, a small number of persons, called _____, recover from typhoid fever but continue to carry the bacteria. Both ill persons and _____ shed S. Typhi in their feces (stool).
A. Salmonella Typhi
B. Typhoid fever
C. Bacterium
D. Carriers
E. None of the above

97. You can get typhoid fever if you eat food or drink beverages that have been handled by a person who is shedding _____ or if sewage contaminated with S. Typhi bacteria gets into the water you use for drinking or washing food. Therefore, typhoid fever is more common in areas of the world where handwashing is less frequent and water is likely to be contaminated with sewage.
A. Salmonella Typhi
B. Typhoid fever
C. Bacterium
D. Carriers
E. None of the above

98. Once S. Typhi bacteria are eaten or drunk, they _____ into the bloodstream. The body reacts with fever and other signs and symptoms.
A. Salmonella Typhi
B. Typhoid fever
C. Bacterium
D. Carriers
E. None of the above

1.A, 2.A, 3.A, 4.B, 5.C, 6.D, 7.A, 8.B, 9.B, 10.A, 11.B, 12. A, 13.A, 14.A, 15.B, 16.B, 17.B, 18.C, 19.D, 20.D, 21.A, 22.B, 23.D, 24.E, 25.E, 26.A, 27.B, 28.B, 29.C, 30.D, 31.B, 32. C, 33.A, 34.A, 35.A, 36.C, 37.E, 38.E, 39.D, 40.A, 41.A, 42.B, 43.A, 44.A, 45.A, 46.A, 47.C, 48.B, 49.E, 50.D, 51.E, 52.D, 53.E, 54.B, 55.C, 56.C, 57.E, 58.E, 59.C, 60.E, 61.E, 62.A, 63.B, 64.E, 65.D, 66.C, 67.D, 68.E, 69.B, 70.C, 71.D, 72.D, 73.C, 74.E, 75.B, 76.B, 77.D, 78.E, 79.B, 80.E, 81.E, 82.B, 83.D, 84.B, 85.D, 86.D, 87.A, 88.B, 89.E, 90.C, 91.E, 92.D, 93.B, 94.B, 95.E, 96.D, 97.A, 98.E

EPA Regulations Chapter 8

Proposed Stage 2 DBP Rule – Compliance

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The proposed Stage 2 Disinfection Byproduct Rule (DBPR) is expected to minimize exposure to disinfection byproducts (DBPs) at individual locations in the distribution system and to shave the peak values of DBPs in the system. The Initial Distribution System Evaluation was discussed in the last issued of the Newsletter. In the IDSE process, utilities are required to complete a study that locates new permanent DBP monitoring sites. When the IDSE is complete, an IDSE Report is submitted to the State which includes the proposed new permanent DBP monitoring sites for the utility. Each State may handle the approval of the monitoring sites in a slightly different manner, but the basic process is that that the State can approve the monitoring sites as recommended in the IDSE report, the State can suggest alternative sites, or the State may request additional information.

Stage 2A

Compliance under the Stage 2 DBPR is divided into two phases, Stage 2A and Stage 2B. During the time when utilities are monitoring for the IDSE, compliance continues to be based on the current Stage 1 DBPR requirements. Recall that that Stage 1 DBPR requires utilities to meet MCLs of 80 µg/L and 60 µg/L for TTHMs and HAA5s, respectively, based on a running annual average calculation across the whole system. During that same period, utilities should be preparing themselves to meet the locational running annual average (LRAA) requirements of the Stage 2A portion of the rule. The relative timing of the parts of the rule are shown in Figure 1.

Stage 2A requires all utilities to calculate the locational running annual average (LRAA) of quarterly sample results for TTHMs and HAA5s at each Stage 1 DBPR sampling site. The LRAA is the running annual average at a specific location in the distribution system. All utilities must meet the MCL of 120 µg/L for TTHMs and 100 µg/L for HAA5s on this LRAA basis. Preparation for meeting these LRAA levels should include evaluating the LRAA at each Stage 1 compliance monitoring site. For any sites that cannot meet the MCLs, development and implementation of a plan to bring them into compliance would be wise. The Stage 2A compliance date is three years from the date when the rule is finalized. At that time, all Stage 1 compliance sites must meet the Stage 2A MCLs based on the LRAA calculation method, in addition to maintaining compliance with the running annual average requirements across the system as required by the Stage 1 rule.

Stage 2B

As soon as the IDSE is complete, utilities can begin to prepare for meeting Stage 2B by monitoring at the newly selected compliance sites identified in the IDSE. Obtaining this data prior to the Stage 2B compliance date will provide information to the utility regarding areas in the system that need changes made that will reduce DBPs. Compliance with Stage 2B is based on meeting the MCLs of 80 µg/L and 60 µg/L for TTHMs and HAA5s, respectively, using the locational running annual average of quarterly sample results at each new monitoring site. Once the utility has a some data from the new sites, plans can be made for alterations in treatment or operations to meet MCLs, if needed.

Sufficient time is allowed in the rule for systems to make changes in their system to meet the compliance date for Stage 2B. The Stage 2B compliance date is six years after the rule is finalized for systems serving 10,000 people or more. For small systems the compliance date for Stage 2B is 7.5 or 8.5 years after the rule is finalized, depending on whether the system is required to sample for *Cryptosporidium* under the LT2ESWTR monitoring. Compliance dates for all system sizes for both stages of the rule are shown in Table 1.

TABLE 1
PROPOSED COMPLIANCE SCHEDULE FOR
STAGE 2A AND STAGE 2B DBPR

System Size	IDSE Report Due	Comply with Stage 2A	Comply with Stage 2B
≥ 10,000	2 years after publication	3 years after publication	6 years after publication
< 10,000 in combined distribution system	2 years after publication	3 years after publication	6 years after publication
< 10,000	4 years after publication	3 years after publication	7.5/8.5 years after publication

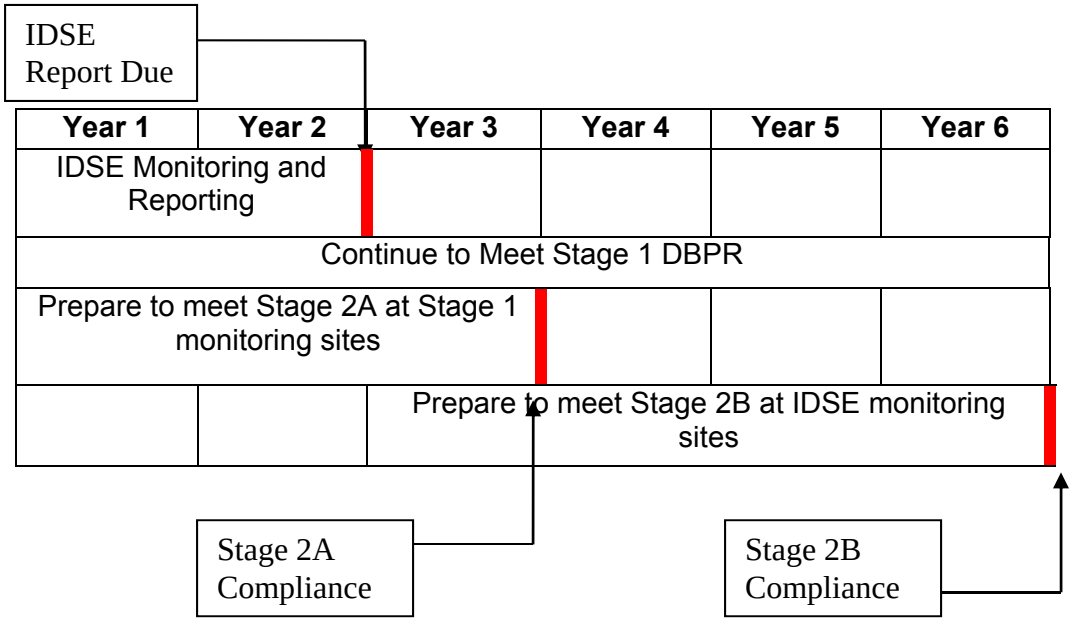
Summary
Utilities should keep in mind that the requirements of the Stage 1 DBPR continue to remain in force. This means that the percent removal requirements for total organic carbon (TOC) continue, as do the alternative compliance criteria for TOC removal. A summary of the Stage 2 DBPR phased compliance requirements is shown in Table 2.

Ground water serving10,000 people or more and surface water systems serving 500 or more people must monitor quarterly, taking at least one sample during the peak historical month for DBP concentration. Very small surface water plants and small ground water plants are on a yearly sampling schedule.

TABLE 2
PROPOSED STAGE 2 DBPR PHASED COMPLIANCE WITH MCLs

	Compliance Monitoring Sites	Compliance Calculation Basis	TTHM MCL □g/L	HAA5 MCL □g/L
Stage 2A	Stage 1	LRAA	120	100
	Stage 1	RAA	80	60
Stage 2B	Stage 2	LRAA	80	60

Figure 1
Schedule For DBP Compliance Monitoring



Notes:

- Applies to Large and Medium Systems and Small Systems in Larger Combined Distribution Systems
- Based on years after Stage 2 DBPR is promulgated.

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Waterborne Diseases

Name	Causative organism	Source of organism	Disease
Viral gastroenteritis	**Rotavirus** (mostly in young children)	Human feces	Diarrhea or vomiting
Norwalk Agent	**Noroviruses** (genus *Norovirus*, family *Caliciviridae*) [*1]	Human feces; also, shellfish; lives in polluted waters	Diarrhea and vomiting
Salmonellosis	***Salmonella*** (bacterium)	Animal or human feces	Diarrhea or vomiting
Gastroenteritis Escherichia *coli*	-- ***E. coli O1* 57:H7** (bacterium): Other *E. coli* organisms:	Human feces	Symptoms vary with type caused
Typhoid	***Salmonella typhi*** (bacterium)	Human feces, urine	Inflamed intestine, enlarged spleen, high temperature-sometimes fatal
Shigellosis	***Shigella*** (bacterium)	Human feces	Diarrhea
Cholera	***Vibrio choleras*** (bacterium)	Human feces; also, shellfish; lives in many coastal waters	Vomiting, severe diarrhea, rapid dehydration, mineral loss-high mortality
Hepatitis A	**Hepatitis A** virus	Human feces; shellfish grown in polluted waters	Yellowed skin, enlarged liver, fever, vomiting, weight loss, abdominal pain-low mortality, lasts up to four months
Amebiasis	***Entamoeba histolytica*** (protozoan)	Human feces	Mild diarrhea, dysentery, extra intestinal infection
Giardiasis	***Giardia lamblia*** (protozoan)	Animal or human feces	Diarrhea, cramps, nausea, and general weakness — lasts one week to months
Cryptosporidiosis	***Cryptosporidium parvum***	Animal or human feces	Diarrhea, stomach pain — lasts (protozoan) days to weeks

Notes:
*1 **http://www.cdc.gov/**

Drinking Water Rules and Disease Relationship

Public water systems are regulated under the Safe Drinking Water Act (SDWA) of 1974 and its subsequent 1986 and 1996 amendments (*7--9*). Under SDWA, the EPA is authorized to set national standards to protect drinking water and its sources against naturally occurring or man-made contaminants. The 1996 SDWA amendments require the EPA to publish a list every 5 years of contaminants that are known or anticipated to occur in public water systems and that might need to be regulated. The first list was called the drinking water Contaminant Candidate List (CCL). CCL contained 60 contaminants/ contaminant groups, included 10 pathogens, and was published in the *Federal Register* on March 2, 1998 (*10*). A decision concerning whether to regulate ≥5 contaminants from the CCL was required by August 2001.

Microbial contamination is regulated under the Total Coliform Rule (TCR) of 1989 and the Surface Water Treatment Rule (SWTR) of 1989 (*11--13*). SWTR covers all water systems that use surface water or groundwater under the direct influence of surface water (Glossary). SWTR is intended to protect against exposure to *Giardia intestinalis*, viruses, and *Legionella*, as well as selected other pathogens. In 1998, the EPA promulgated the Interim Enhanced Surface Water Treatment Rule (IESWTR) (*14*), which provides additional protection against *Cryptosporidium* and other waterborne pathogens for systems that serve ≥10,000 persons.

In 2002, the EPA finalized the Long Term 1 Enhanced SWTR (LT1ESWTR) for public water systems that use surface water or groundwater under the direct influence of surface water and serve <10,000 persons (*15*). LT1ESWTR was proposed in combination with the Filter Backwash Recycling Rule (FBRR), which was finalized in 2001 (*16,17*).

The 1996 Amendments require the EPA to develop regulations that require disinfection of groundwater systems as necessary to protect the public health; the EPA has proposed the Ground Water Rule (GWR) to meet this mandate (*18*). GWR specifies the appropriate use of disinfection in groundwater and addresses other components of groundwater systems to ensure public health protection. GWR applies to public groundwater systems (systems that have ≥15 service connections or regularly serve ≥25 persons/day for ≥60 days/year).

This rule also applies to any system that mixes surface and groundwater if the groundwater is added directly to the distribution system and provided to consumers without treatment. GWR does not apply to privately owned wells. Additional protection of groundwater from both chemical and microbial contamination from shallow wells (including cesspools) is expected to be provided as a result of recent revisions to the Underground Injection Control Regulations, published December 7, 1999 (*19*).

To fill gaps in existing data regarding occurrence of microbial pathogens and other indicators of microbial contamination, occurrence of disinfection byproducts, and characterization of treatment processes, the EPA promulgated the Information Collection Rule in 1996 (*20*), which required systems serving ≥100,000 persons to provide treatment data and monitor disinfection byproducts and source-water--quality parameters.

Surface water systems are also required to monitor for the presence of *Cryptosporidium*, *Giardia*, total culturable viruses, and total* and fecal coliforms or *Escherichia coli* ≥1 time/month for 18 months. The required monitoring ended in December 1998, and data were analyzed.

The EPA also made minor changes in 2000 to the Lead and Copper Rule to streamline requirements, promote consistent national implementation, and in certain cases, reduce the burden for water systems. The action levels of 0.015 mg/L for lead and 1.3 mg/L for copper remain the same (*21*).

Recreational Water
Regulation of recreational water is determined by state and local governments.

Standards for operating, disinfecting, and filtering public swimming and wading pools are regulated by state and local health departments and, as a result, are varied. In 1986, the EPA established a guideline for microbiological water quality for recreational freshwater (e.g., lakes and ponds) and marine water (*22*). The guideline recommends that the monthly geometric mean concentration of organisms in freshwater should be ≤33/100 mL for enterococci or ≤126/100 mL for *Es. coli*.

States have latitude regarding their guidelines or regulations and can post warning signs to alert potential bathers until water quality improves. Unlike treated venues where disinfection can be used to address problems with microbiological quality of the water, contaminated freshwater can require weeks or months to improve or return to normal. Prompt identification of potential sources of contamination and remedial action is necessary to return bathing water to an appropriate quality for recreational use (*23*).

The EPA's Action Plan for Beaches and Recreational Waters (Beach Watch) was developed as part of the Clean Water Action Plan.† The intent of Beach Watch is to assist state, tribal, and local authorities in strengthening and extending programs that specifically protect users of recreational waters. As part of the Beaches Act of 2000, the U.S. Congress directed the EPA to also develop a new set of guidelines for recreational water based on new water-quality indicators.

Data collected as part of the national WBDO surveillance system are used to describe the epidemiology of waterborne diseases in the United States. Data regarding water systems and deficiencies implicated in these outbreaks are used to assess whether regulations for water treatment and monitoring of water quality are adequate to protect the public against disease. Surveillance also enables identifying etiologic agents and environmental or behavioral risk factors that are responsible for these outbreaks.

This information is used to inform public health and regulatory agencies, water utilities, pool operators, and other stakeholders of new or reemerging trends that might necessitate different interventions and changes in policies and resource allotment.

Data Sources
State, territorial, and local public health agencies have primary responsibility for detecting and investigating WBDOs, and they voluntarily report them to CDC on a standard form (CDC form 52.12, which is available at http:// www.cdc.gov/.

The form solicits data related to 1) characteristics of the outbreak, including person, place, time, and location of the outbreak; 2) results from epidemiological studies conducted; 3) specimen and water sample testing; and 4) factors contributing to the outbreak, including environmental factors, water distribution, and disinfection concerns. Each year, CDC requests reports from state and territorial epidemiologists or from persons designated as WBDO surveillance coordinators.

Additional information regarding water quality and treatment is obtained from the state's drinking water agency as needed. Numerical and text data are abstracted from the outbreak form and supporting documents and are entered into a database before analysis.

Definitions[§]
The unit of analysis for the WBDO surveillance system is an outbreak, not an individual case of a waterborne disease. Two criteria must be met for an event to be defined as a WBDO. First, ≥2 persons must have experienced a similar illness after either ingestion of drinking water or exposure to water encountered in recreational or occupational settings. This criterion is waived for single cases of laboratory-confirmed primary amebic meningoencephalitis and for single cases of chemical poisoning if water-quality data indicate contamination by the chemical. Second, epidemiologic evidence (Table 1) must implicate water as the probable source of the illness.

For drinking water, reported outbreaks caused by contaminated water or ice at the point of use (e.g., a contaminated water faucet or serving container) are not classified as WBDOs.

If primary cases (i.e., among persons exposed to contaminated water) and secondary cases (i.e., among persons who became ill after contact with primary persons) are distinguished on the outbreak report form, only primary cases are included in the total number of cases. If both actual and estimated case counts are included on the outbreak report form, the estimated case count can be used if the population was sampled randomly or the estimated count was calculated by applying the attack rate to a standardized population.

Public water systems, which are classified as either community or noncommunity (Glossary), are regulated under SDWA. Of the approximately 170,000 public water systems in the United States, 113,000 (66.5 %) are noncommunity systems, of which 93,000 are transient systems (i.e., public water systems that regularly serve ≥25 of the same persons for ≥6 months/year [e.g., highway rest stations, restaurants, and parks with their own public water systems]) and 20,000 are nontransient systems (Glossary). A total of 54,000 systems (31.8%) are community systems. Community water systems serve approximately 264 million persons in the United States (96.0% of the U.S. population).

Approximately 11 million persons (4.0%) rely on private or individual water systems (*24,25*) (Glossary). These statistics exclude outbreaks associated with these sources

because they are not intended for drinking and are not considered to be public water systems. Also excluded from these statistics are the millions of persons who use noncommunity systems while traveling or working.

In this surveillance system, outbreaks associated with water not intended for drinking (e.g., lakes, springs, and creeks used by campers and boaters; irrigation water and other nonpotable sources with or without taps) are also classified as individual systems (Glossary). Sources used for bottled water are also classified as individual systems; bottled water is not regulated by the EPA but is subject to regulation by the Food and Drug Administration (FDA). Each drinking water system associated with a WBDO is classified as having one of the deficiencies in the following list. If >1 deficiency is noted on the outbreak report form, the deficiency that most likely caused the outbreak is noted.

Deficiency classifications are as follows:
1: untreated surface water;
2: untreated groundwater;
3: treatment deficiency (e.g., temporary interruption of disinfection, chronically inadequate disinfection, or inadequate or no filtration);
4: distribution system deficiency (e.g., cross-connection, contamination of water mains during construction or repair, or contamination of a storage facility); and
5: unknown or miscellaneous deficiency (e.g., contaminated bottled water) or water source not intended for drinking (e.g., irrigation water tap).

Recreational waters include swimming pools, wading pools, whirlpools, hot tubs, spas, water parks, interactive fountains, and fresh and marine surface waters. Although the WBDO surveillance system includes whirlpool- and hot tub-associated outbreaks of dermatitis caused by *Pseudomonas aeruginosa*, wound infections resulting from waterborne organisms are not included.

Outbreak Classification ***All related tables and figures are in the rear of this section***
WBDOs reported to the surveillance system are classified according to the strength of the evidence implicating water as the vehicle of transmission (Table 1). The classification scheme (i.e., Classes I--IV) is based on the epidemiologic and water-quality data provided with the outbreak report form. Epidemiologic data are weighted more than water-quality data. Although outbreaks without water-quality data might be included in this summary, reports that lack epidemiologic data were excluded. Outbreaks of dermatitis and single cases of either primary amebic meningoencephalitis or illness resulting from chemical poisoning were not classified according to this scheme. Weighting of epidemiologic data does not preclude the relative importance of both types of data. The purpose of the outbreak system is not only to implicate water as the vehicle for the outbreak, but also to understand the circumstances that led to the outbreak. A classification of I indicates that adequate epidemiologic and water-quality data were reported (Table 1); however, the classification does not necessarily imply whether an investigation was optimally conducted. Likewise, a classification of II, III, or IV should not be interpreted to mean that the investigations were inadequate or incomplete. Outbreaks and the resulting investigations occur under various circumstances, and not all outbreaks can or should be rigorously investigated. In addition, outbreaks that affect fewer persons are more likely to receive a classification of III, rather than I, on the basis of the relatively limited sample size available for analysis.

Results *All related tables and figures are in the rear of this section*

Outbreaks Associated with Drinking Water

During 1999--2000, a total of 39 outbreaks associated with drinking water were reported by 25 states (see Appendix A for selected case descriptions). One of the 39 outbreaks was a multistate outbreak of *Salmonella* Bareilly that included cases from 10 states. Of the 39 total drinking water outbreaks, 15 outbreaks were reported for 1999 and 24 for 2000. Florida reported the most outbreaks (15) during this period. These 39 outbreaks caused illness among an estimated 2,068 persons; 122 persons were hospitalized, and two died. The median number of persons affected in an outbreak was 13.5 (range: 2--781). Outbreaks peaked during the summer months (Figure 1), June--August.

Nine of the 39 (23.1%) outbreaks were assigned to Class I on the basis of epidemiologic and water-quality data; three (7.7%) were Class II; 25 (64.1%) were Class III; and 1 was Class IV (Table 1). One of two outbreaks associated with a chemical etiology was not assigned a class because that outbreak was a single case of illness resulting from nitrate poisoning associated with consumption of water from a private well. Outbreaks are listed by state (Tables 2 and 3) and are tabulated by the etiologic agent, the water system type (Table 4), and by the type of deficiency and type of water system type (Table 5).

Etiologic Agents
Twenty (51.3%) of the 39 outbreaks were of known infectious etiology; 17 (43.6%) were of unknown etiology; and two (5.1%) were attributed to chemical poisoning. Of the 20 outbreaks with known infectious etiology, seven (35.0%) were caused by parasites; nine (45.0%) were caused by bacteria; and four (20.0%) were caused by viruses (Figure 2) (Appendix A).

Parasites. Seven outbreaks affecting 57 persons were attributed to parasitic infection: six *Giardia* outbreaks and one *Cryptosporidium* outbreak. Six outbreaks of *Giardia* associated with drinking water affected 52 persons from five states: Florida (two outbreaks), New Mexico (one), New Hampshire (one), Minnesota (one), and Colorado (one). These outbreaks occurred in January (one), June (one), July (one), August (one), and September (two). Four outbreaks were associated with well water systems, and two were associated with surface water systems. Two outbreaks caused by *G. intestinalis* involved possible contamination of wells by animal feces. *G. intestinalis* can infect mammalian hosts, which in turn, can serve as reservoirs for human infection. Water treatment failure was a factor in two other outbreaks of *Giardia*.

Bacteria. Nine outbreaks affecting an estimated 1,166 persons were attributed to bacterial infection: four *Es. coli* O157: H7 outbreaks, one *Campylobacter jejuni*, one *Salmonella* Typhimurium, one *Sa.* Bareilly, and two mixed *Ca. jejuni* and shiga toxin-producing *Es. coli* (O157:H7 or O111) outbreaks. The two outbreaks with multiple pathogens caused the two largest bacterial drinking water outbreaks reported during this study period.

Viruses. During this period, four outbreaks involving viral gastroenteritis were reported. A total of 426 persons reported illness; no hospitalizations or deaths were reported in association with these four viral outbreaks. Three of the four outbreaks occurred in camp

facilities in California, New Mexico, and West Virginia. All three water sources were noncommunity groundwater sources.

Chemicals. During 1999, two outbreaks involving chemical contamination were reported. A total of three persons were affected by contamination of drinking water from nitrate and sodium hydroxide.

Unidentified Etiologic Agents. Seventeen outbreaks involving gastroenteritis of unknown etiology were reported from four states, affecting an estimated 416 persons and resulting in five hospitalizations. Testing for certain enteric pathogens (including ova and parasite testing) was attempted in five of the 17 outbreaks. In a June 2000 outbreak affecting 2 persons, stool specimens collected from one person tested negative for *G. intestinalis* but positive for *Blastocystis hominis*. However, whether *B. hominis* was the cause of the reported illness is unclear, and the pathogenicity of *B. hominis* has been debated in the scientific community (*26*). Stool specimens were negative for parasitic and bacterial enteric pathogens in two outbreaks in Washington (July 1999 and August 1999) and in two Florida outbreaks (March 1999 and April 2000) (Appendix A).

In addition, suspected pathogens were noted in four other outbreak reports submitted. On the basis of symptoms of illness, Norwalk-like virus (NLV) was suspected in an Idaho outbreak among firefighters that caused 65 illnesses and four hospitalizations, but the outbreak was not laboratory-confirmed. *G. intestinalis* was suspected in an April 2000 outbreak in a Florida trailer park affecting 21 persons, on the basis of the incubation period and symptoms reported.

In another outbreak in a Florida trailer park in March 2000 among 19 persons, a bacterial pathogen was suspected as the cause of the outbreak on the basis of the symptoms, which included conjunctivitis and dermatitis in addition to gastroenteritis.

A chemical agent was suspected as the cause of illness among four residents in a Florida apartment building who had a cross-connection between their drinking water and a toilet flush-valve. The residents of the apartment had noted blue tap water before onset of illness on multiple occasions before an improper flush valve in the toilet tank was discovered.

Four outbreaks of gastroenteritis were associated with consumption of untreated water from private wells. These four outbreaks occurred in Florida and affected 3--4 persons each. In July 2000, flooding was a possible contributor to two outbreaks. Water in each of the homes tested positive for coliforms and did not have adequate disinfection.

Water-Quality Data

Water-quality data (i.e., information regarding the presence of coliform bacteria, pathogens, or chemical contaminants) were available for 35 (89.7%) of the 39 drinking water outbreaks. Two reports of outbreaks of confirmed or suspected infectious etiology and two reports of outbreaks of confirmed or suspected chemical etiology did not provide water-quality data.

Of the 36 reports of outbreaks with a suspected or confirmed infectious etiology, 33 outbreaks provided water-quality data. Twenty-six (78.8%) of the 33 outbreaks with a suspected or confirmed infectious etiology reported a positive coliform, total coliform, or fecal coliform result. Organisms also were detected in the water in two of these outbreaks. In August 2000, *Ca. jejuni* was detected in the water in a mixed *Ca. jejuni*/*Es. coli* O157:H7 outbreak in Utah, although shiga toxins were not detected. *Es. coli* O157:H7 was found in the water in a July 2000 California outbreak. In a 2000 Colorado outbreak, the presence of *G. intestinalis* was demonstrated in a sample from the water holding tank, despite the lack of coliform data.

Of the three outbreaks with either a confirmed or suspected chemical etiology, only one demonstrated that the chemical had been directly in the water. Tap water was tested after the health department was notified that an infant had methemoglobinemia. Both fecal coliforms and 28 mg/L of nitrate were detected in the water. For an outbreak where burns and gastroenteritis were reported and linked to a sodium hydroxide spill, a pH test of the water that could indicate whether NaOH or another basic substance had spilled into the water was not documented. However, the environmental assessment indicated the tank contents had emptied into the water. A third suspected chemical outbreak involving a cross-connection between a toilet flush-valve and the drinking water system did not have water-quality data available.

In 11 of the 35 outbreaks, water was not sampled for coliforms until >1 month after the first case associated with the outbreak was reported (range: 5--16 weeks). In four of these 11 outbreaks, the water samples did not test positive for coliforms (fecal or total), chemicals, or pathogens. Instead, these were confirmed as outbreaks by epidemiologic data or by reports that treatment deficiencies had occurred.

Water Systems and Water Sources

Eleven (28.2%) of the 39 drinking water outbreaks were associated with community systems, 11 (28.2%) with noncommunity systems, and 17 (43.6%) with individual water systems (Tables 4 and 5). Ten (25.6%) of the 39 drinking water outbreaks were associated with surface water, including three outbreaks that implicated irrigation water not intended for consumption. Twenty-nine (74.4%) of the 39 drinking water outbreaks, including the outbreak associated with bottled water, were associated with groundwater sources (wells and springs).

Five (45.5%) of the 11 outbreaks associated with community water systems were caused by treatment deficiencies; one (9.0%) outbreak was related to contaminated, untreated groundwater, and five (45.5%) outbreaks were related to problems in the water distribution system. Two of the five distribution system problems were related to cross-connections between the distribution system and an irrigation well.

The third outbreak related to a community water source had a household cross-connection between the toilet water and main kitchen tap. One outbreak of *Cr. parvum* (Florida, December 2000) was related to a repeated history of water main breaks. In another outbreak in Ohio in August 2000, deficiencies in the distribution system of a fairgrounds might have allowed back-siphonage of animal manure into the water used by food and beverage vendors.

Ten (90.1%) of 11 outbreaks associated with noncommunity water systems occurred in groundwater systems. Seven of the 10 groundwater outbreaks were linked to untreated wells, and one of the 10 involved consumption of untreated spring water. Two of the 10 outbreaks were related to treatment deficiencies in water taken from wells or a spring and were associated with outbreaks of NLV and a small round-structured virus. An outbreak associated with *G. intestinalis* related to consumption of surface water occurred when a pump failure and a defective filter cartridge resulted in river water entering the drinking water holding tank without filtration. No information concerning chlorine levels from water samples was provided.

Nine (52.9%) of 17 outbreaks associated with individual water systems occurred in groundwater systems. Eight of these groundwater systems were wells that were not treated routinely; one outbreak of giardiasis occurred when the filtration system for a well was inadvertently turned off. Five (31.3%) of the 16 outbreaks occurred when persons drank water not intended for direct consumption from irrigation systems or when they consumed surface water that had been ineffectively or improperly treated. One (6.3%) of the 16 outbreaks in a system occurred in a home where creek water on the property was directly consumed without treatment.

Of the nine bacterial outbreaks, four occurred in groundwater systems (one was associated with a deficiency in the distribution system, one with a treatment deficiency, and two occurred in untreated systems). Six of seven parasitic outbreaks occurred in groundwater systems: three occurred in untreated systems; two involved problems in the distribution system; and one was related to a treatment deficiency. All four viral outbreaks occurred in noncommunity groundwater systems. Two occurred in untreated wells, and two were related to treatment deficiencies in a spring and well. Two chemical outbreaks were related to treatment deficiencies in well water. Fourteen of the 17 outbreaks of unknown etiology were linked to groundwater systems. Ten of these 14 outbreaks occurred in untreated systems; two were related to distribution system problems, and two were related to treatment deficiencies.

Outbreaks Associated with Recreational Water

During 1999--2000, a total of 23 states reported 59 outbreaks associated with recreational water (Tables 6--9) (see Appendix B for selected case descriptions).

Twenty-three outbreaks were reported for 1999, and 36 for 2000. The states that reported the largest number of outbreaks were Florida (14 outbreaks) and Minnesota (eight outbreaks). These 59 outbreaks affected 2,093 persons and resulted in 25 hospitalizations and four deaths. The median size of the outbreak was 10 persons (range: 1--700).

Of the 59 outbreaks, 36 were outbreaks of gastroenteritis (Tables 6 and 7); 15 were outbreaks of dermatitis (Table 9); four were cases of meningoencephalitis; and the remaining four outbreaks were of leptospirosis, chemical keratitis, acute respiratory

infection of unknown etiology, and Pontiac fever (Table 8). Thirty-one (86.1%) of the 36 outbreaks involving gastroenteritis occurred during the summer months (i.e., June--August) (Figure 3). Outbreaks of dermatitis associated with recreational water contact were reported more frequently in February, March, June, and July. The four cases of primary amebic meningoencephalitis occurred in the warmer months (April--October).

Etiologic Agents
Of the 59 recreational water outbreaks, 44 (74.6%) were of known infectious etiology (Tables 6--9). Of the 36 outbreaks involving gastroenteritis, 17 (47.2%) were caused by parasites; nine (25.0%) by bacteria; three (8.3%) by viruses; one (2.8%) by a combination of parasites and bacteria; and the remaining six (16.7%) were of unknown etiology (Figure 4).

Of the 23 nongastroenteritis-related recreational outbreaks, seven were attributed to *P. aeruginosa*, four to free-living amoebae, one to *Leptospira* species, one to *Legionella* species, and one to bromine (Tables 8 and 9). Nine nongastroenteritis-related recreational outbreaks were of unknown etiology, eight of which were suspected but not confirmed to be caused by *P. aeruginosa* or schistosomes. The ninth outbreak of unknown etiology was suspected to be caused by a virus or by *Legionella pneumophila* on the basis of observed symptoms and the epidemiologically implicated vehicle of transmission. Of the 59 recreational water outbreaks, 21 (35.6%) were associated with fresh or surface water, and 37 (62.7%) with treated (e.g., chlorinated) water. Information regarding the water venue for an outbreak of meningoencephalitis was not provided.

Parasites. Sixteen of the 17 parasitic recreational water outbreaks involving gastroenteritis were caused by *Cr. parvum*. The seventeenth outbreak was caused by *G. intestinalis*. Fifteen of the 17 parasitic outbreaks occurred in chlorinated venues; in these outbreaks, inadequate treatment, disrupted chlorine disinfection, or suboptimal pool maintenance were contributing factors to the outbreaks. *Cr. parvum* is highly resistant to chlorine disinfection and can survive for days in adequately chlorinated pools; therefore, suboptimal chlorination of the pool might not be the sole factor contributing to the occurrence of an outbreak.

Three outbreaks of laboratory-confirmed cryptosporidiosis occurred during the 1999 summer swim season. During the 2000 summer swim season, three substantial outbreaks of *Cr. parvum* occurred that were related to swimming in municipal pools. In August 2000, an outbreak occurred in Colorado that affected 112 persons attending a private pool party. In June 2000, the two other cryptosporidiosis outbreaks, one in Ohio affecting 700 persons and the other in Nebraska affecting 225 persons (*27*), occurred among members of private swim clubs. In both outbreaks, the protracted nature of the outbreaks during ≥2 months was the result of repeated recontamination of the pools by infected persons continuing to swim; 37 (18%) of 205 persons interviewed in the Nebraska outbreak admitted to swimming while symptomatic, and 32% swam while ill or during the 2 weeks after their illness (*27*). Another outbreak (Florida, August 2000) was associated with the outbreak that occurred in Ohio.

A family who were members of the implicated swim club in the Ohio outbreak were vacationing with a sick child. While in a pool in Florida, the infant had two fecal accidents. The resulting outbreak caused five cases of diarrheal illness and two hospitalizations.

Eight other outbreaks of cryptosporidiosis occurred in treated venues during the 2000 swim season. Two outbreaks of gastroenteritis occurred in untreated venues: one in a freshwater lake in Minnesota in July 2000 and one in a Massachusetts pond in July 1999 (Appendix B).

Four cases of laboratory-confirmed primary amebic meningoencephalitis attributed to *Naegleria fowleri* occurred during this 2-year reporting period. All four persons were aged ≤19 years. Three of the persons died from infection after having contact with a pond, lake, or mud hole. The fourth person's freshwater exposure could not be determined; that person had fallen from a jet ski into an unspecified body of water, sustained injuries, and died from an infection shortly after it was detected.

Bacteria. Nine recreational outbreaks involving gastroenteritis were attributed to bacterial pathogens, and five of the nine were linked to freshwater sources. Five cases (Wisconsin, August 1999) of *Es. coli* O157:H7 occurred among persons who had visited the same swimming beach. After a review of potential risk factors, the only common link found was swimming at the implicated beach. The popular beach featured a shallow, dammed area that was used for wading. Total and fecal coliforms were detected in water samples collected before and during the outbreak, although the levels detected did not exceed levels of EPA-recommended guidelines for microbiologic quality of water (*22*).

One sample that was tested for *Es. coli* O157:H7 was negative. *Es. coli* O157:H7 was implicated in another outbreak among 36 persons (August 1999) who visited a state park in Washington. *Es. coli* O121:H19 was implicated in an outbreak in a Connecticut community (July 2000). *Shigella sonnei* was implicated in two outbreaks that occurred at swimming beaches in Minnesota (July and August 2000).

Non-freshwater sources were implicated in four bacterial recreational water outbreaks involving gastroenteritis. In March 1999, an outbreak of *Ca. jejuni* was associated with a private pool in Florida that did not have continuous chlorine disinfection and reportedly had ducks swimming in the pool. Outbreaks of *Shigella flexneri* and *Es. coli* O157:H7 (Missouri, September 2000 and Nebraska, June 1999) occurred among children using unchlorinated wading pools. Fecal accidents were factors contributing to the contamination of the water in both outbreaks. *Es. coli* O157:H7 also was implicated as a cause of illness in an outbreak (Florida, September 1999) among two young children who had been playing in ditch water. Both clinical specimens and water samples tested positive for *Es. coli* O157:H7.

Two nongastroenteritis-related recreational water outbreaks were also reported. One outbreak of leptospirosis was reported among 21 persons who participated in an adventure race in Guam in July 2000 (Table 8). These persons reported multiple outdoor exposures, including running through jungles and savannahs, swimming in a river and a reservoir, and bicycling and kayaking in the ocean. *Leptospira* was confirmed by serology, and an epidemiologic investigation demonstrated that swimming in the reservoir, submerging one's head in the water, and swallowing water while swimming were risk factors for illness. Water samples were not tested, and an environmental assessment of the reservoir was not conducted. The second nongastroenteritis-related recreational water outbreak was an outbreak of Pontiac fever epidemiologically linked to use of a whirlpool at a hotel.

Viruses. During 1999--2000, three outbreaks of NLV (Calicivirus) that affected a total of 202 persons were reported. Two NLV outbreaks occurred in untreated systems; one outbreak of NLV occurred (Idaho, June 1999) at a resort and water park and affected 25 persons. The pool implicated in the investigation was untreated because the source of the pool's water was a natural hot springs that was high in mineral content. The investigators noted that geothermal pools used for swimming are not required to be regulated by public health officials in that locale. The pool implicated by the investigation also had been implicated in a previous outbreak of NLV in June 1996.

Other. During 1999--2000, six recreational water outbreaks involving gastroenteritis of unknown etiology were reported. One outbreak (Florida, August) involved a motel pool that was cloudy and dirty at the time of exposure. Nine persons who swam in this pool and did not share any other common exposure became ill with gastroenteritis. Disinfectant residuals and operation of the filtration system at the time of the investigation were deficient. Problems were also noted with the equipment used for adjusting pH.

Another outbreak (Florida, August 1999) among 38 persons who visited a beach park was attributed to both *Sh. sonnei* and *Cr. parvum* (*28*). Illness was epidemiologically linked to playing in an interactive fountain at the park, ingesting water, and consuming food and beverages at the fountain. The fountain's recirculation, filtration, and disinfection systems were not approved by the health department and were inadequate or not completely operational at the time of its use. Samples of the fountain water tested positive for coliforms but did not test positive for fecal coliforms. Nevertheless, the cause of the outbreak was determined to be the fountain, which was closed until the health department's concerns could be remedied.

Three cases of chemical keratitis (Vermont, February 2000) resulted from exposure to bromine in a hotel swimming pool. Bromine levels were >5 ppm (acceptable bromine levels are 1--3 ppm), and the pH level was >8.5. Patrons who spent time with their heads underwater with their eyes open were affected.

Twelve persons affected in an outbreak (Texas, September 1999) reported symptoms that included exhaustion, sore muscles, headache, chills, and fever after attending a conference at a guest ranch. One woman reported a miscarriage during her illness. Exposure to a hot tub, defined as either immersion or being near the hot tub, was associated with illness. Although clinical specimens (urine, blood, sputum, and throat swabs) were tested for organisms, including *Leg. pneumophila* serogroups 1 and 6, influenza virus, parainfluenza virus, and adenovirus, no infectious agent was identified. No testing for biologic or chemical agents was performed on water samples because the hot tub had already been drained, refilled, and hyperchlorinated before the environmental investigation.

During the 1999--2000 reporting period, 15 outbreaks of dermatitis were identified (Table 9). Three of these outbreaks were associated with swimming in freshwater and were assumed to be cercarial dermatitis caused by contact with the larval form (cercariae) of schistosomes, which are present in freshwater environments. Two of these dermatitis outbreaks occurred in lakes in California that were associated with past cases of cercarial dermatitis. The onset of dermatitis occurred within hours after swimming in the lake and resolved after a limited number of days (median days of illness were 2 and 3 days [range: 2--3 and 3--5 days], respectively).

The 12 remaining outbreaks were associated with pool and hot tub use and affected 5--29 persons each. *P. aeruginosa* was confirmed in clinical isolates in 3 of the 12 outbreaks and was confirmed in water/filter samples in five outbreaks, two of which also had a clinical isolate. In eight of these outbreaks of dermatitis, specific treatment deficiencies or problems were identified. Outbreaks in Arkansas (June 1999), Florida (August 2000), Colorado (December 1999), and Washington (March 2000) were attributed to deficiencies in treatment.

In one outbreak of dermatitis (Maine, February 2000), nine persons reported rash in addition to headache, fever, fatigue, and sore throat (*29*). Swimming in the hot tub or swimming in the pool was a risk factor. The pool and hot tub were on separate filtration systems, and both were used by the majority of persons in the outbreak. Low levels of free chlorine were found in the pool and hot tub, but the presence of chlorinate isocyanurates (chlorine stabilizers) might have influenced measured levels of free chlorine. A clinical isolate of *P. eruginosa* was obtained from an ill person; *P. aeruginosa* also was isolated from the pool filter even after the pool had been cleaned twice.

P. aeruginosa was isolated from clinical specimens and water samples in an outbreak at a Colorado hotel that affected 19 persons in February 1999 (*28*), 13 of whom were children aged <15 years. Symptoms were not limited to rash; they included diarrhea, vomiting, nausea, fever, fatigue, muscle aches, joint pain, swollen lymph nodes, and subcutaneous nodules on hands and feet. Because of the severity and range of symptoms, clinical specimens were examined for enteric bacterial and parasitic pathogens as well as *Legionella* species, *Leptospira* species, and *Entamoeba histolytica* but did not test positive for any of these etiologic agents. Swabs taken from the hot tub floor and rail were positive for *P. aeruginosa* and other *Pseudomonas* species.

Pool and hot tub records indicated that chlorine and pH had declined below the state-mandated levels at the time of exposure. Epidemiologic evidence implicated the hot tub as the likely vehicle of exposure for the outbreak. In both the Colorado outbreak and the Maine outbreak that occurred in February 2000, an offsite contractor had been engaged to monitor disinfectant and pH levels. Insufficient communication between pool staff and the remote monitoring company might have contributed to extended periods of usage with inadequate disinfection (*28*).

Outbreaks Associated with Occupational Exposure to Water

Two outbreaks not associated with drinking or recreational water exposure were reported during this period (Table 10). One outbreak of leptospirosis (Hawaii, August 1999) occurred among persons landscaping a pond. Leptospirosis was confirmed serologically for the two persons who had contact with the pond. Both persons reported multiple skin abrasions and were exposed to the pond water for a period of 5--10 days. One of the two persons was hospitalized.

An outbreak of acute respiratory illness occurred among sugar beet processing plant workers (Minnesota, August 2000). Of the 15 cases identified, 13 were hospitalized. Serology for 4 (26.7%) of the 15 persons tested positive for *Leg. pneumophila*; three (20.0%) persons were confirmed positive for *Leg. pneumophila* by sputum polymerase chain reaction (PCR). Fourteen (93.3%) of the 15 persons worked on a crew that had performed high-pressure cleaning in one area of the plant; the fifteenth patient had conducted high-pressure cleaning elsewhere in the plant.

The sources of water for the high-pressure cleaning contained 10^5 colony-forming unit (CFU)/mL of *Leg. pneumophila* and endotoxin levels of 22,200 endotoxin units/mL. Although the attack rate, symptoms, and laboratory findings were consistent with an outbreak of Pontiac fever, endotoxin exposure might have contributed to this outbreak.

Previously Unreported Outbreaks

Three previously unreported drinking water outbreaks that occurred in 1995 and 1997 were submitted during this reporting period (Table 11). An illegal cross-connection (Washington, July 1995) between a domestic water supply and an irrigation system at a plant nursery resulted in contamination of multiple wells in a community. Eighty-seven cases of gastroenteritis were reported, and one hospitalization was recorded. *G. intestinalis* was determined in 33 (52.4%) of 63 stool specimens; *Entamoeba coli* and *B. hominis* were each found in one stool specimen. One (7.1%) of 14 stool specimens that were cultured for *Ca. jejuni* tested positive.

NLV was implicated as the cause of an outbreak (New York, December 1997) of 1,450 cases at a restaurant at a ski resort. Epidemiologic data implicated water or consumption of ice made from water as the cause of the outbreak. The environmental assessment revealed possible problems with the well operation and location. The chlorinator for the well had been malfunctioning and had already been disconnected before the assessment. Testing of the water by the local health department determined that neither a free nor total chlorine residual was detectable in the potable water supply and indicated the presence of fecal coliforms. In addition, the well was located <24 inches away from a stream. During the period the chlorinator was not functioning, the pump for the well had been continuously pumping water. Surface water that might have been introduced into the water supply, plus a deficiency in treatment of the water, played a key role in the outbreak.

Seven persons who were either employees or visitors at a hospital (California, November 1997) were symptomatic for methemoglobinemia in one outbreak. An epidemiologic investigation indicated that the only shared exposure among these persons was a visit to the hospital cafeteria and the consumption of a carbonated beverage with ice from the self-service soda dispenser. The onset of symptoms occurred 1--5 minutes after or while drinking a carbonated beverage. One person was hospitalized, and no deaths occurred. The environmental investigation discovered a cross-connection in the plumbing system that might have allowed water from the cooling tower, which had been recently shock-treated with sodium metaborate, to be drawn into the drinking water system. Sodium metaborate has been associated with nitrate poisoning and methemoglobinemia in past incidents (*30*).

Outbreaks Not Classified as WBDOs

Outbreaks attributed to drinking water that was contaminated or potentially contaminated at the point of use rather than at the source or in the distribution system are not classified as WBDOs. Six outbreaks, causing illness among a total of 102 persons, are in this category. None of the six outbreaks reflected a common vehicle of contamination: one outbreak of *Cr. parvum* was epidemiologically associated with ice consumption; a school-based outbreak of *Sh. sonnei* was related to consumption of water from a dispenser stored in a bathroom facility; a third outbreak involved water taken from a garden hose (the water had been stored in an ice chest before consumption at a private residence); and a fourth outbreak was associated with bottled water that might have been contaminated at the point of use.

Two of the six reported point-of-use outbreaks involving a suspected chemical exposure occurred in food service facilities, but water testing was not performed to verify the presence of the chemical; and, because of the relatively limited number of cases associated with these incidents, the epidemiologic information was not adequate to include these incidents as outbreaks.

Data from six other possible or confirmed outbreaks were also not included in this analysis. One confirmed outbreak of leptospirosis was related to travel outside the United States or its territories and therefore was excluded. This outbreak occurred among student travelers who became ill after their return from Ecuador. Three cases of leptospirosis were confirmed by laboratory testing among the cohort, and four additional cases were suspected. Three other outbreaks of *G. intestinalis, Cr. parvum,* and NLV could not be included in the analysis. Although these outbreaks were probably caused by a recreational water exposure, the data provided did not meet the criteria for inclusion (i.e., the outbreaks did not meet the criteria for Classes I--IV).

Two additional outbreaks were excluded because of inadequate information: one outbreak of dermatitis caused by in-home bathing and one potential drinking water outbreak of *Cr. parvum* in a New England community. This outbreak of *Cr. parvum* occurred in a community near another reported community outbreak of *G. intestinalis* and *Cr. parvum* in 1999. The pond implicated in the recreational water outbreak of *G. intestinalis* also served as a surface water source, which was intermittently mixed into the municipal drinking water that supplied the community. However, not all the persons received their drinking water from the municipal water source. Although raw surface water samples later tested positive for *Cr. parvum* by immunomagnetic spectroscopy (IMS), household water samples either tested negative, were not tested, or the results were not provided. The epidemiologic information and water-quality information provided were not conclusive.

Discussion

Considerations Regarding Reported Results

The WBDO surveillance system provides information concerning epidemiologic and etiologic trends in outbreaks. In previous years, a decrease in the number of drinking water-associated outbreaks had been observed. However, the cumulative number of drinking water outbreaks reported for the 1999--2000 period demonstrates a reversal of this trend (Figures 5 and 6). The number of recreational water outbreaks has been gradually increasing for the past 15 years and is at the highest level since CDC began receiving such reports in 1978.

Although the number of outbreaks reported through the surveillance system has increased, the significance of this increase is unclear. Whether this indeed reflects a true increase in the number of outbreaks that occurred in the United States is unknown.

Not all outbreaks are recognized, investigated, and then reported to CDC or the EPA, and studies have not been performed that assess the sensitivity of this system and indicate what percentage of actual outbreaks this system is able to detect. Multiple factors exist that can influence whether WBDOs are recognized and investigated by local, territorial, and state public health agencies: the size of the outbreak; severity of disease caused by the outbreak; public awareness of the outbreak; routine laboratory testing for organisms; requirements for reporting cases of diseases; and resources

available to the local health departments for surveillance and investigation of probable outbreaks.
This surveillance system probably underreports the true number of outbreaks because of the multiple steps required before an outbreak is identified and investigated. In addition, changes in the capacity of local, county, and state public health agencies and laboratories to detect an outbreak might influence the numbers of outbreaks reported in each state relative to other states. The states with the majority of outbreaks reported during this period might not be the states where the majority of outbreaks actually occurred. An increase in the number of outbreaks reported could either reflect an actual increase in outbreaks or an improved sensitivity in surveillance practices.

Recognition of WBDOs is also dependent on certain outbreak characteristics; outbreaks associated with serious illness or affecting a substantial number of persons are more likely to receive attention from health authorities. Outbreaks involving acute diseases, including those characterized by a short incubation period, are more readily identified than outbreaks associated with chronic, low-level exposure to an agent (e.g., certain chemicals) or are associated with organisms that have a longer incubation period (e.g., certain parasitic organisms).

Larger Drinking Water Systems
Outbreaks involving larger drinking water systems (e.g., community systems) are more likely to be detected than outbreaks that involve noncommunity systems because these systems serve mostly nonresidential areas and transient populations. Outbreaks associated with individual systems are the most likely to be underreported because they typically involve a limited number of persons. Recreational outbreaks where persons congregate in one venue and then are geographically dispersed can be difficult to document.

The identification of the etiologic agent of a WBDO depends on the timely recognition of the outbreak so that appropriate clinical and environmental samples can be collected. The laboratory involved in the testing of specimens must have the capacity and capability to test for a particular organism. In certain cases, specific tests must be requested. Routine testing of stool specimens at laboratories will include tests for the presence of enteric bacterial pathogens and might also include an ova and parasite examination.

However, *Cr. parvum*, one of the most commonly reported waterborne parasites, is not often included in standard ova and parasite examinations and in certain instances, must be specifically requested (*31*). During 1999--2000, tests for NLV and other possible agents of viral origin were rarely performed or documented in the outbreaks that were reported to CDC. Collection of water-quality data depends primarily on local and state statutory requirements, the availability of investigative personnel, and the technical capacity of the laboratories that test the water. Furthermore, certain outbreaks can substantially alter the relative proportion of cases of waterborne disease attributed to a particular agent. The number of reported cases is typically an approximate figure, and the method and accuracy of the approximation vary among outbreaks.

One key limitation of the data collected as part of the WBDO surveillance system is that the information collected pertains only to outbreaks of waterborne illness. The epidemiologic trends and water-quality concerns observed in outbreaks might not

necessarily reflect or correspond with trends associated with endemic waterborne illness.

Epidemiologic Studies
CDC and the EPA are collaborating on a series of epidemiologic studies to assess the magnitude of non-outbreak waterborne illness associated with consumption of municipal drinking water and with exposure to recreational marine and freshwaters.

Outbreaks Associated with Drinking Water
The number of outbreaks reported during 1999 (15) and 2000 (24) is higher than the number reported during 1997 (7) and 1998 (10). As described previously, the number of drinking water outbreaks had declined (*2*,*3*). The increase in reported outbreaks should be carefully interpreted. Although the number of drinking water outbreaks has changed, the total number of persons affected by a drinking water outbreak during 1999--2000 (n = 2,027) is comparable to what was initially reported in 1997--1998 (n = 2,038) and 1995--1996 (n = 2,567) (*2*,*3*).

Changes in surveillance and reporting of outbreaks might have improved detection of outbreaks affecting limited, private systems that in turn, affect a relatively limited number of persons. However, the increase in outbreaks that affect persons in limited, private systems merits further investigation by public health and water-quality agencies.

Certain states reported drinking water outbreaks for the first time in >10 years (e.g., Connecticut since 1976 or Utah since 1986). California reported multiple drinking water outbreaks after reporting no outbreaks in 1997 and 1998, and compared with other years during 1990--2000, the number of reported outbreaks in California increased slightly.

The number of outbreaks reported by Florida also increased. Although the numbers of reported outbreaks increased overall, the seasonality of the drinking water outbreaks is consistent with previous years, with the number peaking during the summer months. The observed increase in the number of outbreaks is associated with an increase in outbreaks associated with consumption of untreated water from both surface and groundwater sources, but specifically private wells.

The percentage of drinking water outbreaks associated with surface water during 1999--2000 was 17.9% (i.e., seven outbreaks) (Figure 2). This percentage is higher than the 11.8% reported during 1997--1998 period (i.e., two outbreaks). However, three of the seven surface water outbreaks reported during 1999--2000 were associated with the direct ingestion of surface water without any treatment or with inadequate individual treatment. Two of these outbreaks were associated with consumption of water during outdoor excursions where point-of-use treatment (e.g., filtration or disinfection) might have been attempted and was either inadequate to protect health or was inconsistently or incorrectly applied. The third outbreak occurred after a household had run out of potable water and instead served untreated creek water to their guests.

These three outbreaks illustrate that the public might be unaware that surface water, despite its clarity, is prone to contamination by organisms. Surface waters should not be directly consumed without being treated at the point of use or boiled. Manufacturers of point-of-use devices and the National Sanitation Foundation (NSF) provide information regarding different devices, instructions for use, and their ability to make water safe for human consumption.

The remaining four outbreaks comprise approximately 11% of all drinking water outbreaks, an equivalent percentage to that reported in 1997--1998. These four outbreaks were associated with systems that routinely received treatment. One outbreak of giardiasis occurred at a resort (Colorado, August 2000) served by a noncommunity system. The increased demand for water during the summer, coupled with multiple treatment failures, resulted in the delivery of unfiltered and non-disinfected water to the resort.

Cross-connections

These multiple failures illustrate the importance of routine maintenance, specifically among noncommunity systems, which do not have consistent demand for water year-round. Two outbreaks (Florida, March 1999 and August 1999) were associated with cross-connections: one to an irrigation well and another to a toilet. Another surface water outbreak (Ohio, August 2000) at a fairgrounds was suspected to have resulted from back-siphonage into the drinking water from an animal manure site. These outbreaks indicate that even when treatment of water at the source is adequate, deficiencies in the distribution system or at the home can result in illness. Such deficiencies are preventable, and the public should be informed of how to detect and avoid creating cross-connections.

Twenty-eight (71.8%) of the 39 outbreaks related to drinking water were associated with groundwater sources. This number is an 87% increase from the number reported in the previous period (i.e., 15). Seventeen of the 28 outbreaks (60.7%) were linked to consumption of untreated groundwater; eight of 28 (28.6%) outbreaks were associated with treatment deficiencies; and three (10.7%) were linked to deficiencies in the distribution system. The observed pattern of deficiencies is contrary to what was observed in the previous reporting period, where the majority of groundwater outbreaks were associated with treatment or distribution system problems.

This pattern indicates that untreated groundwater systems are increasingly associated with outbreaks of illness. Groundwater systems, with the exception of systems influenced by surface water, are not routinely required to use filtration or treatment that would be expected to reduce the number of pathogens in the water. The EPA's pending GWR** is expected to establish multiple barriers in groundwater systems to protect against bacteria and viruses in drinking water from groundwater sources and should establish a targeted strategy to identify groundwater systems at high risk for fecal contamination.

Twenty-six of these 28 groundwater outbreaks had a well as the implicated water source, and two were linked to a spring. The percentages of outbreaks associated with wells and springs were similar during this reporting period to the 1997--1998 period. Although GWR is expected to have public health benefits, these protections extend primarily to community groundwater systems. Of the 26 well-related outbreaks that occurred during the 1999--2000 period, only eight of 26 were associated with community wells. Ten were associated with individual private wells, and eight were associated with noncommunity wells.

These systems would not necessarily benefit from the promulgation of GWR, and therefore, the quality of water in wells remains a public health concern. Approximately 14--15 million households in the United States rely on a private, household well for drinking water each year, and >90,000 new wells are drilled throughout the United

States each year (*32*). In addition, contamination of a private well is not only a health concern for the household served by the well, but can impact households using other nearby water supplies and could potentially contaminate the aquifer.[††] Additional education efforts should be targeted towards well owners, users, well drillers, and local and state drinking water personnel to encourage practices that best ensure safe drinking water for private well users.

Irrigation Waters

Three outbreaks were associated with direct consumption of water from irrigation systems, comprising approximately 8% of drinking water outbreaks (Figure 2). Cross-connections to irrigation systems were implicated as contaminating factors in three other irrigation-related outbreaks. Irrigation waters are not regulated under the Safe Drinking Water Act, because they are typically intended for agricultural purposes, not for human consumption.

Therefore, irrigation water would not be expected to be treated to reduce the level of microorganisms or other contaminants potentially in the water to the same standards as water intended for consumption. In one outbreak, children drank directly from an irrigation canal while playing outside a home. In two other outbreaks, water was directly consumed from an irrigation tap by sports team members. In the first instance, the sports team consumed water from a labeled irrigation tap despite being informed that the water was not intended for consumption. In the second instance, two teams drank from taps on the field because no other source of potable water was available on field.

The multistate outbreak of *Sa.* Bareilly, which was detected through CDC's *Salmonella* Outbreak Surveillance Algorithm (SODA), epidemiologically implicated the consumption of bottled water as a risk factor for illness. This is the first widespread outbreak implicating bottled water in the United States. Previous bottled water outbreaks occurred in New Jersey in 1973 (*33*), Pennsylvania in 1980 (*34*), and in the Northern Mariana Islands in 1993 (*4*). Bottled water standards and regulations, unlike the majority of drinking water standards, are not set and enforced by the EPA, but by the FDA. The FDA regulates bottled water as a packaged food product and bases their bottled water standards on the EPA's tap water standards.

In addition, bottled water might be subject to state and voluntary industry regulation. Bottled water, before this outbreak, had not been identified as a vehicle for transmission of infectious organisms in the United States, although a bottled water outbreak of *Ca. jejuni* associated with consumption of water bottled in Greece was documented during the 1997--1998 surveillance period (*2*). Because of the wide geographic distribution of bottled water products, an outbreak associated with the consumption of bottled water would be difficult to recognize. FDA, EPA, CDC, and the bottled water industry together should address concerns regarding consumption of bottled water and public health.

Overall, the number of outbreaks associated with the five drinking water deficiencies (untreated surface water, untreated groundwater, treatment deficiency, distribution system deficiency, and unknown/miscellaneous deficiency) increased in each category from the 1997--1998 levels. The percentage of outbreaks caused by a treatment deficiency and distribution system problem decreased relative to reported increases in the other three categories.

Although problems with treatment and with distribution systems remain critical concerns for safe drinking water, the public's lack of understanding of the risk associated with consumption of untreated water and the assumption that all water is suitable for consumption is a concern also.

The relative proportion and number of outbreaks associated with different water systems also differs from the figures from the 1997--1998 period (Figure 2). Outbreaks in community systems increased from 8 to 11 outbreaks (37.5% increase); noncommunity outbreaks doubled from 5 to 11; and individual system outbreaks quadrupled, increasing from 4 to 17 outbreaks. However, the proportion of outbreaks in community systems decreased from 47.1% during 1997--1998 to 28.2% during 1999--2000, whereas the relative proportion of outbreaks in individual systems increased from 23.5% during 1997--1998 to 43.6 during 1999--2000. In addition, the number of outbreaks reported that were associated with individual systems during this period is the highest reported level since 1984.

The drinking water quality of community systems, which typically have been the focus of increased EPA regulation, has continually improved. But noncommunity systems and individual systems, which are not regulated to the same extent, are continuing problems. The majority of these individual system outbreaks are linked to currently unregulated groundwater supplies, specifically private wells. The populations served by these systems merit increased attention by public health officials.

Unknown Etiology

The etiologic agent was not identified in 17 (43.6%) of 39 outbreaks (Figure 2). These outbreaks of unknown etiology comprised the largest group of outbreaks, followed by outbreaks caused by bacteria (nine), parasites (seven), viruses (four), and chemicals (two). During 1997--1998, parasites accounted for the largest percentage of the 17 outbreaks (six [35.3%]), followed by unidentified pathogens (five [29.4%]), bacteria (four [23.5%]), chemicals (two [11.6%]), and viruses (zero [0%]). The number of outbreaks per type of agent were increased for all categories during 1999--2000, with the exception of chemicals.

Although the number of reported viral outbreaks increased, indicating an improvement in the availability and usage of laboratory detection methods during previous years, viral outbreaks are probably substantially underreported. Although viruses were suspected in other outbreaks, specifically in those of unknown etiology, testing for viruses was not performed. The technology for detection of viruses in stool and water samples has improved, but testing for viruses is not widely practiced. Investigators are encouraged to submit clinical specimens to CDC or state laboratories that conduct these tests.

Stool Specimens for Identification

Guidelines for collecting stool specimens for identification of viral organisms are available from CDC (*35*). Investigators are also encouraged to contact CDC and EPA regarding testing of water samples.

Only two outbreaks of chemical origin were identified during this surveillance period, the same number as was reported during the 1997--1998 period. One outbreak related to a spill of sodium hydroxide at a community water treatment plant demonstrated the need for safe water treatment practices.

The other outbreak was a single case of methemoglobinemia in an infant who required hospitalization after having been fed boiled water taken from a private well.

Coordination of public health messages is critical; an intervention that was intended to reduce the transmission of infections agents concentrated the chemicals present in the water. These figures, as in the past, probably under represent the actual waterborne chemical poisonings that occur.

Multiple factors can explain the low reporting rate, including the likelihood that:
1) the majority of waterborne chemical poisonings typically occur in private residences and affect a relatively limited number of persons;
2) exposures to chemicals through drinking water might cause illness that is difficult to link to a chemical exposure;
3) the mechanisms for reporting waterborne chemical poisonings to the WBDO surveillance system are not as established for chemicals as they are for WBDOs attributed to infectious agents; and 4) health-care providers and those affected might not as easily recognize chemical poisonings. As a result of these factors, WBDOs of chemical poisonings are less likely to be reported to public health officials.

We Need To Do More
Strengthening the capacity of local and state public health epidemiologists and environmental health specialists to detect and investigate outbreaks remains a priority at CDC and the EPA. As part of that effort, CDC and the EPA should partner with the states, CSTE, and the Association of Public Health Laboratories to develop training materials and online resources that would be useful and easily accessible to local and state public health personnel.

Although no federal regulation exists for monitoring private wells, developing educational materials targeted towards the general public, informing them of ways to maintain the safety and water quality of their wells would be valuable. In addition, health messages regarding the consumption of nonpotable water and appropriate point-of-use treatment should be developed and distributed to the public.

Outbreaks Associated with Recreational Water
Of the 59 recreational WBDOs, those involving gastroenteritis were most frequently reported (n = 36). The 15 outbreaks reported in 1999 and 21 outbreaks reported in 2000 equal or surpass the number reported in 1998, which previously was the highest number of outbreaks involving recreational water-related gastroenteritis reported in one year since the inception of the surveillance system. Together, the outbreaks involving gastroenteritis reported during the 1999--2000 period are higher than the 18 outbreaks documented in the previous reporting period (Figures 7 and 8). Since 1989, the number of gastroenteritis-related outbreaks has been gradually increasing, and this increase is statistically significant ($p = 0.01$).

Because swimming is essentially a shared water activity or communal bathing, rinsing of soiled bodies and overt fecal accidents cause contamination of the water. Unintentional ingestion of recreational water contaminated with pathogens can then lead to gastrointestinal illness, even in non-outbreak settings (*36,37*). Fresh and marine waters are also subject to other modes of contamination from point sources (i.e., sewage releases), watersheds (i.e., runoff from agriculture and residential areas), and floods.

Outbreaks involving gastroenteritis are more frequently observed during the swimming season, which usually starts on Memorial Day weekend (the last weekend in May) and ends Labor Day weekend (the first weekend in September). However, swimming also occurs year-round in indoor venues and in states with more temperate climates. Outbreaks of illness by month (Figure 3) include two outbreaks that occurred noticeably outside the summer months: one outbreak in a Florida pool in March and another outbreak in an indoor pool in Wisconsin in January.

As during the previous reporting period, *Cr. parvum* accounted for the largest percentage of outbreaks involving gastroenteritis (44.4%), followed by *Es. coli* O157:H7 (11.1%), NLV (8.3%), and *Shigella* (8.3%). An outbreak of *G. intestinalis* was also reported in 1999. The last reported recreational water outbreak of *Giardia* occurred in 1996. Outbreaks of *Ca. jejuni*, *Es. coli* O121:H19, and a mixed *Sh. sonnei*/*Cr. parvum* outbreak were also reported for the first time to the surveillance system. Outbreaks of unknown etiology comprised 16.7% of the recreational water outbreaks involving gastroenteritis.

Twenty-two (61.1%) of the 36 outbreaks of gastroenteritis occurred in treated systems (i.e., pools) that would usually be expected to be chlorinated or disinfected to prevent transmission of infectious agents after unintentional ingestion. However, the term *treated system* might pertain to systems not routinely treated, including wading pools, interactive fountains, and in one case, an untreated pool that was served by a natural hot springs source.

Multiple interrelated factors can impede disinfection in treated venues, including an increased bather load in a pool, high levels of organic material (e.g., fecal material or environmental or skin debris) and ultraviolet light, all of which deplete chlorine residuals that usually maintain protection in the system. In certain outbreaks, fecal material was indicated on the report as a contributing factor to the outbreak; the majority of fecal accidents were attributed to young children who were in or near the water at the time the accident occurred.

Unlike previous years, a substantial number of different bacterial and viral organisms were reported as causing gastrointestinal illness in these treated recreational water venues (Figure 9). Nevertheless, >66% of these outbreaks were attributed to *Cr. parvum* (Figure 4). Unlike other organisms, which are more susceptible to the levels of chlorine typically found in a pool, *Cr. parvum* is highly chlorine-resistant and requires increased levels of chlorine and longer contact times with chlorine for inactivation. *Cr. parvum* can survive for days in public health-mandated chlorine concentrations required for pools. In addition, its relatively limited size (4--6 µm) can allow it to pass through particulate filtration systems during recirculation of water in the pool.

Because a low number of oocysts might cause illness in a person, even ingestion of a limited amount of water can cause infection. Although the number of *Cr. parvum* outbreaks has been steadily increasing during 1990--2000, multiple explanations could exist for the increase. The properties of the organism, coupled with the popularity of swimming and the tendency of persons to aggregate in larger water venues, increases the likelihood that swimming water can become contaminated and that swimmers will ingest the water and become infected.

However, the increases in outbreaks could be explained by a higher awareness of *Cr. parvum* as a potential cause of illness among swimmers by the public health community and the recreational water industry and, as a result, are more likely to be detected.

The majority of these *Cr. parvum* outbreak investigations noted inadequate pool maintenance. Although low chlorine levels are unlikely to have been the cause of the outbreaks, the frequent reporting of low chlorine levels in these outbreaks indicates a disturbing lack of awareness concerning the role of chlorine and pH control as the major protective barrier against infectious disease transmission in pools.

Inadequate disinfectant levels in any pool increases the risk for transmission of chlorine-sensitive pathogens (e.g., *Es. coli* O157:H7 or *Shigella* species) if an infected swimmer contaminates the pool. Pool operators and staff should be appropriately trained regarding the spread of recreational water illnesses and the critical role of pool maintenance (i.e., disinfection, pH control, and filtration) in preventing WBDOs.[§§]

Gastroenteritis

Fourteen outbreaks involving gastroenteritis after freshwater exposure were reported during 1999--2000, compared with eight during 1997--1998. *Es. coli* O157:H7 accounted for the most outbreaks of known etiology (three), followed by NLV (two), *Shigella* species (two), *Es. coli* O121:H19 (one), *G. intestinalis* (one), and *Cr. parvum* (one). Four outbreaks were of unknown etiology. Certain outbreaks occurred in beach areas that had substantial numbers of families bathing and swimming in the water. Again, a common element noted in these reports was the presence of diaper-aged children in the water, diaper-changing on the beach, and even washing off young children in the water. One incident involved persons who swam in a lake that was had posted signs indicating that the lake was unsafe for swimming.

Reports of infants and children swimming when they have diarrhea is a problem common to both freshwater systems and treated venues. Although health communication messages have been targeted in the past for treated venues, similar messages should be provided to those who swimming in freshwater venues. EPA, as part of the Beaches Action Plan, is developing guidelines and information for users of freshwaters.[¶¶]

Geothermal Pools and Hot Springs

Geothermal pools and hot springs should be examined closely. In one outbreak, pools in a complex were exempt from public health regulation because they were naturally occurring hot springs and mineral waters. Hot springs, which feature high levels of minerals and elevated temperatures, are potentially ideal venues for microbial growth or contamination. These springs and geothermal pools pose an increased risk to swimmers, compared with treated pools because of their lack of disinfection and filtration. Improved consumer and staff education and supplementary treatment might be necessary to prevent future outbreaks in these enclosed freshwater pools.

Twelve of the 15 outbreaks of dermatitis were associated with hot tub or pool use. The majority of these reports of dermatitis are associated with deficient maintenance and inadequate disinfection of the water. The higher temperatures commonly found in hot tubs deplete disinfectant levels at a more rapid rate; hot tub operators should be encouraged to actively check and maintain adequate disinfectant levels. In addition to rashes, reports have been received of other symptoms.

In Alaska, three of 29 persons reported nausea. In the two Maine outbreaks, persons also reported headache, fatigue, and other symptoms. The Colorado outbreak was notable for its severe symptomatology and an extended duration of illness. Extended and painful rashes associated with *P. aeruginosa* outbreaks are unusual but have been documented (*38,39*).

One report (*39*) also indicates that a substantial number of children are being affected by these outbreaks. In the Colorado outbreak of *P. aeruginosa*, the persons affected were primarily children, but no indication was provided that age was a risk factor for infection. More remarkable is the observed duration of illness. Certain persons reported chronic illness (i.e., rash, joint pain, abdominal pain, and chest pain) that lasted ≥6 weeks. Using remote pool monitoring services in two of these outbreaks underscores the need for training pool staff regarding the role of monitoring service and prompt communication between service and pool operators when problems are detected.

Three outbreaks of dermatitis that occurred after persons swam in fresh or marine water were presumed to be caused by an allergic reaction to the cercariae, the larval form of certain nonhuman species of schistosomes. Cercarial dermatitis was an identified problem in two of these lakes, and signs posted by the health department regarding this problem were ignored by swimmers. The extent of the problem of cercarial dermatitis caused by freshwater exposure is unknown, although it probably occurs more frequently than what is reported to the surveillance system. As schistosomes occur naturally in ecosystems that bring snails and birds or aquatic mammals close together, a substantial number of freshwater lakes in the United States might cause illness among swimmers. Swimmers should pay careful attention to where they swim, avoid shallow swimming areas known to be appropriate snail habitats in lakes associated with cercarial dermatitis, and report any incidents to their local health department to prevent further illnesses.

The four deaths associated with primary amebic meningoencephalitis (PAM) reported during the 1999--2000 period were all linked to freshwater exposure. Typically, these cases are associated with swimming in freshwater bodies in the late summer months because *N. fowleri*, which has been implicated in >90% of the cases reported to CDC, proliferate in warm, stagnant waters. Previous cases of PAM have been reported from states with more temperate climates (e.g., California, Florida, and Texas) or from areas with hot springs.

PAM

The amoebas associated with PAM are believed to enter through the nasal passage. Preventing forceful entry of water up the nasal passages during jumping or diving by holding one's nose or wearing nose plugs could reduce the risk for infection.

Swimming in waters contaminated by animal urine was the likely explanation for an outbreak of leptospirosis among persons participating in an adventure race in Guam. *Leptospira* species can be found frequently in wild animal urine, and can be contracted through inhalation of aerosolized water or ingestion of water while swimming.

Leptospirosis
Leptospirosis can also be acquired through abrasions. In this instance, the exposure was associated with immersion of persons' heads in a body of water while they swam and swallowed water. Although outdoor swimming is not necessarily dangerous, swimmers should be educated regarding the potential risks resulting from swimming in areas that are not secured from wild animal use.

An increased level of bromine, which is used to disinfect pools and hot tubs, caused certain cases of chemical keratitis. Inadequate disinfection of a whirlpool resulted in an outbreak of legionellosis among 20 persons who stayed at a motel. Safe disinfection practices and appropriate pool maintenance protocols should be communicated to operators and managers of facilities that treat recreational water.

Outbreaks Associated with Occupational Exposures to Water
Two outbreaks that do not fit into the previous categories were reported to CDC by Minnesota and Hawaii. Outbreaks associated with exposure to aerosolized water have previously occurred but have not been reported to the WBDO surveillance system (*40*,*41*). These outbreaks are discussed in this report to demonstrate that water exposures are not limited to ingestion and contact (e.g., through swimming), and these outbreaks are preventable. Using barrier masks to prevent inhalation of aerosolized water or disinfection of water that is not being used for drinking or swimming purposes could have prevented the respiratory illnesses associated with these two outbreaks.

Conclusion
Data collected as part of the national WBDO surveillance system are used to describe the epidemiology of waterborne diseases in the United States. Data regarding water systems and deficiencies implicated in these outbreaks are used to assess whether regulations for water treatment and monitoring of water quality are adequate to protect the public's health. Identification of the etiologic agents responsible for these outbreaks is also critical because new trends might necessitate different interventions and changes in policies and resource allotment.

Surveillance for waterborne agents and outbreaks occurs primarily at the local and state level. Local and state public health agencies need to detect and recognize WBDOs and implement appropriate prevention and control measures. Improved communication among local and state public health departments, regulatory agencies, water utilities, and recreational water facilities would aide the detection and control of outbreaks.

Share Water-Quality Data
Routine reporting or sharing of water-quality data with the health department is recommended. Other means of improving surveillance at the local, state, and federal level could include the additional review and follow-up of information gathered through other mechanisms (e.g., issuances of boil-water advisories or reports of illness associated with agents thought to be waterborne).

One repeated observation regarding outbreak data collected as part of the WBDO system was that the timely collection of clinical specimens and water samples for testing and commencement of an environmental investigation would have resulted in an improved ability to detect the outbreak's etiologic agent and the source of water contamination.

However, the course of an investigation is influenced by the ability and capacity of public health departments and laboratories to recognize and investigate potential outbreaks of illness. Even when personnel are available to investigate a potential outbreak in a timely manner, a common observation is that investigations cannot always be completed thoroughly. WBDO outbreak investigations typically require input from different disciplines, including infectious disease epidemiology, environmental epidemiology, clinical medicine, sanitation, water engineering, and microbiology. Either further cross-training of existing personnel needs to be implemented or additional personnel and resources need to be made available or linked to those who typically investigate reports of WBDOs.

Epidemiologic Assistance

State health departments can request epidemiologic assistance and laboratory testing from CDC to investigate WBDOs. CDC and the EPA can be consulted regarding engineering and environmental aspects of drinking water and recreational water treatment and regarding collection of large-volume water samples to identify pathogenic viruses and parasites, which require special protocols for their recovery.

Requests for tests for viral organisms should be made to CDC's Viral Gastroenteritis Section, Respiratory and Enterovirus Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases (NCID), at 404-639-3577. Requests for tests for parasites should be made to CDC's Division of Parasitic Diseases, NCID, at 770-488-7760.

Additional information is available from

- EPA's Safe Drinking Water Hotline at 800-426-4791, on the Internet at http://www.epa.gov/safewater, or by e-mail at hotline-sdwa@epa.gov;
- CDC's NCID website at http://www.cdc.gov/ncidod;
- CDC's Healthy Swimming website at http://ww.cdc.gov/healthyswimming; includes recreational water health communication materials for the general public and pool maintenance staff (e.g., information regarding disinfection, guidelines on response to fecal accidents [*42*], fact sheets concerning recreational water illnesses), and an outbreak investigation toolkit that can be used by public health professionals;
- CDC's Voice and Fax Information System, 888-232-3228 (voice) or 888-232-3299 (fax). Choose cryptosporidiosis in the disease category; and
- for reporting WBDOs, CDC's Division of Parasitic Diseases, NCID, at 770-488-7760 or by fax at 770-488-7761.

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* Total coliforms are considered indicator organisms that typically do not cause disease but might be associated with the presence of other disease-causing organisms. Additional information regarding total coliforms is available at http://www.epa.gov/safewater/dwa/electronic/tcr.pdf.
† Additional information is available at http://www.cleanwater.gov.
§ Additional terms are defined in the glossary.
¶ Additional information is available at http://www.nsf.org.
** Additional information is available at http://www.epa.gov/safewater/gwr.html.
†† Although EPA does not regulate private wells and will not regulate them as part of the proposed GWR, EPA lists recommendations for protecting private water supplies at http://www.epa.gov/safewater/pwells1.html and provides links to other sources of information.
§§ Guidelines for pool operators and other information related to recreational water illnesses is available at http://www.cdc.gov/healthyswimming.
¶¶ Additional information is available at http://www.epa.gov/waterscience/beaches.

Table 1

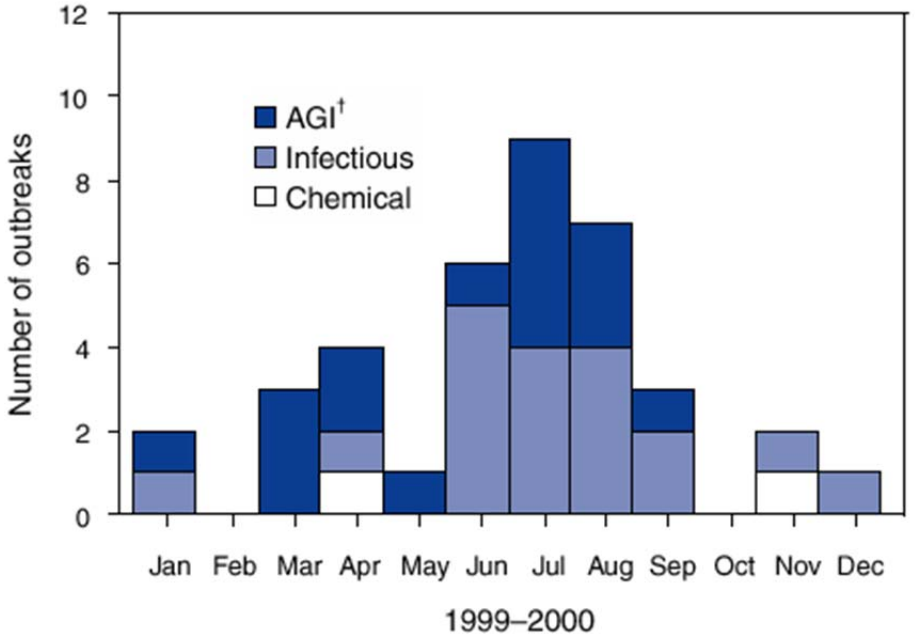
TABLE 1. Classification of investigations of waterborne-disease outbreaks — United States*

Class†	Epidemiologic data	Water-quality data
I	Adequate§ Data were provided regarding exposed and unexposed persons, and the relative risk or odds ratio was ≥2 or the p-value was <0.05	Provided and adequate Historical information or laboratory data (e.g., the history that a chlorinator malfunctioned or a water main broke, no detectable free-chlorine residual, or the presence of coliforms in the water)
II	Adequate	Not provided or inadequate (e.g., stating that a lake was crowded)
III	Provided, but limited Epidemiologic data were provided that did not meet the criteria for Class I, or the claim was made that ill persons had no exposures in common besides water, but no data were provided	Provided and adequate
IV	Provided, but limited	Not provided or inadequate

* Outbreaks of *Pseudomonas* and other water-related dermatitis and single cases of primary amebic meningoencephalitis or of illness resulting from chemical poisoning are not classified according to this scheme.
† On the basis of epidemiologic and water-quality data that were provided on CDC form 52.12.
§ Adequate data were provided to implicate water as the source of the outbreak.

Figure 1

FIGURE 1. Number of waterborne-disease outbreaks associated with drinking water, by etiologic agent and month — United States, 1999–2000 (n = 38)*



* One outbreak of *Salmonella* Bareilly was not included.
† Acute gastrointestinal illness of unknown etiology.

Table 2

TABLE 2. Waterborne-disease outbreaks associated with drinking water — United States, 1999 (n = 15)*

State	Month	Class†	Etiologic agent	Number of cases	Type of system§	Deficiency¶	Source	Setting
California	Jul	III	AGI**	31	Ncom	2	Well	Camp
Florida	Jan	III	AGI	4	Com	2	Well	Community
Florida	Jan	III	*Giardia intestinalis*	2	Ind	2	Well	Household
Florida	Mar	III	AGI	6	Com	4	River/stream	Apartment
Florida	Mar	III	AGI	3	Com	4	Well	Community
Florida	May	III	AGI	3	Ind	2	Well	Household
Florida	Aug	III	AGI††	4	Com	4	River/stream	Apartment
Missouri	Jun	II	*Salmonella* Typhimurium	124	Com	3	Well	Community
New Jersey	Nov	IV	Sodium hydroxide	2	Com	3	Well	Community
New Mexico	Jul	I	Small round-structured virus§§	70	Ncom	3	Spring	Camp
New York	Aug	I	*Escherichia coli* O157:H7, *Campylobacter jejuni*¶¶	781	Ncom	2	Well	Fairgrounds
Texas	Nov	I	*Es. coli* O157:H7	22	Com	3	Well	Community
Washington	Jul	II	AGI	46	Ind	1	River/creek	Household
Washington	Aug	I	AGI	68	Ncom	2	Well	Soccer match
Wisconsin	Apr	NA***	Nitrate	1	Ind	2	Well	Household

* An outbreak is defined as 1) ≥2 persons experiencing a similar illness after ingestion of drinking water and 2) epidemiologic evidence that implicates water as the probable source of the illness.

† On the basis of epidemiologic and water-quality data provided on CDC form 52.12.

§ Com = community; Ncom = noncommunity; Ind = individual; community and noncommunity water systems are public water systems that serve ≥15 connections or an average of ≥25 residents for ≥60 days/year. A community water system serves year-round residents of a community, subdivision, or mobile home park with ≥15 service connections or an average of ≥25 residents. A noncommunity water system can be nontransient or transient. Nontransient systems serve ≥25 of the same persons for ≥6 months/year (e.g., factories or schools), whereas transient systems do not (e.g., restaurants, highway rest stations, or parks). Individual water systems are not owned or operated by a water utility and serve <15 connections or <25 persons. Outbreaks associated with water not intended for drinking (e.g., lakes, springs, and creeks used by campers and boaters; irrigation water; and other nonpotable sources with or without taps) are also classified as individual systems.

¶ 1 = untreated surface water; 2 = untreated groundwater; 3 = treatment deficiency (e.g., temporary interruption of disinfection, chronically inadequate disinfection, and inadequate or no filtration); 4 = distribution system deficiency (e.g., cross-connection, contamination of water mains during construction or repair, and contamination of a storage facility); and 5 = unknown or miscellaneous deficiency (e.g., contaminated bottled water).

** Acute gastrointestinal illness of unknown etiology.

†† Unidentified chemical poisoning.

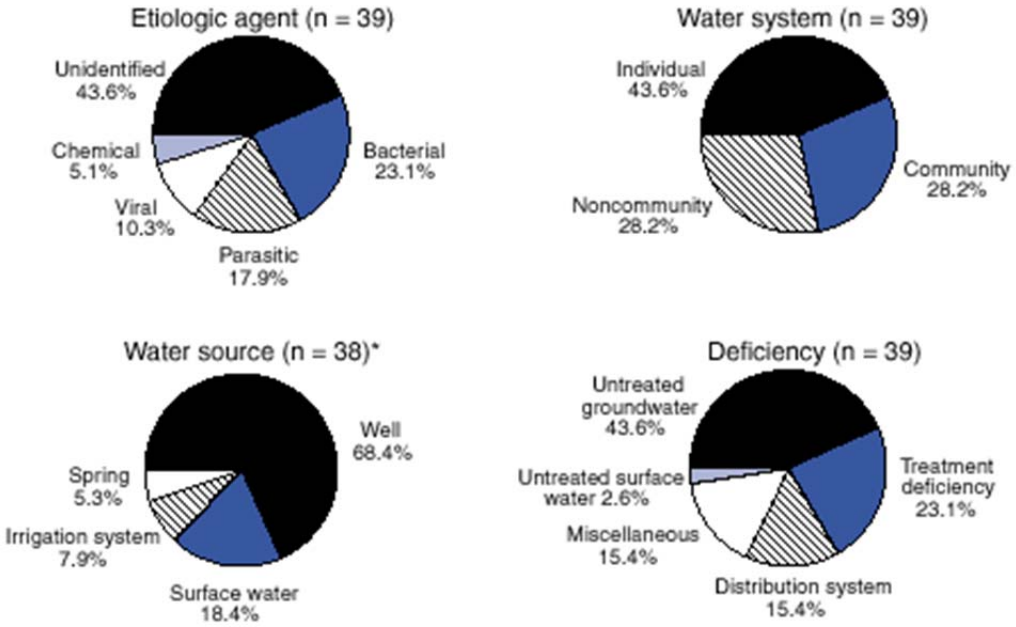
§§ Three persons had stool specimens that tested positive for small round-structured virus, and one person's stool specimen tested positive for *Ca. jejuni*.

¶¶ A total of 126 persons had stool specimens that tested positive for *Es. coli* O157:H7; 43 persons had stool specimens that tested positive for *Ca. jejuni*. One person's stool specimen tested positive for both organisms.

*** Not applicable, see Table 1.

Figure 2

FIGURE 2. Waterborne-disease outbreaks associated with drinking water, by etiologic agent, water system, water source, and deficiency — United States, 1999–2000 (n = 39)



* One outbreak of *Salmonella* Bareilly was not included.

Table 3

TABLE 3. Waterborne-disease outbreaks associated with drinking water — United States, 2000 (n = 24)*

State	Month	Class†	Etiologic agent	Number of cases	Type of system§	Deficiency¶	Source	Setting
California	Jul	I	Norwalk-like virus	147	Ncom	2	Well	Camp
California	Jul	I	*Escherichia coli* O157:H7	5	Ind	5	River/creek	Camp
California	Sep	III	AGI**	63	Ind	5	Irrigation system	Football game
Colorado	Aug	III	*Giardia intestinalis*	27	Ncom	3	River	Resort
Florida	Mar	III	AGI††	19	Com	3	Well	Trailer park
Florida	Apr	III	AGI	21	Com	3	Well	Trailer park
Florida	Apr	I	AGI	71	Ind	2	Well	Community
Florida	Jun	III	AGI§§	2	Ind	2	Well	Household
Florida	Jul	III	AGI	3	Ind	2	Well	Household
Florida	Jul	III	AGI	3	Ind	2	Well	Household
Florida	Aug	III	AGI	4	Ind	2	Well	Household
Florida	Sep	III	*G. intestinalis*	2	Ind	4	Well	Household
Florida	Dec	III	*Cryptosporidium parvum*	5	Com	4	Well	Community
Idaho	Apr	III	*Es. coli* O157:H7	4	Ind	5	Irrigation canal	Household
Idaho	Jun	III	*Campylobacter jejuni*	15	Ncom	2	Spring	Camp
Idaho	Jul	III	AGI	65	Ncom	2	Well	Restaurant
Kansas	Jun	III	Norwalk-like virus	86	Ncom	2	Well	Reception hall
Minnesota	Jun	III	*G. intestinalis*¶¶	12	Ncom	2	Well	Camp
New Hampshire	Sep	III	*G. intestinalis*	5	Ind	3	Well	Household
New Mexico	Jul	II	*G. intestinalis*	4	Ind	5	River	Rafting trip
Ohio	Aug	I	*Es. coli* O157:H7	29	Com	4	Surface water***	Fairgrounds
Utah	Aug	III	*Ca. jejuni*†††	102	Ind	5	Irrigation water	Football camp
West Virginia	Jun	III	Norwalk-like virus	123	Ncom	3	Wells	Camp
Multistate	Apr–Aug	I	*Salmonella* Bareilly	84	Ind	5§§§	Municipal/spring§§§	Wells/bottled water***

* An outbreak is defined as 1) ≥2 persons experiencing a similar illness after ingestion of drinking water and 2) epidemiologic evidence that implicates water as the probable source of the illness.

† On the basis of epidemiologic and water-quality data provided on CDC form 52.12.

§ Com = community; Ncom = noncommunity; Ind = individual; community and noncommunity water systems are public water systems that serve ≥15 service connections or an average of ≥25 residents for ≥60 days/year. A community water system serves year-round residents of a community, subdivision, or mobile home park with ≥15 service connections or an average of ≥25 residents. A noncommunity water system can be nontransient or transient. Nontransient systems serve ≥25 of the same persons for ≥6 months/year (e.g., factories or schools), whereas transient systems do not (e.g., restaurants, highway rest stations, or parks). Individual water systems are not owned or operated by a water utility and serve <15 connections or <25 persons. Outbreaks associated with water not intended for drinking (e.g., lakes, springs, and creeks used by campers and boaters; irrigation water; and other nonpotable sources with or without taps) are also classified as individual systems.

¶ 1 = untreated surface water; 2 = untreated groundwater; 3 = treatment deficiency (e.g., temporary interruption of disinfection, chronically inadequate disinfection, and inadequate or no filtration); 4 = distribution system deficiency (e.g., cross-connection, contamination of water mains during construction or repair, and contamination of a storage facility); and 5 = unknown or miscellaneous deficiency (e.g., contaminated bottled water).

** Acute gastrointestinal illness of unknown etiology.

†† Persons also reported rashes in addition to acute gastrointestinal illness.

§§ One person had a stool specimen that tested positive for *Blastocystis hominis*.

¶¶ Eight persons had stool specimens that tested positive for *G. intestinalis*; one stool specimen tested positive for *Dientamoeba fragilis*.

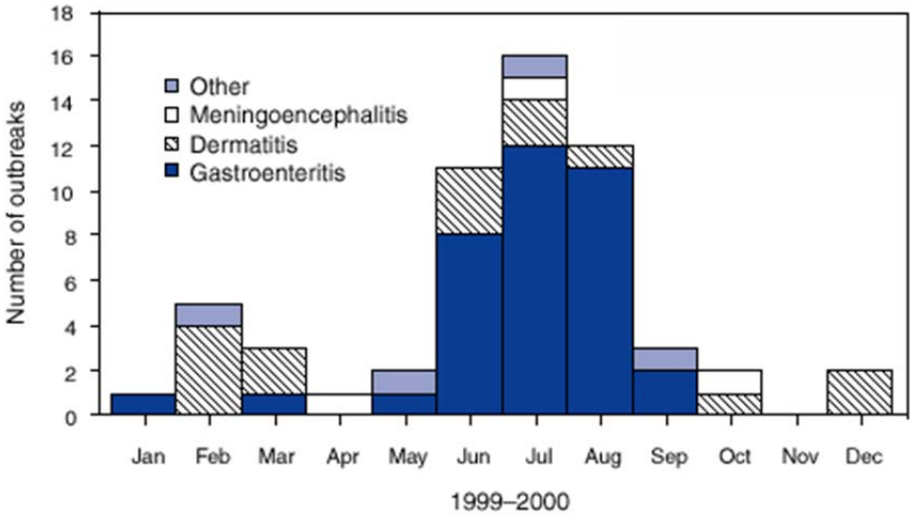
*** Type of water was not specified on report form.

††† Thirty-seven persons had stool specimens that tested positive for *Ca. jejuni*; four persons' stool specimens tested positive for *Es. coli* O157:H7, and three persons had stool that tested positive for *Es. coli* O111.

§§§ The outbreak implicated both drinking water from private wells and springs and water bottled by one facility. The bottling facility used two sources of water.

Figure 3

FIGURE 3. Number of waterborne-disease outbreaks associated with recreational water, by illness and month — United States, 1999–2000 (n = 58)*



* Information regarding the month was not provided for one outbreak of meningoencephalitis.

Table 4

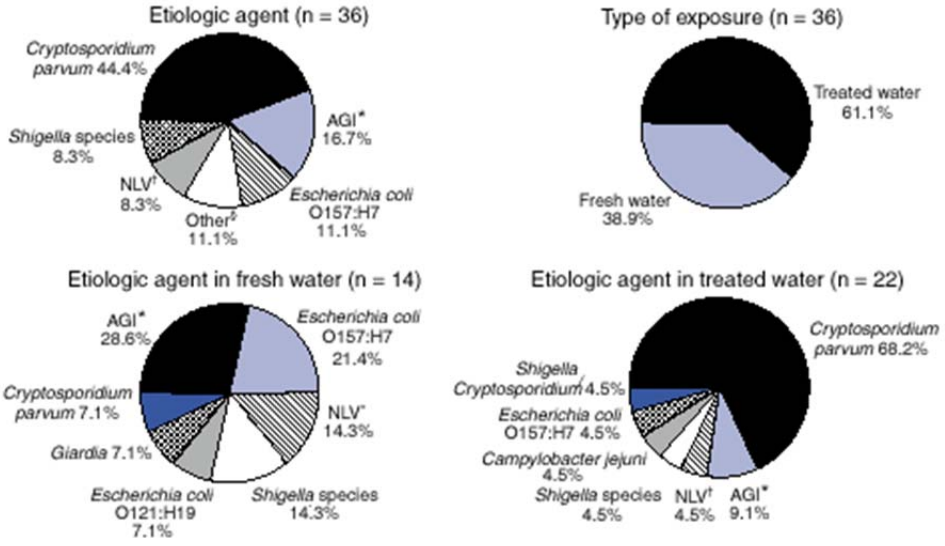
TABLE 4. Waterborne-disease outbreaks associated with drinking water, by etiologic agent and type of water system — United States, 1999–2000 (n = 39)

	Type of water system*							
	Community		Noncommunity		Individual		Total	
Etiologic agent	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases
AGI†	6§	57	3	164	8	195	**17**	**416**
Giardia intestinalis	0	0	2	39	4	13	**6**	**52**
Escherichia coli O157:H7	2	51	0	0	2	9	**4**	**60**
Norwalk-like viruses (NLV)	0	0	3	356	0	0	**3**	**356**
Salmonella species¶	1	124	0	0	1	84	**2**	**208**
Campylobacter jejuni	0	0	1	15	1	102	**2**	**117**
Es. coli O157:H7/*Ca. jejuni*	0	0	1	781	0	0	**1**	**781**
Small round-structured virus	0	0	1	70	0	0	**1**	**70**
Cryptosporidium parvum	1	5	0	0	0	0	**1**	**5**
Sodium hydroxide	1	2	0	0	0	0	**1**	**2**
Nitrate	0	0	0	0	1	1	**1**	**1**
Total	**11**	**239**	**11**	**1,425**	**17**	**404**	**39**	**2,068**
Percentage	**28.2**	**11.6**	**28.2**	**68.9**	**43.6**	**19.5**	**100.0**	**100.0**

* Community and noncommunity water systems are public water systems that serve ≥15 service connections or an average of ≥25 residents for ≥60 days/year. A community water system serves year-round residents of a community, subdivision, or mobile home park with ≥15 service connections or an average of ≥25 residents. A noncommunity water system can be nontransient or transient. Nontransient systems serve ≥25 of the same persons for ≥6 months/year (e.g., factories or schools), whereas transient systems do not (e.g., restaurants, highway rest stations, or parks). Individual water systems are not owned or operated by a water utility and serve <15 connections or <25 persons. Outbreaks associated with water not intended for drinking (e.g., lakes, springs, and creeks used by campers and boaters; irrigation water; and other nonpotable sources with or without taps) are also classified as individual systems.
† Acute gastrointestinal illness of unknown etiology.
§ One outbreak of four cases was caused by an unidentified chemical.
¶ One outbreak was serotype Typhimurium, and one outbreak was serotype Bareilly.

Figure 4

FIGURE 4. Waterborne-disease outbreaks of gastroenteritis associated with recreational water, by etiologic agent and type of exposure — United States, 1999–2000



* Acute gastrointestinal illness of unknown etiology.
† Norwalk-like virus.
§ These included outbreaks of *Campylobacter jejuni*, *Giardia*, *Escherichia coli* O121:H19 and one mixed *Shigella/Cryptosporidium* outbreak.

Table 5

TABLE 5. Waterborne-disease outbreaks associated with drinking water, by type of deficiency and type of water system — United States, 1999–2000 (n = 39)

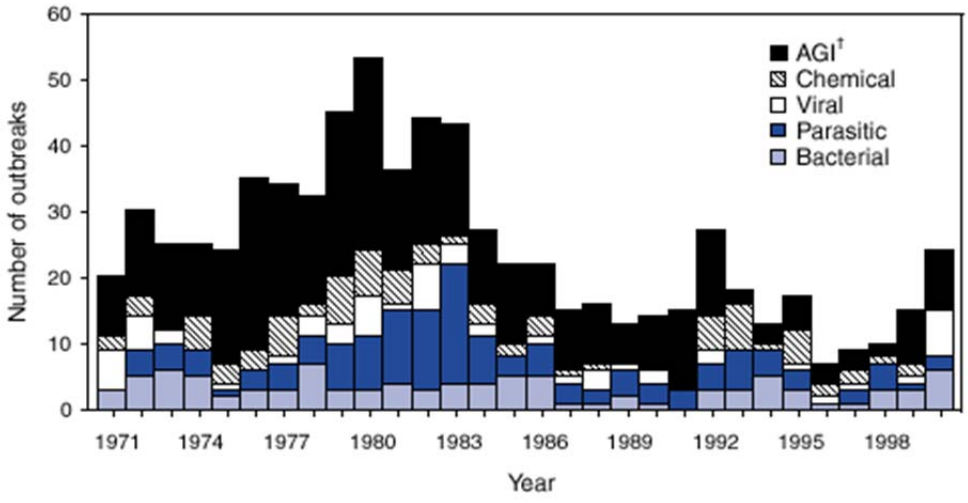
	Type of water system*							
	Community		Noncommunity		Individual		Total	
Type of deficiency†	Outbreaks	%	Outbreaks	%	Outbreaks	%	Outbreaks	%
Untreated surface water	0	0	0	0	1	5.9	**1**	**2.6**
Untreated groundwater	1	9.0	8	72.7	8	47.0	**17**	**43.6**
Inadequate treatment	5	45.5	3	27.3	1	5.9	**9**	**23.1**
Distribution system	5	45.5	0	0	1	5.9	**6**	**15.4**
Miscellaneous or unknown	0	0	0	0	6	35.3	**6**	**15.4**
Total	**11**	**100.0**	**11**	**100.0**	**17**	**100.0**	**39**	**100.0**

* Community and noncommunity water systems are public water systems that serve ≥15 service connections or an average of ≥25 residents for ≥60 days/year. A community water system serves year-round residents of a community, subdivision, or mobile home park with ≥15 service connections or an average of ≥25 residents. A noncommunity water system can be nontransient or transient. Nontransient systems serve ≥25 of the same persons for ≥6 months/year (e.g., factories or schools), whereas transient systems do not (e.g., restaurants, highway rest stations, or parks). Individual water systems are not owned or operated by a water utility and serve <15 connections or <25 persons. Outbreaks associated with water not intended for drinking (e.g., lakes, springs, and creeks used by campers and boaters; irrigation water; and other nonpotable sources with or without taps) are also classified as individual systems.

† Examples of treatment deficiencies include temporary interruption of disinfection, chronically inadequate disinfection, or inadequate or no filtration; examples of distribution system deficiencies include cross-connection, contamination of water mains during construction or repair, or contamination of a storage facility; and examples of unknown or miscellaneous deficiencies include contaminated bottled water.

Figure 5

FIGURE 5. Number of waterborne-disease outbreaks associated with drinking water, by year and etiologic agent — United States, 1971–2000 (n = 730)*



* The total from previous reports has been corrected from n = 691 to n = 688.
† Acute gastrointestinal illness of unknown etiology.

Table 6

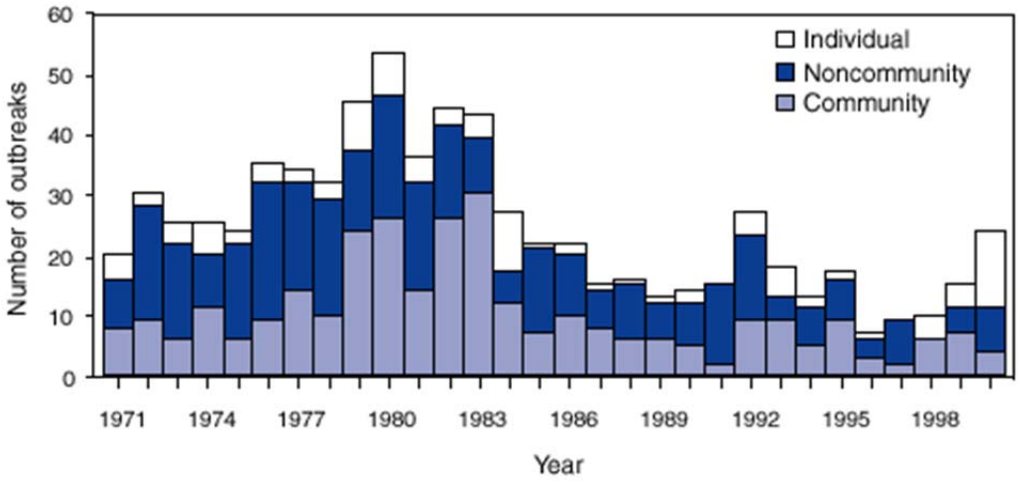
TABLE 6. Waterborne-disease outbreaks of gastroenteritis associated with recreational water — United States, 1999 (n = 15)

State	Month	Class*	Etiologic agent	Illness	Number of cases	Source	Setting
California	Jun	III	AGI†	Gastroenteritis	23	Pool	Apartment complex
Connecticut	Jul	II	*Escherichia coli* O121:H19	Gastroenteritis	11	Lake	Lake
Florida	Mar	III	*Campylobacter jejuni*	Gastroenteritis	6	Pool	Private home
Florida	Aug	I	*Shigella sonnei*, *Cryptosporidium parvum*§	Gastroenteritis	38	Interactive fountain	Beach park
Florida	Aug	IV	*Cr. parvum*	Gastroenteritis	6	Pool	Private home
Florida	Sep	III	*Es. coli* O157:H7	Gastroenteritis	2	Ditch water	Community
Idaho	Jun	IV	Norwalk-like virus	Gastroenteritis	25	Hot springs	Resort
Illinois	Jun	III	AGI	Gastroenteritis	25	Lake	Lake
Massachusetts	Jul	III	*Giardia intestinalis*	Gastroenteritis	18	Pond	Pond
Minnesota	Jul	III	*Cr. parvum*	Gastroenteritis	10	Pool	Trailer park
Nebraska	Jun	IV	*Es. coli* O157:H7	Gastroenteritis	7	Wading pool	Child care center
New York	Jun	II	Norwalk-like virus	Gastroenteritis	168	Lake	County park
Washington	Aug	I	*Es. coli* O157:H7	Gastroenteritis	36	Lake	State park
Wisconsin	Jul	IV	*Cr. parvum*	Gastroenteritis	10	Pool	Municipal pool
Wisconsin	Aug	II	*Es. coli* O157:H7	Gastroenteritis	5	Lake/pond	Swimming beach

* On the basis of epidemiologic and water-quality data provided on CDC form 52.12.
† Acute gastrointestinal illness of unknown etiology.
§ Five persons had stool specimens that tested positive for *Shigella sonnei*; two stools tested positive for *Cryptosporidium parvum*.

Figure 6

FIGURE 6. Number of waterborne-disease outbreaks associated with drinking water, by year and type of water system — United States, 1971–2000 (n = 730)*



* The total from previous reports has been corrected from n = 691 to n = 688.

Table 7

TABLE 7. Waterborne-disease outbreaks of gastroenteritis associated with recreational water — United States, 2000 (n = 21)

State	Month	Class*	Etiologic agent	Illness	Number of cases	Source	Setting
Colorado	Aug	I	*Cryptosporidium parvum*	Gastroenteritis	112	Pool	Municipal pool
Florida	May	IV	AGI†	Gastroenteritis	2	Lake	Lake
Florida	Jul	III	AGI	Gastroenteritis	4	Outdoor spring	County park
Florida	Jul	III	*Cr. parvum*	Gastroenteritis	3	Pool	Apartment complex
Florida	Aug	III	*Cr. parvum*	Gastroenteritis	5	Pool	Country club
Florida	Aug	I	*Cr. parvum*	Gastroenteritis	19	Pool	Resort
Florida	Aug	III	AGI	Gastroenteritis	9	Pool	Motel
Florida	Aug	III	*Cr. parvum*	Gastroenteritis	5	Pool	Condominium
Georgia	Jun	II	*Cr. parvum*	Gastroenteritis	36	Pools§	Community
Maine	Jul	II	AGI	Gastroenteritis	32	Lake/pond	Swimming beach
Minnesota	Jul	II	*Cr. parvum*¶	Gastroenteritis	220	Lake	Swimming beach
Minnesota	Jul	IV	*Shigella sonnei***	Gastroenteritis	15	Lake/pond	Swimming beach
Minnesota	Jul	III	*Cr. parvum*	Gastroenteritis	7	Pool	Day camp
Minnesota	Jul	II	*Cr. parvum*	Gastroenteritis	6	Pool	Hotel
Minnesota	Aug	II	*Sh. sonnei*	Gastroenteritis	25	Lake	Public beach
Minnesota	Aug	IV	*Cr. parvum*	Gastroenteritis	4	Pool	Municipal pool
Missouri	Sep	III	*Shigella flexneri*	Gastroenteritis	6	Wading pool	Community
Nebraska	Jun	I	*Cr. parvum*	Gastroenteritis	225	Pools	Community
Ohio	Jun	I	*Cr. parvum*	Gastroenteritis	700	Pool	Private swim club
South Carolina	Jul	IV	*Cr. parvum*	Gastroenteritis	26	Pool	Community
Wisconsin	Jan	IV	Norwalk-like virus	Gastroenteritis	9	Pool	Motel

* On the basis of epidemiologic and water-quality data provided on CDC form 52.12.

† Acute gastrointestinal illness of unknown etiology.

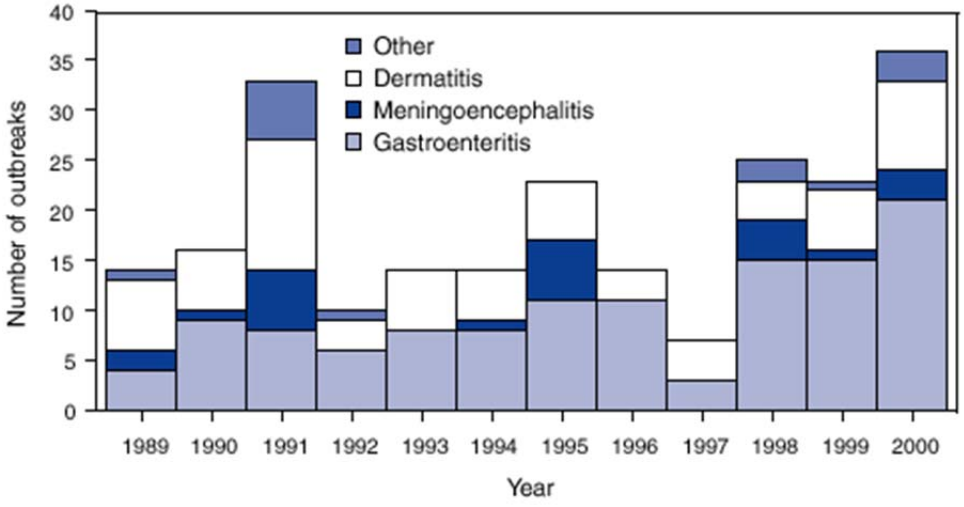
§ Persons swam in a community pool and an inflatable pool.

¶ Seventeen persons had stool specimens that tested positive for *Cr. parvum*. One person had a stool specimen that tested positive for *Giardia intestinalis*. One person had a stool specimen that tested positive for both organisms.

** Fourteen of 15 stool specimens tested positive for *Shigella*; one person tested positive for *Cr. parvum*; and one tested positive for both.

Figure 7

FIGURE 7. Number of waterborne-disease outbreaks associated with recreational water, by year and illness — United States, 1989–2000 (n = 229)*



*The total from previous reports has been corrected from n = 171 to n = 170.

Table 8

TABLE 8. Waterborne-disease outbreaks of meningoencephalitis, keratitis, leptospirosis, and Pontiac fever associated with recreational water — United States, 1999–2000 (n = 8)

State	Year	Month	Class*	Etiologic agent	Illness	Number of cases	Source	Setting
California	2000	Apr	NA†	*Naegleria fowleri*	Meningoencephalitis	1	Mudhole	Mudhole
Florida	1999	Oct	NA	*N. fowleri*	Meningoencephalitis	1	Pond	Pond
Florida	2000	—§	NA	*N. fowleri*	Meningoencephalitis	1	—	—
Guam	2000	Jul	II	*Leptospira interrogans*	Leptospirosis	21	Lake	Adventure race
Vermont	2000	Feb	NA	Bromine	Chemical keratitis	3	Pool	Pool
Texas	1999	Sep	II	Unknown¶	Acute respiratory infection	12	Hot tub	Ranch
Texas	2000	Jul	NA	*N. fowleri*	Meningoencephalitis	1	Lake	Lake
Wisconsin	2000	May	I	*Legionella pneumophila*	Pontiac fever	20	Whirlpool	Motel

* On the basis of epidemiologic and water-quality data provided on CDC form 52.12.
† Not applicable.
§ The month the outbreak occurred was not reported; the source and setting were not reported.
¶ Clinical specimens tested negative for *Legionella pneumophila* serotypes 1 and 6, adenovirus, influenza virus, and parainfluenza virus.

Figure 8

FIGURE 8. Number of outbreaks involving gastroenteritis associated with recreational water, by year and illness — United States, 1978–2000 (n = 146)

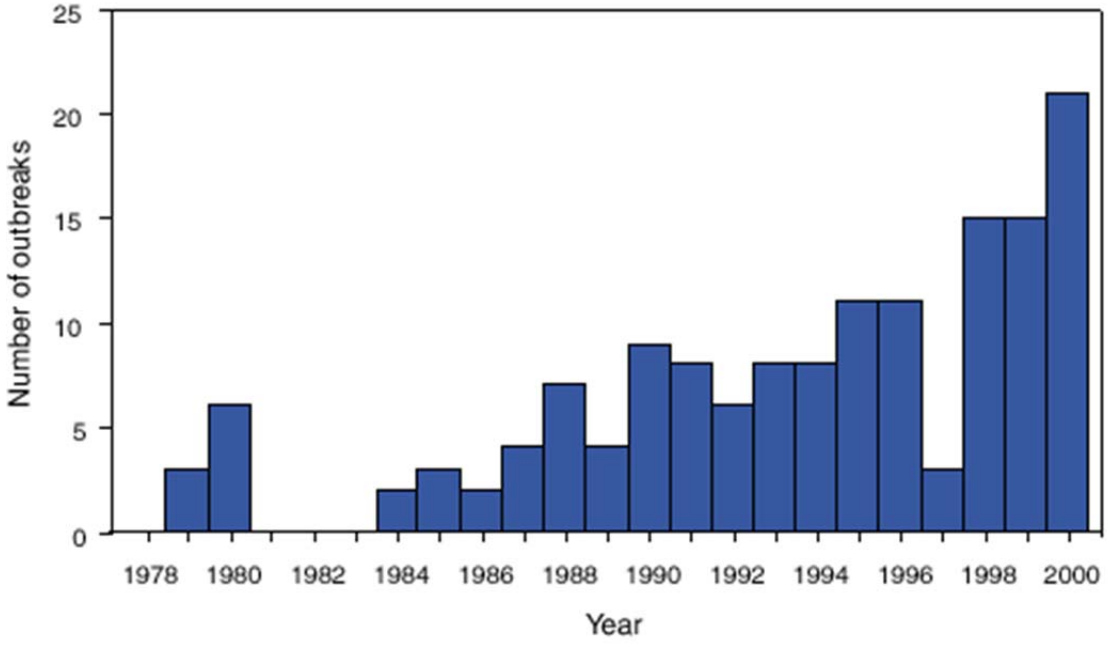


Table 9

TABLE 9. Waterborne-disease outbreaks of dermatitis associated with recreational water — United States, 1999–2000 (n = 15)

State	Year	Month	Class*	Etiologic agent	Number of cases	Source	Setting
Alaska	2000	Oct	NA†	*Pseudomonas aeruginosa*§	29	Pool/hot tub	Hotel
Arkansas	1999	Jun	NA	*P. aeruginosa*§	10	Pool	Community
Arkansas	2000	Feb	NA	*P. aeruginosa*§	26	Pool/ hot tub	Motel
California	2000	Jun	IV	Schistosomes**	6	Pond	Pond
California	2000	Jul	IV	Schistosomes**	4	Pond	Pond
Colorado	1999	Feb	NA	*P. aeruginosa*¶§	19	Hot tub	Hotel
Colorado	1999	Dec	NA	*P. aeruginosa*††	5	Hot tub	Ski lodge
Florida	2000	Aug	NA	*P. aeruginosa*††	6	Hot tub	Apartment complex
Maine	2000	Feb	NA	*P. aeruginosa*¶	9	Hot tub/pool	Hotel
Maine	2000	Mar	NA	*P. aeruginosa*§	11	Hot tub	Hotel
Minnesota	2000	Dec	NA	*P. aeruginosa*¶	16	Hot tub	Private
Oregon	1999	Jul	IV	Schistosomes**	2	Lake	Lake
Vermont	1999	Jun	NA	*P. aeruginosa*††	9	Hot tub	Hotel
Vermont	1999	Feb	NA	*P. aeruginosa*††	11	Hot tub	Vacation home
Washington	2000	Mar	NA	*P. aeruginosa*††	10	Pool/hot tub	Motel

* On the basis of epidemiologic and water-quality data provided on CDC form 52.12.
† Not applicable.
§ Organism isolated from water.
¶ Laboratory-confirmed case.
** Suspected etiology on the basis of clinical syndrome and setting.
†† Suspected etiology on the basis of clinical syndrome.

Figure 9

FIGURE 9. Number of outbreaks involving gastroenteritis associated with recreational water, by water type — United States, 1985–2000 (n = 135)

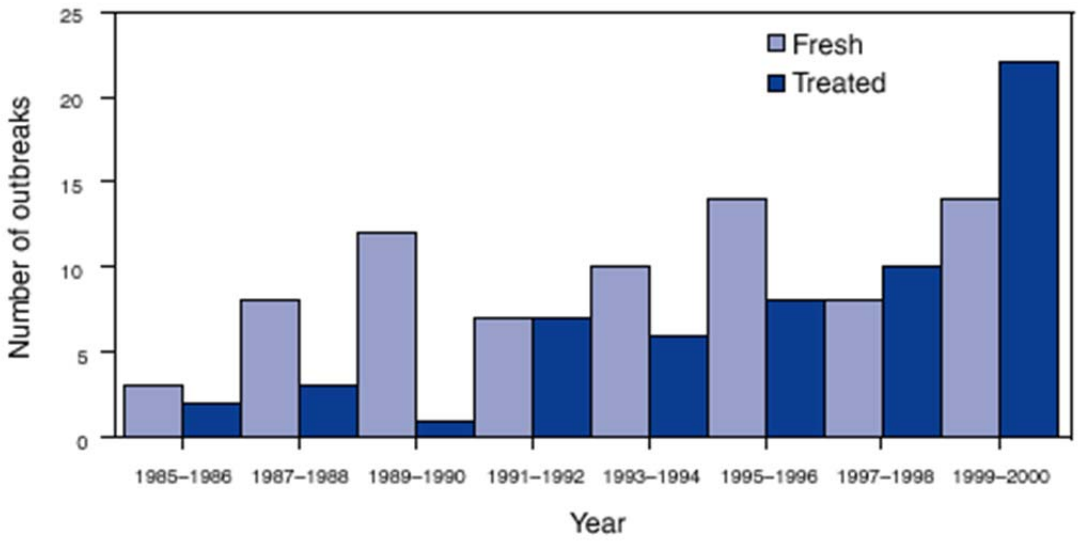


Table 10

TABLE 10. Waterborne-disease outbreaks associated with occupational exposures — United States, 1999–2000 (n = 2)

State	Year	Month	Class*	Etiologic agent	Exposure	Number of cases	Source	Setting
Hawaii	1999	Aug	IV	*Leptospira*	Contact with pond water	2	Pond	Outdoor landscaping
Minnesota	2000	Aug	III	Pontiac fever†	High-pressure cleaning using lagoon water	15	Plant lagoon	Sugar beet plant

* On the basis of epidemiologic and water-quality data provided on CDC form 52.12.
† Endotoxin was also isolated from environmental samples; the role of endotoxin is unclear.

Table 11

TABLE 11. Waterborne-disease outbreaks associated with drinking water that were not included in the previous surveillance summaries — United States, 1995–1997 (n = 3)*

State	Year	Month	Class*	Etiologic agent	Number of cases	Type of system§	Deficiency¶	Source	Setting
Washington	1995	Jul	III	*Giardia intestinalis***	87	Com	4	Well	Community
California	1997	Nov	III	Nitrite (sodium metaborite)	7	Com	4	Mixed river/groundwater	Hospital cafeteria
New York	1997	Dec	I	Norwalk-like virus	1,450	Ncom	3	Well	Ski resort

* An outbreak is defined as 1) ≥2 persons experiencing a similar illness after either ingestion of drinking water or exposure to water used for recreational purposes and 2) epidemiologic evidence that implicates water as the probable source of illness.
† On the basis of epidemiologic and water-quality data provided on CDC form 52.12.
§ Com = community; Ncom = noncommunity; Ind = individual; Community and noncommunity water systems are public water systems that serve ≥15 connections or an average of ≥25 residents for ≥60 days/year. A community water system serves year-round residents of a community, subdivision, or mobile home park with ≥15 service connections or an average of ≥25 residents. A noncommunity water system can be nontransient or transient. Nontransient systems serve ≥25 of the same persons for ≥6 months/year (e.g., factories or schools), whereas transient systems do not (e.g., restaurants, highway rest stations, or parks). Individual water systems are not owned or operated by a water utility and serve <15 connections or <25 persons. Outbreaks associated with water not intended for drinking (e.g., lakes, springs, and creeks used by campers and boaters; irrigation water; and other nonpotable sources with or without taps) are also classified as individual systems.
¶ 1 = untreated surface water; 2 = untreated groundwater; 3 = treatment deficiency (e.g., temporary interruption of disinfection, chronically inadequate disinfection, and inadequate or no filtration); 4 = distribution system deficiency (e.g., cross-connection, contamination of water mains during construction or repair, and contamination of a storage facility); and 5 = unknown or miscellaneous deficiency (e.g., contaminated bottled water).
** Thirty-three persons had stool specimens that tested positive for *G. intestinalis*. One specimen tested positive for *Entamoeba coli*. One other specimen tested positive for *Blastocystis hominis*. One cultured specimen tested positive for *Campylobacter jejuni*.

BOX. Environmental Protection Agency (EPA) regulations regarding drinking water, 1974–2003

Regulation/date	Description
Safe Drinking Water Act/1974 and 1986 and 1996 amendments	Authorizes EPA to set national standards to protect drinking water and its sources
Total Coliform Rule (TCR)/and Maximum Contaminant Level (MCL)/1989	Requires routine monitoring for total coliforms of all public water systems plus periodic on-site inspections for systems that take <5 samples/month to evaluate and document treatment, storage, distribution network, operation and maintenance, and overall management. Systems that collect ≥40 samples/month (i.e., typically, systems that serve >33,000 persons) violate MCL if >5.0% of the samples collected during each month are positive for total coliforms; systems that collect <40 samples/month violate MCL if two samples during the month are positive for total coliforms. If a system has a total coliform-positive sample, then 1) that sample must be tested for the presence of fecal coliforms or *Escherichia coli*, and 2) three repeat samples must be collected (four, if the system collects ≤1 routine sample/month) within 24 hours and analyzed for total coliforms. If positive, the sample must be analyzed for fecal coliforms or *Es. coli*. In addition, ≥5 routine samples must be collected during the next month of sampling, regardless of system size. For any size system, if two consecutive total coliform-positive samples occur at one site during a month, and one of these samples is also fecal coliform-positive or *Es. coli*-positive, the system has an acute violation of the Maximum Contaminant Level and must notify the state and the public immediately.
Surface Water Treatment Rule (SWTR)/1989	Covers all water systems that use surface water or groundwater under the direct influence of surface water; all systems must disinfect their water, and the majority of systems must filter their water also, unless they meet EPA-specified filter-avoidance criteria that define high-quality source water. Specific requirements include • a combined filter-effluent–performance standard for turbidity (i.e., for rapid granular filters, 0.5 nephelometric turbidity unit [NTU] maximum for 95% of measurements [taken every 4 hours] during a month) and no single NTU reading >5.0; • watershed protection, redundant disinfection capability, and other requirements for unfiltered systems; • a 0.2-mg/L disinfectant residual entering the distribution system; and • maintenance of a detectable disinfectant residual in all parts of the distribution system. This rule requires that all such systems reduce the level of *Giardia* by 99.9% (3-log reduction) and viruses by 99.99% (4-log reduction) through a combination of removal (filtration) and inactivation (disinfection).
Information Collection Rule/ 1996–1998	Requires systems serving ≥100,000 persons to provide treatment data and monitor disinfection byproducts and source water quality parameters. Surface water systems are also required to monitor *Cryptosporidium*, *Giardia*, total culturable viruses, and total and fecal coliforms or *Es. coli* ≥1 time/month for 18 months. Results provided information to facilitate development of the Long Term 2 Enhanced SWTR, which is intended to protect against microbial risks by targeting those systems with suboptimal quality source water and to balance the health risks associated with disinfection byproducts and the anticipated Stage 2 Disinfection Byproduct Rule.
Interim Enhanced Surface Water Treatment Rule (IESWTR)/1998	Follow-up to SWTR that covers all public systems using surface water or groundwater under the direct influence of surface water and serving ≥10,000 persons. Key provisions include • a 2-log *Cryptosporidium*-removal requirement for filtered systems; • strengthened combined filter-effluent–turbidity performance standards for systems using conventional filtration treatment or direct filtration (0.3 NTU maximum for 95% of measurements during a month and no single NTU reading >1.0); • individual filter turbidity monitoring provisions;

BOX (*Continued*). Environmental Protection Agency (EPA) regulations regarding drinking water, 1974–2003

Regulation/date	Description
	• disinfection profile and benchmark provisions to ensure continued levels of microbial protection while facilities take necessary steps to comply with new disinfection byproduct standards; • revision of the definition of groundwater under the influence of surface water and the watershed-control requirements for unfiltered public water systems to include detection of *Cryptosporidium*; • requirements for covers on newly finished water reservoirs; • sanitary surveys for all surface water systems regardless of size; and • an MCL goal of zero oocysts for *Cryptosporidium*.
Lead and Copper Rule/2000 changes	Streamlines requirements, promotes consistent national implementation, and reduces the burden for water systems.
Long Term 1 Enhanced SWTR (LT1ESWTR)/2002 and the Filter Backwash Recycling Rule (FBRR)/2001	Companion regulations for IESWTR; LT1ESWTR applies to public water systems that use surface water or groundwater under the direct influence of surface water and that serve <10,000 persons. FBRR regulates how treatment plants recycle water that has been used to backwash a filter or that has been extracted from treatment plant sludge. FBRR regulates the point in the treatment plant at which the contaminated recycle water may be introduced, assuring that the water is subject to the entire particle and *Cryptosporidium parvum* removal process.
Long Term 2 Enhanced SWTR (LT2ESWTR)/expected in 2003	Applies to all systems using surface water or groundwater under the influence of surface water; will provide additional protection against *Cryptosporidium*. Systems will be assigned to a treatment category on the basis of their source-water *Cryptosporidium* levels; the category then determines how much additional treatment is required.
Stage 2 Disinfection Byproduct Rule (DBPR)/expected in 2003	Will apply to community water systems and nontransient noncommunity water systems that use an alternative to ultraviolet disinfection or deliver disinfected water; systems will be required to monitor for total trihalomethanes and the sum of five haloacetic acids and comply with MCLs at each monitoring location as a locational running annual average.
Ground Water Rule (GWR) (1996 amendment to EPA's Safe Drinking Water Act)/expected to be finalized in 2003	Applies to public groundwater systems (i.e., systems that have ≥15 service connections, or regularly serve ≥25 persons daily for ≥60 days/year) or any system that mixes surface and groundwater if the groundwater is added directly to the distribution system and provided to consumers without treatment. Establishes multiple barriers to protect against bacteria and viruses in drinking water from groundwater sources; establishes targeted strategy to identify groundwater systems at high risk for fecal contamination. Key areas include • system sanitary surveys; • hydrogeologic sensitivity assessments for nondisinfected systems; • source-water microbial monitoring by systems that do not disinfect and that draw from hydrogeologically sensitive aquifers or have detected fecal indicators within the system's distribution system; • corrective action by any system with substantial deficiencies or positive microbial samples indicating fecal contamination; and • compliance monitoring for systems that disinfect to ensure that they reliably achieve 4-log (99.99%) inactivation or removal of viruses. GWR does not apply to privately owned wells that serve <25 persons (e.g., individual homeowner wells).

Drinking Water Rules and Disease Chapter 8 Review

1. The first list was called the drinking water _____. CCL contained 60 contaminants/contaminant groups, included 10 pathogens, and was published in the Federal Register on March 2, 1998. A decision concerning whether to regulate ≥5 contaminants from CCL was required by August 2001.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Safe Drinking Water Act (SDWA) of 1974
D. Contaminant Candidate List (CCL)
E. Surface Water Treatment Rule (SWTR)

2. Public water systems are regulated under the _____and its subsequent 1986 and 1996 amendments. Under it, the EPA is authorized to set national standards to protect drinking water and its sources against naturally occurring or man-made contaminants.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Safe Drinking Water Act (SDWA) of 1974
D. Contaminant Candidate List (CCL)
E. Surface Water Treatment Rule (SWTR)

3. The _____ require the EPA to publish a list every 5 years of contaminants that are known or anticipated to occur in public water systems and that might need to be regulated.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Safe Drinking Water Act (SDWA) of 1974
D. Contaminant Candidate List (CCL)
E. Surface Water Treatment Rule (SWTR)

4. Microbial contamination is regulated under the Total Coliform Rule (TCR) of 1989 and the _____ of 1989.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Safe Drinking Water Act (SDWA) of 1974
D. Contaminant Candidate List (CCL)
E. Surface Water Treatment Rule (SWTR)

5. _____ covers all water systems that use surface water or groundwater under the direct influence of surface water.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Safe Drinking Water Act (SDWA) of 1974
D. Contaminant Candidate List (CCL)
E. Surface Water Treatment Rule (SWTR)

6. _____ is intended to protect against exposure to Giardia intestinalis, viruses, and Legionella, as well as selected other pathogens.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Safe Drinking Water Act (SDWA) of 1974
D. Contaminant Candidate List (CCL)
E. Surface Water Treatment Rule (SWTR)

7. In 1998, the EPA promulgated the _____, which provides additional protection against Cryptosporidium and other waterborne pathogens for systems that serve ≥10,000 persons.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Long Term 1 Enhanced SWTR (LT1ESWTR)
E. Surface Water Treatment Rule (SWTR)

8. In 2002, the EPA finalized the _____ for public water systems that use surface water or groundwater under the direct influence of surface water and serve <10,000 persons.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Long Term 1 Enhanced SWTR (LT1ESWTR)
E. Surface Water Treatment Rule (SWTR)

9. _____ was proposed in combination with the Filter Backwash Recycling Rule (FBRR), which was finalized in 2002.
A. Total Coliform Rule (TCR)
B. Filter Backwash Recycling Rule (FBRR)
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Long Term 1 Enhanced SWTR (LT1ESWTR)
E. Surface Water Treatment Rule (SWTR)

10. The _____ require the EPA to develop regulations that require disinfection of groundwater systems as necessary to protect the public health; EPA has proposed the Ground Water Rule (GWR) to meet this mandate.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Long Term 1 Enhanced SWTR (LT1ESWTR)
E. Surface Water Treatment Rule (SWTR)

11. _____ specifies the appropriate use of disinfection in groundwater and addresses other components of groundwater systems to ensure public health protection. GWR applies to public groundwater systems (systems that have ≥15 service connections or regularly serve ≥25 persons/day for ≥60 days/year).
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Ground Water Rule (GWR)

12. This rule also applies to any system that mixes surface and groundwater if the groundwater is added directly to the distribution system and provided to consumers without treatment. _____ does not apply to privately owned wells.
A. Total Coliform Rule (TCR)
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Ground Water Rule (GWR)
E. Surface Water Treatment Rule (SWTR)

13. To fill gaps in existing data regarding occurrence of microbial pathogens and other indicators of microbial contamination, occurrence of disinfection byproducts, and characterization of treatment processes, the EPA promulgated the _____in 1996, which required systems serving ≥100,000 persons to provide treatment data and monitor disinfection byproducts and source-water--quality parameters.
A. Information Collection Rule
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Ground Water Rule (GWR)
E. Surface Water Treatment Rule (SWTR)

14. _____ are also required to monitor for the presence of Cryptosporidium, Giardia, total culturable viruses, and total* and fecal coliforms or Escherichia coli ≥1 time/month for 18 months. The required monitoring ended in December 1998, and data were analyzed.
A. Information Collection Rule
B. 1996 SDWA amendments
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Ground Water Rule (GWR)
E. None of the above

15. The EPA also made minor changes in 2000 to the _____ to streamline requirements, promote consistent national implementation, and in certain cases, reduce the burden for water systems. The action levels of 0.015 mg/L for lead and 1.3 mg/L for copper remain the same.
A. Information Collection Rule
B. Lead and Copper Rule
C. Interim Enhanced Surface Water Treatment Rule (IESWTR)
D. Ground Water Rule (GWR)
E. None of the above

1.D, 2.C, 3.B, 4.E, 5.E, 6.E, 7.C, 8.D, 9.D, 10.B, 11.D, 12.D, 13.A, 14.E, 15.B



A huge high pressure sewer line has ruptured. This looks like a water line break, but it isn't. It is a nightmare and it happens throughout our nation. Most of us will not report this event to the government agencies because we are fearful of the penalties. It seems strange that we are required to report these events but we do not want to lose our jobs or be punished with fines. So instead of reporting, most of us, kind of hide or make-up an excuse not to report the event. Nevertheless, you will be caught one day and you will pay a huge price for not reporting a massive sewage spill. My suggestion is that we as water/wastewater professionals need to have a better relationship with the governing agencies. I know this is very difficult to develop, hopefully the governing agencies will open their eyes and become reasonable towards us and receive these reports as professionals and treat us as so.

Are you prepared to deal with a terrorist damaging your system? Well the Government believes that this event could happen. Plans for this type of destruction have been found and currently information suggests that the sewer system is the most likely and most vulnerable target in the U.S. Think of the diseases that would spread within a very short period of time. Plus, this event would impact the entire potable water system.

Water Sampling and Laboratory Procedures Chapter 9

Before we can identify our waterborne disease, we first must sample the water. Most of you are very familiar with water sampling and may skip this section and proceed to the other chapters. Proper collection and handling of a water sample is critical for obtaining a valid water test. Sample containers should always be obtained from the testing laboratory because containers may be specially prepared for a specific contaminant. Sampling and handling procedures will depend on the specific water quality concern and should be followed carefully. If the water is being treated, it may be necessary to sample both before and after the water goes through the treatment equipment.

Clean sample containers, preservatives and coolers are generally provided by most laboratories. Contact the laboratory about a month before the sampling date to schedule analyses and container shipment or pickup. Collecting water-quality samples involves not only the process of physically acquiring the best possible sample for the intended analysis, but also characterizing the environment from which the sample was drawn, and handling the sample so as to protect its value for its intended purpose. The goal of sample collection and field measurements is to accurately represent the water resource being sampled at that time. This means obtaining a series of measurements (field parameters or in situ measurements) in a prescribed manner, preserving and maintaining water-quality and QA/QC samples according to established guidelines, and observing chain of custody requirements.

Obtaining a representative sample means being careful in your choice of equipment. If you are sampling for the presence of heavy metals, do not use samplers with metal components. When sampling for organics, avoid using samplers with plastic components, as the plastic may adsorb and contaminate the samples. Most importantly, always decontaminate equipment before use. Once the equipment is decontaminated, wrap inorganic equipment in plastic and organic equipment in aluminum foil for transport to the site.



Colilert tests simultaneously detects and confirms coliforms and E. coli in water samples in 24 hours or less.

Simply add the Colilert reagent to the sample, incubate for 24 hours, and read results.

Colilert is easy to read, as positive coliform samples turn yellow or blue, and when E. coli is present, samples fluoresce under UV light.

Bacteriological Monitoring, more detailed information in Lab Section

Most of us have gathered samples and the primary reason is that most waterborne diseases and illnesses have been related to the microbiological quality of drinking water. The routine microbiological analysis of your water is for coliform bacteria. The coliform bacteria group is used as an indicator organism to determine the biological quality of your water.

The presence of an indicator or pathogenic bacteria in your drinking water is an important health concern. Indicator bacteria signal possible fecal contamination, and therefore, the potential presence of pathogens. They are used to monitor for pathogens because of the difficulties in determining the presence of specific disease-causing microorganisms.

Indicator bacteria are usually harmless, occur in high densities in their natural environment, and are easily cultured in relatively simple bacteriological media. Indicators in common use today for routine monitoring of drinking water include total coliforms, fecal coliforms, and *Escherichia coli (E. coli).*

Bacteria Sampling
Water samples for bacteria tests must always be collected in a sterile container. Take the sample from an inside faucet with the aerator removed. Sterilize by spraying a 5% household bleach or alcohol solution or flaming the end of the tap with disposable butane lighter or propane torch.

Run the water for five minutes to clear the water lines and bring in fresh water. Do not touch or contaminate the inside of the bottle or cap. Carefully open the sample container and hold the outside of the cap. Fill the container and replace the top.

Refrigerate the sample and transport it to the testing laboratory within six hours (in an ice chest). Many labs will not accept bacteria samples on Friday so check the lab's schedule. Mailing bacteria samples is not recommended because laboratory analysis results are not as reliable.

Iron bacteria forms an obvious slime on the inside of pipes and fixtures. A water test is not needed for identification. Check for a reddish-brown slime inside a toilet tank or where water stands for several days.



Standard Sample Coliform Bacteria Bac-T.

Bac-T Sample Bottle, often referred to as a Standard Sample, 100 mls, Notice the white powder inside the bottle. That is Sodium Thiosulfate, a de-chlorination agent. Be careful not to wash-out this chemical while sampling. Notice the custody seal on the bottle.

Coliform bacteria are common in the environment and are generally not harmful. However, the presence of these bacteria in drinking water is usually a result of a problem with the treatment system or the pipes which distribute water, and indicates that the water may be contaminated with germs that can cause disease.

Laboratory Procedures, more detailed information in the next section

The laboratory may perform the total coliform analysis in one of four methods approved by the U.S. EPA and your local environmental or health division.

Methods
The MMO-MUG test, a product marketed as Colilert is the most common. The sample results will be reported by the laboratories as simply coliforms present or absent. If coliforms are present, the laboratory will analyze the sample further to determine if these are fecal coliforms or E. coli and report their presence or absence.

Types of Water Samples
It is important to properly identify the type of sample you are collecting. Please indicate in the space provided on the laboratory form the type of sample.

The three (3) types of samples are:
1. **Routine:** Samples collected on a routine basis to monitor for contamination. Collection should be in accordance with an approved sampling plan.
2. **Repeat:** Samples collected following a '***coliform present'*** routine sample. The number of repeat samples to be collected is based on the number of routine samples you normally collect.
3. **Special:** Samples collected for other reasons.
Examples would be a sample collected after repairs to the system and before it is placed back into operation, or a sample collected at a wellhead prior to a disinfection injection point.

Routine Coliform Sampling (*Check with your governmental environmental or health agency for more information*)
The number of routine samples and frequency of collection for community public water systems is shown in **Table 3-1** next page.

Noncommunity and nontransient noncommunity public water systems will sample at the same frequency as a like sized community public water system if:
1. It has more than 1,000 daily population and has ground water as a source, or

2. It serves 25 or more daily population and utilizes surface water as a source or ground water under the direct influence of surface water as its source.

Noncommunity and nontransient, noncommunity water systems with less than 1,000 daily population and groundwater as a source will sample on a quarterly basis.

Table 3 Number of Samples per System Population

Persons served	*Samples per month*
up to 1,000	1
1,001-2,500	2
2,501-3,300	3
3,301 to 4,100	4
4,101 to 4,900	5
4,901 to 5,800	6
5,801 to 6,700	7
6,701 to 7,600	8
7,601 to 8,500	9
8,501 to 12,900	10
12,901 to 17,200	15
17,201 to 21,500	20
21,501 to 25,000	25
25,001 to 33,000	30
33,001 to 41,000	40
41,001 to 50,000	50
50,001 to 59,000	60
59,001 to 70,000	70
70,001 to 83,000	80
83,001 to 96,000	90
96,001 to 130,000	100
130,001 to 220,000	120
220,001 to 320,000	150
320,001 to 450,000	180
450,001 to 600,000	210
600,001 to 780,000	240



Repeat Sampling

Repeat sampling replaces the old check sampling with a more comprehensive procedure to try to identify problem areas in the system. Whenever a routine sample is positive for total coliform or fecal coliform present, a set of repeat samples must be collected within 24 hours after being notified by the laboratory.

Before I start out to re-sample, I always call the Distribution Section and have their personnel flush the area and obtain a good Chlorine residual.

The follow-up for repeat sampling is:

1. If only one routine sample per month or quarter is required, four (4) repeat samples must be collected.
2. For systems collecting two (2) or more routine samples per month, three (3) repeat samples must be collected.
3. Repeat samples must be collected from:

a. The original sampling location of the coliform present sample.
b. Within five (5) service connections upstream from the original sampling location.
c. Within five (5) service connections downstream from the original sampling location.
d. Elsewhere in the distribution system or at the wellhead, if necessary.

4. If the system has only one service connection, the repeat samples must be collected from the same sampling location over a four-day period or on the same day.
5. All repeat samples are included in the MCL compliance calculation.
6. If a system which normally collects fewer than five (5) routine samples per month has a coliform present sample, it must collect five (5) routine samples the following month or quarter regardless of whether an MCL violation occurred or if repeat sampling was coliform absent. **(*Check with your governmental environmental or health agency for more information*).**

Positive or Coliform Present Results

What do you do when your sample is positive or coliform present?

When you are notified of a positive test result you need to contact either the Drinking Water Program or your local county health department within 24 hours, or by the next business day after the results are reported to you. The Drinking Water Program contracts with many of the local health departments to provide assistance to water systems. Most of us have had this problem. It can be stressful, and people have lost their jobs over these problems. The best option is to have a SOP in place because positive samples or false positives will come to your section.

Assistance

After you have contacted an agency for assistance, you will be instructed as to the proper repeat sampling procedures and possible corrective measures for solving the problem. It is very important to initiate the repeat sampling immediately as the corrective measures will be based on those results.

Some examples of typical corrective measures to coliform problems are:

1. Shock chlorination of a ground water well. The recommended dose of 5% household bleach is 2 cups per 100 gallons of water in the well. This should be done anytime the bell is opened for repair (pump replacement, etc.). If you plan to shock the entire system, calculate the total gallonage of storage and distribution.
2. Conduct routine distribution line flushing. Install blowoffs on all dead end lines.
3. Conduct a cross connection program to identify all connections with non-potable water sources. Eliminate all of these connections or provide approved backflow prevention devices.
4. Upgrade the wellhead area to meet current construction standards as set by your state environmental or health agency.
5. If you continuously chlorinate, review your operation and be sure to maintain a detectable residual (**0.2 mg/l free chlorine**) at all times in the distribution system.
6. Perform routine cleaning of the storage system.



This list provides some basic operation and maintenance procedures that could help eliminate potential bacteriological problems, check with your state drinking water section or health department for further instructions.

Maximum Contaminant Levels (MCLs)

State and federal laws establish standards for drinking water quality. Under normal circumstances when these standards are being met, the water is safe to drink with no threat to human health. These standards are known as maximum contaminant levels (**MCL**). When a particular contaminant exceeds its MCL a potential health threat may occur.

The MCLs are based on extensive research of the toxicological properties of the contaminants, risk assessments and factors, short term (**acute**) exposure, and long term (**chronic**) exposure. You conduct the monitoring to make sure your water is in compliance with the MCL. There are two types of MCL violations for coliform bacteria. The first is for total coliform; the second is an acute risk to health violation characterized by the confirmed presence of fecal coliform or E. coli.

(Check with your governmental environmental or health agency for more information.)



Common Sample bottles, *Radiochems, VOCs, (Volatile Organic Compounds), TTHMs, Total Trihalomethanes), Nitrate, Nitrite.*

Most of these sample bottles will come with the preservative already inside the bottle. Some bottles will come with a separate preservative (acid) for the field preservation. Slowly add the acid or other preservative to the water sample; not water to the acid or preservative. Put a new label on the preservative dispensing bottle with the current date.

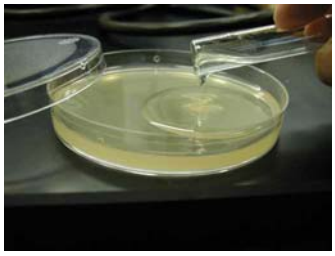
Heterotrophic Plate Count, more detailed information in the next section

(Check with your governmental environmental or health agency for more information.)

Heterotrophic Plate Count (**HPC**) --- formerly known as the standard plate count, is a procedure for estimating the number of live heterotrophic bacteria and measuring changes during water treatment and distribution in water or in swimming pools. Colonies may arise from pairs, chains, clusters, or single cells, all of which are included in the term "***colony-forming units***" (**CFU**).

Method:

There are three methods for standard plate count:



1. **Pour Plate Method**

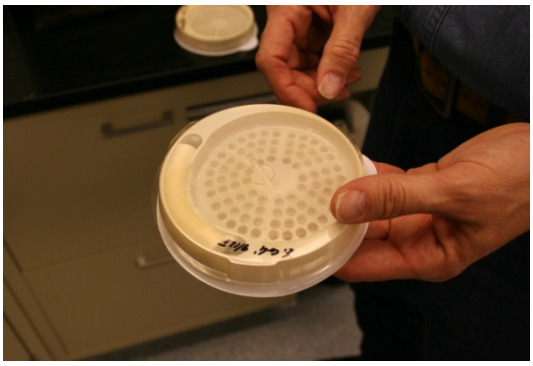
The colonies produced are relatively small and compact, showing less tendency to encroach on each other than those produced by surface growth. On the other hand, submerged colonies often are slower growing and are difficult to transfer.

2. Spread Plate Method

All colonies are on the agar surface where they can be distinguished readily from particles and bubbles. Colonies can be transferred quickly, and colony morphology easily can be discerned and compared to published descriptions.

3. Membrane Filter Method

This method permits testing large volumes of low-turbidity water and is the method of choice for low-count waters.



Material:

i) Apparatus
- Glass rod
- Erlenmeyer flask
- Graduated Cylinder
- Pipette
- Petri dish
- Incubator

ii) Reagent and sample
- Reagent-grade water
- Nutrient agar
- Sample

Procedure*

1.Boil mixture of nutrient agar and nutrient broth for 15 minutes, then cool for about 20 minutes.
2.Pour approximately 15 ml of medium in each Petri dish, let medium solidify.
3.Pipet 0.1 ml of each dilution onto surface of pre-dried plate, starting with the highest dilution.
4.Distribute inoculum over surface of the medium using a sterile bent glass rod.
5.Incubate plates at 35^{o}C for 48h.

6.Count all colonies on selected plates promptly after incubation, consider only plates having 30 to 300 colonies in determining the plate count.
*Duplicate samples

Computing and Reporting: Compute bacterial count per milliliter by the following equation:
CFU/ml = colonies counted / actual volume of sample in dish
a) If there is no plate with 30 to 300 colonies, and one or more plates have more than 300 colonies, use the plate(s) having a count nearest 300 colonies. (*Check with your governmental environmental or health agency for more information*)
b) If plates from all dilutions of any sample have no colony, report the count as less than 1/actual volume of sample in dish estimated CFU/ml.
c) Avoid creating fictitious precision and accuracy when computing CFU by recording only the first two left-hand digits.

Heterotrophic Plate Count

(Spread Plate Method) ***(Check with your governmental environmental or health agency for more information.)***
Heterotrophic organisms utilize organic compounds as their carbon source (food or substrate). In contrast, autotrophic organisms use inorganic carbon sources. The Heterotrophic Plate Count provides a technique to quantify the bacteriological activity of a sample. The R2A agar provides a medium that will support a large variety of heterotrophic bacteria. After an incubation period, a bacteriological colony count provides an estimate of the concentration of heterotrophs in the sample of interest.

Required Laboratory Equipment

100 x 15 Petri Dishes
Turntable
Glass Rods: Bend fire polished glass rod 45 degrees about 40 mm from one end. Sterilize before using.
Pipette: Glass, 1.1 mL. Sterilize before using.
Quebec Colony Counter
Hand Tally Counter

Reagents

1) R2A Agar: Dissolve and dilute 0.5 g of yeast extract, 0.5 g of proteose peptone No. 3, 0.5 g of casamino acids, 0.5 g of glucose, 0.5 g of soluble starch, 0.3 g of dipotassium hydrogen phosphate, 0.05 g of magnesium sulfate heptahydrate, 0.3 g of sodium pyruvate, 15.0 g of agar to 1 L. Adjust pH to 7.2 with dipotassium hydrogen phosphate **before adding agar.** Heat to dissolve agar and sterilize at 121 C for 15 minutes.
2) Ethanol: As needed for flame sterilization.

Preparation of Spread Plates
Immediately after agar sterilization, pour 15 mL of R2A agar into sterile 100 x 15 Petri dishes; let agar solidify. Pre-dry plates inverted so that there is a 2 to 3 g water loss overnight with the lids on. Use pre-dried plates immediately or store up to two weeks in sealed plastic bags at 4 degrees C.

Sample Preparation
Mark each plate with sample type, dilution, date, and any other information before sample application. Prepare at least duplicate plates for each volume of sample or dilution examined. Thoroughly mix all samples by rapidly making about 25 complete up-and-down movements.

Sample Application
Uncover pre-dried agar plate. Minimize time plate remains uncovered. Pipette 0.1 or 0.5 mL sample onto surface of pre-dried agar plate.

Record volume of sample used. Using a sterile bent glass rod, distribute the sample over the surface of the medium by rotating the dish by hand on a turntable. Let the sample be absorbed completely into the medium before incubating. Put cover back on Petri dish and invert for duration of incubation time. Incubate at 28°C for 7 days. Remove Petri dishes from incubator for counting.

Counting and Recording
After incubation period, promptly count all colonies on the plates. To count, uncover plate and place on Quebec colony counter. Use hand tally counter to maintain count. Count all colonies on the plate, regardless of size. Compute bacterial count per milliliter by the following equation:

$$\text{CFU} \Big/ \text{mL} = \frac{\text{colonies counted}}{\text{actual volume of sample in dish, mL}}$$

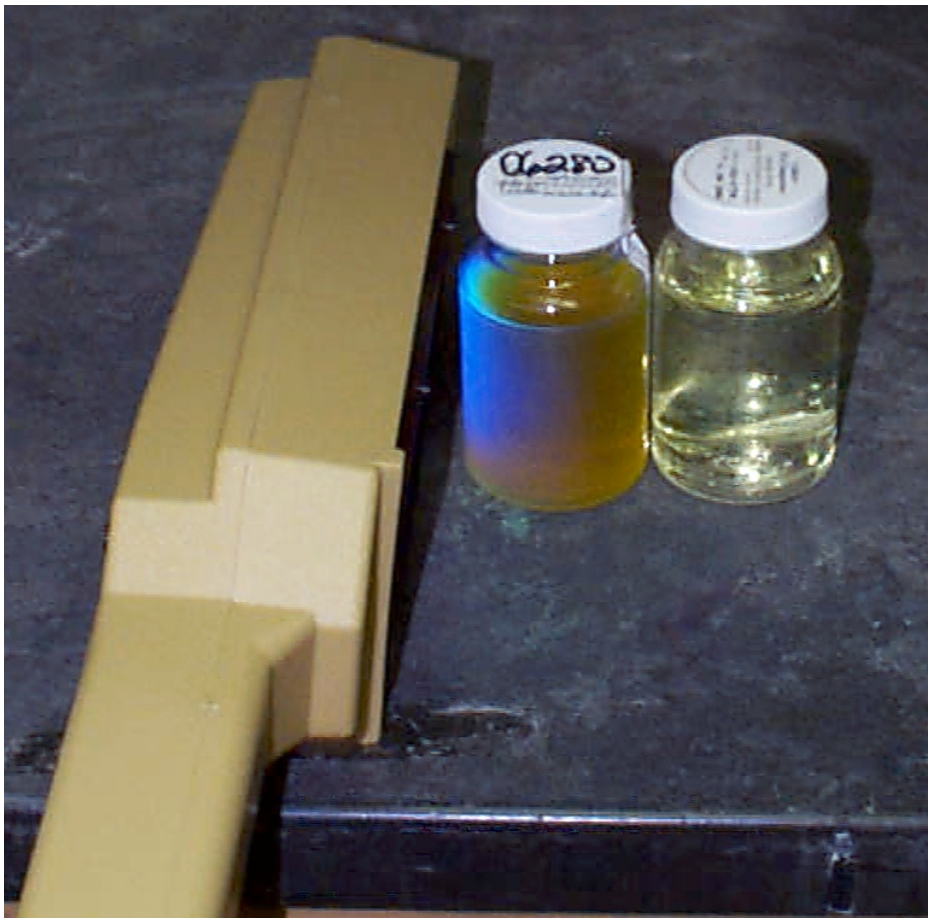
To report counts on a plate with no colonies, report the count as less than one (<1) divided by the sample volume put on that plate (remember to account for any dilution of that sample.)

If plates of all dilutions for a sample have no colonies, report the count as less than one (<1) divided by the largest sample volume used. Example: if 0.1 mL of a 100:1 and 10000:1 dilution of a sample both turned up with no colonies formed, the reported result would be <1 divided by the largest sample volume 0.001 mL (0.1 mL divided by 100). The final reported result for the sample is <1000 CFU per mL.

Assignment
1. Report the number of colony forming units (**CFU**) found on each plate.
2. Calculate the **CFU** per mL for each plate.
3. The aim of diluting samples is to produce a plate having 30 to 300 colonies, which plates meet these criteria. If no sample produces a plate with a count in this range, use the plate(s) with a count closest to 300. Based on these criteria, use your calculated results to report the **CFU** per mL for each sample. In the conclusion of your lab report, comment on your final results for each sample type as well as the quality of your application of this analysis technique. Feel free to justify your comments using statistical analysis. Also, comment on the general accuracy of this analytical technique and the factors that affect its accuracy and or applicability.

Data Table for Samples

Sample ID	Volume of Sample, mL	Colonies Counted per plate



E. coli or fecal bacteria will fluoresce.

Total Coliforms, more detailed information in the next section

This MCL is based on the presence of total coliforms, and compliance is on a monthly or quarterly basis, depending on your water system type and state rule. For systems which collect *fewer* than 40 samples per month, no more than one sample per month may be positive. In other words, the second positive result (repeat or routine) in a month or quarter results in an MCL violation.

For systems which collect 40 or more samples per month, no more than five (5) percent may be positive. Check with your state drinking water section or health department for further instructions.

Acute Risk to Health (Fecal coliforms and E. coli)
An acute risk to human health violation occurs if either one of the following happen:
1. A routine analysis shows total coliform present and is followed by a repeat analysis which indicates fecal coliform or E. coli present.
2. A routine analysis shows total and fecal coliform or E. coli present and is followed by a repeat analysis which indicates total coliform present. An acute health risk violation requires the water system to provide public notice via radio and television stations in the area. This type of contamination can pose an immediate threat to human health and notice must be given as soon as possible, but no later than 72 hours after notification from your laboratory of the test results.

Certain language may be mandatory for both these violations and is included in your state drinking water rule.

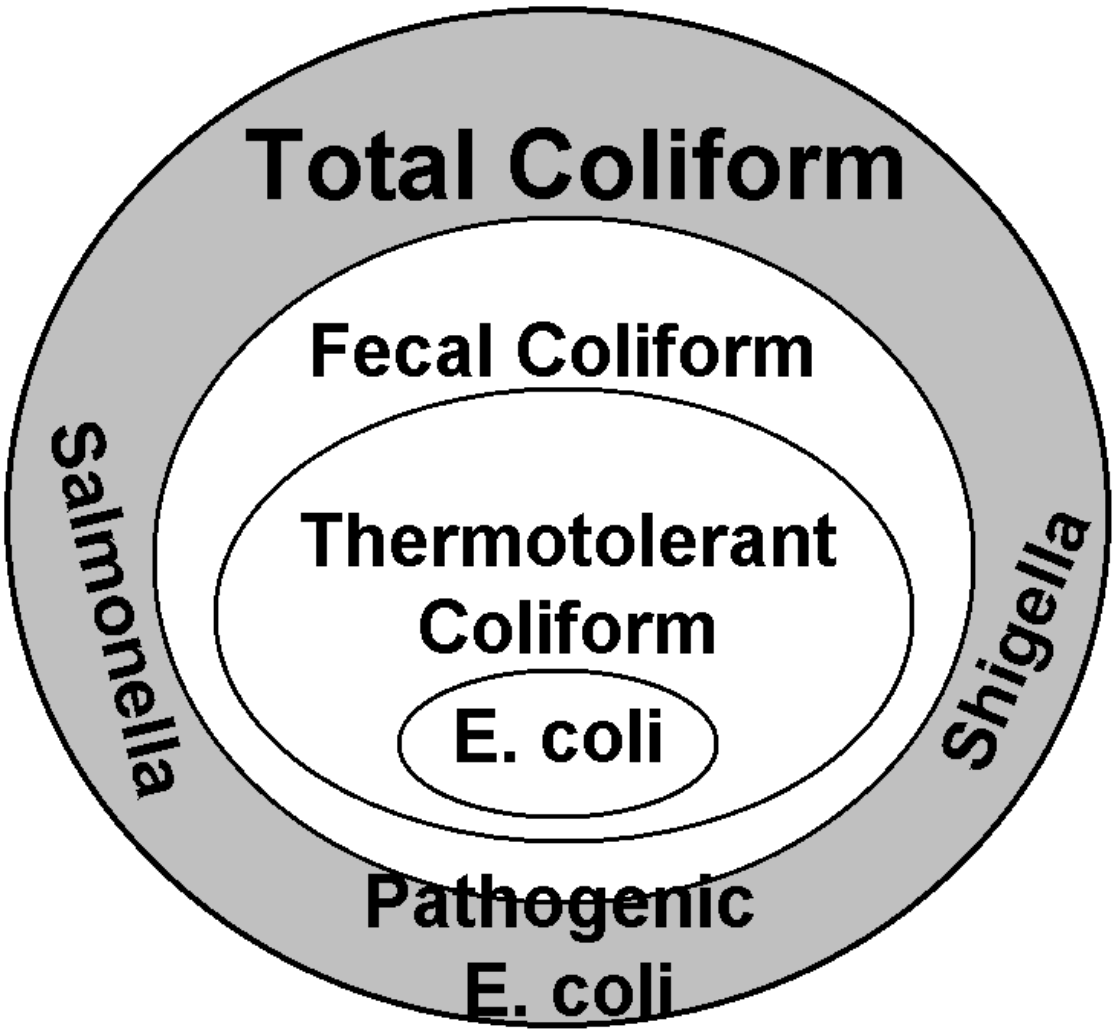
Public Notice
A public notice is required to be issued by a water system whenever it fails to comply with an applicable MCL or treatment technique, or fails to comply with the requirements of any scheduled variance or permit. This will inform users when there is a problem with the system and give them information.

A public notice is also required whenever a water system fails to comply with its monitoring and/or reporting requirements or testing procedure. Each public notice must contain certain information, be issued properly and in a timely manner, and contain certain mandatory language.

The timing and place of posting of the public notice depends on whether an acute risk is present to users. Check with your state drinking water section or health department for further instructions.

The following are acute violations:
1. Violation of the MCL for nitrate.
2. Any violation of the MCL for total coliforms, when fecal coliforms or E. coli are present in the distribution system.
3. Any outbreak of waterborne disease, as defined by the rules.

(Check with your governmental environmental or health agency for more information.)


Total Coliform
Fecal Coliform
Thermotolerant Coliform
E. coli
Salmonella
Shigella
Pathogenic E. coli

General Questions about Coliform

Always check with your State to ensure this information is correct, for many States have stricter laws than what is in this section.

What is the Total Coliform Rule (TCR)?

The Total Coliform Rule (TCR) requires all public water supplies to monitor for the presence of total coliforms in the distribution system. For drinking water, total coliforms are used to monitor the sanitary quality of the water, adequacy of water treatment, and the integrity of the distribution system. The absence of total coliforms in the distribution system minimizes the likelihood that fecal pathogens are present. Thus, total coliforms are used to determine the vulnerability of a system to fecal contamination**.**

What is total coliform bacteria?

Total coliforms are a group of closely related bacteria that are (with few exceptions) not harmful to humans. They are an indicator of other pathogens that can be present in water. Coliform bacteria are present in the intestinal tract of warm-blooded animals. They are shed from the body in the feces. Because these organisms are shed from the body in large numbers and are relatively easy to detect in the laboratory, they have been accepted as an indicator of contamination. All bacteriological samples are analyzed for the coliform group; however, a positive reaction to these coliform analyses may be from sources other than fecal. In order to differentiate between these sources, all samples that are total coliform positive must be analyzed again to determine if fecal coliform or *E. coli* are present.

What are fecal coliform and *E. coli*?

Fecal coliform is bacteria whose presence indicates that the water may be contaminated with human or animal wastes. *E. coli* is a member of the fecal coliform group. Microbes in these wastes can cause diarrhea, cramps, nausea, headaches, or other symptoms. They may pose a special health risk for infants, young children, and people with severely compromised immune systems. The presence of fecal coliform or *E. coli* in drinking water sample indicate recent contamination and signals a greater possibility that pathogenic organisms are also in the water.

Why must a water system monitor for total coliform, fecal coliform, and *E. coli*?

Total coliforms serve as indicators of the efficiency of water treatment, of the integrity of the pipes in the distribution system, and as a screen for the presence of fecal contamination. Usually, coliforms are a sign that there could be a problem with the system's treatment or distribution system. Fecal and/or *E. coli* indicates that pathogenic organisms may be present that can cause short-term health effects, such as diarrhea, cramps, nausea, headaches, fatigue and jaundice. They may pose a special health risk for infants, young children, and people with severely compromised immune systems.

Monitoring Requirements

How many coliform samples must a water system collect?

Every Public Water System (WS or PWS) must collect a given number of "routine" coliform samples from the distribution system per month generally based on the population served.

Additional raw, finished, and distribution water samples may also be required depending on your source of water, water treatment facilities, service area of the distribution system, and any related sanitary survey deficiencies. After a WS or PWS sanitary survey is conducted by the official State water or health agency inspector, the total number of coliform samples required each month is listed on the follow-up inspection report that is mailed to the WS or PWS. If you are not sure how many samples you are required to collect, please contact your **Drinking Water Agency.**

Introduction.
If a WS or PWS has a positive coliform sample, confirmation (repeat) samples must be collected immediately. The number of repeats will vary.

Does the number of monthly routine distribution coliform samples ever change?
Yes. Two events may change your monthly distribution monitoring requirements:
1) A change in population may cause a change in the number of samples. If a change occurs, your official State Drinking Water Agency will notify the WS or PWS.
2) One coliform positive routine finished or routine distribution system sample result during any month requires a minimum of **FIVE** routine distribution samples be collected the following month. If your system is already required to collect a minimum of five distribution samples each month, this does not affect you.

Do I have to collect my monthly routine coliform samples from specific locations?
Yes. Failure to do so may result in a monitoring violation. Every WS or PWS must have on file an approved coliform sample site plan at the appropriate **Drinking Water Agency**. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

What is a coliform sample site plan?
A coliform sample site plan is a list of sites by street address, lot number, or other permanent description, that identifies all the approved locations where your routine (monthly) coliform samples may be collected. The list of sites must be plotted on a map of your service area. Larger water systems will divide their distribution system into specific sample areas.

Does the site plan have to be approved by the official State water or health agency?
Yes. The list of sites and the map is reviewed by the official State water or health agency Drinking Water Agency serving your facility to insure representative sites have been selected. A site number is then assigned to each sample location. All reporting forms must be completed using the sample site number rather than the street address. Your approved site plan MUST be followed each time you collect routine samples. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

How can I find out the water system's sampling locations?
A copy of the WS or PWS's coliform site plan (and sample site numbers) is on file at your official State water or health agency

How often and when must I collect my routine samples?
Routine compliance samples must be collected every month. Each month is a sampling period.
A WS or PWS with only a **groundwater source** (not under the direct influence of surface water) that **serves 4,900 persons or fewer**, may collect all required samples on a single day if they are taken from each area of the approved sample site plan. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Surface water supplies, supplies using groundwater under the direct influence of surface water and groundwater supplies that serve more than 4,900 people must collect samples throughout the month. WS or PWS that collect weekly or more frequently should continue this practice. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Samples Should be Collected as Early as Possible

Samples should be collected as early each month as possible, but not before the first day of each month. Prompt collection allows WS or PWS sufficient time for replacement sampling if samples are discarded. Reasons for discarding samples include: failure to record date or time of collection, sample(s) more than 30 hours old upon arrival at the certified laboratory, and sample(s) broken or frozen in transit. If the sample(s) and/or any necessary replacement samples are not collected within the monthly monitoring period, the WS or PWS will be in violation of their monitoring requirements and must make public notice. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Collecting Samples
How do I choose a proper sample faucet?
Since coliform samples must be representative of the water quality in the distribution system, it is important to select proper sampling locations. Visit the site before selecting it for your coliform sample site plan. See if a smooth-nosed cold-water faucet is available that will allow the collector to run the faucet at a constant flushing rate for 30 to 60 seconds without flooding the sink. You may choose to install a smooth nosed faucet at your designated sample locations.

The sampling faucet should be conveniently located and readily accessible to the collector. Avoid faucets that are connected to private water treatment equipment such as water softeners or filters. Faucets that are subject to exterior contamination because they are too close to a sink bottom or to the ground must be avoided. It is difficult to place a bottle beneath a low faucet without touching the interior of the bottle's neck against the outside of the faucet. Threaded faucets that might harbor bacteria around the threads should not be used. Leaking faucets that allow water to flow around the stem and over the outside of the faucet should be avoided. If an even stream of water cannot be sustained, a more suitable tap should be found. Failure to follow these precautions could result in a contaminated sample.

Keep in mind that in the event of a positive coliform sample, two (or three) additional distribution sites meeting the above criteria, one within five (5) service connections upstream and one within five (5) service connections downstream, will be needed for the collection of repeat samples. These additional sites should be included in your coliform sample site plan. You may wish to install dedicated sampling faucets or sampling stations to assure access and satisfactory sampling conditions at all times. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

How should a coliform sample be collected?
Use great care when collecting coliform samples. Only bottles received from the certified laboratory should be used to collect samples. It is extremely important that the sample collector uses only the approved sampling locations and follow proper sampling techniques.

Contamination from the sampling faucet can easily occur if extreme caution is not used. Should an incident occur during sample collection that may result in contamination, the sample should be discarded and a new bottle requested. **It is assumed that all samples submitted for testing are properly collected. Sample error will not be accepted as an excuse to avoid repeat sampling.**

Generally, the following protocol should be followed:
1. Sample bottles should be examined when received. If for any reason (loose caps, caps off, etc.) the sterility of the bottle is in question, the bottles should not be used.
2. Open an approved sampling faucet so that a smooth flow of water at moderate pressure is obtained. Be sure that there is no splashing. Allow the water to flow for sufficient time to clear the service line. Depending on time of year and water source you may notice a water temperature change when the line has been cleared.

3. **If your WS or PWS is chlorinated,** check for residual chlorine. These results MUST be included on the reporting form. Indicate whether the residual measured is free (F) or total (T) chlorine.
4. The bottle cap should not be removed until you are ready to collect the sample. Do not lay the bottle cap down or put it in a pocket. Hold the bottle in one hand and the cap in the other, keeping the bottle cap right side up (threads down) and taking care not to touch the inside of the cap. Avoid touching the inside of the sterile bottle(s) with your fingers or the faucet nose.
5. Once you start filling the bottles do not adjust the stream flow. Do not allow splashing drops of water from the ground or sink to enter the bottle. Fill the bottle to the 100 ml mark on the side of the bottle. Cap the bottle immediately. Then turn off the faucet.

Can I use any container to collect the sample?
No. You must use sterile 100 ml plastic (or glass) bottles obtained from a certified laboratory. These bottles contain a small amount of sodium thiosulfate to neutralize the residual chlorine in the sample. The sodium thiosulfate may appear as a tablet or as a white residue in the bottle. Do NOT rinse this material out of the bottle.

Do we need to use any special reporting forms when submitting samples to the laboratory?
Yes. These forms can be obtained by calling your **certified laboratory**. The reporting forms should be included with the bottles. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

How do I complete the reporting/collection form?
For the most part, the form is self-explanatory. A few reminders:
1. Always use your official State water or health agency assigned sample site numbers (not sample address). If a site number does not exist for a location, please add it to your site plan by calling your **official Drinking Water Agency**. Simply record the address if it's a "one-time" sample location.
2. Be-sure you include all "Contact Person" information including telephone number and cellular number. This information is very important in case there is a positive sample.
3. Clearly mark sample purpose.
4. If the WS or PWS is chlorinated, you must include the chlorine residual on the form. Failure to do so will result in a violation.

After I collect the sample, how long does it have to reach the laboratory?
Always check with your Agency to ensure this rule is correct, for this rule is different in some States. In order for the laboratory to analyze the sample(s), it must be received within 24 or 30 hours of collection. If not, a replacement must be collected. It is strongly recommended that the monthly routine samples be collected within the first few days of each month. This will allow ample time for the collection of replacement sample(s) if they are required.

What days of the week should I collect my samples?
It is preferred that routine samples be collected and shipped to the laboratory on Mondays or Tuesdays. If routine samples are hand delivered to the laboratory, they should be delivered no later than Thursday. However, emergency or repeat samples may be submitted at any time. If you anticipate the samples will arrive on a weekend or holiday, you should contact your certified laboratory and make the necessary arrangements. It is recommended that you call your certified laboratory for a contact person, business hours, and any special delivery instructions. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Do all coliform samples collected during the month count towards meeting our compliance monitoring requirements?
No. Special purpose samples, such as those taken to lift a boil order, or new construction samples to determine whether disinfection practices are sufficient following pipe placement, replacement or repair, will not be used to determine compliance with the Maximum Contaminant Level (MCL) or towards the routine number of samples required each month.

Routine raw and/or finished water entry point samples do not count towards meeting the total number of distribution samples required each month. However, finished water entry point samples will be used to determine compliance with the MCL. Thus, if you have a positive finished water entry point sample, you must follow-up with the collection of repeat samples.

Our water system is chlorine exempt. Are there any special monitoring requirements?
Yes. In past years, some very small systems have been granted an exemption from chlorination by State water or health agencies. New exemptions are no longer granted. Official State water or health agency may still honors past chlorine exemptions, but recognizes that the water lacks the protection of the residual chlorine.

Consequently, it is especially important that the bacterial quality of the water be monitored at frequent intervals. One of the conditions of the exemption is that samples be collected and analyzed at twice the frequency required of a chlorinated WS or PWS (two times a month). Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Interpretation of Results
How do I know if the sample is satisfactory?
The laboratory will determine if any bacteria are present in your sample(s). If no bacteria are present the sample is considered satisfactory with a (S) recorded on the reporting form.

If bacteria are detected in the sample, the laboratory will record the number of colonies (if using the membrane filter technique) and analyze the sample to see if the bacteria are coliform. If the bacteria are determined to be coliform the laboratory will use the designation of “P” (positive).If no coliform is detected, the designation will be given as “N” (negative). *Any sample with a non-coliform bacteria count of 200 colonies or less is considered a satisfactory (S) sample.*

If any coliform bacteria are found, the sample is positive and requires collection of repeat samples as described in **– Repeat Samples / Follow-up to Coliform Positive Samples**. Additional tests are performed on the original total coliform positive sample to determine if fecal coliform or *E. coli* is present. This result is also recorded on the reporting form with a P or N.

A sample analyzed by the membrane filter technique is deemed invalid (I) in the following scenarios:
1. Samples are negative for coliform but exhibit confluent growth. Confluent growth is continuous bacterial growth covering all or part of a membrane filter.
2. Samples are negative for coliform but bacteria colonies are too numerous to count (reported as G- or TNTC by the laboratory). Submission of replacement sample(s) is required. When using the multiple fermentation tube procedure or the presence-absence procedure, if the media is turbid with no production of gas or acid, the sample also is invalidated with replacement sample(s) required.

How long must I keep coliform results and other related paperwork?
5 years. Records of total coliform analyses must be kept for no less than five (5) years. The actual laboratory reports may be kept or the data may be transferred to tabular summaries. Local procedures may contain requirements concerning the retention of records. The most stringent requirement should be followed. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Laboratory Related Questions

Can we have our coliform samples analyzed anywhere?
No. A laboratory certified by your State Drinking Water Agency or Health Department must analyze samples. A sample analyzed at a laboratory that is not certified cannot be used for compliance.

What laboratory methods are used for analyzing coliform samples?
WS or PWS must conduct total coliform analyses in accordance with one of the analytical methods in the following table:

Organism Methodology
Total Coliforms Total Coliform Fermentation Technique
Total Coliform Membrane Filter Technique
Presence-Absence (P-A) Coliform Test
ONPG-MUG Test (Colilert)
Colisure Test
E*Colite® Test
m-ColiBlue24® Test
Readycult coliform 100 Presence/Absence
Colitag® Test

Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Do we need to use any special reporting forms when submitting samples to the laboratory?
Yes. These forms can be obtained by calling your **certified laboratory**. The reporting forms should be included with the bottles.

Is the water system or the laboratory responsible for getting results to the State water or health agency?
The water system. Regardless of whether a State of private laboratory is used, the WS or PWS is ultimately held accountable. It is very important that the WS or PWS is in frequent contact with the laboratory to confirm: the samples reach the laboratory, the status of results (positive vs. negative), and the results are sent to the official State water or health agency in a timely manner (within 10 days of the sampling period).

Repeat Samples / Follow-up to Coliform Positive Samples

How am I notified of positive coliform samples?
If a routine or replacement sample is total coliform positive, the certified laboratory and the Drinking Water Agency will try to contact the WS or PWS by telephone using the contact name and number provided on the reporting form. All Water Systems should keep a small number of extra coliform bottles/reporting forms in case of required repeat sampling. If for some unforeseen reason, the WS or PWS does not have extra coliform bottles on hand, the laboratory will overnight coliform bottles to the facility. However, this is not a preferred situation. Remember, responsibility for timely sampling ultimately falls on the WS or PWS. Therefore, waiting for bottles to arrive in the mail incurs risk on the WS or PWS. Obviously, to receive "repeat" sample bottles in the mail is an indication that there is a positive sample and immediate action is needed.

What is a repeat sample?
Any sample that is analyzed as a follow-up to an initial positive result is referred to as "repeat" sample. After a routine coliform sample is found to be total coliform positive, repeat samples are required to confirm the initial positive result(s), to determine if the contamination is ongoing, and to evaluate the extent of the contamination within the distribution system.

How many repeat samples must I collect?
The number of repeats samples is dependent on the number of routine samples collected for the month. **Three** repeat samples are required for each distribution sample that is coliform positive; **however**, if the WS or PWS sends in only one routine distribution sample per month, **four** repeat samples are required for each sample that is coliform positive. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Where must repeat samples be collected?
If **three** repeat samples are required, one repeat sample must be collected from a tap within five (5) service connections upstream from the original sample, another repeat sample must be collected within five (5) service connections downstream from the original sampling site, and the last must be collected at the original site.
If **four** repeat samples are required, one repeat sample must be collected from a tap within five (5) service connections upstream from the original sample, another repeat sample must be collected within five (5) service connections downstream from the original sampling site, another must be collected at the original site, and the fourth may be collected anywhere within the distribution system (this may aid in identifying the possible source of contamination).

All repeat samples (or sample set) must be collected on the same day. Exceptions to this rule are WS or PWS with a single service connection. In this case the official State water or health agency may allow the WS or PWS to collect the repeat samples over a four-day period or to collect a single sample of at least 400 ml (300 ml if the system collects more than one sample per month). Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Where should the repeat samples be collected if the positive sample was collected at the end of the distribution system?
The WS or PWS is still required to collect three (or four when applicable) repeat samples. If the original sampling site is at the end of the distribution system (or one tap away from the end) the State Drinking Water agency may waive the requirement to collect one of the repeat samples downstream. An additional sample will be required upstream or from the same building. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

How many repeats are required if a finished water entry point sample or raw well sample is positive?
One. Only one repeat sample should be collected from the positive finished water entry point location or raw positive location. Raw or finished entry point samples (or raw/entry point repeat samples) are not used when determining compliance. Prior to August 2007, a routine coliform positive finished water (entry point) sample required three or four repeats. This has changed. Only one repeat is now required and it is to be collected from the same entry point location as the positive (downstream repeat samples are no longer required). Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

How much time do I have to collect repeat sample(s)?
24 Hours. If a routine or replacement sample is total coliform positive, the WS or PWS must collect a set of repeat samples within 24 hours of being notified of the positive result. Again, **all WS or PWS should keep a small number of extra coliform bottles/reporting forms in case of required repeat sampling.** If the WS or PWS is waiting for bottles to be shipped from the laboratory, repeat samples must be collected on the day of bottle receipt.

All repeat samples (or sample set) must be collected on the same day. Exceptions to this rule are WS or PWS with a single service connection. In this case the official State water or health agency may allow the WS or PWS to collect the repeat samples over a four-day period or to collect a single sample of at least 400 ml (300 ml if the system collects more than one sample per month). Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

When does the repeat sample collection "start clock" begin?
The 24-hour clock starts when the laboratory (or State) notifies the water system of the initial positive coliform result. You have 24 hours from the time of notification to collect your repeat samples and return them to a laboratory for analysis. If you fail to meet this window, a violation will be issued, provided that no extension had been granted.

If the WS or PWS cannot be reached via phone, the official State water or health agency has defined "notified" as the date that the laboratory initiates shipment of repeat sample bottles. The laboratory records this date on the coliform reporting form. The repeat samples must be collected on the on the day that the bottles are received at the WS or PWS. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

What if I cannot meet the 24-hour repeat collection requirement?
your official State water or health agency acknowledges that some circumstances may arise, totally beyond the control of the WS or PWS, which prevent repeat samples from being collected within the 24-hour period. Therefore, extensions may be granted by your **Drinking Water Agency**. A valid reason for the extension must be provided. Extensions cannot be granted after the fact. Therefore, requests for extensions MUST be made prior to or at the same time the repeat samples are being collected. Extensions will always specify exactly how much time the WS or PWS has to collect and return the repeat samples. Failure to obtain the extension or failure to meet the terms of the extension will result in a monitoring violation. Please remember that an extension on the 24-hour repeat collection requirement is NOT a waiver for actually collecting the repeat samples. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

What happens if I am notified on a Friday of positive routine results (or receive repeat bottles on a Friday or Holiday)?
The 24-hour collection requirement must still be met. You should contact your **certified laboratory** to arrange a time on Saturday to collect the repeat samples and drive them to the laboratory. If this is not possible, the WS or PWS must request an extension the first business day following the weekend or holiday. Please call the official State water or health agency at at the earliest possible time to request an extension on the 24-hour requirement.

It is strongly recommended that all routine coliform samples be collected and mailed on a Monday or Tuesday to avoid this situation.

What happens if any of the repeats are positive or invalid?
If one or more repeat samples in the set are total coliform positive or invalid, the whole repeat monitoring process must start over. A new "set" of three or four (if only one routine sample is collected per month) repeats must be collected within 24 hours of being notified of the positive or invalid repeat.

If a repeat sample location is positive and is not from the same location as the original positive sample (and/or the original site is negative), the next repeat collection should be based on the original positive site location and NOT the positive repeat location. Every consecutive set of repeat samples must be collected at the same locations as the 1st set of repeat samples.

The WS or PWS must repeat this process until either total coliform are not detected in one complete "set" of repeat samples or the WS or PWS determines that the total coliform Maximum Contaminant Level(MCL) has been exceeded and notifies your official State water or health agency. It is highly recommended that sampling be repeated until a "set" is satisfactory. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Does one (or more) positive routine or repeat sample change the following month's monitoring requirements?
Yes. If you collect less than 5 routine distribution samples per month and have at least one positive routine, repeat or replacement sample, 5 routine distribution samples MUST be collected the following month. The samples can be collected from other approved coliform sites or from other locations in the distribution system. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

Who is responsible for notifying the official State water or health agency if results are positive?
The WS or PWS is responsible for notifying your official State water or health agency when a total coliform positive sample is found and for having the appropriate repeat samples analyzed. In most cases, an agreement has been made between the certified laboratory and the WS or PWS that the laboratory will notify the State Agency; however, even in this case, the WS or PWS is held accountable for this notification requirement.

Invalidating Sample Results
Can a sample result be invalidated?
A coliform positive can be invalidated when there is a significant reason to believe the test results are not accurate or not representative of the water quality. These samples are not used in compliance calculations and a replacement must be collected within the same monitoring period (same month) at the same location to avoid a possible monitoring violation.

There are three conditions in which a total coliform positive sample result may be invalidated:
1. The laboratory establishes that an error in its analytical procedure caused the total coliform positive result.
2. The State water or health agency, on the basis of the results of repeat samples collected determines that the total coliform positive sample resulted from a domestic or other non-distribution system-plumbing problem.
3. The State water or health agency determines that there are substantial grounds to believe that a total coliform positive result is due to a circumstance or condition that does not reflect water quality in the distribution system.

The laboratory will invalidate the results if they are unable to obtain a true result according to the test method used to analyze the sample. If a laboratory invalidates a routine sample due to interference, the WS or PWS must collect another sample from the same location as the original sample within 24 hours of being notified of the interference problem.

How can I have a positive coliform result invalidated?
Always check with your Agency to ensure this rule is correct, for this rule is different in some States. The invalidation process involves 3 steps:
1. All repeat samples must have been collected in accordance with the repeat sampling requirements.
2. As soon as you feel a sample should be invalidated, the **Drinking Water Agency** should be contacted by telephone and the situation discussed. If the Drinking Water Agency verbally agrees that the sample is not representative of the water quality, they will direct you as to what certain steps need to be taken (e.g., proof, additional samples, etc.). Ultimately, the Drinking Water Agency will recommend to the Compliance or Regulatory division whether or not a sample should be invalidated. Failure to get the Drinking Water Agency's concurrence will result in your request being rejected by the Compliance or Regulatory division.
3. A formal written requested must be mailed to the **Drinking Water Agency** and Compliance or Regulatory division within four weeks of the original routine sample collection date. The written documentation must state the specific cause of the total coliform positive sample and what action the supplier has taken, or will take, to correct this problem. The official State water or health agency will provide a written notification to the WS or PWS as to whether or not the request for invalidation was granted.

The State water or health agency will not invalidate a total coliform positive sample solely on the grounds that all repeat samples are total coliform negative.

How soon do I have to initiate the invalidation process?
At the time of receiving notice of positive results, the WS or PWS should immediately begin an investigation and collect repeat samples. At that time, any reason to question validity of a result should be acted on promptly while the situation is fresh at hand.

If a sample is invalidated by the State water or health agency or the certified laboratory does it still count towards meeting the monthly monitoring requirements?
No. You MUST collect another routine sample to replace any invalidated routine sample. To confirm the sample purpose look at the reporting form. Remember that all routine samples are marked with a sample purpose of "Routine" on the reporting form. The replacement sample(s) MUST be collected within the same monitoring period (same month). Repeat samples cannot be used to meet this requirement. Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

My repeat sample is invalid. Now what?
If one or more repeat samples in a set are invalid, the whole repeat monitoring process must begin over starting with the collection of a new "set" (3 or 4) of repeat samples within 24 hours. Every consecutive set of repeat samples should be collected at the same locations as the 1st set of repeats.

Compliance, Violations, and Follow-up Actions

What is an MCL?
Maximum Contaminant Level. State and federal regulations set maximum contaminant levels (MCLs) on contaminants that have been determined to cause possible health effects. When MCLs are exceeded, the WS or PWS must accomplish all the required actions that generally include public notice within a specified time period. There are two types of coliform MCL violations, monthly and acute.

What is a MONTHLY coliform MCL exceedance and how is it determined?
A monthly coliform MCL violation occurs when a WS or PWS exceeds the number of allowed coliform positive samples. The number allowed depends on the number of routine distribution samples collected during the monthly sample period.

What is an ACUTE coliform MCL exceedance and how is it determined?
Acute MCLs are extremely serious and require immediate action. Coliform sample results have indicated an immediate threat to the WS or PWS and serious health effects can result. Therefore, water customers must be notified without delay. All samples that are total coliform positive must be examined for fecal coliform or *E. coli.* Always check with your Agency to ensure this rule is correct, for this rule is different in some States.

What is a monitoring violation and how can I avoid one?
A monitoring violation occurs when the coliform monitoring requirements for a month are not met. All WS or PWS are required to submit samples for bacteriological analysis during each calendar month. Do NOT sample before the beginning of the month.

The WS or PWS must meet several criteria to avoid a monitoring violation:
1. At a minimum, collect the required number of distribution samples each month. The number of distribution samples required is based on population served. It is crucial that every WS or PWS operator know the coliform monitoring requirements for his or her WS or PWS.

2. Sampling locations used must be in accordance with the approved written coliform sample site plan. All sampling areas must be represented during each monthly sampling period.

3. All replacement and repeat samples must be returned promptly to the laboratory for analysis. These samples will be included in determining compliance.

4. Repeat samples and replacement samples for invalid (negative coliform growth) samples must be collected within 24 hours of notification. If these samples cannot be collected within 24 hours, you must contact the official State water or health agency for an extension. The time extension will be given on a case-by-case basis and will always specify exactly how much time the WS or PWS has to collect and return the repeat samples. Failure to obtain the extension or failure to meet the terms of the extension will result in a monitoring violation.

5. If a private certified laboratory analyzes samples, it is the responsibility of the WS or PWS to make sure that copies of the reports are sent to the official State water or health agency within 10 days of the end of the sampling period (month).

6. Collect at least five coliform distribution system samples the month following a coliform positive finished or distribution system sample.

How are repeat samples used in determining compliance?
Repeat samples are not counted towards determining monthly distribution monitoring compliance. However, failure to collect repeats within 24-hours (following notification of a positive routine sample) will result in a monitoring violation (and possible MCL violation) unless the WS or PWS obtained a waiver from the 24-hour collection requirement. In addition, if a WS or PWS fails to collect any of the repeat samples for a positive routine sample, the missing repeats will be "assumed" positive and included when totaling the number of positive samples for the month. In most cases, this will result in an MCL violation and require public notification.

Do I need to notify the water customers of a violation?
Yes. Each violation and situation requiring notice has been assigned to one of three categories, or tiers, based on the risk of adverse health effects. Public notice is required for any of the following:

Tier 1 Violations or other Situations
Tier 1 violations or problems may result in an immediate adverse health problem for some consumers. Violation of the MCL for total coliform, when ***fecal coliform or E. coli*** are present in the water distribution system, or ***failure to test*** for fecal coliform or *E. coli* when any repeat sample tests positive for coliform.

Tier 2 Violations
All violations of the ***MCL***, ***MRDL***, and ***treatment technique*** requirements ***except*** where ***Tier 1 notice*** is required Violations of the ***monitoring*** requirements **when** required by the official State water or health agency (see Tier 3).

Tier 3 Violations or other Situations
Monitoring violations, except where Tier 1 or Tier 2 notice is required as determined by your official State water or health agency.

Common Mistakes to Avoid
Below describes some common errors that may result in a violation.
1. WS or PWS does not collect the correct number of repeat samples following a routine positive sample.
2. Failure of small WS or PWS (that serve a population 4,100 or less) to collect a minimum of 5 routine distribution coliform samples the month following a coliform positive sample.
3. Marking the wrong sample purpose box on the reporting form. Most often, this occurs when collecting a repeat (due to positive sample) sample and marking it as an invalid replacement or replacement (due to invalid, TNTC, or broken) sample and vice versa.
4. Failure to use sample site numbers.
5. Failure to report the chlorine residual (if chlorine/chloramines is added) on the reporting form.
6. Failure to monitor early in the month. Waiting until the end of the month to collect the routine samples does not allow enough time for follow-up actions if required.

Proper Sampling Handling

The proper handling of water quality samples also includes wearing gloves. Gloves not only protect field personnel, but also prevent potential contamination to the water sample. Always wear powderless, disposable gloves. When sampling for inorganics, wear latex gloves. Nitrile gloves are appropriate for organics.

The following will provide a field reference for chain of custody procedures, sampling surface water and ground water, and further provide procedures for measuring field parameters and handling water-quality samples.

Use chain-of-custody procedures when coolers and containers are prepared, sealed and shipped. They will remain sealed until used in the field. When making arrangements with the laboratory, make sure you request enough containers, including those for blank and duplicate samples. Order extra sample bottles to allow for breakage or contamination in the field.

Some samples require low-temperature storage and/or preservation with chemicals to maintain their integrity during shipment and before analysis in the laboratory. The most common preservatives are hydrochloric, nitric, sulfuric and ascorbic acids, sodium hydroxide, sodium thiosulfate, and biocides. Many laboratories provide pre-preserved bottles filled with measured amounts of preservatives.

Although most federal and state agencies allow the use of pre-preserved sample containers, some may require either cool temperatures or added preservatives in the field.

When the containers and preservatives are received from the laboratory, check to see that none have leaked. Be aware that many preservatives can burn eyes and skin, and must be handled carefully.

Sampling bottles should be labeled with type of preservative used, type of analysis to be done and be accompanied by A Material Safety Data Sheet (**MSDS**). Make sure you can tell which containers are pre-preserved, because extra care must be taken not to overfill them when collecting samples in the field. Check with the laboratory about quality control procedures when using pre-preserved bottles.

Coolers used for sample shipment must be large enough to store containers, packing materials and ice. Obtain extra coolers, if necessary. Never store coolers and containers near solvents, fuels or other sources of contamination or combustion. In warm weather, keep coolers and samples in the shade.

Field Parameters
Measure and record the field parameters of temperature, electrical conductivity, pH and dissolved oxygen in an undisturbed section of streamflow. Other parameters may be measured, if desired.

***(Check with your governmental environmental or health agency for more information*).**

QA/QC Measures

In addition to standard samples, the field technicians collect equipment blanks (**EB**), field cleaned equipment blanks (**FB**), split samples (**SS**), and field duplicate samples (**FD**).

Overall care must be taken in regards to equipment handling, container handling/storage, decontamination, and record keeping. Sample collection equipment and non preserved sample containers must be rinsed three times with sample water before the actual sample is taken. Exceptions to this are any pre-preserved container or bac-t type samples.

If protective gloves are used, they shall be clean, new and disposable. These should be changed upon arrival at a new sampling point.

Highly contaminated samples shall never be placed in the same ice chest as environmental samples. It is good practice to enclose highly contaminated samples in a plastic bag before placing them in ice chests. The same is true for wastewater and drinking water samples.

Ice chests or shipping containers with samples suspected of being highly contaminated shall be lined with new, clean, plastic bags.

If possible, one member of the field team should take all the notes, fill out labels, etc., while the other member does all of the sampling.

Preservation of Samples
Proper sample preservation is the responsibility of the sampling team, not the lab providing sample containers. The best reference for preservatives is a current edition of ***Standard Methods*** or your local sampling laboratory.

It is the responsibility of the field team to assure that all samples are appropriately preserved.

- Follow the preservative solution preparation instructions.
- Always use strong safety precautions diluting the acid.
- Put a new label on the dispensing bottle with the current date.
- Slowly add the acid or other preservative to the water sample; not water to the acid or preservative.
- Wait 3-4 hours for the preservative to cool most samples down to 4°C.
- Most preservatives have a shelf life of one year from the preparation date.

When samples are analyzed for TKN, TP, NH_4 and NOx, 1 mL of 50% Trace Metal grade sulfuric acid is added to the each discrete auto sampler bottles/bags in the field lab before sampling collection. The preservative maintains the sample at 1.5<pH<2 after collection. To meet maximum holding time for these preserved samples (28 days), pull and ship samples every 14 days. Narrow range pH paper (test strips) can be used to test an aliquot of the preserved sample. Place the pH paper into the container and compare the color with the manufacturer's color chart.

Collection of Surface Water Samples

Representative samples may be collected from rivers, streams and lakes if certain rules are followed:

1. Watch out for flash floods! If a flooding event is likely and samples must be obtained, always go in two-person teams for safety. Look for an easy route of escape;

2. Select a sampling location at or near a gauging station so that stream discharge can be related to water-quality loading. If no gauging station exists, then measure the flow rate at the time of sampling, using the streamflow method described below;

3. Locate a straight and uniform channel for sampling;

4. Unless specified in the sampling plan, avoid sampling locations next to confluences or point sources of contamination;

5. Use bridges or boats for deep rivers and lakes where wading is dangerous or impractical;

6. Do not collect samples along a bank as they may not be representative of the surface water body as a whole; and

7. Use appropriate gloves when collecting the sample.

Streamflow Measurement

Before collecting water quality samples, record the stream's flow rate at the selected station. The flow rate measurement is important for estimating contaminant loading and other impacts. The first step in streamflow measurement is selecting a cross-section. Select a straight reach where the stream bed is uniform and relatively free of boulders and aquatic growth. Be certain that the flow is uniform and free of eddies, slack water and excessive turbulence. After the cross-section has been selected, determine the width of the stream by stringing a measuring tape from bank-to-bank at right angles to the direction of flow. Next, determine the spacing of the verticals. Space the verticals so that no partial section has more than 5 percent of the total discharge within it.



At the first vertical, face upstream and lower the velocity meter to the channel bottom, record its depth, then raise the meter to 0.8 and 0.2 of the distance from the stream surface, measure the water velocities at each level, and average them.

Move to the next vertical and repeat the procedure until you reach the opposite bank. Once the velocity, depth and distance of the cross-section have been determined, the mid-section method can be used for determining discharge. Calculate the discharge in each increment by multiplying the averaged velocity in each increment by the increment width and averaged depth.

(Note that the first and last stations are located at the edge of the waterway and have a depth and velocity of zero.) Add up the discharges for each increment to calculate total stream discharge. Record the flow in liters (or cubic feet) per second in your field book.

(*Check with your governmental environmental or health agency for more information*)

River and Stream Sampling

Collection of samples from rivers and stream involves transporting all necessary items to the water-quality station and setting up field notes, instrumentation, filtration equipment (if not performed elsewhere as with microbiologicals), sample containers and decontamination washes near the channel. The first step is to measure all field parameters and then measure streamflow. After collecting and preserving the samples, equipment storage and decontamination will follow. Avoid spills when decontaminating equipment. For remote sites, extra collections equipment may be used to eliminate the need for field decontamination. Your governmental agencies have written procedures covering all aspects of surface-water characterization and sampling.

Composite Sampling
Composite sampling is intended to produce a water quality sample representative of the total stream discharge at the sampling station. If your sampling plan calls for composite sampling, use an automatic type sampler.

River or Channel Grab Sampling
Grab sampling is performed when uniform mixing in the river or stream channel makes composite sampling unnecessary, when point samples are desired, when sample degassing may occur, or when the water is too shallow for composite sampling. Record any decision to use grab sampling in the sampling plan. For streams at least 4 inches (10 cm) deep, collect grab samples in the middle of the channel using a laboratory cleaned or decontaminated glass or plastic container, and add the required preservatives.



An automatic refrigerator sampler with a Pickle Jar, this automatic sampler can also do grab type samples.

Laboratory
123 W. Main St
Sun City, Arizona 85541

LAB I.D. NUMBER

Sampler: DATE: PAGE 1 OF 1

Company:
Department:
Address:
Contact:
Telephone:

Sample Identification	Date	Time	Matrix	Lab ID	Metals* See Attached	TSS	Lead/Copper	BOD/COD	Nitrate	Nitrate + Nitrite	TKN / Ammonia	VOC / THM's	Semi Volital Organics (625)	Chloride		Cyanide	Florida	Surfactants (MBAS)	Tot. Coliform MPN	Fecal Coliform MPN-HPC	Organo-Phosphorus Pest. (8141)	Sulfate	EC Conductivity	Number/Containers

Project Name

Project Number

Field Measurements:

pH:
Temp:

Sample Receipt

No. Containers: _____
Custody Seals: Yes No
Received Intact: Yes No
Received Cold: Yes No
Temperature:
PRIORITY:

RELINQUISHED BY:

Signature: Time:
Printed Name: Date:
Company

RELINQUISHED BY:

Signature: Time:
Printed Name: Date:
Company

SAMPLED RECEIVED BY:

Signature: Time:
Printed Name: Date:
Company

SAMPLED RECEIVED BY:

Signature: Time:
Printed Name: Date:
Company

Chain-of-Custody Report Example

Chain of Custody Procedures

Because a sample is physical evidence, chain of custody procedures are used to maintain and document sample possession from the time the sample is collected until it is introduced as evidence. Chain of custody requirements will vary from agency to agency. However, these procedures are similar and the chain of custody outlined in this manual is only a guideline. Consult your project manager for specific requirements.

If you have physical possession of a sample, have it in view, or have it physically secured to prevent tampering, then it is defined as being in "***custody***." A chain of custody record, therefore, begins when the sample containers are obtained from the laboratory. From this point on, a chain of custody record will accompany the sample containers.

Handle the samples as little as possible in the field. Each custody sample requires a chain of custody record and may require a seal. If you do not seal individual samples, then seal the containers in which the samples are shipped.

When the samples transfer possession, both parties involved in the transfer must sign, date and note the time on the chain of custody record. If a shipper refuses to sign, you must seal the samples and chain of custody documents inside a box or cooler with bottle seals or evidence tape. The recipient will then attach the shipping invoices showing the transfer dates and times to the custody sheets.

If the samples are split and sent to more than one laboratory, prepare a separate chain of custody record for each sample. If the samples are delivered to after hours night drop-off boxes, the custody record should note such a transfer and be locked with the sealed samples inside sealed boxes.

Laboratory Analysis Section

Although development of an acceptable immunomagnetic separation system for *Giardia* lagged behind development of an acceptable system for *Cryptosporidium*, an acceptable system was identified in October 1998, and EPA validated a method for simultaneous detection of *Cryptosporidium* and *Giardia* in February 1999 and developed quality control (QC) acceptance criteria for the method based on this validation study. To avoid confusion with Method 1622, which already had been validated and was in use both domestically and internationally as a stand-alone *Cryptosporidium*-only detection method, EPA designated the new combined procedure EPA Method 1623.

The interlaboratory validated versions of Method 1622 (January 1999; EPA-821-R-99-001) and Method 1623 (April 1999; EPA-821-R-99-006) were used to analyze approximately 3,000 field and QC samples during the Information Collection Rule Supplemental Surveys (ICRSS) between March 1999 and February 2000. Method 1622 was used to analyze samples from March 1999 to mid-July 1999; Method 1623 was used from mid-July 1999 to February 2000. The April 2001 revision of both methods include updated QC acceptance criteria based on analysis of the QC samples analyzed during the ICRSS.

EPA Method 1623 is a performance-based method applicable to the determination of *Cryptosporidium* and *Giardia* in aqueous matrices. EPA Method 1623 requires filtration, immunomagnetic separation of the oocysts and cysts from the material captured, and an immunofluorescence assay for determination of oocyst and cyst concentrations, with confirmation through vital dye staining and differential interference contrast microscopy.

The interlaboratory validation of EPA Method 1623 conducted by the EPA used the Pall Gelman capsule filtration procedure, Dynal immunomagnetic separation (IMS) procedure, and Meridian sample staining procedure are described in this document. Alternate procedures are allowed, provided that required quality control tests are performed and all quality control acceptance criteria in this method are met.

Since the interlaboratory validation of EPA Method 1623, interlaboratory validation studies have been performed to demonstrate the equivalency of modified versions of the method using the following components:
- Whatman Nuclepore CryptTest™ filter
- IDEXX Filta-Max™ filter
- Waterborne Aqua-Glo™ G/C Direct FL antibody stain
- Waterborne Crypt-a-Glo™ and Giardi-a-Glo™ antibody stains

The validation studies for these modified versions of the method met EPA performance-based measurement system Tier 2 validation for nationwide use (see Section 9.1.2 for details), and have been accepted by the EPA as equivalent in performance to the original version of the method validated by the EPA.

The equipment and reagents used in these modified versions of the method are noted in Sections 6 and 7 of the method; the procedures for using these equipment and reagent options are available from the manufacturers.

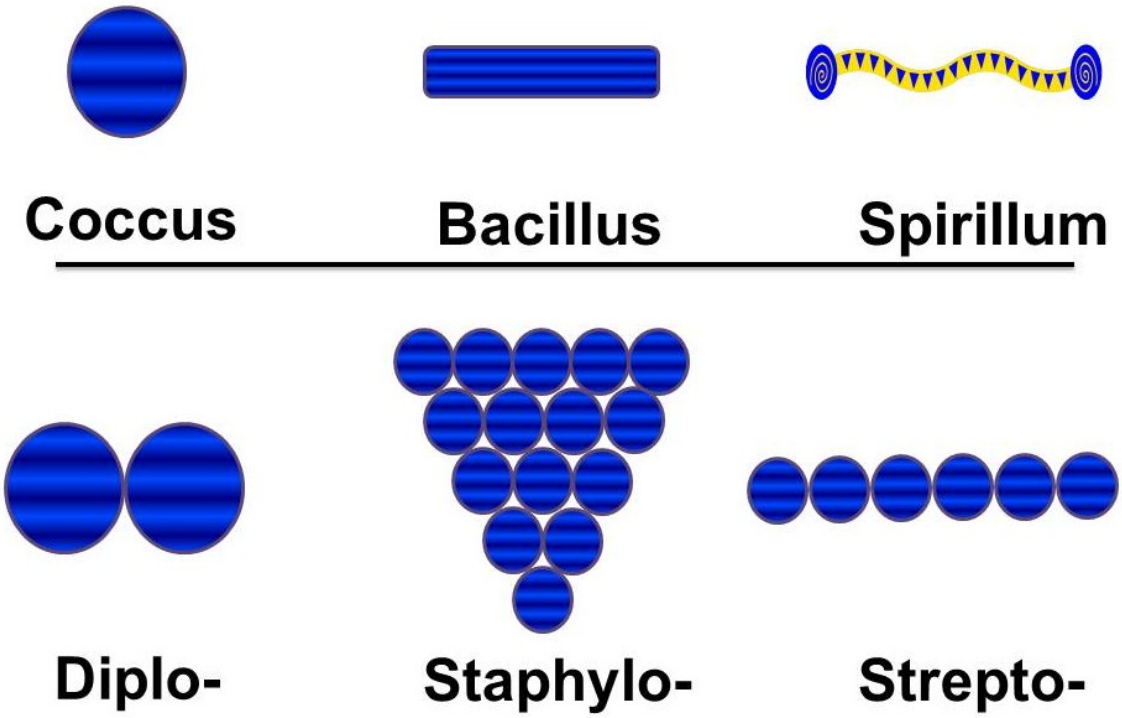
Because this is a performance-based method, other alternative components not listed in the method may be available for evaluation and use by the laboratory. Confirming the acceptable performance of the modified version of the method using alternate components in a single laboratory does not require an interlaboratory validation study be conducted.

However, method modifications validated only in a single laboratory have not undergone sufficient testing to merit inclusion in the method.

Only those modified versions of the method that have been demonstrated as equivalent at multiple laboratories and multiple water sources through a Tier 2 interlaboratory study will be cited in the method.

The EPA initiated an effort in 1996 to identify new and innovative technologies for protozoan monitoring and analysis. After evaluating potential alternatives to the then-current method through literature searches, discussions with research and commercial laboratories, and meetings with experts in the field, the Engineering and Analysis Division within the Office of Science and Technology within the EPA's Office of Water developed draft Method 1622 for *Cryptosporidium* detection in December 1996.

This *Cryptosporidium*-only method was validated through an interlaboratory study in August 1998, and was revised as a final, valid method for detecting *Cryptosporidium* in water in January 1999.



Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration IMS/FA

1.0 Scope and Application

1.1 This method is for determination of the identity and concentration of *Cryptosporidium* (CAS Registry number 137259-50-8) and *Giardia* (CAS Registry number 137259-49-5) in water by filtration, immunomagnetic separation (IMS), and immunofluorescence assay (FA) microscopy. *Cryptosporidium* and *Giardia* may be confirmed using 4',6-diamidino-2-phenylindole (DAPI) staining and differential interference contrast (DIC) microscopy. The method has been validated in surface water, but may be used in other waters, provided the laboratory demonstrates that the method's performance acceptance criteria are met.

1.2 This method is designed to meet the survey and monitoring requirements of the U.S. Environmental Protection Agency (EPA). It is based on laboratory testing of recommendations by a panel of experts convened by EPA. The panel was charged with recommending an improved protocol for recovery and detection of protozoa that could be tested and implemented with minimal additional research.

1.3 This method will not identify the species of *Cryptosporidium* or *Giardia* or the host species of origin, nor can it determine the viability or infectivity of detected oocysts and cysts.

1.4 This method is for use only by persons experienced in the determination of *Cryptosporidium* and *Giardia* by filtration, IMS, and FA. Experienced persons are defined in Section 22.2 as analysts. Laboratories unfamiliar with analyses of environmental samples by the techniques in this method should gain experience using water filtration techniques, IMS, fluorescent antibody staining with monoclonal antibodies, and microscopic examination of biological particulates using bright-field and DIC microscopy.

1.5 Any modification of the method beyond those expressly permitted is subject to the application and approval of alternative test procedures under 40 *CFR* Part 141.27.

2.0 Summary of Method

2.1 A water sample is filtered and the oocysts, cysts, and extraneous materials are retained on the filter. Although EPA has only validated the method using laboratory filtration of bulk water samples shipped from the field, field-filtration also can be used.

2.2 Elution and separation

2.2.1 Materials on the filter are eluted and the eluate is centrifuged to pellet the oocysts and cysts, and the supernatant fluid is aspirated.

2.2.2 The oocysts and cysts are magnetized by attachment of magnetic beads conjugated to anti-*Cryptosporidium* and anti-*Giardia* antibodies. The magnetized oocysts and cysts are separated from the extraneous materials using a magnet, and the extraneous materials are discarded. The magnetic bead complex is then detached from the oocysts and cysts.

2.3 Enumeration

2.3.1 The oocysts and cysts are stained on well slides with fluorescently labeled monoclonal antibodies and 4',6-diamidino-2-phenylindole (DAPI). The stained sample is examined using fluorescence and differential interference contrast (DIC) microscopy.

2.3.2 Qualitative analysis is performed by scanning each slide well for objects that meet the size, shape, and fluorescence characteristics of Cryptosporidium oocysts or Giardia cysts. Potential oocysts or cysts are confirmed through DAPI staining characteristics and DIC microscopy. Oocysts and cysts are identified when the size, shape, color, and morphology agree with specified criteria and examples in a photographic library.

2.3.3 Quantitative analysis is performed by counting the total number of objects on the slide confirmed as oocysts or cysts.

2.4 Quality is assured through reproducible calibration and testing of the filtration, immunomagnetic separation (IMS), staining, and microscopy systems. Detailed information on these tests is provided in Section 9.0.

3.0 Definitions

3.1 *Cryptosporidium* is defined as a protozoan parasite potentially found in water and other media. The six species of *Cryptosporidium* and their potential hosts are *C. parvum* (mammals, including humans); *C. baileyi* and *C. meleagridis* (birds); *C. muris* (rodents); *C. serpentis* (reptiles); and *C. nasorum* (fish).

3.2 *Giardia* is defined as a protozoan parasite potentially found in water and other media. The two species of *Giardia* and their potential hosts are *G. intestinalis* (humans) and *G. muris* (mice).

3.3 Definitions for other terms used in this method are given in the glossary (Section 22.0).

4.0 Contamination, Interferences, and Organism Degradation

4.1 Turbidity caused by inorganic and organic debris can interfere with the concentration, separation, and examination of the sample for *Cryptosporidium* oocysts and *Giardia* cysts. In addition to naturally-occurring debris, such as clays and algae, chemicals, such as iron and alum coagulants and polymers, may be added to finished waters during the treatment process, which may result in additional interference.

4.2 Organisms and debris that autofluoresce or demonstrate non-specific fluorescence, such as algal and yeast cells, when examined by epifluorescent microscopy, may interfere with the detection of oocysts and cysts and contribute to false positives by immunofluorescence assay (FA).

4.3 Solvents, reagents, labware, and other sample-processing hardware may yield artifacts that may cause misinterpretation of microscopic examinations for oocysts and cysts. All materials used shall be demonstrated to be free from interferences under the conditions of analysis by running a method blank (negative control sample) initially and a minimum of every week or after changes in source of reagent water. Specific selection of reagents and purification of solvents and other materials may be required.

4.4 Interferences co-extracted from samples will vary considerably from source to source, depending on the water being sampled. Experience suggests that high levels of algae, bacteria, and other protozoa can interfere in the identification of oocysts and cysts (Reference 20.1).

4.5 Freezing samples, filters, eluates, concentrates, or slides may interfere with the detection and/or identification of oocysts and cysts.

4.6 All equipment should be cleaned according to manufacturers' instructions. Disposable supplies should be used wherever possible.

5.0 Safety

5.1 The biohazard associated with, and the risk of infection from, oocysts and cysts is high in this method because live organisms are handled. This method does not purport to address all of the safety problems associated with its use. It is the responsibility of the laboratory to establish appropriate safety and health practices prior to use of this method. In particular, laboratory staff must know and observe the safety procedures required in a microbiology laboratory that handles pathogenic organisms while preparing, using, and disposing of sample concentrates, reagents and materials, and while operating sterilization equipment.

5.2 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of Occupational Safety and Health Administration regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets should be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 20.2 through 20.5.

5.3 Samples may contain high concentrations of biohazards and toxic compounds, and must be handled with gloves and opened in a biological safety cabinet to prevent exposure. Reference materials and standards containing oocysts and cysts must also be handled with gloves and laboratory staff must never place gloves in or near the face after exposure to solutions known or suspected to contain oocysts and cysts. Do not mouth-pipette.

5.4 Laboratory personnel must change gloves after handling filters and other contaminant-prone equipment and reagents. Gloves must be removed or changed before touching any other laboratory surfaces or equipment.

5.5 Centers for Disease Control (CDC) regulations (42 CFR 72) prohibit interstate shipment of more than 4 L of solution known to contain infectious materials. State regulations may contain similar regulations for intrastate commerce. Unless the sample is known or suspected to contain *Cryptosporidium*, *Giardia*, or other infectious agents (e.g., during an outbreak), samples should be shipped as noninfectious and should not be marked as infectious. If a sample is known or suspected to be infectious, and the sample must be shipped to a laboratory by a transportation means affected by CDC or state regulations, the sample should be shipped in accordance with these regulations.

6.0 Equipment and Supplies

NOTE*: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.*

6.1 Sample collection equipment for shipment of bulk water samples for laboratory filtration. Collapsible LDPE cubitainer for collection of 10-L bulk sample(s)—Cole Parmer cat. no. U-06100-30 or equivalent. Fill completely to ensure collection of a full 10-L sample. Discard after one use.

6.2 Equipment for sample filtration. Three options have been demonstrated to be acceptable for use with Method 1623. Other options may be used if their acceptability is demonstrated according to the procedures outlined in Section 9.1.2.

6.2.1 Cubitainer spigot to facilitate laboratory filtration of sample (for use with any filtration option)—Cole Parmer cat. no. U-06061-01, or equivalent.

6.2.2 Envirochek™ sampling capsule equipment requirements for use with the procedure described in Section 12.0. The version of the method using this filter was validated using 10-L sample volumes; alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and source water samples (Section 9.1.2).

6.2.2.1 Sampling capsule—Envirochek™, Pall Gelman Laboratory, Ann Arbor, MI, product 12110

6.2.2.2 Laboratory shaker with arms for agitation of sampling capsules

6.2.2.2.1 Laboratory shaker—Lab-Line model 3589, VWR Scientific cat. no. 57039-055, Fisher cat. no. 14260-11, or equivalent

6.2.2.2.2 Side arms for laboratory shaker—Lab-Line Model 3587-4, VWR Scientific cat. no. 57039-045, Fisher cat. no. 14260-13, or equivalent

6.2.3 CrypTest™ capsule filter equipment requirements. Follow the manufacturer's instructions when using this filtration option. The version of the method using this filter was validated using 10-L sample volumes; alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and matrix samples (Section 9.1.2).

6.2.3.1 Capsule filter—CrypTest™, Whatman Inc, Clifton, NJ, product no. 610064

6.2.3.2 Cartridge housing—Ametek 5-in. clear polycarbonate, Whatman cat. no. 71503, or equivalent

6.2.3.3 Ultrasonic bath—VWR Model 75T#21811-808, or equivalent

6.2.3.4 Laboratory tubing—Tygon formula R-3603, or equivalent

6.2.4 Filta-Max™ foam filter equipment requirements. Follow the manufacturer's instructions when using this filtration option. The version of the method using this filter was validated using 50-L sample volumes; alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and matrix samples (Section 9.1.2).

6.2.4.1 Foam filter—Filta-Max™, IDEXX, Westbrook, ME. Filter module and membrane: product code FMC 10601; filter membranes (100 pack), product code FMC 10800

NOTE: Check at least one filter per batch to ensure that the filters have not been affected by improper storage or other factors that could result in brittleness or other problems. At a minimum confirm that the test filter expands properly in water before using the batch or shipping filters to the field.

6.2.4.2 Filter processing equipment—Filta-Max starter kit, IDEXX, Westbrook, ME, cat. no. FMC 11002. Includes all equipment required to run and process Filta-Max filter modules (manual wash station (FMC 10102) including plunger head (FMC 12001), elution tubing set (FMC 10301), vacuum set (FMC 10401), filter housing (FMC 10501), and magnetic stirrer (FMC 10901).

6.3 Ancillary sampling equipment

6.3.1 Tubing—Glass, polytetrafluoroethylene (PTFE), high-density polyethylene (HDPE), or other tubing to which oocysts and cysts will not easily adhere—Tygon formula R-3603, or equivalent. If rigid tubing (glass, PTFE, HDPE) is used and the sampling system uses a peristaltic pump, a minimum length of compressible tubing may be used in the pump. Before use, the tubing must be autoclaved, thoroughly rinsed with detergent solution, followed by repeated rinsing with reagent water to minimize sample contamination. Alternately, decontaminate using hypochlorite solution, sodium thiosulfate, and multiple reagent water rinses; dispose of tubing when wear is evident. Dispose of tubing after one use whenever possible.

6.3.2 Flow control valve—0.5 gpm (0.03 L/s), Bertram Controls, Plast-O-Matic cat. no. FC050B½-PV, or equivalent; or 0.4- to 4-Lpm flow meter with valve—Alamo Water Treatment, San Antonio, TX, cat. no. R5310, or equivalent.

6.3.3 Centrifugal pump—Grainger, Springfield, VA, cat. no. 2P613, or equivalent

6.3.4 Flow meter—Sameco cold water totalizer, E. Clark and Associates, Northboro, MA, product no. WFU 10.110, or equivalent.

6.4 Equipment for spiking samples in the laboratory

6.4.1 10-L carboy with bottom delivery port (½")—Cole-Palmer cat. no. 06080-42, or equivalent; calibrate to 10.0 L and mark level with waterproof marker.

6.4.2 Stir bar—Fisher cat. no. 14-511-93, or equivalent.

6.4.3 Stir plate—Fisher cat. no. 14-493-120S, or equivalent.

6.4.4 Hemacytometer—Neubauer type, Hauser Scientific, Horsham, PA, cat. no. 3200 or 1475, or equivalent.

6.4.5 Hemacytometer coverslip—Hauser Scientific, cat. no. 5000 (for hemacytometer cat. no. 3200) or 1461 (for hemacytometer cat. no 1475), or equivalent.

6.4.6 Lens paper without silicone—Fisher cat. no. 11-995, or equivalent.

6.4.7 Polystyrene or polypropylene conical tubes with screw caps—15- and 50-mL.

6.4.8 Equipment required for enumeration of spiking suspensions using membrane filters.

6.4.8.1 Glass microanalysis filter holder—25-mm-diameter, with fritted glass support, Fisher cat. no. 09-753E, or equivalent. Replace stopper with size 8, one-hole rubber stopper, Fisher Cat. No. 14-135M, or equivalent.

6.4.8.2 Three-port vacuum filtration manifold and vacuum source—Fisher Cat. No. 09-753-39A, or equivalent.

6.4.8.3 Cellulose acetate support membrane—1.2-µm-pore-size, 25-mm-diameter, Fisher cat. no. A12SP02500, or equivalent.

6.4.8.4 Polycarbonate track-etch hydrophilic membrane filter—1-µm-pore-size, 25-mm-diameter, Fisher cat. no. K10CP02500, or equivalent.

6.4.8.5 100 × 15 mm polystyrene Petri dishes (bottoms only).

6.4.8.6 60 × 15 mm polystyrene Petri dishes.

6.4.8.7 Glass microscope slides—1 in. × 3 in or 2 in. × 3 in.

6.4.8.8 Coverslips—25 mm^2

6.5 Immunomagnetic separation (IMS) apparatus

6.5.1 Sample mixer—Dynal Inc., Lake Success, NY, cat. no. 947.01, or equivalent.

6.5.2 Magnetic particle concentrator for 10-mL test tubes—Dynal MPC-1® , cat. no. 120.01, or equivalent.

6.5.3 Magnetic particle concentrator for microcentrifuge tubes—Dynal MPC-M®, cat. no. 120.09, or equivalent.

6.5.4 Flat-sided sample tubes—16 × 125 mm Leighton-type tubes with 60 × 10 mm flat-sided magnetic capture area, Dynal L10, cat. no. 740.03, or equivalent.

6.6 Powder-free latex gloves—Fisher cat no. 113945B, or equivalent.

6.7 Graduated cylinders, autoclavable—10-, 100-, and 1000-mL.

6.8 Centrifuges

6.8.1 Centrifuge capable of accepting 15- to 250-mL conical centrifuge tubes and achieving 1500 × G—International Equipment Company, Needham Heights, MA, Centrifuge Size 2, Model K with swinging bucket, or equivalent.

6.8.2 Centrifuge tubes—Conical, graduated, 1.5-, 50-, and 250-mL.

6.9 Microscope

6.9.1 Epifluorescence/differential interference contrast (DIC) with stage and ocular micrometers and 20X (N.A.=0.4) to 100X (N.A.=1.3) objectives—Zeiss™ Axioskop, Olympus™ BH, or equivalent.

6.9.2 Excitation/band-pass filters for immunofluorescence assay (FA)—Zeiss™ 487909 or equivalent, including, 450- to 490-nm exciter filter, 510-nm dicroic beam-splitting mirror, and 515- to 520-nm barrier or suppression filter.

6.9.3 Excitation/band-pass filters for DAPI—Filters cited below (Chroma Technology, Brattleboro, VT), or equivalent.

Microscope model	Fluoro-chrome	Excitation filter (nm)	Dichroic beam-splitting mirror (nm)	Barrier or suppression filter (nm)	Chroma catalog number
Zeiss™ - Axioskop	DAPI (UV)	340-380	400	420	CZ902
Zeiss™ -IM35	DAPI (UV)	340-380	400	420	CZ702
Olympus™ BH	DAPI (UV)	340-380	400	420	11000
			Filter holder		91002
Olympus™ BX	DAPI (UV)	340-380	400	420	11000
			Filter holder		91008
Olympus™ IMT2	DAPI (UV)	340-380	400	420	11000
			Filter holder		91003

6.10 Ancillary equipment for microscopy

6.10.1 Well slides— Spot-On well slides, Dynal cat. no. 740.04; treated, 12-mm diameter well slides, Meridian Diagnostics Inc., Cincinnati, OH, cat. no. R2206; or equivalent.

6.10.2 Glass coverslips—22 × 50 mm.

6.10.3 Nonfluorescing immersion oil.

6.10.4 Micropipette, adjustable: 0- to 10-µL with 0- to 10-µL tips 10- to 100-µL, with 10- to 200-µL tips 100- to 1000-µL with 100- to 1000-µL tips

6.10.5 Forceps—Splinter, fine tip.

6.10.6 Forceps—Blunt-end.

6.10.7 Desiccant—Drierite™ Absorbent, Fisher cat. no. 07-577-1A, or equivalent

6.10.8 Humid chamber—A tightly sealed plastic container containing damp paper towels on top of which the slides are placed.

6.11 Pipettes—Glass or plastic

6.11.1 5-, 10-, and 25-mL.

6.11.2 Pasteur, disposable.

6.12 Balances

6.12.1 Analytical—Capable of weighing 0.1 mg.

6.12.2 Top loading—Capable of weighing 10 mg.

6.13 pH meter

6.14 Incubator—Fisher Scientific Isotemp™, or equivalent.

6.15 Vortex mixer—Fisons Whirlmixer, or equivalent.

6.16 Vacuum source—Capable of maintaining 25 in. Hg, equipped with shutoff valve and vacuum gauge.

6.17 Miscellaneous labware and supplies

6.17.1 Test tubes and rack.

6.17.2 Flasks—Suction, Erlenmeyer, and volumetric, various sizes.

6.17.3 Beakers—Glass or plastic, 5-, 10-, 50-, 100-, 500-, 1000-, and 2000-mL.

6.17.4 Lint-free tissues.

6.18 10- to 15-L graduated container—Fisher cat. no. 02-961-50B, or equivalent; calibrate to 9.0, 9.5, 10.0, 10.5, and 11.0 L and mark levels with waterproof marker.

6.19 Filters for filter-sterilizing reagents—Sterile Acrodisc, 0.45 µm , Gelman Sciences cat no. 4184, or equivalent.

7.0 Reagents and Standards

7.1 Reagents for adjusting pH

7.1.1 Sodium hydroxide (NaOH)—ACS reagent grade, 6.0 N and 1.0 N in reagent water

7.1.2 Hydrochloric acid (HCl)—ACS reagent grade, 6.0 N, 1.0 N, and 0.1 N in reagent water.

NOTE: Due to the low volumes of pH-adjusting reagents used in this method, and the impact that changes in pH have on the immunofluorescence assay, the laboratory should purchase standards at the required normality directly from a vendor. Normality should not be adjusted by the laboratory.

7.2 Solvents—Acetone, glycerol, ethanol, and methanol, ACS reagent grade

7.3 Reagent water—Water in which oocysts and cysts and interfering materials and substances, including magnetic minerals, are not detected by this method.

7.4 Reagents for eluting filters

7.4.1 Reagents for eluting Envirochek™ sampling capsules (Section 6.2.2)

7.4.1.1 Laureth-12—PPG Industries, Gurnee, IL, cat. no. 06194, or equivalent. Store Laureth-12 as a 10% solution in reagent water. Weigh 10 g of Laureth-12 and dissolve using a microwave or hot plate in 90 mL of reagent water. Dispense 10-mL aliquots into sterile vials and store at room temperature for up to 2 months, or in the freezer for up to a year.

7.4.1.2 1 M Tris, pH 7.4—Dissolve 121.1 g Tris (Fisher cat. no. BP152) in 700 mL of reagent water and adjust pH to 7.4 with 1 N HCl or NaOH. Dilute to a final 1000 mL with reagent water and adjust the final pH. Filter-sterilize through a 0.2-µm membrane into a sterile plastic container and store at room temperature.

7.4.1.3 0.5 M EDTA, 2 Na, pH 8.0—Dissolve 186.1 g ethylenediamine tetraacetic acid, disodium salt dihydrate (Fisher cat. no. S311) in 800 mL and adjust pH to 8.0 with 6.0 N HCl or NaOH. Dilute to a final volume of 1000 mL with reagent water and adjust to pH 8.0 with 1.0 N HCl or NaOH.

7.4.1.4 Antifoam A—Sigma Chemical Co. cat. no. A5758, or equivalent

7.4.1.5 Preparation of elution buffer solution—Add the contents of a pre-prepared Laureth-12 vial (Section 7.4.1.1) to a 1000-mL graduated cylinder. Rinse the vial several times to ensure the transfer of the detergent to the cylinder. Add 10 mL of Tris solution (Section 7.4.1.2), 2 mL of EDTA solution (Section 7.4.1.3), and 150 µL Antifoam A (Section 7.4.1.4). Dilute to 1000 mL with reagent water.

7.4.2 Reagents for eluting CrypTest™ capsule filters (Section 6.2.3). To 900 mL of reagent water add 8.0 g NaCl, 0.2 g KH_2PO_4, 2.9 g Na_2HPO_4 ($12H_2O$) 0.2 g KCl, 0.2 g sodium lauryl sulfate (SDS), 0.2 mL Tween 80, and 0.02 mL Antifoam A (Sigma Chemical Co. cat. no. A5758, or equivalent). Adjust volume to 1 L with reagent water and adjust pH to 7.4 with 1 N NaOH or HCl.

7.4.3 Reagents for eluting Filta-Max™ foam filters (Section 6.2.4)

7.4.3.1 Phosphate buffered saline (PBS), pH 7.4—Sigma Chemical Co. cat. no. P-3813, or equivalent. Alternately, prepare PBS by adding the following to 1 L of reagent water: 8 g NaCl; 0.2 g KCl; 1.15 g Na_2HPO_4, anhydrous; and 0.2 g KH_2PO_4.

7.4.3.2 Tween 20—Sigma Chemical Co. cat. no. P-7949, or equivalent.

7.4.3.3 High-vacuum grease—BDH/Merck. cat. no. 636082B, or equivalent.

7.4.3.4 Preparation of PBST elution buffer. Add the contents of one sachet of PBS to 1.0 L of reagent water. Dissolve by stirring for 30 minutes. Add 100 µL of Tween 20. Mix by stirring for 5 minutes.

7.5 Reagents for immunomagnetic separation (IMS)—Dynabeads® GC-Combo, Dynal cat. nos. 730.02, 730.12, or equivalent.

7.6 Direct antibody labeling reagents for detection of oocysts and cysts. Store reagents at 0 ^{o}C to 8 ^{o}C and return promptly to this temperature after each use. Do not allow any of the reagents to freeze. The reagents should be protected from exposure to light. Diluted, unused working reagents should be discarded after 48 hours. Discard reagents after the expiration date is reached. The labeling reagents in Sections 7.6.1-7.6.3 have been approved for use with this method.

7.6.1 Merifluor Cryptosporidium/Giardia, Meridian Diagnostics cat. no. 250050, Cincinnati, OH, or equivalent.

7.6.2 Aqua-Glo™ G/C Direct FL, Waterborne cat. no. A100FLR, New Orleans, LA, or equivalent.

7.6.3 Crypt-a-Glo™ and Giardi-a-Glo™, Waterborne cat. nos. A400FLR and A300FLR, respectively, New Orleans, LA, or equivalent.

> **NOTE**: If a laboratory will use multiple types of labeling reagents, the laboratory must demonstrate acceptable performance through an initial precision and recovery test (Section 9.4) for each type, and must perform positive and negative staining controls for each batch of slides stained using each product. However, the laboratory is not required to analyze additional ongoing precision and recovery samples or method blank samples for each type.

7.6.4 Diluent for labeling reagents—Phosphate buffered saline (PBS), pH 7.4—Sigma Chemical Co. cat. no. P-3813, or equivalent. Alternately, prepare PBS by adding the following to 1 L of reagent water: 8 g NaCl; 0.2 g KCl; 1.15 g Na_2HPO_4, anhydrous; and 0.2 g KH_2PO_4. Filter-sterilize (Section 6.19) or autoclave. Discard if growth is detected or after 6 months, whichever comes first.

7.7 4',6-diamidino-2-phenylindole (DAPI) stain—Sigma Chemical Co. cat. no. A5758, or equivalent.

7.7.1 Stock solution—Dissolve 2 mg/mL DAPI in absolute methanol. Prepare volume consistent with minimum use. Store at 0 ^{o}C to 8 ^{o}C in the dark. Do not allow to freeze. Discard unused solution when positive staining control fails.

7.7.2 Staining solution (1/5000 dilution in PBS [Section 7.6.4])—Add 10 µL of 2 mg/mL DAPI stock solution to 50 mL of PBS. Prepare daily. Store at 0 ^{o}C to 8 ^{o}C in the dark except when staining. Do not allow to freeze. The solution concentration may be increased up to 1µg /mL if fading/diffusion of DAPI staining is encountered, but the staining solution must be tested first on expendable environmental samples to confirm that staining intensity is appropriate.

7.8 Mounting medium

7.8.1 DABCO/glycerol mounting medium (2%)—Dissolve 2 g of DABCO (Sigma Chemical Co. cat no. D-2522, or equivalent) in 95 mL of warm glycerol/PBS (60% glycerol, 40% PBS [Section 7.6.4]). After the DABCO has dissolved completely, adjust the solution volume to 100 mL by adding an appropriate volume of glycerol/PBS solution. Alternately, dissolve the DABCO in 40 mL of PBS, then add azide (1 mL of 100X, or 10% solution), then 60 mL of glycerol.

7.8.2 Mounting medium supplied with Merifluor direct labeling kit (Section 7.6.1)

7.9 Clear fingernail polish or clear fixative, PGC Scientifics, Gaithersburg, MD, cat. no. 60-4890, or equivalent.

7.10 Oocyst and cyst suspensions for spiking

7.10.1 Enumerated spiking suspensions prepared by flow cytometer—not heat-fixed or formalin fixed: Wisconsin State Laboratory of Hygiene Flow Cytometry Unit or equivalent

7.10.2 Materials for manual enumeration of spiking suspensions

7.10.2.1 Purified Cryptosporidium oocyst stock suspension for manual enumeration—not heat-fixed or formalin-fixed: Sterling Parasitology Laboratory, University of Arizona, Tucson, or equivalent

7.10.2.2 Purified Giardia cyst stock suspension for manual enumeration—not heat-fixed or formalin-fixed: Waterborne, Inc., New Orleans, LA; Hyperion Research, Medicine Hat, Alberta, Canada; or equivalent

7.10.2.3 Tween-20, 0.01%—Dissolve 1.0 mL of a 10% solution of Tween-20 in 1 L of reagent water

7.10.2.4 Storage procedure—Store oocyst and cyst suspensions at 0 °C to 8 °C, until ready to use; do not allow to freeze

7.11 Additional reagents for enumeration of spiking suspensions using membrane filtration (Section 11.3.6)—Sigmacote® Sigma Company Product No. SL-2, or equivalent

8.0 Sample Collection and Storage

8.1 Samples are collected as bulk samples and shipped to the laboratory for processing through the entire method, or are filtered in the field and shipped to the laboratory for processing from elution (Section 12.2.6) onward. Samples must be shipped via overnight service on the day they are collected. Chill samples as much as possible between collection and shipment by storing in a refrigerator or pre-icing the sample in a cooler. If the sample is pre-iced before shipping, replace with fresh ice immediately before shipment. Samples should be shipped at 0 °C to 8 °C, unless the time required to chill the sample to 8 °C would prevent the sample from being shipped overnight for receipt at the laboratory the day after collection. Samples must not be allowed to freeze. Upon receipt, the laboratory should record the temperature of the samples and store them refrigerated at 0 °C to 8 °C until processed. Results from samples shipped overnight to the laboratory and received at >8 °C should be qualified by the laboratory.

NOTE: See transportation precautions in Section 5.5.

8.2 Sample holding times. Sample processing should be completed as soon as possible by the laboratory. The laboratory should complete sample filtration, elution, concentration, purification, and staining the day the sample is received wherever possible. However, the laboratory is permitted to split up the sample processing steps if processing a sample completely in one day is not possible. If this is necessary, sample processing can be halted after filtration, application of the purified sample onto the slide, or staining. Table 1, in Section 21.0 provides a breakdown of the holding times for each set of steps. Sections 8.2.1 through 8.2.4 provide descriptions of these holding times.

8.2.1 Sample collection and filtration. Sample elution must be initiated within 96 hours of sample collection (if shipped to the laboratory as a bulk sample) or filtration (if filtered in the field).

8.2.2 Sample elution, concentration, and purification. The laboratory must complete the elution, concentration, and purification (Sections 12.2.6 through 13.3.3.11) in one work day. It is critical that these steps be completed in one work day to minimize the time that any target organisms present in the sample sit in eluate or concentrated matrix. This process ends with the application of the purified sample on the slide for drying.
8.2.3 Staining. The sample must be stained within 72 hours of application of the purified sample to the slide.
8.2.4 Examination. Although immunofluorescence assay (FA) and 4',6-diamidino-2-phenylindole (DAPI) and differential interference contrast (DIC) microscopy examination and confirmation should be performed immediately after staining is complete, laboratories have up to 7 days from completion of sample staining to complete the examination and confirmation of samples. However, if fading/diffusion of FITC or DAPI staining is noticed, the laboratory must reduce this holding time. In addition the laboratory may adjust the concentration of the DAPI staining solution (Sections 7.7.2) so that fading/diffusion does not occur.
8.5 Spiking suspension enumeration holding times. Flow-cytometer-sorted spiking suspensions (Sections 7.10.1 and 11.2) used for spiked quality control (QC) samples (Section 9) must be used within the expiration date noted on the suspension. Laboratories should use flow-cytometersorted spiking suspensions containing live organisms within two weeks of preparation at the flow cytometry laboratory. Manually enumerated spiking suspensions must be used within 24 hours of enumeration of the spiking suspension if the hemacytometer chamber technique is used (Section 11.3.4); or within 24 hours of application of the spiking suspension to the slides if the well slide or membrane filter enumeration technique is used (Sections 11.3.5 and 11.3.6).

9.0 Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance (QA) program (Reference 20.6). The minimum requirements of this program consist of an initial demonstration of laboratory capability through performance of the initial precision and recovery (IPR) test (Section 9.4), analysis of spiked samples to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.
9.1.1 A test of the microscope used for detection of oocysts and cysts is performed prior to examination of slides. This test is described in Section 10.0.
9.1.2 In recognition of advances that are occurring in analytical technology, the laboratory is permitted to modify certain method procedures to improve recovery or lower the costs of measurements, provided that all required quality control (QC) tests are performed and all QC acceptance criteria are met. Method procedures that can be modified include front-end techniques, such as filtration or immunomagnetic separation (IMS). The laboratory is not permitted to use an alternate determinative technique to replace immunofluorescence assay in this method (the use of different determinative techniques are considered to be different methods, rather than modified version of this method). However, the laboratory is permitted to modify the immunofluorescence assay procedure, provided that all required QC tests are performed (Section 9.1.2.1) and all QC acceptance criteria are met (see guidance on the use of multiple labeling reagents in Section 7.6).

9.1.2.1 Method modification validation/equivalency demonstration requirements.
9.1.2.1.1 Method modifications at a single laboratory. Each time a modification is made to this method for use in a single laboratory, the laboratory is required to validate the modification according to Tier 1 of EPA's performance-based measurement system (PBMS) (Table 2 and Reference 20.7) to demonstrate that the modification produces results equivalent or superior to results produced by this method as written. Briefly, each time a modification is made to this method, the laboratory is required to demonstrate acceptable modified method performance through the IPR test (Section 9.4). IPR results must meet the QC acceptance criteria in Tables 3 and 4 in Section 21.0, and should be comparable to previous results using the unmodified procedure. Although not required, the laboratory also should perform a matrix spike/matrix spike duplicate (MS/MSD) test to demonstrate the performance of the modified method in at least one real-world matrix before analyzing field samples using the modified method. The laboratory is required to perform MS samples using the modified method at the frequency noted in Section 9.1.8.
9.1.2.1.2 Method modifications for nationwide approval. If the laboratory or a manufacturer seeks EPA approval of a method modification for nationwide use, the laboratory or manufacturer must validate the modification according to Tier 2 of EPA's PBMS (Table 2 and Reference 20.7). Briefly, at least three laboratories must perform IPR tests (Section 9.4) and MS/MSD (Section 9.5) tests using the modified method, and all tests must meet the QC acceptance criteria specified in Tables 3 and 4 in Section 21.0. Upon nationwide approval, laboratories electing to use the modified method still must demonstrate acceptable performance in their own laboratory according to the requirements in Section 9.1.2.1.1.
9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the following, at a minimum:
9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modification.
9.1.2.2.2 A listing of the analyte(s) measured (Cryptosporidium and Giardia).
9.1.2.2.3 9.1.2.2.4 A narrative stating reason(s) for the modification.
9.1.2.2.5 Results from all QC tests comparing the modified method to this method, including: (a) IPR (Section 9.4) (b) MS/MSD (Section 9.5) (c) Analysis of method blanks (Section 9.6) Data that will allow an independent reviewer to validate each determination by tracing the following processing and analysis steps leading to the final result:

9.1.2.2.5 Data that will allow an independent reviewer to validate each determination by tracing the following processing and analysis steps leading to the final result:
(a) Sample numbers and other identifiers
(b) Source of spiking suspensions, as well as lot number and date received (Section 7.10)
(c) Spike enumeration date and time
(d) All spiking suspension enumeration counts and calculations (Section 11.0)
(e) Sample spiking dates and times
(f) Volume filtered (Section 12.2.5.2)
(g) Filtration and elution dates and times
(h) Pellet volume, resuspended concentrate volume, resuspended concentrate volume transferred to IMS, and all calculations required to verify the percent of concentrate examined (Section 13.2)
(i) Purification completion dates and times (Section 3.3.3.11)
(j) Staining completion dates and times (Section 14.10)
(k) Staining control results (Section 15.2.1)

(l) All required examination information (Section 15.2.2)
(m) Examination completion dates and times (Section 15.2.4)
(n) Analysis sequence/run chronology
(o) Lot numbers of elution, IMS, and staining reagents
(p) Copies of bench sheets, logbooks, and other recordings of raw data
(q) Data system outputs, and other data to link the raw data to the results reported

9.1.3 The laboratory shall spike a separate sample aliquot from the same source to monitor method performance. This MS test is described in Section 9.5.1.

9.1.4 Analysis of method blanks is required to demonstrate freedom from contamination. The procedures and criteria for analysis of a method blank are described in Section 9.6.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through analysis of the ongoing precision and recovery (OPR) sample that the analysis system is in control. These procedures are described in Section 9.7.

9.1.6 The laboratory shall maintain records to define the quality of data that are generated. Development of accuracy statements is described in Sections 9.5.1.4 and 9.7.3.

9.1.7 The laboratory shall analyze one method blank (Section 9.6) and one OPR sample (Section 9.7) each week during which samples are analyzed if 20 or fewer field samples are analyzed during this period. The laboratory shall analyze one laboratory blank and one OPR sample for every 20 samples if more than 20 samples are analyzed in a week.

9.1.8 The laboratory shall analyze one MS sample (Section 9.5.1) when samples are first received from a utility for which the laboratory has never before analyzed samples. The MS analysis is performed on an additional (second) sample sent from the utility. If the laboratory routinely analyzes samples from 1 or more utilities, 1 MS analysis must be performed per 20 field samples. For example, when a laboratory receives the first sample from a given site, the laboratory must obtain a second aliquot of this sample to be used for the MS. When the laboratory receives the 21st sample from this site, a separate aliquot of this 21st sample must be collected and spiked.

9.2 Micropipette calibration

9.2.1 Micropipettes must be sent to the manufacturer for calibration annually. Alternately, a qualified independent technician specializing in micropipette calibration can be used. Documentation on the precision of the recalibrated micropipette must be obtained from the manufacturer or technician.

9.2.2 Internal and external calibration records must be kept on file in the laboratory's QA logbook.

9.2.3 If a micropipette calibration problem is suspected, the laboratory shall tare an empty weighing boat on the analytical balance and pipette the following volumes of reagent water into the weigh boat using the pipette in question: 100% of the maximum dispensing capacity of the micropipette, 50% of the capacity, and 10% of the capacity. Ten replicates should be performed at each weight. Record the weight of the water (assume that 1.00 mL of reagent water weighs 1.00 g) and calculate the relative standard deviation (RSD) for each. If the weight of the reagent water is within 1% of the desired weight (mL) and the RSD of the replicates at each weight is within 1%, then the pipette remains acceptable for use.

9.2.4 If the weight of the reagent water is outside the acceptable limits, consult the manufacturer's instruction manual troubleshooting section and repeat steps described in Section 9.2.3. If problems with the pipette persist, the laboratory must send the pipette to the manufacturer for recalibration.

9.3 Microscope adjustment and certification: Adjust the microscope as specified in Section 10.0. All of the requirements in Section 10.0 must be met prior to analysis of IPRs, blanks, OPRs, field samples, and MS/MSDs.

9.4 Initial precision and recovery (IPR)—To establish the ability to demonstrate control over the analytical system and to generate acceptable precision and recovery, the laboratory shall perform the following operations:

9.4.1 Using the spiking procedure in Section 11.4 and enumerated spiking suspensions (Section 7.10.1 or Section 11.3), spike, filter, elute, concentrate, separate (purify), stain, and examine four reagent water samples spiked with 100 to 500 oocysts and 100 to 500 cysts. If more than one process will be used for filtration and/or separation of samples, a separate set of IPR samples must be prepared for each process.

NOTE: IPR tests must be accompanied by analysis of a method blank (Section 9.6).

9.4.2 Using results of the four analyses, calculate the average percent recovery and the relative standard deviation (RSD) of the recoveries for Cryptosporidium and for Giardia. The RSD is the standard deviation divided by the mean times 100.

9.4.3 Compare RSD and the mean with the corresponding limits for initial precision and recovery in Tables 3 and 4 in Section 21.0. If the RSD and the mean meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If the RSD or the mean falls outside the range for recovery, system performance is unacceptable. In this event, correct the problem and repeat the test (Section 9.4.1).

9.5 Matrix spike (MS) and matrix spike duplicate (MSD):

9.5.1 Matrix spike—The laboratory shall spike and analyze a separate field sample aliquot to determine the effect of the matrix on the method's oocyst and cyst recovery. The MS shall be analyzed according to the frequency in Section 9.1.8.

9.5.1.1 Analyze an unspiked field sample according to the procedures in Sections 12.0 to 15.0. Using the spiking procedure in Section 11.4 and enumerated spiking suspensions (Section 7.10.1 or Section 11.3), spike, filter, elute, concentrate, separate (purify), stain, and examine a second field sample aliquot with the number of organisms used in the IPR or OPR tests (Sections 9.4 and 9.7).

9.5.1.2 For each organism, calculate the percent recovery (R) using the following equation.

$$R = 100 \times \frac{Nsp - Ns}{T}$$

where
R is the percent recovery
N_{sp} is the number of oocysts or cysts detected in the spiked sample

N_s is the number of oocysts or cysts detected in the unspiked sample T is the true value of the oocysts or cysts spiked

9.5.1.3 Compare the recovery for each organism with the corresponding limits in Tables 3 and 4 in Section 21.0.

NOTE: Some sample matrices may prevent the acceptance criteria in Tables 3 and 4 from being met. An assessment of the distribution of MS recoveries across 430 MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.
9.5.1.4 As part of the QA program for the laboratory, method precision for samples should be assessed and records maintained. After the analysis of five samples for which the spike recovery for each organism passes the tests in Section 9.5.1.3, the laboratory should calculate the average percent recovery (P) and the standard deviation of the percent recovery (s_r). Express the precision assessment as a percent recovery interval from P ¯2 s_r to P + 2 s_r for each matrix. For example, if P = 80% and s_r = 30%, the accuracy interval is expressed as 20% to 140%. The precision assessment should be updated regularly across all MS samples and stratified by MS samples for each source.
9.5.2 Matrix spike duplicate—MSD analysis is required as part of nationwide approval of a modified version of this method to demonstrate that the modified version of this method produces results equal or superior to results produced by the method as written (Section 9.1.2.1.2). At the same time the laboratory spikes and analyzes the second field sample aliquot in Section 9.5.1.1, the laboratory shall spike and analyze a third, identical field sample aliquot.

NOTE: Matrix spike duplicate samples are only required for Tier 2 validation studies. They are recommended for Tier 1 validation, but not required.

9.5.2.1 For each organism, calculate the percent recovery (R) using the equation in Section 9.5.1.2.

9.5.2.2 Calculate the mean of the number of oocysts or cysts in the MS and MSD (X_{mean}) (= [MS+MSD]/2).

9.5.2.3 Calculate the relative percent difference (RPD) of the recoveries using the following equation:

$$RPD = 100 \frac{| NMS - NMSD |}{Xmean}$$

where
RPD is the relative percent difference
N_{MS} is the number of oocysts or cysts detected in the MS
N_{MSD} is the number of oocysts or cysts detected in the MSD
X_{mean} is the mean number of oocysts or cysts detected in the MS and MSD

9.5.2.4 Compare the mean MS/MSD recovery and RPD with the corresponding limits in Tables 3 and 4 in Section 21.0 for each organism.

9.6 Method blank (negative control sample, laboratory blank): Reagent water blanks are analyzed to demonstrate freedom from contamination. Analyze the blank immediately prior to analysis of the IPR test (Section 9.4) and OPR test (Section 9.7) and prior to analysis of samples for the week to demonstrate freedom from contamination.
9.6.1 Filter, elute, concentrate, separate (purify), stain, and examine at least one reagent water blank per week (Section 9.1.7) according to the procedures in Sections 12.0 to 15.0. If more than 20 samples are analyzed in a week, process and analyze one reagent water blank for every 20 samples.
9.6.2 If *Cryptosporidium* oocysts, *Giardia* cysts, or any potentially interfering organism or material is found in the blank, analysis of additional samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination. Any sample in a batch associated with a contaminated blank that shows the presence of one or more oocysts or cysts is assumed to be contaminated and should be recollected, if possible. Any method blank in which oocysts or cysts are not detected is assumed to be uncontaminated and may be reported.

9.7 Ongoing precision and recovery ([OPR]; positive control sample; laboratory control sample): Using the spiking procedure in Section 11.4 and enumerated spiking suspensions (Section 7.10.1 or Section 11.3), filter, elute, concentrate, separate (purify), stain, and examine at least one reagent water sample spiked with 100 to 500 oocysts and 100 to 500 cysts each week to verify all performance criteria. The laboratory must analyze one OPR sample for every 20 samples if more than 20 samples are analyzed in a week. If multiple method variations are used, separate OPR samples must be prepared for each method variation. Adjustment and/or recalibration of the analytical system shall be performed until all performance criteria are met. Only after all performance criteria are met may samples be analyzed.
9.7.1 Examine the slide from the OPR prior to analysis of samples from the same batch.
9.7.1.1 Using 200X to 400X magnification, more than 50% of the oocysts or cysts must appear undamaged and morphologically intact; otherwise, the analytical process is damaging the organisms. Determine the step or reagent that is causing damage to the organisms. Correct the problem and repeat the OPR test.

9.7.1.2 Identify and enumerate each organism using epifluorescence microscopy. The first three presumptive *Cryptosporidium* oocysts and three *Giardia* cysts identified in the OPR sample must be examined using FITC, DAPI, and DIC, as per Section 15.2, and the detailed characteristics (size, shape, DAPI category, and DIC category) reported on the *Cryptosporidium* and *Giardia* report form, as well as any additional comments on organism appearance, if notable.

9.7.2 For each organism, calculate the percent recovery (R) using the following equation:

$$R = 100 \times \frac{N}{T}$$

where:
R = the percent recovery
N = the number of oocysts or cysts detected
T = the number of oocysts or cysts spiked

9.7.3 Compare the recovery with the limits for ongoing precision and recovery in Tables 3 and 4 in Section 21.0. If the recovery meets the acceptance criteria, system performance is acceptable and analysis of blanks and samples may proceed. If, however, the recovery falls outside of the range given, system performance is unacceptable. In this event, there may be a problem with the microscope or with the filtration or separation systems. Troubleshoot the problem using the procedures at Section 9.7.4 as a guide. After assessing the issue, reanalyze the OPR sample. All samples must be associated with an OPR that passes the criteria in Section 21.0. Samples that are not associated with an acceptable OPR must be flagged accordingly.

9.7.4 Troubleshooting. If an OPR sample has failed, and the cause of the failure is not known, the laboratory generally should identify the problem working backward in the analytical process from the microscopic examination to filtration.
9.7.4.1 Microscope system and antibody stain: To determine if the failure of the OPR test is due to changes in the microscope or problems with the antibody stain, re-examine the positive staining control (Section 15.2.1), check Köhler illumination, and check the fluorescence of the fluorescein-labeled monoclonal antibodies (Mabs) and 4',6-diamidino-2-phenylindole (DAPI). If results are unacceptable, re-examine the previously-prepared positive staining control to determine whether the problem is associated with the microscope or the antibody stain.
9.7.4.2 Separation (purification) system: To determine if the failure of the OPR test is attributable to the separation system, check system performance by spiking a 10-mL volume of reagent water with 100 - 500 oocysts and cysts and processing the sample through the IMS, staining, and examination procedures in Sections 13.3 through 15.0.
9.7.4.3 Filtration/elution/concentration system: If the failure of the OPR test is attributable to the filtration/elution/concentration system, check system performance by processing spiked reagent water according to the procedures in Section 12.2 through 13.2.2.1, and filter, stain, and examine the sample concentrate according to Section 11.3.6.

9.7.5 The laboratory should add results that pass the specifications in Section 9.7.3 to initial and previous ongoing data and update the QC chart to form a graphic representation of continued laboratory performance. The laboratory should develop a statement of laboratory accuracy (reagent water, raw surface water) by calculating the average percent recovery (R) and the standard deviation of percent recovery (s_r). Express the accuracy as a recovery interval from R ¯2 s_r to R + 2 s_r. For example, if R = 95% and s_r = 25%, the accuracy is 45% to 145%.

9.8 The laboratory should periodically analyze an external QC sample, such as a performance evaluation or standard reference material, when available.

The laboratory also should periodically participate in interlaboratory comparison studies using the method.

9.9 The specifications contained in this method can be met if the analytical system is under control. The standards used for initial (Section 9.4) and ongoing (Section 9.7) precision and recovery should be identical, so that the most precise results will be obtained. The microscope in particular will provide the most reproducible results if dedicated to the settings and conditions required for the determination of Cryptosporidium and Giardia by this method.

9.10 Depending on specific program requirements, field replicates may be collected to determine the precision of the sampling technique, and duplicate spiked samples may be required to determine the precision of the analysis.

10.0 Microscope Calibration and Analyst Verification

10.1 In a room capable of being darkened to near-complete darkness, assemble the microscope, all filters, and attachments. The microscope should be placed on a solid surface free from vibration. Adequate workspace should be provided on either side of the microscope for taking notes and placement of slides and ancillary materials.

10.2 Using the manuals provided with the microscope, all analysts must familiarize themselves with operation of the microscope.

10.3 Microscope adjustment and calibration (adapted from Reference 20.6)

10.3.1 Preparations for adjustment

10.3.1.1 The microscopy portion of this procedure depends upon proper alignment and adjustment of very sophisticated optics. Without proper alignment and adjustment, the microscope will not function at maximal efficiency, and reliable identification and enumeration of oocysts and cysts will not be possible. Consequently, it is imperative that all portions of the microscope from the light sources to the oculars are properly adjusted.

10.3.1.2 While microscopes from various vendors are configured somewhat differently, they all operate on the same general physical principles. Therefore, slight deviations or adjustments may be required to make the procedures below work for a particular instrument.

10.3.1.3 The sections below assume that the mercury bulb has not exceeded time limits of operation, that the lamp socket is connected to the lamp house, and that the condenser is adjusted to produce Köhler illumination.

10.3.1.4 Persons with astigmatism should always wear contact lenses or glasses when using the microscope.

CAUTION: In the procedures below, do not touch the quartz portion of the mercury bulb with your bare fingers. Finger oils can cause rapid degradation of the quartz and premature failure of the bulb.
WARNING: Never look at the ultraviolet (UV) light from the mercury lamp, lamp house, or the UV image without a barrier filter in place. UV radiation can cause serious eye damage.

10.3.2 Epifluorescent mercury bulb adjustment: The purpose of this procedure is to ensure even field illumination. This procedure must be followed when the microscope is first used, when replacing bulbs, and if problems such as diminished fluorescence or uneven field illumination are experienced.
10.3.2.1 Remove the diffuser lens between the lamp and microscope or swing it out of the transmitted light path.
10.3.2.2 Using a prepared microscope slide, adjust the focus so the image in the oculars is sharply defined.
10.3.2.3 Replace the slide with a business card or a piece of lens paper.
10.3.2.4 Close the field diaphragm (iris diaphragm in the microscope base) so only a small point of light is visible on the card. This dot of light indicates the location of the center of the field of view.
10.3.2.5 Mount the mercury lamp house on the microscope without the UV diffuser lens in place and turn on the mercury bulb.
10.3.2.6 Remove the objective in the light path from the nosepiece. A primary (brighter) and secondary image (dimmer) of the mercury bulb arc should appear on the card after focusing the image with the appropriate adjustment.
10.3.2.7 Using the lamp house adjustments, adjust the primary and secondary mercury bulb images so they are side by side (parallel to each other) with the transmitted light dot in between them.
10.3.2.8 Reattach the objective to the nosepiece.
10.3.2.9 Insert the diffuser lens into the light path between the mercury lamp house and the microscope.
10.3.2.10 Turn off the transmitted light and replace the card with a slide of fluorescent material. Check the field for even fluorescent illumination. Adjustment of the diffuser lens probably will be required. Additional slight adjustments as in Section 10.3.2.7 above may be required.
10.3.2.11 Maintain a log of the number of hours the UV bulb has been used. Never use the bulb for longer than it has been rated. Fifty-watt bulbs should not be used longer than 100 hours; 100-watt bulbs should not be used longer than 200 hours.

10.3.3 Transmitted bulb adjustment: The purpose of this procedure is to center the filament and ensure even field illumination. This procedure must be followed when the bulb is changed.
10.3.3.1 Remove the diffuser lens between the lamp and microscope or swing it out of the transmitted light path.
10.3.3.2 Using a prepared microscope slide and a 40X (or similar) objective, adjust the focus so the image in the oculars is sharply defined.

10.3.3.3 Without the ocular or Bertrand optics in place, view the pupil and filament image at the bottom of the tube.
10.3.3.4 Focus the lamp filament image with the appropriate adjustment on the lamp house.
10.3.3.5 Similarly, center the lamp filament image within the pupil with the appropriate adjustment(s) on the lamp house.
10.3.3.6 Insert the diffuser lens into the light path between the transmitted lamp house and the microscope.

10.3.4 Adjustment of the interpupillary distance and oculars for each eye: These adjustments are necessary so that eye strain is reduced to a minimum, and must be made for each individual using the microscope. Section 10.3.4.2 assumes use of a microscope with both oculars adjustable; Section 10.3.4.3 assumes use of a microscope with a single adjustable ocular. The procedure must be followed each time an analyst uses the microscope.

10.3.4.1 Interpupillary distance

10.3.4.1.1 Place a prepared slide on the microscope stage, turn on the transmitted light, and focus the specimen image using the coarse and fine adjustment knobs.

10.3.4.1.2 Using both hands, move the oculars closer together or farther apart until a single circle of light is observed while looking through the oculars with both eyes. Note interpupillary distance.

10.3.4.2 Ocular adjustment for microscopes capable of viewing a photographic frame through the viewing binoculars: This procedure assumes both oculars are adjustable.

10.3.4.2.1 Place a card between the right ocular and eye keeping both eyes open. Adjust the correction (focusing) collar on the left ocular by focusing the left ocular until it reads the same as the interpupillary distance. Bring an image located in the center of the field of view into as sharp a focus as possible.

10.3.4.2.2 Transfer the card to between the left eye and ocular. Again keeping both eyes open, bring the same image into as sharp a focus for the right eye as possible by adjusting the ocular correction (focusing) collar at the top of the right ocular.

10.3.4.3 Ocular adjustment for microscopes without binocular capability: This procedure assumes a single focusing ocular. The following procedure assumes that only the right ocular is capable of adjustment.

10.3.4.3.1 Place a card between the right ocular and eye keeping both eyes open. Using the fine adjustment, focus the image for the left eye to its sharpest point.

10.3.4.3.2 Transfer the card to between the left eye and ocular. Keeping both eyes open, bring the image for the right eye into sharp focus by adjusting the ocular collar at the top of the ocular without touching the coarse or fine adjustment.

10.3.5 Calibration of an ocular micrometer: This section assumes that a reticle has been installed in one of the oculars by a microscopy specialist and that a stage micrometer is available for calibrating the ocular micrometer (reticle). Once installed, the ocular reticle should be left in place. The more an ocular is manipulated the greater the probability is for it to become contaminated with dust particles. This calibration should be done for each objective in use on the microscope. If there is a top lens on the microscope, the calibration procedure must be done for the respective objective at each top lens setting. The procedure must be followed when the microscope is first used and each time the objective is changed.

10.3.5.1 Place the stage micrometer on the microscope stage, turn on the transmitted light, and focus the micrometer image using the coarse and fine adjustment knobs for the objective to be calibrated. Continue adjusting the focus on the stage micrometer so you can distinguish between the large (0.1 mm) and the small (0.01 mm) divisions.

10.3.5.2 Adjust the stage and ocular with the micrometer so the 0 line on the ocular micrometer is exactly superimposed on the 0 line on the stage micrometer.

10.3.5.3 Without changing the stage adjustment, find a point as distant as possible from the two 0 lines where two other lines are exactly superimposed.

10.3.5.4 Determine the number of ocular micrometer spaces as well as the number of millimeters on the stage micrometer between the two points of superimposition. For example: Suppose 48 ocular micrometer spaces equal 0.6 mm.

10.3.5.5 Calculate the number of mm/ocular micrometer space. For example:

0.6 *mm* 0.0125 *mm* = 48 *ocular micrometer spaces ocular micrometer space*

10.3.5.6 Because most measurements of microorganisms are given in µm rather than mm, the value calculated above must be converted to µm by multiplying it by 1000 µm /mm. For example:

0.0125 mm 1,000 µm 12.5 µm x =
ocular micrometer space mm ocular micrometer space

10.3.5.7 Follow the procedure below for each objective. Record the information as shown in the example below and keep the information available at the microscope.

Item no.	Objective power	Description	No. of ocular micrometer spaces	No. of stage micrometer mm[1]	µm/ocular micrometer space[2]
1		10X		N.A.[3]=	
2		20X		N.A.=	
3		40X		N.A.=	
4		100X		N.A.=	

[1]100 µm /mm [2](Stage micrometer length in mm × (1000 µm /mm)) ÷ no. ocular micrometer spaces [3]N.A. refers to numerical aperature. The numerical aperature value is engraved on the barrel of the objective.

10.3.6 Köhler illumination: This section assumes that Köhler illumination will be established for only the 100X oil DIC objective that will be used to identify internal morphological characteristics in Cryptosporidium oocysts and Giardia cysts. If more than one objective is to be used for DIC, then each time the objective is changed, Köhler illumination must be reestablished for the new objective lens. Previous sections have adjusted oculars and light sources. This section aligns and focuses the light going through the condenser underneath the stage at the specimen to be observed. If Köhler illumination is not properly established, then DIC will not work to its maximal potential. These steps need to become second nature and must be practiced regularly until they are a matter of reflex rather than a chore. The procedure must be followed each time an analyst uses the microscope and each time the objective is changed.

10.3.6.1 Place a prepared slide on the microscope stage, place oil on the slide, move the 100X oil objective into place, turn on the transmitted light, and focus the specimen image using the coarse and fine adjustment knobs.

10.3.6.2 At this point both the radiant field diaphragm in the microscope base and the aperture diaphragm in the condenser should be wide open. Now close down the radiant field diaphragm in the microscope base until the lighted field is reduced to a small opening.

10.3.6.3 Using the condenser centering screws on the front right and left of the condenser, move the small lighted portion of the field to the center of the visual field.

10.3.6.4 Now look to see whether the leaves of the iris field diaphragm are sharply defined (focused) or not. If they are not sharply defined, then they can be focused distinctly by changing the height of the condenser up and down with the condenser focusing knob while you are looking through the binoculars. Once you have accomplished the precise focusing of the radiant field diaphragm leaves, open the radiant field diaphragm until the leaves just disappear from view.

10.3.6.5 The aperture diaphragm of the condenser is now adjusted to make it compatible with the total numerical aperture of the optical system. This is done by removing an ocular, looking into the tube at the rear focal plane of the objective, and stopping down the aperture diaphragm iris leaves until they are visible just inside the rear plane of the objective.

10.3.6.6 After completing the adjustment of the aperture diaphragm in the condenser, return the ocular to its tube and proceed with the adjustments required to establish DIC

10.4 Protozoa libraries: Each laboratory is encouraged to develop libraries of photographs and drawings for identification of protozoa.

10.4.1 Take color photographs of Cryptosporidium oocysts and Giardia cysts by FA and 4',6-diamidino-2-phenylindole (DAPI) that the analysts (Section 22.2) determine are accurate (Section 15.2).

10.4.2 Similarly, take color photographs of interfering organisms and materials by FA and DAPI that the analysts believe are not Cryptosporidium oocysts or Giardia cysts. Quantify the size, shape, microscope settings, and other characteristics that can be used to differentiate oocysts and cysts from interfering debris and that will result in positive identification of DAPI positive or negative organisms.

10.5 Verification of performance: Until standard reference materials, such as National Institute of Standards and Technology standard reference materials, are available that contain a reliable number of DAPI positive or negative oocysts and cysts, this method shall rely upon the ability of the analyst for identification and enumeration of oocysts and cysts.

10.5.1 At least monthly when microscopic examinations are being performed, the laboratory shall prepare a slide containing 40 to 100 oocysts and 40 to 100 cysts. More than 50% of the oocysts and cysts must be DAPI positive.

10.5.2 Each analyst shall determine the total number of oocysts and cysts and the number that are DAPI positive or negative using the slide prepared in Section 10.5.1.

10.5.3 The total number and the number of DAPI positive or negative oocysts and cysts determined by each analyst (Section 10.5.2.) must be within ±10% of each other. If the number is not within this range, the analysts must identify the source of any variability between analysts' examination criteria, prepare a new slide, and repeat the performance verification (Sections 10.5.1 to 10.5.2).

10.5.4 Document the date, name(s) of analyst(s), number of total, DAPI positive or negative oocysts and cysts determined by the analyst(s), whether the test was passed/failed and the results of attempts before the test was passed.

10.5.5 Only after an analyst has passed the criteria in Section 10.5.3, may oocysts and cysts in QC samples and field samples be identified and enumerated.

11.0 Oocyst and Cyst Suspension Enumeration and Spiking

11.1 This method requires routine analysis of spiked QC samples to demonstrate acceptable initial and ongoing laboratory and method performance (initial precision and recovery samples [Section 9.4], matrix spike and matrix spike duplicate samples [Section 9.5], and ongoing precision and recovery samples [Section 9.7]). The organisms used for these samples must be enumerated to calculate recoveries and precision. EPA recommends that flow cytometry be used for this enumeration, rather than manual techniques. Flow cytometer–sorted spikes generally are characterized by a relative standard deviation of ≤ 2.5%, versus greater variability for manual enumeration techniques (Reference 20.8). Guidance on preparing spiking suspensions using a flow cytometer is provided in Section 11.2. Manual enumeration procedures are provided in Section 11.3. The procedure for spiking bulk samples in the laboratory is provided in Section 11.4.

11.2 Flow cytometry enumeration guidelines. Although it is unlikely that many laboratories performing Method 1623 will have direct access to a flow cytometer for preparing spiking suspensions, flow-sorted suspensions are available from commercial vendors and other sources (Section 7.10.1). The information provided in Sections 11.2.1 through 11.2.4 is simply meant as a guideline for preparing spiking suspensions using a flow cytometer. Laboratories performing flow cytometry must develop and implement detailed standardized protocols for calibration and operation of the flow cytometer.

11.2.1 Spiking suspensions should be prepared using unstained organisms that have not been heat-fixed or formalin-fixed.

11.2.2 Spiking suspensions should be prepared using Cryptosporidium parvum oocysts <3 months old, and Giardia intestinalis cysts <2 weeks old.

11.2.3 Initial calibration. Immediately before sorting spiking suspensions, an initial calibration of the flow cytometer should be performed by conducting 10 sequential sorts directly onto membranes or well slides. The oocyst and cyst levels used for the initial calibration should be the same as the levels used for the spiking suspensions. Each initial calibration sample should be stained and manually counted microscopically and the manual counts used to verify the accuracy of the system. The relative standard deviation (RSD) of the 10 counts should be ≤ 2.5%. If the RSD is > 2.5%, the laboratory should perform the initial calibration again, until the RSD of the 10 counts is ≤ 2.5%. In addition to counting the organisms, the laboratory also should evaluate the quality of the organisms using DAPI and DIC to confirm that the organisms are in good condition.

11.2.4 Ongoing calibration. When sorting the spiking suspensions for use in QC samples, the laboratory should perform ongoing calibration samples at a 10% frequency, at a minimum. The laboratory should sort the first run and every eleventh sample directly onto a membrane or well slide. Each ongoing calibration sample should be stained and manually counted microscopically and the manual counts used to verify the accuracy of the system. The mean of the ongoing calibration counts also should be used as the estimated spike dose, if the relative standard deviation (RSD) of the ongoing calibration counts is ≤ 2.5%. If the RSD is > 2.5%, the laboratory should discard the batch.

11.2.5 Method blanks. Depending on the operation of the flow cytometer, method blanks should be prepared and examined at the same frequency as the ongoing calibration samples (Section 11.2.4).

11.2.6 Holding time criteria. Flow-cytometer-sorted spiking suspensions (Sections 7.10.1 and 11.2) used for spiked quality control (QC) samples (Section 9) must be used within the expiration date noted on the suspension. Laboratories should use flow-cytometer-sorted spiking suspensions containing live organisms within two weeks of preparation at the flow cytometry laboratory.

11.3 Manual enumeration procedures. Two sets of manual enumerations are required per organism before purified Cryptosporidium oocyst and Giardia cyst stock suspensions (Sections 7.9.2.1 and 7.9.2.2) received from suppliers can be used to spike samples in the laboratory. First, the stock suspension must be diluted and enumerated (Section 11.3.3) to yield a suspension at the appropriate oocyst or cyst concentration for spiking (spiking suspension). Then, 10 aliquots of spiking suspension must be enumerated to calculate a mean spike dose. Spiking suspensions can be enumerated using hemacytometer chamber counting (Section 11.3.4), well slide counting (Section 11.3.5), or membrane filter counting (Section 11.3.6).

11.3.1 Precision criteria. The relative standard deviation (RSD) of the calculated mean spike dose for manually enumerated spiking suspensions must be ≤16% for *Cryptosporidium* and ≤19% for *Giardia* before proceeding (these criteria are based on the pooled RSDs of 105 manual *Cryptosporidium* enumerations and 104 manual *Giardia* enumerations submitted by 20 different laboratories under the EPA Protozoa Performance Evaluation Program).

11.3.2 Holding time criteria. Manually enumerated spiking suspensions must be used within 24 hours of enumeration of the spiking suspension if the hemacytometer chamber technique is used (Section 11.3.4); or within 24 hours of application of the spiking suspension or membrane filter to the slides if the well slide or membrane filter enumeration technique is used (Sections 11.3.5 and 11.3.6).

11.3.3 Enumerating and diluting stock suspensions

11.3.3.1 Purified, concentrated stock suspensions (Sections 7.10.2.1 and 7.10.2.2) must be diluted and enumerated before the diluted suspensions are used to spike samples in the laboratory. Stock suspensions should be diluted with reagent water/Tween-20, 0.01% (Section 7.10.2.3), to a concentration of 20 to 50 organisms per large hemacytometer square before proceeding to Section 11.3.3.2.

11.3.3.2 Apply a clean hemacytometer coverslip (Section 6.4.5) to the hemacytometer and load the hemacytometer chamber with 10 µL of vortexed suspension per chamber. If this operation has been properly executed, the liquid should amply fill the entire chamber without bubbles or overflowing into the surrounding moats. Repeat this step with a clean, dry hemacytometer and coverslip if loading has been incorrectly performed. See Section 11.3.3.13, below, for the hemacytometer cleaning procedure.

11.3.3.3 Place the hemacytometer on the microscope stage and allow the oocysts or cysts to settle for 2 minutes Do not attempt to adjust the coverslip, apply clips, or in any way disturb the chamber after it has been filled.

11.3.3.4 Use 200X magnification.

11.3.3.5 Move the chamber so the ruled area is centered underneath it.

11.3.3.6 Move the objective close to the coverslip while watching it from the side of the microscope, rather than through the microscope.

11.3.3.7 Focus up from the coverslip until the hemacytometer ruling appears.

11.3.3.8 At each of the four corners of the chamber is a 1-square-mm area divided into 16 squares in which organisms are to be counted (Figure 1). Beginning with the top row of four squares, count with a hand-tally counter in the directions indicated in Figure 2. Avoid counting organisms twice by counting only those touching the top and left boundary lines. Count each square millimeter in this fashion.

11.3.3.9 Use the following formula to determine the number of organisms per mL of suspension:

11.3.3.10 Record the result on a hemacytometer data sheet.

11.3.3.11 A total of six different hemacytometer chambers must be loaded, counted, and averaged for each suspension to achieve optimal counting accuracy.

11.3.3.12 Based on the hemacytometer counts, the stock suspension should be diluted to a final concentration of between 8000 and 12,000 organisms per mL (80 to 120 organisms per 10 $_{\mu L}$); however, ranges as great as 5000 to 15,000 organisms per mL (50 to 150 organisms per 10 µL) can be used.

NOTE: If the diluted stock suspensions (the spiking suspensions) will be enumerated using hemacytometer chamber counts (Section 11.3.4) or membrane filter counts (Section 11.3.6), then the stock suspensions should be diluted with 0.01% Tween-20. If the spiking suspensions will be enumerated using well slide counts (Section 11.3.3), then the stock suspensions should be diluted in reagent water.

To calculate the volume (in µL) of stock suspension required per mL of reagent water (or reagent water/Tween-20, 0.01%), use the following formula:

required number of organisms x 1000 µLvolume of stock suspension (µL)
required = number of organisms/mL of Stock suspension

If the volume is less than 10 µL , an additional dilution of the stock suspension is recommended before proceeding.

To calculate the dilution factor needed to achieve the required number of organisms per 10 µL , use the following formula:
Total volume (µL) number of organisms required x 10 µL
predicted number of organisms per 10 µL (80 to 120)

To calculate the volume of reagent water (or reagent water/Tween-20, 0.01%) needed, use the following formula:
reagent water volume (µL) = total volume (µL) -stock suspension volume required (µL)

11.3.3.13 After each use, the hemacytometer and coverslip must be cleaned immediately to prevent the organisms and debris from drying on it. Since this apparatus is precisely machined, abrasives cannot be used to clean it, as they will disturb the flooding and volume relationships.

11.3.3.13.1 Rinse the hemacytometer and cover glass first with tap water, then 70% ethanol, and finally with acetone.

11.3.3.13.2 Dry and polish the hemacytometer chamber and cover glass with lens paper. Store it in a secure place.

11.3.3.14 Several factors are known to introduce errors into hemacytometer counts, including:

• Inadequate mixing of suspension before flooding the chamber.
• Irregular filling of the chamber, trapped air bubbles, dust, or oil on the chamber or coverslip.
• Total number of organisms counted is too low to provide statistical confidence in the result
• Error in recording tally.
• Calculation error; failure to consider dilution factor, or area counted.
• Inadequate cleaning and removal of organisms from the previous count.
• Allowing filled chamber to sit too long, so that the chamber suspension dries and concentrates.

11.3.4 Enumerating spiking suspensions using a hemacytometer chamber

NOTE: Spiking suspensions enumerated using a hemacytometer chamber must be used within 24 hours of enumeration.

11.3.4.1 Vortex the tube containing the spiking suspension (diluted stock suspension; Section 11.3.3) for a minimum of 2 minutes. Gently invert the tube three times.

11.3.4.2 To an appropriate-size beaker containing a stir bar, add enough spiking suspension to perform all spike testing and the enumeration as described. The liquid volume and beaker relationship should be such that a spinning stir bar does not splash the sides of the beaker, the stir bar has unimpeded rotation, and there is enough room to draw sample from the beaker with a 10-µL micropipette without touching the stir bar. Cover the beaker with a watch glass or Petri dish to prevent evaporation between sample withdrawals.

11.3.4.3 Allow the beaker contents to stir for a minimum of 30 minutes before beginning enumeration.

11.3.4.4 While the stir bar is still spinning, remove a 10-µL aliquot and carefully load one side of the hemacytometer. Count all organisms on the platform, at 200X magnification using phase-contrast or darkfield microscopy. The count must include the entire area under the hemacytometer, not just the four outer 1-mm^2 squares. Repeat this procedure nine times. This step allows confirmation of the number of organisms per 10 µL (Section 11.3.3.12). Based on the 10 counts, calculate the mean, standard deviation, and RSD of the counts. Record the counts and the calculations on a spiking suspension enumeration form. The relative standard deviation (RSD) of the calculated mean spike dose must be ≤16% for Cryptosporidium and ≤19% for Giardia before proceeding. If the RSD is unacceptable, or the mean number is outside the expected range, add additional oocysts from stock suspension or dilute the contents of the beaker appropriately with reagent water. Repeat the process to confirm counts. Refer to Section 11.3.3.14 for factors that may introduce errors.

Enumerating spiking suspensions using well slides

NOTE: Spiking suspensions enumerated using well slides must be used within 24 hours of application of the spiking suspension to the slides.

11.3.5.1 Remove well slides from cold storage and lay the slides on a flat surface for 15 minutes to allow them to warm to room temperature.

11.3.5.2 Vortex the tube containing the spiking suspension (diluted stock suspension; Section 11.3.3) for a minimum of 2 minutes. Gently invert the tube three times.

11.3.5.3 Remove a 10-µL aliquot from the spiking suspension and apply it to the center of a well.

11.3.5.4 Before removing subsequent aliquots, cap the tube and gently invert it three times to ensure that the oocysts or cysts are in suspension.

11.3.5.5 Ten wells must be prepared and counted, and the counts averaged, to sufficiently enumerate the spike dose. Air-dry the well slides. Because temperature and humidity varies from laboratory to laboratory, no minimum time is specified. However, the laboratory must take care to ensure that the sample has dried completely before staining to prevent losses during the rinse steps. A slide warmer set at 35 ^{o}C to 42 ^{o}C also can be used.

11.3.5.6 Positive and negative controls must be prepared.

11.3.5.6.1 For the positive control, pipette 10 µL of positive antigen or 200 to 400 intact oocysts or cysts to the center of a well and distribute evenly over the well area.

11.3.5.6.2 For the negative control, pipette 50 µL of PBS onto the center of a well and spread it over the well area with a pipette tip.
11.3.5.6.3 Air-dry the control slides.

11.3.5.7 Apply 50-µL of absolute methanol to each well containing the dried sample and allow to air-dry for 3 to 5 minutes.

11.3.5.8 Follow the manufacturer's instructions (Section 7.6) in applying the stain to the slide.

11.3.5.9 Place the slides in a humid chamber in the dark and incubate at room temperature for approximately 30 minutes. The humid chamber consists of a tightly sealed plastic container containing damp paper towels on top of which the slides are placed.

11.3.5.10 Apply one drop of wash buffer (prepared according to the manufacturer's instructions [Section 7.6]) to each well. Tilt each slide on a clean paper towel, long edge down. Gently aspirate the excess detection reagent from below the well using a clean Pasteur pipette or absorb with a paper towel or other absorbent material. Avoid disturbing the sample.

NOTE: If using the Merifluor stain (Section 7.6.1), do not allow slides to dry completely.

11.3.5.11 Add mounting medium (Section 7.8) to each well.

11.3.5.12 Apply a cover slip. Use a tissue to remove excess mounting fluid from the edges of the coverslip. Seal the edges of the coverslip onto the slide using clear nail polish.

11.3.5.13 Record the date and time that staining was completed. If slides will not be read immediately, store in a humid chamber in the dark at 0 ^{o}C to 8 ^{o}C until ready for examination.

11.3.5.14 After examination of the 10 wells, calculate the mean, standard deviation, and RSD of the 10 replicates. Record the counts and the calculations on a spiking suspension enumeration form. The relative standard deviation (RSD) of the calculated mean spike dose must be ≤16% for Cryptosporidium and ≤19% for Giardia before proceeding. If the RSD is unacceptable, or the mean number is outside the expected range, add additional oocysts from stock suspension or dilute the contents of the beaker appropriately with reagent water. Repeat the process to confirm counts.

11.3.6 Enumeration of spiking suspensions using membrane filters

NOTE: Spiking suspensions enumerated using membrane filters must be used within 24 hours of application of the filters to the slides.

11.3.6.1 Pre-coat the glass funnels with Sigmacote® by placing the funnel in a large Petri dish and applying 5-mL of Sigmacoat® to the funnel opening using a pipette and allowing it to run down the inside of the funnel. Repeat for all funnels to be used. The pooled Sigmacoat® may be returned to the bottle for re-use. Place the funnels at 35 ^{o}C or 41 ^{o}C for approximately 5 minutes to dry.

11.3.6.2 Place foil around the bottoms of the 100 × 15 mm Petri dishes.

11.3.6.3 Filter-sterilize (Section 6.19) approximately 10 mL of PBS pH

7.2 (Section 7. 9. 4). Dilute detection reagent (Section 7.7) as per manufacturer's instructions using sterile PBS. Multiply the anticipated number of filters to be stained by 100 mL to calculate total volume of stain required. Divide the total volume required by 5 to obtain the microliters of antibody necessary. Subtract the volume of antibody from the total stain volume to obtain the required microliters of sterile PBS to add to the antibody.

11.3.6.4 Label the tops of foil-covered, 60 × 15 mm Petri dishes for 10 spiking suspensions plus positive and negative staining controls and multiple filter blanks controls (one negative control, plus a blank after every five sample filters to control for carry-over). Create a humid chamber by laying damp paper towels on the bottom of a stain tray (the inverted foil-lined Petri dishes will protect filters from light and prevent evaporation during incubation).

11.3.6.5 Place a decontaminated and cleaned filter holder base (Section 6.4.8.1) into each of the three ports of the vacuum manifold (Section 6.4.8.2).

11.3.6.6 Pour approximately 10 mL of 0.01% Tween 20 into a 60 × 15 mm Petri dish.

11.3.6.7 Using forceps, moisten a 1.2-µm cellulose-acetate support membrane (Section 6.4.8.3) in the 0.01% Tween 20 and place it on the fritted glass support of one of the filter bases. Moisten a polycarbonate filter (Section

6.4.8.4) the same way and position it on top of the cellulose-acetate support membrane. Carefully clamp the glass funnel to the loaded filter support. Repeat for the other two filters.

11.3.6.8 Add 5 mL of 0.01% Tween 20 to each of the three filtration units and allow to stand.

11.3.6.9 Vortex the tube containing the spiking suspension (diluted stock suspension; Section 11.3.3) for a minimum of 2 minutes. Gently invert the tube three times.

11.3.6.10 Using a micropipettor, sequentially remove two, 10-µL aliquots from the spiking suspension and pipet into the 5 mL of 0.01% Tween 20 standing in the unit. Rinse the pipet tip twice after each addition. Apply 10 µL of 0.01% Tween 20 to the third unit to serve as the negative control. Apply vacuum at 2" Hg and allow liquid to drain to miniscus, then close off vacuum. Pipet 10 mL of reagent water into each funnel and drain to miniscus, closing off the vacuum. Repeat the rinse and drain all fluid, close off the vacuum.

11.3.6.11 Pipet 100 mL of diluted antibody to the center of the bottom of a 60 × 15 mm Petri dish for each sample.

11.3.6.12 Unclamp the top funnel and transfer each cellulose acetate support membrane/ polycarbonate filter combination onto the drop of stain using forceps (apply each membrane/filter combination to a different Petri dish containing stain). Roll the filter into the drop to exclude air. Place the small Petri dish containing the filter onto the damp towel and cover with the corresponding labeled foil-covered top. Incubate for approximately 45 minutes at room temperature.

11.3.6.13 Reclamp the top funnels, apply vacuum and rinse each three times, each time with 20 mL of reagent water.

11.3.6.14 Repeat Sections 11.3.6.4 through 11.3.6.10 for the next three samples (if that the diluted spiking suspension has sat less than 15 minutes, reduce the suspension vortex time to 60 seconds). Ten, 10-µL spiking suspension aliquots must be prepared and counted, and the counts averaged, to sufficiently enumerate the spike dose.

Include a filter blank sample at a frequency of every five samples; rotate the position of filter blank to eventually include all three filter placements.
11.3.6.15 Repeat Sections 11.3.6.4 through 11.3.6.10 until the 10-µL spiking suspensions have been filtered. The last batch should include a 10-µL 0.01 Tween 20 blank control and 20 µL of positive control antigen as a positive staining control.
11.3.6.16 Label slides. After incubation is complete, for each sample, transfer the cellulose acetate filter support and polycarbonate filter from drop of stain and place on fritted glass support. Cycle vacuum on and off briefly to remove excess fluid. Peel the top polycarbonate filter off the supporting filter and place on labeled slide. Discard cellulose acetate filter support. Mount and apply coverslips to the filters immediately to avoid drying.
11.3.6.17 To each slide, add 20 µL of mounting medium (Section 7.8).
11.3.6.18 Apply a coverslip. Seal the edges of the coverslip onto the slide using clear nail polish. (Sealing may be delayed until cover slips are applied to all slides.)

11.3.6.19 Record the date and time that staining was completed. If slides will not be read immediately, store sealed slides in a closed container in the dark at 0 ^{o}C to 8 ^{o}C until ready for examination.

11.3.6.20 After examination of the 10 slides, calculate the mean, standard deviation, and RSD of the 10 replicates. Record the counts and the calculations on a spiking suspension enumeration form. The relative standard deviation (RSD) of the calculated mean spike dose must be ≤16% for Cryptosporidium and ≤19% for Giardia before proceeding. If the RSD is unacceptable, or the mean number is outside the expected range, add additional oocysts from stock suspension or dilute the contents of the beaker appropriately with reagent water. Repeat the process to confirm counts.

11.3.6.21 If oocysts or cysts are detected on the filter blanks, modify the rinse procedure to ensure that no carryover occurs and repeat enumeration.

11.4 Procedure for spiking samples in the laboratory with enumerated spiking suspensions.

11.4.1 Arrange a bottom-dispensing container to feed the filter.

11.4.2 For initial precision and recovery (Section 9.4) and ongoing precision and recovery (Section 9.7) samples, fill the container with a volume of reagent water equal to the volume of the field samples analyzed in the analytical batch. For matrix spike samples (Section 9.5), fill the container with the field sample to be spiked. Continuously mix the sample (using a stir bar and stir plate for smaller-volume samples and alternate means for larger-volume samples).

11.4.3 Vortex the spiking suspension(s) (Section 11.2 or Section 11.3) for a minimum of 2 minutes.

11.4.3.1 For flow cytometer–enumerated suspensions (where the entire volume of a spiking suspension tube will be used):

11.4.3.1.1 Add 500 µL of the diluted antifoam to the tube containing the spiking suspension and vortex for 2 minutes.
11.4.3.1.2 Pour the suspension into the sample container.
11.4.3.1.3 Add 20 mL of reagent water to the empty tube, cap, vortex 10 seconds to rinse, and add the rinsate to the carboy.
11.4.3.1.4 Repeat this rinse using another 20 mL of reagent water.
11.4.3.1.5 Record the estimated number of organisms spiked, the date and time the sample was spiked, and the sample volume spiked on a bench sheet.

11.4.3.1.6 Proceed to Section 11.4.4.

11.4.3.2 For manually enumerated spiking suspensions:

11.4.3.2.1 Rinse a pipette tip with 0.01% Tween-20 once, then rinse with the well-mixed spiking suspension a minimum of five times before pulling an aliquot to be used to spike the container.

11.4.3.2.2 Add the spiking suspension(s) to the carboy, delivering the aliquot below the surface of the water.

11.4.3.2.3 Record the estimated number of organisms spiked, the date and time the sample was spiked, and the sample volume spiked on a bench sheet. Proceed to Section 11.4.4

11.4.4 Allow the spiking suspensions to mix for approximately 1 minute in the container.

11.4.5 Turn on the pump and allow the flow rate to stabilize. Set flow at the rate designated for the filter being used. As the carboy is depleted, check the flow rate and adjust if necessary.

11.4.6 When the water level approaches the discharge port of the carboy, tilt the container so that it is completely emptied. At that time, turn off the pump and add sufficient reagent water to the container to rinse. Swirl the contents to rinse down the sides.

11.4.7 Turn on the pump. Allow all of the water to flow through the filter and turn off the pump.

12.0 Sample Filtration and Elution

12.1 A water sample is filtered according to the procedures in Section 12.2. Alternate procedures may be used if the laboratory first demonstrates that the alternate procedure provides equivalent or superior performance per Section 9.1.2.

NOTE: Sample elution must be initiated within 96 hours of sample collection (if shipped to the laboratory as a bulk sample) or filtration (if filtered in the field).

12.2 Capsule filtration (adapted from Reference 20.9). This procedure was validated using 10-L sample volumes. Alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and source water samples (Section 9.1.2).

NOTE: The filtration procedures specified in Section 12.2.1 - 12.2.5.3 are specific to laboratory filtration of a bulk sample, and reflect the procedures used during the interlaboratory validation of this method (Reference 20.10). These procedures may require modification if samples will be filtered in the field.

12.2.1 Flow rate adjustment

12.2.1.1 Connect the sampling system, minus the capsule, to a carboy filled with reagent water (Figure 3).

12.2.1.2 Turn on the pump and adjust the flow rate to 2.0 L/min.

12.2.1.3 Allow 2 to 10 L of reagent water to flush the system. Adjust the pump speed as required during this period. Turn off the pump when the flow rate has been adjusted.

12.2.2 Install the capsule filter in the line, securing the inlet and outlet ends with the appropriate clamps/fittings.

12.2.3 Record the sample number, sample turbidity (if not provided with the field sample), sample type, and sample filtration start date and time on a bench sheet.

12.2.4 Filtration

12.2.4.1 Connect the sampling system to the field carboy of sample water, or transfer the sample water to the laboratory carboy used in Section
12.2.1.1. If the sample will be filtered from a field carboy, a spigot (Section 6.2.1) can be used with the carboy to facilitate sample filtration.

NOTE: If the bulk field sample is transferred to a laboratory carboy, the laboratory carboy must be cleaned and disinfected before it is used with another field sample.

12.2.4.2 Place the drain end of the sampling system tubing into an empty graduated container with a capacity of 10 to 15 L, calibrated at 9.0, 9.5, 10.0, 10.5, and 11.0 L (Section 6.18). This container will be used to determine the sample volume filtered. Alternately, connect a flow meter (Section 6.3.4) downstream of the filter, and record the initial meter reading.

12.2.4.3 Allow the carboy discharge tube and capsule to fill with sample water. Vent residual air using the bleed valve/vent port, gently shaking or tapping the capsule, if necessary. Turn on the pump to start water flowing through the filter. Verify that the flow rate is 2 L/min.

12.2.4.4 After all of the sample has passed through the filter, turn off the pump. Allow the pressure to decrease until flow stops. (If the sample was filtered in the field, and excess sample remains in the filter upon receipt in the laboratory, pull the remaining sample volume through the filter before eluting the filter [Section 12.2.6].)

12.2.5 Disassembly

12.2.5.1 Disconnect the inlet end of the capsule filter assembly while maintaining the level of the inlet fitting above the level of the outlet fitting to prevent backwashing and the loss of oocysts and cysts from the filter. Restart the pump and allow as much water to drain as possible. Turn off the pump.

12.2.5.2 Based on the water level in the graduated container or meter reading, record the volume filtered on the bench sheet to the nearest quarter liter. Discard the contents of the graduated container.

12.2.5.3 Loosen the outlet fitting, then cap the inlet and outlet fittings.

12.2.6 Elution

NOTE: The laboratory must complete the elution, concentration, and purification (Sections 12.2.6 through 13.3.3.11) in one work day. It is critical that these steps be completed in one work day to minimize the time that any target organisms present in the sample sit in eluate or concentrated matrix. This process ends with the application of the purified sample on the slide for drying.

12.2.6.1 Setup

12.2.6.1.1 Assemble the laboratory shaker with the clamps aligned vertically so that the filters will be aligned horizontally. Extend the clamp arms to their maximum distance from the horizontal shaker rods to maximize the shaking action.

12.2.6.1.2 Prepare sufficient elution buffer so that all samples to be eluted that day can be eluted with the same batch of buffer. Elution may require up to 275 mL of buffer per sample.

12.2.6.1.3 Designate at least one 250-mL conical centrifuge tube for each sample and label with the sample number.

12.2.6.2 Elution

12.2.6.2.1 Record the elution date and time on the bench sheet. Using a ring stand or other means, clamp each capsule in a vertical position with the inlet end up. Remove the inlet cap and allow the liquid level to stabilize.

12.2.6.2.2 Pour elution buffer through the inlet fitting. Sufficient elution buffer must be added to cover the pleated white membrane with buffer solution. Replace the inlet cap and clamp the cap in place.

12.2.6.2.3 Securely clamp the capsule in one of the clamps on the laboratory shaker with the bleed valve positioned at the top on a vertical axis (in the 12 o'clock position). Turn on the shaker and set the speed to maximum (approximately 900 rpm). Agitate the capsule for approximately 5 minutes. Time the agitation using a lab timer, rather than the timer on the shaker to ensure accurate time measurement.

12.2.6.2.4 Remove the filter from the shaker, remove the inlet cap, and pour the contents of the capsule into the 250-mL conical centrifuge tube.

12.2.6.2.5 Clamp the capsule vertically with the inlet end up and add sufficient volume of elution buffer through the inlet fitting to cover the pleated membrane. Replace the inlet cap.

12.2.6.2.6 Return the capsule to the shaker with the bleed valve positioned at the 4 o'clock position. Turn on the shaker and agitate the capsule for approximately 5 minutes.

12.2.6.2.7 Remove the filter from the shaker, but leave the elution buffer in the capsule. Re-clamp the capsule to the shaker at the 8 o'clock position. Turn on the shaker and agitate the capsule for a final 5 minutes.

12.2.6.2.8 Remove the filter from the shaker and pour the contents into the 250-mL centrifuge tube. Rinse down the inside of the capsule filter walls with reagent water or elution buffer using a squirt bottle inserted in the inlet end of the capsule. Invert the capsule filter over the centrifuge tube and ensure that as much of the eluate as possible has been transferred.

12.2.7 Proceed to Section 13.0 for concentration and separation (purification).

13.0 Sample Concentration and Separation (Purification)

13.1 During concentration and separation, the filter eluate is concentrated through centrifugation, and the oocysts and cysts in the sample are separated from other particulates through immunomagnetic separation (IMS). Alternate procedures and products may be used if the laboratory first demonstrates equivalent or superior performance as per Section 9.1.2.

13.2 Adjustment of pellet volume

13.2.1 Centrifuge the 250-mL centrifuge tube containing the capsule filter eluate at 1500 × G for 15 minutes. Allow the centrifuge to coast to a stop—do not use the brake. Record the pellet volume (volume of solids) on the bench sheet.

NOTE: Recoveries may be improved if centrifugation force is increased to 2000 × G. However, do not use this higher force if the sample contains sand or other gritty material that may degrade the condition of any oocysts and/or cysts in the sample.

13.2.2 Using a Pasteur pipette, carefully aspirate the supernatant to 5 mL above the pellet. Extra care must be taken to avoid aspirating oocysts and cysts during this step, particularly if the sample is reagent water (e.g. initial or ongoing precision and recovery sample).
13.2.3 If the packed pellet volume is ≤ 0.5 mL, vortex the tube vigorously until pellet is completely resuspended. Swirl the centrifuge tube gently to reduce any foaming after vortexing. Record the resuspended pellet volume on the bench sheet. Proceed to Section 13.3.

NOTE: Extra care must be taken with samples containing sand or other gritty material when vortexing to ensure that the condition of any oocysts and/or cysts in the sample is not compromised.

13.2.4 If the packed pellet volume is > 0.5 mL, the concentrate needs to be separated into multiple subsamples (a subsample is equivalent to no greater than 0.5 mL of packed pellet material, the recommended maximum amount of particulate material to process through the subsequent purification and examination steps in the method). Use the following formula to determine the total volume required in the centrifuge tube before separating the concentrate into two or more subsamples:

$$\text{total volume (mL) required} = \frac{\text{pellet volume}}{0.5\ \text{mL}} \times 5\ \text{mL}$$

(For example, if the packed pellet volume is 1.2 mL, the total volume required is 12 mL.) Add reagent water to the centrifuge tube to bring the total volume to the level calculated above. Vortex the tube vigorously for 10 to 15 seconds to completely resuspend the pellet. Record the resuspended pellet volume on the bench sheet.

NOTE: Extra care must be taken with samples containing sand or other gritty material when vortexing to ensure that the condition of any oocysts in the sample is not compromised.

13.2.4.1 Analysis of entire sample. If analysis of the entire sample is required, determine the number of subsamples to be processed independently through the remainder of the method:

13.2.4.1.1 Calculate number of subsamples: Divide the total volume in the centrifuge tube by 5 mL and round up to the nearest integer (for example, if the resuspended volume in Section 13.2.4 is 12 mL, then the number of subsamples would be 12 mL / 5 mL = 2.4, rounded = 3 subsamples).

13.2.4.1.2 Determine volume of resuspended concentrate per subsample. Divide the total volume in the centrifuge tube by the calculated number of subsamples (for 13.2.4.1.3 example, if the resuspended volume in Section 13.2.4 is 12 mL, then the volume to use for each subsample = 12 mL / 3 subsamples = 4 mL).

Process sub-samples through IMS. Proceed to Section 13.3, and transfer aliquots of the resuspended concentrate equivalent to the volume in the previous step to multiple, flat-sided sample tubes in Section 13.3.2.1. Process the sample as multiple, independent subsamples from Section 13.3 onward, including the preparation and examination of separate slides for each aliquot. Record the volume of resuspended concentrate transferred to IMS on the bench sheet (this will be equal to the volume recorded in Section 13.2.4). Also record the number of subsamples processed independently through the method on the bench sheet.

13.2.4.2 Analysis of partial sample. If not all of the concentrate will be examined, proceed to Section 13.3, and transfer one or more 5-mL aliquots of the resuspended concentrate to one or more flat-sided sample tubes in Section 13.3.2.1. Record the volume of resuspended concentrate transferred to IMS on the bench sheet. To determine the volume analyzed, calculate the percent of the concentrate examined using the following formula:

$$\text{percent examined} = \frac{\text{total volume of resuspended concentrate transferred to IMS}}{\text{total volume of resuspended concentrate in Section 13.2.4}} \times 100\%$$

Then multiply the volume filtered (Section 12.2.5.2) by this percentage to determine the volume analyzed.

13.3 IMS procedure (adapted from Reference 20.11)

NOTE: The IMS procedure should be performed on a bench top with all materials at room temperature, ranging from 15 ^{o}C to 25 ^{o}C.

13.3.1 Preparation and addition of reagents

13.3.1.1 Prepare a 1X dilution of SL-buffer-A from the 10X SL-buffer-A (clear, colorless solution) supplied. Use reagent water (demineralized; Section 7.3) as the diluent. For every 1 mL of 1X SL-buffer-A required, take 100 µL of 10X SL-buffer-A and make up to 1 mL with the diluent water. A volume of 1.5 mL of 1X SL-buffer-A will be required per sample or subsample on which the Dynal IMS procedure is performed.

13.3.1.2 For each sample or subsample (Section 13.2) to be processed through IMS, add 1 mL of the 10X SL-buffer-A (supplied—not the diluted 1X SL-buffer-A) to a flat-sided tube (Section 6.5.4).

13.3.1.3 For each subsample, add 1 mL of the 10X SL-buffer-B (supplied— magenta solution) to the flat-sided tube containing the 10X SL-buffer-A.

13.3.2 Oocyst and cyst capture

13.3.2.1 Use a graduated, 10-mL pipette that has been pre-rinsed with elution buffer to transfer the water sample concentrate from Section 13.2 to the flat-sided tube(s) containing the SL-buffer. If all of the concentrate is used, rinse the centrifuge tube twice with reagent water and add the rinsate to the flat-sided tube containing the concentrate (or to the tube containing the first subsample, if multiple subsamples will be processed). Each of the two rinses should be half the volume needed to bring the total volume in the flat-sided sample tube to 10 mL. (For example, if 5 mL was

transferred after resuspension of the pellet, the centrifuge tube would be rinsed twice with 2.5 mL of reagent water to bring the total volume in the flat-sided tube to 10 mL.) Visually inspect the centrifuge tube after completing the transfer to ensure that no concentrate remains. If multiple subsamples will be processed, bring the volume in the remaining flat-sided tubes to 10 mL with reagent water. Label the flat-sided tube(s) with the sample number (and subsample letters).

13.3.2.2 Vortex the Dynabeads®Crypto-Combo vial from the IMS kit for approximately 10 seconds to suspend the beads. Ensure that the beads are fully resuspended by inverting the sample tube and making sure that there is no residual pellet at the bottom.

13.3.2.3 Add 100 µL of the resuspended Dynabeads®Crypto-Combo (Section 13.3.2.2) to the sample tube(s) containing the water sample concentrate and SL-buffer.

13.3.2.4 Vortex the Dynabeads®Giardia-Combo vial from the IMS kit for approximately 10 seconds to suspend the beads. Ensure that the beads are fully resuspended by inverting the tube and making sure that there is no residual pellet at the bottom.

13.3.2.5 Add 100 µL of the resuspended Dynabeads®Giardia-Combo (Section 13.3.2.4) to the sample tube(s) containing the water sample concentrate, Dynabeads®Crypto-Combo, and SL-buffer.

13.3.2.6 Affix the sample tube(s) to a rotating mixer and rotate at approximately 18 rpm for 1 hour at room temperature.

13.3.2.7 After rotating for 1 hour, remove each sample tube from the mixer and place the tube in the magnetic particle concentrator (MPC-1) with flat side of the tube toward the magnet.

13.3.2.8 Without removing the sample tube from the MPC-1, place the magnet side of the MPC-1 downwards, so the tube is horizontal and the flat side of the tube is facing down.

13.3.2.9 Gently rock the sample tube by hand end-to-end through approximately 90^{o}, tilting the cap-end and base-end of the tube up and down in turn. Continue the tilting action for 2 minutes with approximately one tilt per second.

13.3.2.10 Ensure that the tilting action is continued throughout this period to prevent binding of low-mass, magnetic or magnetizable material. If the sample in the MPC-1 is allowed to stand motionless for more than 10 seconds, repeat Section 13.3.2.9 before continuing to Section 13.3.2.11.

13.3.2.11 Return the MPC-1 to the upright position, sample tube vertical, with cap at top. Immediately remove the cap and, keeping the flat side of the tube on top, pour off all of the supernatant from the tube held in the MPC-1 into a suitable container. Do not shake the tube and do not remove the tube from MPC-1 during this step.

13.3.2.12 Remove the sample tube from the MPC-1 and resuspend the sample in 1-mL 1X SL-buffer-A (prepared from 10X SL-buffer-A stock—supplied). Mix very gently to resuspend all material in the tube. Do not vortex.

13.3.2.13 Quantitatively transfer (transfer followed by two rinses) all the liquid from the sample tube to a labeled, 1.5-mL microcentrifuge tube. Use 1 mL of 1X SL-buffer-A to perform the first rinse and 0.5 mL of

reagent water for the second rinse. Liberally rinse down the sides of the Leighton tube before transferring. Allow the flat-sided sample tube to sit for a minimum of 1 minute after transfer of the second rinse volume, then use a pipette to collect any residual volume that drips down to the bottom of the tube to ensure that as much sample volume is recovered as possible. Ensure that all of the liquid and beads are transferred.

13.3.2.14 Place the microcentrifuge tube into the second magnetic particle concentrator (MPC-M), with its magnetic strip in place.

13.3.2.15 Without removing the microcentrifuge tube from MPC-M, gently rock/roll the tube through 180° by hand. Continue for approximately 1 minute with approximately one 180° roll/rock per second. At the end of this step, the beads should produce a distinct brown dot at the back of the tube.

13.3.2.16 Immediately aspirate the supernatant from the tube and cap held in the MPC-M. If more than one sample is being processed, conduct three 90° rock/roll actions before removing the supernatant from each tube. Take care not to disturb the material attached to the wall of the tube adjacent to the magnet. Do not shake the tube. Do not remove the tube from MPC-M while conducting these steps.

13.3.3 Dissociation of beads/oocyst/cyst complex

NOTE: Two acid dissociations are required.

13.3.3.1 Remove the magnetic strip from the MPC-M.

13.3.3.2 Add 50 µL of 0.1 N HCl, then vortex at the highest setting for approximately 50 seconds.

NOTE: The laboratory should use 0.1-N standards purchased directly from a vendor, rather than adjusting the normality in-house.

13.3.3.3 Place the tube in the MPC-M without the magnetic strip in place and allow to stand in a vertical position for at least 10 minutes at room temperature.

13.3.3.4 Vortex vigorously for approximately 30 seconds.

13.3.3.5 Ensure that all of the sample is at the base of the tube. Place the microcentrifuge tube in the MPC-M.

13.3.3.6 Replace magnetic strip in MPC-M and allow the tube to stand undisturbed for a minimum of 10 seconds.

13.3.3.7 Prepare a well slide for sample screening and label the slide.

13.3.3.8 Add 5 µL of 1.0 N NaOH to the sample wells of two well slides (add 10 µL to the sample well of one well slide if the volume from the two required dissociations will be added to the same slide).

NOTE: The laboratory should use 1.0-N standards purchased directly from a vendor rather than adjusting the normality in-house.

13.3.3.9 Without removing the microcentrifuge tube from the MPC-M, transfer all of the sample from the microcentrifuge tube in the MPC-M to the sample well with the NaOH. Do not disturb the beads at the back wall of the tube. Ensure that all of the fluid is transferred.

13.3.3.10 Do not discard the beads or microcentrifuge tube after transferring the volume from the first acid dissociation to the well slide. Perform the steps in Sections 13.3.3.1 through 13.3.3.9 a second time. The volume from the second dissociation can be added to the slide containing the volume from the first dissociation, or can be applied to a second slide.

NOTE: If one slide is used, exert extra care when using Dynal Spot-On slides to ensure that the sample stays within the smaller-diameter wells on these slides.

13.3.3.11 Record the date and time the purified sample was applied to the slide(s).

13.3.3.12 Air-dry the sample on the well slide(s). Because temperature and humidity varies from laboratory to laboratory, no minimum time is specified. However, the laboratory must take care to ensure that the sample has dried completely before staining to prevent losses during the rinse steps. A slide warmer set at 35 ^{o}C to 42 ^{o}C also can be used.

14.0 Sample Staining

NOTE: The sample must be stained within 72 hours of application of the purified sample to the slide.

14.1 Prepare positive and negative controls.

14.1.1 For the positive control, pipette 10 µL of positive antigen or 200 to 400 intact oocysts and 200 to 400 cysts to the center of a well.

14.1.2 For the negative control, pipette 50 µL of 150 mM PBS (Section 7.6.4) into the center of a well and spread it over the well area with a pipette tip.

14.1.3 Air-dry the control slides (see Section 13.3.3.12 for guidance).

14.2 Apply 50-µL of absolute methanol to each well containing the dried sample and allow to air-dry for 3 to 5 minutes.

14.3 Follow manufacturer's instructions in applying stain to slide.

14.4 Place the slides in a humid chamber in the dark and incubate at room temperature for approximately 30 minutes. The humid chamber consists of a tightly sealed plastic container containing damp paper towels on top of which the slides are placed.

14.5 Apply one drop of wash buffer (prepared according to the manufacturer's instructions [Section 7.6]) to each well. Tilt each slide on a clean paper towel, long edge down. Gently aspirate the excess detection reagent from below the well using a clean Pasteur pipette or absorb with paper towel or other absorbent material placed at edge of slide. Avoid disturbing the sample.

NOTE: If using the Merifluor stain (Section 7.6.1), do not allow slides to dry completely.

14.6 Apply 50 µL of 4',6-diamidino-2-phenylindole (DAPI) staining solution (Section 7.7.2) to each well. Allow to stand at room temperature for a minimum of 1 minute. (The solution concentration may be increased up to 1 µg /mL if fading/diffusion of DAPI staining is encountered, but the staining solution must be tested first on expendable environmental samples to confirm that staining intensity is appropriate.)

14.7 Apply one drop of wash buffer (prepared according to the manufacturer's instructions [Section 7.6]) to each well. Tilt each slide on a clean paper towel, long edge down. Gently aspirate the excess DAPI staining solution from below the well using a clean Pasteur pipette or absorb with paper towel or other absorbent material placed at edge of slide. Avoid disturbing the sample.

NOTE: If using the Merifluor stain (Section 7.6.1), do not allow slides to dry completely.

14.8 Add mounting medium (Section 7.8) to each well.
14.9 Apply a cover slip. Use a tissue to remove excess mounting fluid from the edges of the coverslip. Seal the edges of the coverslip onto the slide using clear nail polish.
14.10 Record the date and time that staining was completed on the bench sheet. If slides will not be read immediately, store in a humid chamber in the dark at 0 ^{o}C to 8 ^{o}C until ready for examination.

15.0 Examination

NOTE: Although immunofluorescence assay (FA) and 4',6-diamidino-2-phenylindole (DAPI) and differential interference contrast (DIC) microscopy examination and confirmation should be performed immediately after staining is complete, laboratories have up to 7 days from completion of sample staining to complete the examination and confirmation of samples. However, if fading/diffusion of FITC or DAPI staining is noticed, the laboratory must reduce this holding time. In addition the laboratory may adjust the concentration of the DAPI staining solution (Sections 7.7.2) so that fading/diffusion does not occur.

15.1 Scanning technique: Scan each well in a systematic fashion. An up-and-down or a side-to-side scanning pattern may be used (Figure 4).
15.2 Examination using immunofluorescence assay (FA), 4',6-diamidino-2-phenylindole (DAPI) staining characteristics, and differential interference contrast (DIC) microscopy. The minimum magnification requirements for each type of examination are noted below.
NOTE: All shape and measurements must be determined using 1000X magnification and reported to the nearest 0.5 µm.

Record examination results for Cryptosporidium oocysts on a Cryptosporidium report form; record examination results for Giardia cysts on a Giardia report form. All oocysts and cysts that meet the criteria specified in Sections 15.2.2 and 15.2.3, less atypical organisms specifically identified as non-target organisms by DIC or DAPI (e.g. possessing spikes, stalks, appendages, pores, one or two large nuclei filling the cell, red fluorescing chloroplasts, crystals, spores, etc), must be reported.

15.2.1 Positive and negative staining control.

15.2.1.1 Each analyst must characterize a minimum of three Cryptosporidium oocysts and three Giardia cysts on the positive staining control slide before examining field sample slides. This characterization must be performed by each analyst during each microscope examination session.

FITC examination must be conducted at a minimum of 200X total magnification, DAPI examination must be conducted at a minimum of 400X, and DIC examination must be conducted at a minimum of 1000X. Size, shape, and DIC and DAPI characteristics of the three Cryptosporidium oocysts and Giardia cysts must be recorded by the analyst on a microscope log. The analyst also must indicate on each sample report form whether the positive staining control was acceptable.

15.2.1.2 Examine the negative staining control to confirm that it does not contain any oocysts or cysts (Section 14.1). Indicate on each sample report form whether the negative staining control was acceptable.

15.2.1.3 If the positive staining control contains oocysts and cysts within the expected range and at the appropriate fluorescence for both FA and DAPI, and the negative staining control does not contain any oocysts or cysts (Section 14.1), proceed to Sections 15.2.2 and 15.2.3.

15.2.2 Sample examination—Cryptosporidium

15.2.2.1 FITC examination (the analyst must use a minimum of 200X total magnification). Use epifluorescence to scan the entire well for apple-green fluorescence of oocyst and cyst shapes. When brilliant apple-green fluorescing ovoid or spherical objects 4 to 6 µm in diameter are observed with brightly highlighted edges, increase magnification to 400X and switch the microscope to the UV filter block for DAPI (Section 15.2.2.2), then to DIC (Section 15.2.2.3).

15.2.2.2 DAPI examination (the analyst must use a minimum of 400X total magnification). Using the UV filter block for DAPI, the object will exhibit one of the following characteristics: (a) Light blue internal staining (no distinct nuclei) with a green rim (b) Intense blue internal staining (c) Up to four distinct, sky-blue nuclei Record oocysts in category (a) as DAPI negative; record oocysts in categories (b) and (c) as DAPI positive.

15.2.2.3 DIC examination (the analyst must use a minimum of 1000X total magnification). Using DIC, look for external or internal morphological characteristics atypical of Cryptosporidium oocysts (e.g., spikes, stalks, appendages, pores, one or two large nuclei filling the cell, red fluorescing chloroplasts, crystals, spores, etc.) (adapted from Reference 20.6). If atypical structures are not observed, then categorize each apple-green fluorescing object as: (a) An empty Cryptosporidium oocyst (b) A Cryptosporidium oocyst with amorphous structure (c) A Cryptosporidium oocyst with internal structure (one to four sporozoites/oocyst)

Using 1000X total magnification, record the shape, measurements (to the nearest 0.5 µm), and number of sporozoites (if applicable) for each apple-green fluorescing object meeting the size and shape characteristics. Although not a defining characteristic, surface oocyst folds may be observed in some specimens.

NOTE: All measurements must be made at 1000X magnification.

15.2.3 Sample examination—Giardia

15.2.3.1 FITC examination (the analyst must use a minimum of 200X total magnification). When brilliant apple-green fluorescing round to oval objects (8 - 18 µm long by 5 - 15 µm wide) are observed, increase magnification to 400X and switch the microscope to the UV filter block for DAPI (Section 15.2.3.2) then to DIC (Section 15.2.3.3).

15.2.3.2 DAPI examination (the analyst must use a minimum of 400X total magnification). Using the UV filter block for DAPI, the object will exhibit one or more of the following characteristics: (a) Light blue internal staining (no distinct nuclei) and a green rim (b) Intense blue internal staining (c) Two to four sky-blue nuclei Record cysts in category (a) as DAPI negative; record cysts in categories (b) and (c) as DAPI positive.

15.2.3.3 DIC examination (the analyst must use a minimum of 1000X total magnification). Using DIC, look for external or internal morphological characteristics atypical of Giardia cysts (e.g., spikes, stalks, appendages, pores, one or two large nuclei filling the cell, red fluorescing chloroplasts, crystals, spores, etc.) (adapted from Reference 20.6). If atypical structures are not observed, then categorize each object meeting the criteria specified in Sections 15.2.3.1 - 15.2.3.3 as one of the following, based on DIC examination: (a) An empty Giardia cyst (b) A Giardia cyst with amorphous structure (c) A Giardia cyst with one type of internal structure (nuclei, median body, or axonemes), or (d) A Giardia cyst with more than one type of internal structure.

Using 1000X total magnification, record the shape, measurements (to the nearest 0.5 µm), and number of nuclei and presence of median body or axonemes (if applicable) for each apple-green fluorescing object meeting the size and shape characteristics.

NOTE: All measurements must be made at 1000X magnification.

15.2.4 Record the date and time that sample examination was completed on the report form. 15.2.5 Report Cryptosporidium and Giardia concentrations as oocysts/L and cysts/L.

16.0 Analysis of Complex Samples

16.1 Some samples may contain high levels (>1000/L) of oocysts and cysts and/or interfering organisms, substances, or materials. Some samples may clog the filter (Section 12.0); others will not allow separation of the oocysts and cysts from the retentate or eluate; and others may contain materials that preclude or confuse microscopic examination.

16.2 If the sample holding time has not been exceeded and a full-volume sample cannot be filtered, dilute an aliquot of sample with reagent water and filter this smaller aliquot (Section 12.0). This dilution must be recorded and reported with the results.

16.3 If the holding times for the sample and for microscopic examination of the cleaned up retentate/eluate have been exceeded, the site should be re-sampled. If this is not possible, the results should be qualified accordingly.

17.0 Method Performance

17.1 Method acceptance criteria are shown in Tables 3 and 4 in Section 21.0. The initial and ongoing precision and recovery criteria are based on the results of spiked reagent water samples analyzed during the Information Collection Rule Supplemental Surveys (Reference 20.12). The matrix spike and matrix spike duplicate criteria are based on spiked source water data generated during the interlaboratory validation study of Method 1623 involving 11 laboratories and 11 raw surface water matrices across the U.S. (Reference 20.10).

NOTE: Some sample matrices may prevent the MS acceptance criteria in Tables 3 and 4 to be met. An assessment of the distribution of MS recoveries across 430 MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.

18.0 Pollution Prevention

18.1 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.

18.2 Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

19.0 Waste Management

19.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the biohazard and hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required. An overview of these requirements can be found in the *Environmental Management Guide for Small Laboratories* (EPA 233-B-98-001).

19.2 Samples, reference materials, and equipment known or suspected to have viable oocysts or cysts attached or contained must be sterilized prior to disposal.

19.3 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better: Laboratory Chemical Management for Waste Reduction,* both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

20.0 References

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20.11 "Dynabeads® GC-Combo," Dynal Microbiology R&D, P.O. Box 8146 Dep., 0212 Oslo, Norway (September 1998, Revision no. 01).
20.12 USEPA. Implementation and Results of the Information Collection Rule Supplemental Surveys. EPA-815-R-01-003. Office of Water, Office of Ground Water and Drinking Water, Standards and Risk Management Division, Washington, DC (2001).
20.13 Connell, K., J. Scheller, K. Miller, and C.C. Rodgers, 2000. Performance of Methods 1622 and 1623 in the ICR Supplemental Surveys. Proceedings, American Water Works Association Water Quality Technology Conference, November 5 - 9, 2000, Salt Lake City, UT.

21.0 Tables and Figures

Table 1. Method Holding Times (See Section 8.2 for details)

Sample Processing Step	Maximum Allowable Time between Breaks
Collection	
Filtration	
> Up to 96 hours are permitted between sample collection (if shipped to the laboratory as a bulk sample) or filtration (if filtered in the field) and initiation of elution	
Elution	
	These steps must be completed in 1 working day
Concentration	
Purification	
Application of purified sample to slide	
Drying of sample	
> Up to 72 hours are permitted from application of the purified sample to the slide to staining	
Staining	
> Up to 7 days are permitted between sample staining and examination	
Examination	

Table 2. Tier 1 and Tier 2 Validation/Equivalency Demonstration Requirements

Test	Description	Tier 1 modification(1)	Tier 2 modification(2)
IPR (Section 9.4)	4 replicates of spiked reagent water	Required. Must be accompanied by a method blank.	Required per laboratory
Method blank (Section 9.6)	Unspiked reagent water	Required	Required per laboratory
MS (Section 9.5.1)	Spiked matrix water	Required on each water to which the modification will be applied and on every 20th sample of that water thereafter. Must be accompanied by an unspiked field sample collected at the same time as the MS sample	Not required
MS/MSD (Section 9.5)	2 replicates of spiked matrix water	Recommended, but not required. Must be accompanied by an unspiked field sample collected at the same time as the MS sample	Required per laboratory. Each laboratory must analyze a different water.

(1) If a modification will be used only in one laboratory, these tests must be performed and the results must meet all of the QC acceptance criteria in the method (these tests also are required the first time a laboratory uses the validated version of the method).
(2) If nationwide approval of a modification is sought for one type of water matrix (such as surface water), a minimum of 3 laboratories must perform the tests and the results from each lab individually must meet all QC acceptance criteria in the method. If more than 3 laboratories are used in a study, a minimum of 75% of the laboratories must meet all QC acceptance criteria.

NOTE: The initial precision and recovery and ongoing precision and recovery (OPR) acceptance criteria listed in Tables 3 and 4 are based on results from 293 Cryptosporidium OPR samples and 186 Giardia OPR samples analyzed by six laboratories during the Information Collection Rule Supplemental Surveys (Reference 20.12). The matrix spike acceptance criteria are based on data generated through interlaboratory validation of Method 1623 (Reference 20.10).

Table 3. Quality Control Acceptance Criteria for *Cryptosporidium*

Performance test	Section	Acceptance criteria
	9.4 9.4.2 9.4.2	
Initial precision and recovery Mean recovery (percent) Precision (as maximum relative standard deviation)		24 - 100 55
Ongoing precision and recovery (percent)	9.7	11 - 100
Matrix spike/matrix spike duplicate (for method modifications) Mean recovery[1, 2](as percent) Precision (as maximum relative percent difference)	9.5 9.5.2 9.5.2	13 - 111 61

(1) The acceptance criteria for mean MS/MSD recovery serves as the acceptance criteria for MS recovery during routine use of the method (Section 9.5.1).
(2) Some sample matrices may prevent the acceptance criteria from being met. An assessment of the distribution of MS recoveries from multiple MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.

Table 4.
Quality Control Acceptance Criteria for *Giardia*

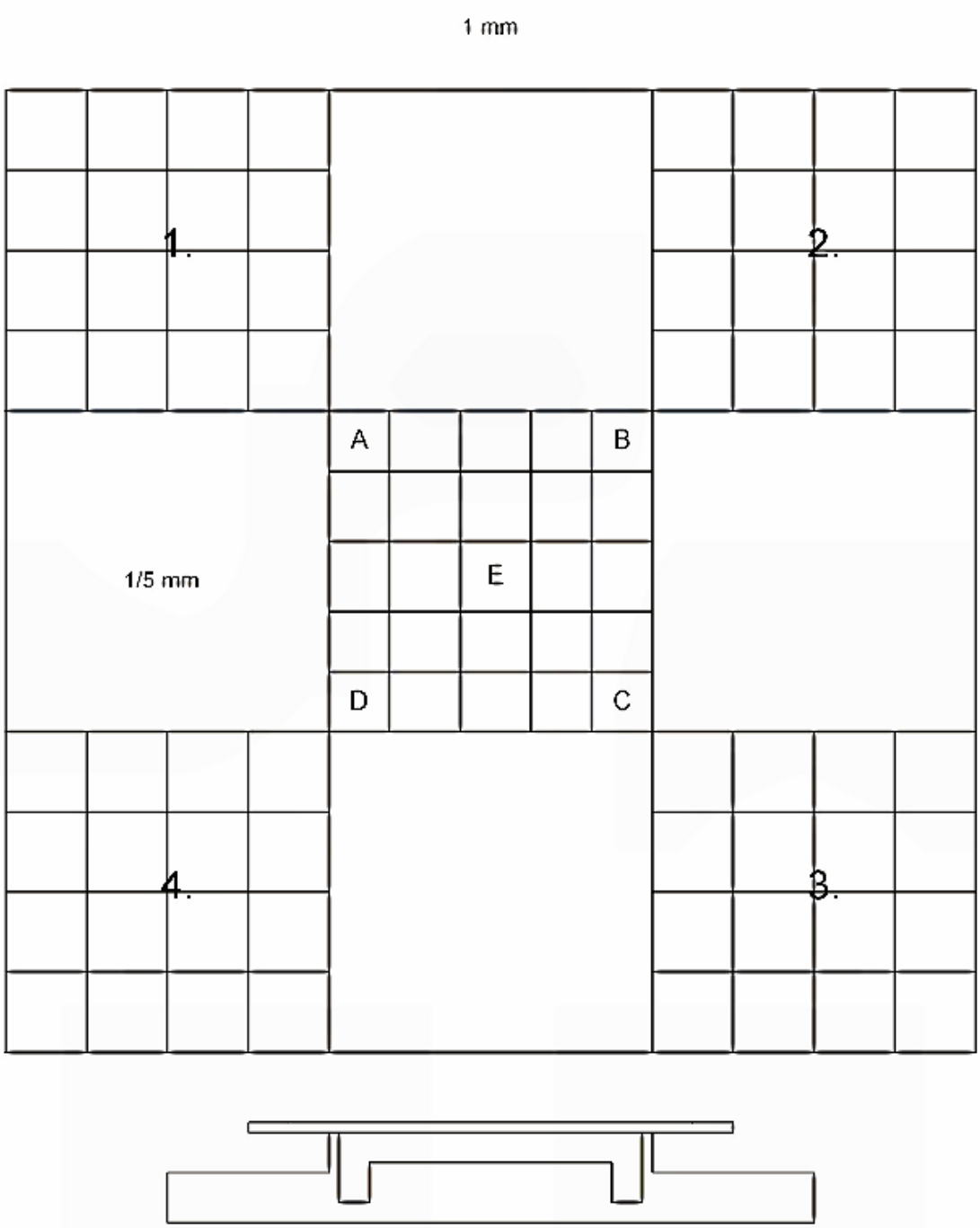
Quality Control Acceptance Criteria for *Giardia* Performance test	Section	Acceptance criteria
	9.4 9.4.2 9.4.2	
Initial precision and recovery Mean recovery (percent) Precision (as maximum relative standard deviation)		24 - 100 49
Ongoing precision and recovery (percent)	9.7 9.5 9.5.2 9.5.2	14 - 100
Matrix spike/matrix spike duplicate (for method modifications) Mean recovery* (as percent) Precision (as maximum relative percent difference)		15 - 118 30

(1) The acceptance criteria for mean MS/MSD recovery serves as the acceptance criteria for MS recovery during routine use of the method (Section 9.5.1).
(2) Some sample matrices may prevent the acceptance criteria from being met. An assessment of the distribution of MS recoveries across multiple MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.

Table 5. Distribution of Matrix Spike Recoveries from Multiple Samples Collected from 87 Source Waters During the ICR Supplemental Surveys (Adapted from Reference 20.13)

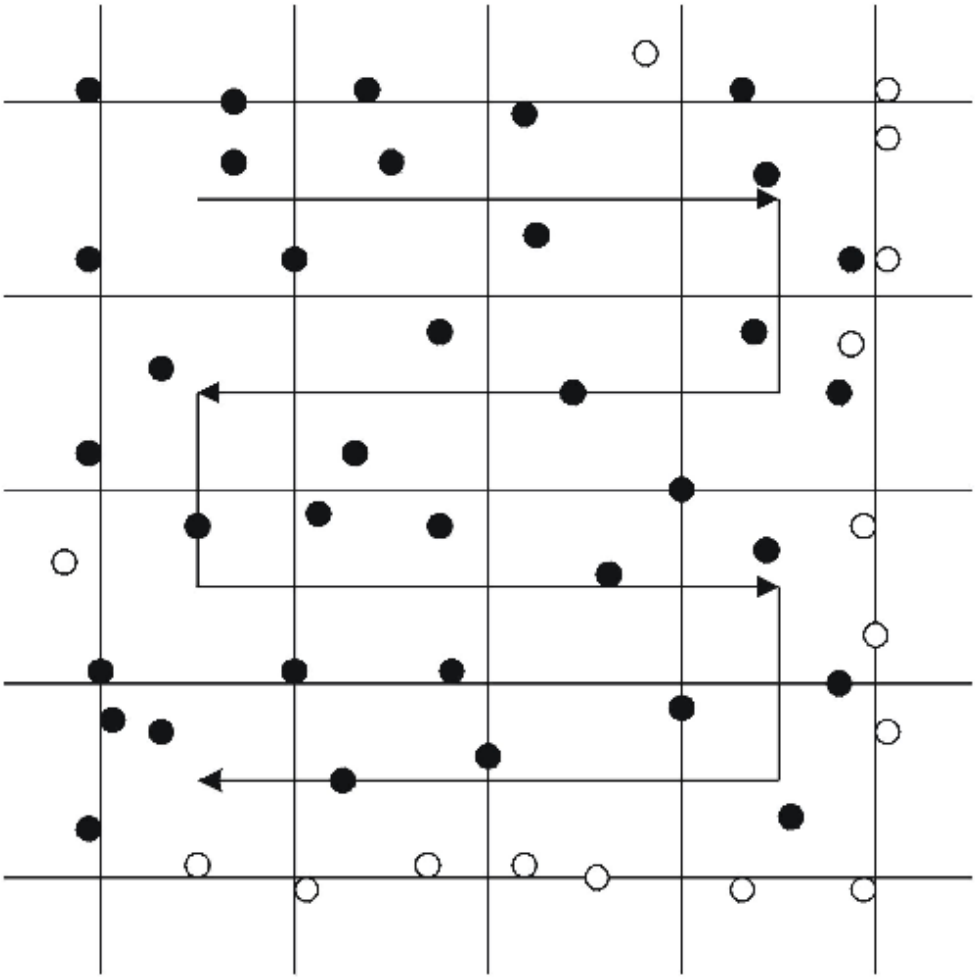
Source Waters During the ICR Supplemental Surveys (Adapted from Reference 20.13) MS Recovery Range	Percent of 430 *Cryptosporidium*MS Samples in Recovery Range	Percent of 270 *Giardia*MS Samples in Recovery Range
<10%	6.7%	5.2%
>10% - 20%	6.3%	4.8%
>20% - 30%	14.9%	7.0%
>30% - 40%	14.2%	8.5%
>40% - 50%	18.4%	17.4%
>50% - 60%	17.4%	16.3%
>60% - 70%	11.2%	16.7%
>70% - 80%	8.4%	14.1%
>80% - 90%	2.3%	6.3%
>90%	0.2%	3.7%

Figure 1.



Hemacytometer Platform Ruling. Squares 1, 2, 3, and 4 are used to count stock suspensions of *Cryptosporidium*oocysts and *Giardia* cysts (after Miale, 1967)

Figure 2.



Manner of Counting Oocysts and Cysts in 1 Square mm. Dark organisms are counted and light organisms are omitted (after Miale, 1967).

Figure 3. Laboratory Filtration System

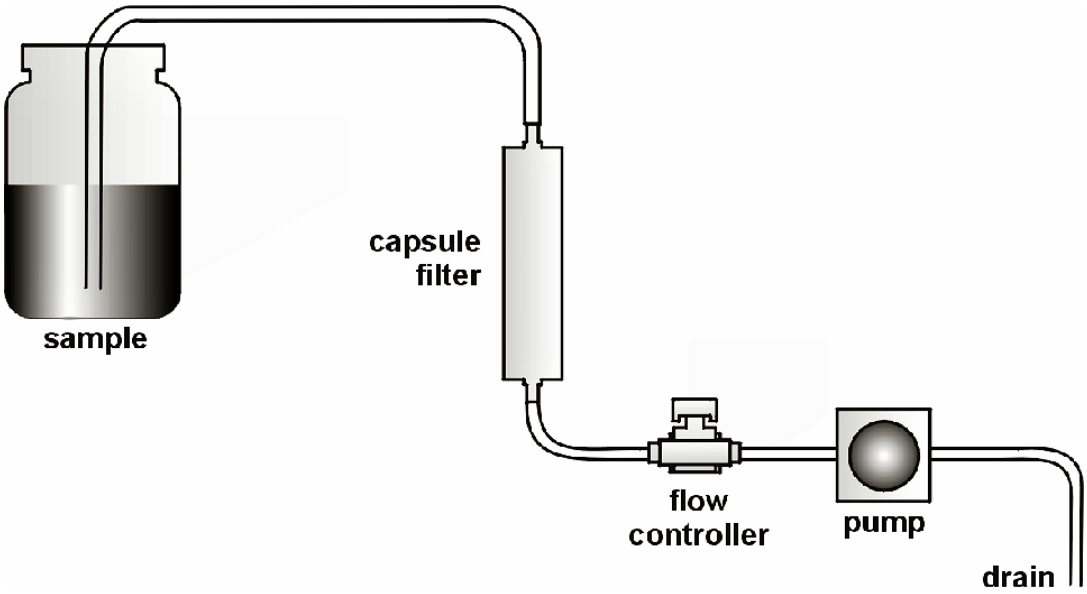
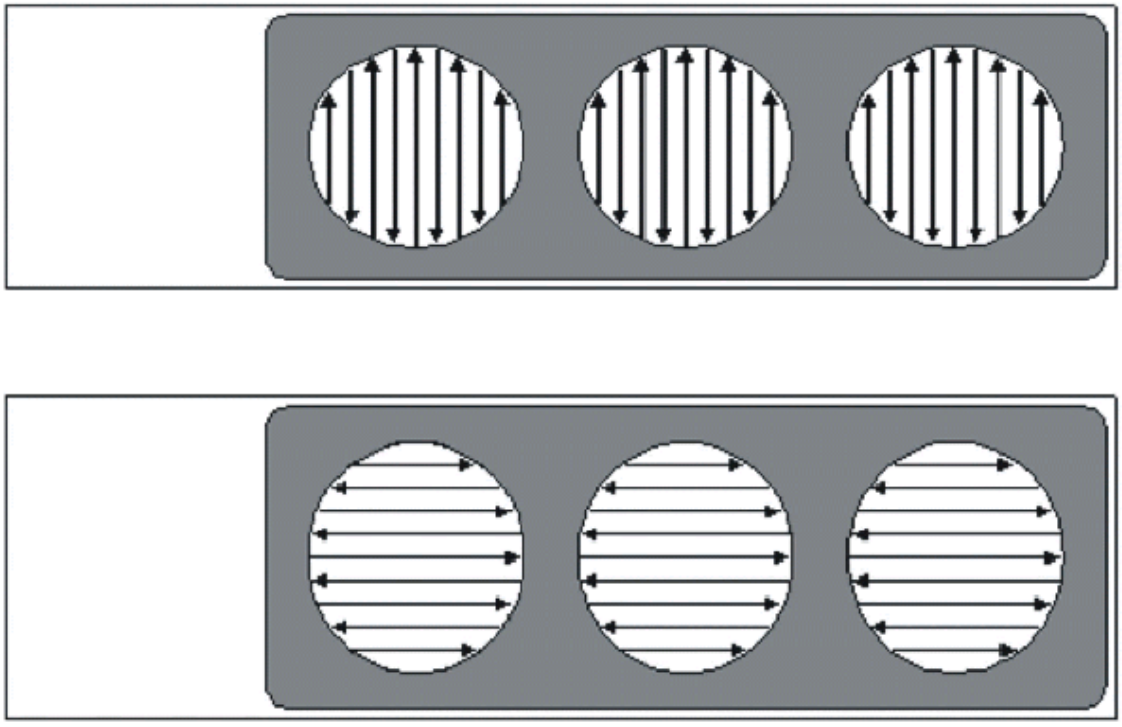


Figure 4. Methods for Scanning a Well Slide



Method 1604: Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium)

1.0 Scope and Application

1.1 This test method describes a sensitive and differential membrane filter (MF) medium, using MI agar or MI broth, for the simultaneous detection and enumeration of both total coliforms (TC) and *Escherichia coli* (*E. coli*) in water samples in 24 hours or less on the basis of their specific enzyme activities. Two enzyme substrates, the fluorogen 4-Methylumbelliferyl- β -D-galactopyranoside (MUGal) and a chromogen Indoxyl-β-D-glucuronide (IBDG), are included in the medium to detect the enzymes β -galactosidase and β -glucuronidase, respectively, produced by TC and *E. coli*, respectively.

1.2 Total coliforms include species that may inhabit the intestines of warm-blooded animals or occur naturally in soil, vegetation, and water. They are usually found in fecally-polluted water and are often associated with disease outbreaks. Although they are not usually pathogenic themselves, their presence in drinking water indicates the possible presence of pathogens. *E. coli*, one species of the coliform group, is always found in feces and is, therefore, a more direct indicator of fecal contamination and the possible presence of enteric pathogens. In addition, some strains of *E. coli* are pathogenic (Reference 16.12).

1.3 This method, which has been validated for use with drinking water in single-lab and multi-lab studies (References 16.8 - 16.10), will be used primarily by certified drinking water laboratories for microbial analysis of potable water. Other uses include recreational, surface or marine water, bottled water, groundwater, well water, treatment plant effluents, water from drinking water distribution lines, drinking water source water, and possibly foods, pharmaceuticals, clinical specimens (human or veterinary), other environmental samples (*e.g.*, aerosols, soil, runoff, or sludge) and/or isolation and separation of transformants though the use of *E. coli lac Z* or *gus* A/*uid* reporter genes (Reference 16.11).

1.4 Since a wide range of sample volumes or dilutions can be analyzed by the MF technique, a wide range of *E. coli* and TC levels in water can be detected and enumerated.

2.0 Summary of Method

2.1 An appropriate volume of a water sample (100 mL for drinking water) is filtered through a 47-mm, 0.45-µm pore size cellulose ester membrane filter that retains the bacteria present in the sample. The filter is placed on a 5-mL plate of MI agar or on an absorbent pad saturated with 2-3 mL of MI broth, and the plate is incubated at 35°C for up to 24 hours. The bacterial colonies that grow on the plate are inspected for the presence of blue color from the breakdown of IBDG by the *E. coli* enzyme β -glucuronidase and fluorescence under long wave ultraviolet light (366 nm) from the breakdown of MUGal by the TC enzyme β -galactosidase (Reference 16.8).

3.0 Definitions

3.1 Total coliforms (TC) - In this method, TC are those bacteria that produce fluorescent colonies upon exposure to long wave ultraviolet light (366 nm) after primary culturing on MI agar or broth (See Figure 1.). The fluorescent colonies can be completely blue-white (TC other than *E. coli*) or blue-green (*E. coli*) in color or fluorescent halos may be observed around the edges of the blue-green E. coli colonies. In addition, non-fluorescent blue colonies, which rarely occur, are added to the total count because the fluorescence is masked by the blue color from the breakdown of IBDG (Reference 16.8).

3.2 Escherichia coli - In this method, the E. coli are those bacteria that produce blue colonies under ambient light after primary culturing on MI agar or broth (See Figures 1 and 2.). These colonies can be fluorescent or non-fluorescent under long wave ultraviolet light (366 nm) (Reference 16.8).

4.0 Interferences and Contamination

4.1 Water samples containing colloidal or suspended particulate material can clog the membrane filter, thereby preventing filtration, or cause spreading of bacterial colonies which could interfere with identification of target colonies. However, the blue E. coli colonies can often be counted on plates with heavy particulates or high concentrations of total bacteria (See Figures 2 and 3.) (Reference 16.8).

4.2 The presence of some lateral diffusion of blue color away from the target E. coli colonies can affect enumeration and colony picking on plates with high concentrations of E. coli. This problem should not affect filters with low counts, such as those obtained with drinking water or properly diluted samples (Reference 16.8).

4.3 Tiny, flat or peaked pinpoint blue colonies (# 0.5-mm in diameter on filters containing # 200 colonies) may be due to species other than E. coli. These colonies occur occasionally in low numbers and should be excluded from the count of the E. coli colonies, which are usually much larger in size (1-3-mm in diameter). The small colonies have never been observed in the absence of typical E. coli, but, if such should occur, the sample should not be considered E. coli-positive unless at least one colony has been verified by another method [e.g., EC medium with 4-Methylumbelliferyl- β -D-glucuronide (MUG) or API 20E strips] (Reference 16.8).

4.4 Bright green, fluorescent, non-blue colonies, observed along with the typical blue/white or blue-green fluorescent TC colonies, may be species other than coliforms. These colonies, which generally occur in low numbers (# 5%) and can usually be distinguished from the TC, should be eliminated from the TC count. An increase in the number of bright green colonies may indicate an unusual sample population or a breakdown of the cefsulodin in the medium (Reference 16.8).

5.0 Safety

5.1 The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents, and materials, and while operating sterilization equipment.

5.2 Mouth-pipetting is prohibited.

5.3 Avoid prolonged exposure to long wave or germicidal ultraviolet light.

5.4 Autoclave all contaminated plates and materials at the end of the analysis.

6.0 Equipment and Supplies

6.1 Incubator set at 35°C ± 0.5°C, with approximately 90% humidity if loose-lidded Petri dishes are used.

6.2 Stereoscopic microscope, with magnification of 10-15x, wide-field type.

6.3 A microscope lamp producing diffuse light from cool, white fluorescent lamps adjusted to give maximum color.

6.4 Hand tally.

6.5 Pipet container of stainless steel, aluminum, or Pyrex glass, for pipets.

6.6 Graduated cylinders (100-mL for drinking water), covered with aluminum foil or kraft paper and sterilized.

6.7 Membrane filtration units (filter base and funnel), glass, plastic or stainless steel. These are wrapped with aluminum foil or kraft paper and sterilized.

6.8 Germicidal ultraviolet (254 nm) light box for sanitizing the filter funnels is desirable, but optional.

6.9 Line vacuum, electric vacuum pump, or aspirator is used as a vacuum source. In an emergency, a hand pump or a syringe can be used. Such vacuum-producing devices should be equipped with a check valve to prevent the return flow of air.

6.10 Vacuum filter flask, usually 1 liter, with appropriate tubing. Filter manifolds to hold a number of filter bases are desirable, but optional.

6.11 Safety trap flask, placed between the filter flask and the vacuum source.

6.12 Forceps, straight (preferred) or curved, with smooth tips to permit easy handling of filters without damage.

6.13 Alcohol, 95% ethanol, in small wide-mouthed vials, for sterilizing forceps.

6.14 Bunsen or Fisher-type burner or electric incinerator unit.

6.15 Sterile T.D. (To Deliver) bacteriological or Mohr pipets, glass or plastic (1-mL and 10-mLvolumes).

6.16 Membrane Filters (MF), white, grid-marked, cellulose ester, 47-mm diameter, 0.45 µm ± 0.02-µm pore size, pretrial or sterilized for 10 minutes at 121°C (15-lb pressure).

6.17 Long wave ultraviolet lamp (366 nm), handheld 4-watt (preferred) or 6-watt, or microscope attachment.

6.18 Dilution water: Sterile phosphate-buffered dilution water, prepared in large volumes (e.g., 1 liter)for wetting membranes before addition of the sample and for rinsing the funnel after sample filtration or in 99-mL dilution blanks [Section 9050C in Standard Methods (Reference 16.2)].

6.19 Indelible ink marker for labeling plates.

6.20 Thermometer, checked against a National Institute of Science and Technology (NIST)-certified thermometer, or one traceable to an NIST thermometer.

6.21 Petri dishes, sterile, plastic, 9 x 50 mm, with tight-fitting lids, or 15 x 60 mm, glass or plastic, with loose-fitting lids; 15 x 100 mm dishes may also be used.

6.22 Bottles, milk dilution, borosilicate glass, screw-cap with neoprene liners, marked at 99 mL for 1:100 dilutions (if needed). Dilution bottles marked at 90 mL, or tubes marked at 9 mL may be used for 1:10 dilutions.

6.23 Flasks, borosilicate glass, screw-cap, 250- to 2000-mL volume, for agar preparation.

6.24 Waterbath maintained at 50°C for tempering agar.

6.25 Syringe filter, sterile, disposable, 25-mm diameter, 0.22-µm pore size, to filter cefsulodin for MI agar.

6.26 Syringe, sterile, plastic, disposable, 20-cc capacity. Autoclaved glass syringes are also acceptable.

6.27 Test tubes, sterile, screw-cap, 20 x 150-mm, borosilicate glass or plastic, with lids.

6.28 Sterilization filter units, presterile, disposable, 500- or 1000-mL capacity, 0.2-µm pore size, to filter stock buffer solutions.
6.29 Sterile 47-mm diameter absorbent pads (used with MI broth).
Note: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

7.0 Reagents and Standards
7.1 Purity of Reagents: Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society (Reference 16.1). The agar used in preparation of culture media must be of microbiological grade.
7.2 Whenever possible, use commercial culture media as a means of quality control.
7.3 Purity of Water: Reagent-grade distilled water conforming to Specification D1193, Type II water or better, ASTM Annual Book of Standards (Reference 16.3).
7.4 Buffered Dilution Water (Reference 16.2)
7.4.1 Stock Phosphate Buffer Solution (Reference 16.2):
Potassium Dihydrogen Phosphate (KH_2PO_4) 34.0 g Reagent-Grade Distilled Water 500 mL
7.4.2 Preparation of Stock Buffer Solution: Adjust the pH of the solution to 7.2 with 1 N NaOH, and bring volume to 1000 mL with reagent-grade distilled water. Sterilize by filtration or autoclave for 15 minutes at 121°C (15-lb pressure).
7.4.3 $MgCl_2$ Solution (Reference 16.2): Dissolve 38 g anhydrous $MgCl_2$ (or 81.1 g $MgCl_2C6H_2O$) in one liter of reagent-grade distilled water. Sterilize by filtration or autoclave for 15 minutes at 121°C (15-lb pressure).
7.4.4 Storage of Stock Buffer and $MgCl_2$ Solutions: After sterilization of the stock solutions, store in the refrigerator until used. Handle aseptically. If evidence of mold or other contamination appears in either stock, the solution should be discarded, and a fresh solution should be prepared.
7.4.5 Working Solution (Final pH 7.0 ± 0.2): Add 1.25 mL phosphate buffer stock (Section 7.4.2) and 5 mL $MgCl_2$ stock (Section 7.4.3) for each liter of reagent-grade distilled water prepared. Mix well, and dispense in appropriate amounts for dilutions in screw-cap dilution bottles or culture tubes, and/or into larger containers for use as rinse water. Autoclave at 121°C (15-lb pressure) for 15 minutes. Longer sterilization times may be needed depending on the container and load size and the amount of time needed for the liquid to reach 121°C.

7.5 MI Agar (Reference 16.8)

7.5.1 Composition:

Proteose Peptone #3 5.0 g
Yeast Extract 3.0 g
β -D-Lactose 1.0 g
4-Methylumbelliferyl- β -D-Galactopyranoside (MUGal)
(Final concentration 100µg/mL) 0.1 g
Indoxyl- β -D-Glucuronide (IBDG)
(Final concentration 320 µg/mL) 0.32 g
NaCl 7.5 g
K_2HPO_4 3.3 g
KH_2PO_4 1.0 g
Sodium Lauryl Sulfate 0.2 g
Sodium Desoxycholate 0.1 g
Agar 15.0 g
Reagent-Grade Distilled Water 1000 mL

7.5.2 Cefsulodin Solution (1 mg / 1 mL): Add 0.02 g of cefsulodin to 20 mL reagent-grade distilled water, sterilize using a 0.22-µm syringe filter, and store in a sterile tube at 4°C until needed. Prepare fresh solution each time. Do not save the unused portion.

7.5.3 Preparation: Autoclave the medium for 15 minutes at 121°C (15-lb pressure), and add 5 mL of the freshly-prepared solution of Cefsulodin (5 µg/mL final concentration) per liter of tempered agar medium. Pipet the medium into 9 x 50-mm Petri dishes (5 mL/plate). Store plates at 4°C for up to 2 weeks. The final pH should be 6.95 ± 0.2.

7.6 MI Broth: The composition of MI broth is the same as MI agar, but without the agar. The final pH of MI broth should be 7.05 ± 0.2. The broth is prepared and sterilized by the same methods described for MI agar in Sections 7.5.1, 7.5.2, and 7.5.3, except that absorbent pads are placed in 9 x 50 mm Petri dishes and saturated with 2-3 mL of MI broth containing 5 :g/mL final concentration of Cefsulodin. Alternately, the broth can be filter-sterilized. Excess broth is poured off before using the plates. Plates should be stored in the refrigerator and discarded after 96 hours (Reference 16.15).

7.7 Tryptic Soy Agar/Trypticase Soy Agar (Difco 0369-17-6, BD 4311043, Oxoid CM 0129B, or equivalent) (TSA)

7.7.1 ***Composition*****:**

Tryptone 15.0 g
Soytone 5.0 g
NaCl 5.0 g
Agar 15.0 g

7.7.2 ***Preparation*****:** Add the dry ingredients listed above to 1000 mL of reagent-grade distilled water, and heat to boiling to dissolve the agar completely. Autoclave at 121°C (15-lb pressure) for 15 minutes. Dispense the agar into 9 x 50-mm Petri dishes (5 mL/plate). Incubate the plates for 24 - 48 hours at 35°C to check for contamination. Discard any plates with growth. If > 5% of the plates show contamination, discard all plates, and make new medium. Store at 4°C until needed. The final pH should be 7.3 ± 0.2.

8.0 Sample Collection, Preservation, and Storage

8.1 Water samples are collected in sterile polypropylene sample containers with leakproof lids.

8.2 Sampling procedures are described in detail in Sections 9060A and 9060B of the 18th edition of *Standard Methods for the Examination of Water and Wastewater* (Reference 16.2) or in the *USEPA Microbiology Methods Manual*, Section II, A (Reference 16.6). Residual chlorine in drinking water (or chlorinated effluent) samples should be neutralized with sodium thiosulfate (1 mL of a 10% solution per liter of water) at the time of collection. Adherence to sample preservation procedures and holding time limits are critical to the production of valid data. Samples not collected according to these rules should not be analyzed.

8.2.1 *Storage Temperature and Handling Conditions:* Ice or refrigerate water samples at a temperature of 1-4°C during transit to the laboratory. Use insulated containers to assure proper maintenance of storage temperature. Take care that sample bottles are not totally immersed in water from melted ice during transit or storage.

8.2.2 *Holding Time Limitations:* Analyze samples as soon as possible after collection. Drinking water samples should be analyzed within 30 h of collection (Reference 16.13). Do not hold source water samples longer than 6 h between collection and initiation of analyses, and the analyses should be complete within 8 h of sample collection.

9.0 Calibration and Standardization

9.1 Check temperatures in incubators twice daily to ensure operation within stated limits (Reference 16.14).

9.2 Check thermometers at least annually against an NIST-certified thermometer or one traceable to NIST. Check mercury columns for breaks.

10.0 Quality Control (QC)

10.1 Pretest each batch of MI agar or broth for performance (*i.e.*, correct enzyme reactions) with known cultures (*E. coli*, TC, and a non-coliform).

10.2 Test new lots of membrane filters against an acceptable reference lot using the method of Brenner and Rankin (Reference 16.7).

10.3 Perform specific filtration control tests each time samples are analyzed, and record the results.

> **10.3.1** *Filter Control*: Place one or more membrane filters on TSA plates, and incubate the plates for 24 hours at 35°C. Absence of growth indicates sterility of the filter(s).
>
> **10.3.2** *Phosphate-Buffered Dilution Water Controls*: Filter a 50-mL volume of sterile dilution water before beginning the sample filtrations and a 50-mL volume of dilution water after completing the filtrations. Place the filters on TSA plates, and incubate the plates for 24 hours at 35°C. Absence of growth indicates sterility of the dilution water.
>
> **10.3.3** *Agar or Broth Controls*: Place one or more TSA plates and one or more MI agar plates or MI broth pad plates in the incubator for 24 hours at 35°C. Broth pad plates should be incubated *grid-side up*, not inverted like the agar plates. Absence of growth indicates sterility of the plates.

10.4 See recommendations on quality control for microbiological analyses in the "*Manual for the Certification of Laboratories Analyzing Drinking Water*: *Criteria and Procedures; Quality Assurance*" (Reference 16.15) and the *USEPA Microbiology Methods Manual*, part IV, C (Reference 16.6).

11.0 Procedure

11.1 Prepare MI agar or MI broth and TSA as described in Sections 7.5, 7.6, and 7.7. If plates are made ahead of time and stored in the refrigerator, remove them and allow them to warm to room temperature. The crystals that form on MI agar after refrigeration will disappear as the plates warm up (Reference 16.8).

11.2 Label the bottom of the MI agar or MI broth plates with the sample number/identification and the volume of sample to be analyzed. Label QC TSA plates and the MI agar or MI broth sterility control plate(s).

11.3 Using a flamed forceps, place a membrane filter, grid-side up, on the porous plate of the filter base. If you have difficulties in removing the separation papers from the filters due to static electricity, place a filter with the paper on top of the funnel base and turn on the vacuum. The separation paper will curl up, allowing easier removal.

11.4 Attach the funnel to the base of the filter unit, taking care not to damage or dislodge the filter. The membrane filter is now located between the funnel and the base.

11.5 Put approximately 30 mL of sterile dilution water in the bottom of the funnel.

11.6 Shake the sample container vigorously 25 times.

11.7 Measure an appropriate volume (100 mL for drinking water) or dilution of the sample with a sterile pipette or graduated cylinder, and pour it into the funnel. Turn on the vacuum, and leave it on while rinsing the funnel twice with about 30 mL sterile dilution water.

11.8 Remove the funnel from the base of the filter unit. A germicidal ultraviolet (254 nm) light box can be used to hold and sanitize the funnel between filtrations. At least 2 minutes of exposure time is required for funnel decontamination. Protect eyes from UV irradiation with glasses, goggles, or an enclosed UV chamber.

11.9 Holding the membrane filter at its edge with a flamed forceps, gently lift and place the filter grid-side up on the MI agar plate or MI broth pad plate. Slide the filter onto the agar or pad, using a rolling action to avoid trapping air bubbles between the membrane filter and the underlying agar or absorbent pad. Run the tip of the forceps around the outside edge of the filter to be sure the filter makes contact with the agar or pad. Reseat the membrane if non-wetted areas occur due to air bubbles.

11.10 Invert the agar Petri dish, and incubate the plate at 35°C for 24 hours. Pad plates used with MI broth should be incubated grid-side up at 35°C for 24 hours. If loose-lidded plates are used for MI agar or broth, the plates should be placed in a humid chamber.

11.11 Count all blue colonies on each MI plate under normal/ambient light, and record the results (See Figures 1 and 2.). This is the E. coli count. Positive results that occur in less than 24 hours are valid, but the results cannot be recorded as negative until the 24-hour incubation period is complete (Reference 16.14).

11.12 Expose each MI plate to long wave ultraviolet light (366 nm), and count all fluorescent colonies [blue/green fluorescent E. coli, blue/white fluorescent TC other than E. coli, and blue/green with fluorescent edges (also E. coli)] (See Figure 1.). Record the data.

11.13 Add any blue, non-fluorescent colonies (if any) found on the same plate to the TC count (Reference 16.8).

12.0 Data Analysis and Calculations

12.1 Use the following general rules to calculate the E. coli or TC per 100 mL of sample:

12.1.1 Select and count filters with # 200 total colonies per plate.

12.1.2 Select and count filter with # 100 target colonies (ideally, 20-80).

12.1.3 If the total number of colonies or TC on a filter are too-numerous-to-count or confluent, record the results as "TC^+ (TNTC)" and count the number of E. coli. If both target organisms are $ 200, record the results as "TC^+ EC^+ (TNTC)".

12.1.4 Calculate the final values using the formula:

$$\text{E. coli/100 mL} = \frac{\text{Number of blue colonies}}{\text{Volume of sample filtered (mL)}} \times 100$$

$$\text{TC/100 mL} = \frac{\text{Number of fluorescent colonies + Number of blue, non-fluorescent colonies (if any)}}{\text{Volume of sample filtered (mL)}} \times 100$$

12.2 See the USEPA Microbiology Manual, Part II, Section C, 3.5, for general counting rules (Reference 16.6).

12.3 Report results as E. coli or TC per 100 mL of drinking water.

13.0 Method Performance

13.1 The detection limits of this method are one E. coli and/or one total coliform per sample volume or dilution tested (Reference 16.8).

13.2 The false-positive and false-negative rates for E. coli are both reported to be 4.3% (Reference 16.8).

13.3 The single lab recovery of E. coli is reported (Reference 16.8) to be 97.9% of the Heterotrophic Plate Count (pour plate) (Reference 16.2) and 115% of the R2A spread plate (Reference 16.2). For Klebsiella pneumoniae and Enterobacter aerogenes, two total coliforms, the recoveries are 87.5% and 85.7% of the HPC (Reference 16.8), respectively, and 89.3% and 85.8% of the R2A spread plate, respectively.

13.4 The specificities for *E. coli* and total coliforms are reported to be 95.7% and 93.1% (Reference 16.8), respectively.

13.5 The single lab coefficients of variation for *E. coli* and total coliforms are reported to be 25.1% and 17.6% (Reference 16.8), respectively, for a variety of water types.

13.6 In a collaborative study (References 16.4, 16.5, and 16.9), 19 laboratories concurrently analyzed six wastewater-spiked Cincinnati tap water samples, containing 3 different concentrations of *E. coli* (# 10, 11-30, and > 30 per 100 mL).

13.6.1 The single laboratory precision (coefficient of variation), a measure of the repeatability, ranged from 3.3% to 27.3% for *E. coli* and from 2.5% to 5.1% for TC for the six samples tested, while the overall precision (coefficient of variation), a measure of reproducibility, ranged from 8.6% to 40.5% and from 6.9% to 27.7%, respectively. These values are based on $\log_{10}$-transformed data (Reference 16.5).

13.6.2 Table 1 contains the statistical summary of the collaborative study (Reference 16.9) results.

14.0 Pollution Prevention

14.1 Pollution prevention is any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. It is the environmental management tool preferred over waste disposal or recycling. When feasible, laboratory staff should use a pollution prevention technique, such as preparation of the smallest practical volumes of reagents, standards, and media or downsizing of the test units in a method.

14.2 The laboratory staff should also review the procurement and use of equipment and supplies for other ways to reduce waste and prevent pollution. Recycling should be considered whenever practical.

15.0 Waste Management

15.1 The Environmental Protection Agency requires that laboratory waste management practices be consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling releases from hoods and bench operations, complying with the letter and spirit of sewer discharge permits and regulations and by complying with solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. All infectious wastes should be autoclaved before disposal.

16.0 References

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16.5 Association of Official Analytical Chemists. 1989. Guidelines for Collaborative Study Procedure to Validate Characteristics of a Method of Analysis. Journal of the Association of Official Analytical Chemists 72 (4): 694-704.

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16.11 Buntel, C.J. 1995. E. coli $-Glucuronidase (GUS) as a Marker for Recombinant Vaccinia Viruses. BioTechniques 19 (3); 352-353.
16.12 Federal Register. 1985. National Primary Drinking Water Regulations; Synthetic Organic Chemicals, Inorganic Chemicals and Microorganisms; Proposed Rule. Federal Register 50: 46936-47022.
16.13 Federal Register. 1994. National Primary and Secondary Drinking Water Regulations: Analytical Methods for Regulated Drinking Water Contaminants; Final Rule. *Federal Register* 59: 62456-62471.
16.14 Federal Register. 1999. National Primary and Secondary Drinking Water Regulations: Analytical Methods for Chemical and Microbiological Contaminants and Revisions to Laboratory Certification Requirements; Final Rule. *Federal Register* 64: 67450-67467.
16.15 U.S. Environmental Protection Agency. 1992. Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures, Quality Assurance, Third Edition. EPA-814B-92-002, Office of Ground Water and Drinking Water, Technical Support Division, U.S. Environmental Protection Agency, Cincinnati, OH.

17.0 Tables and Figures

Table 1. Statistical Summary of the Collaborative Study Results[1]

Target Organism	Sample Number	*E. coli* Count Category (Range)[2]	Initial n[3]	Final n[4]	S_r[5]	RSD_r[6] (%)	$\bar{\chi}$[7]	S_R[8]	RSD_R[9] (%)	$\frac{RSD_R}{RSD_r}$ Ratio
Escherichia coli	1	Low (≤ 10)	63	63	0.17	27.3	0.64	0.26	40.5	1.49
	2		63	63	0.21	25.0	0.84	0.33	39.0	1.56
	3	Medium (11-30)	63	63	0.10	7.9	1.27	0.15	12.1	1.52
	4		63	60	0.07	5.6	1.32	0.12	9.2	1.65
	5	High (> 30)	63	60	0.06	3.3	1.87	0.16	8.6	2.62
	6		63	63	0.09	4.3	1.99	0.25	12.6	2.91
Total Coliforms	1	Low (≤ 10)	63	63	0.10	4.3	2.35	0.62	26.4	6.11
	2		63	63	0.09	3.8	2.31	0.64	27.7	7.25
	3	Medium (11-30)	63	63	0.11	5.1	2.17	0.47	21.8	4.28
	4		63	57	0.10	3.3	3.07	0.21	6.9	2.08
	5	High (> 30)	63	63	0.15	4.8	3.10	0.43	14.0	2.96
	6		63	63	0.08	2.5	3.14	0.46	14.7	5.97

[1] The values are based on $\log_{10}$ transformed data (Reference 16.5).

[2] The samples were grouped by their *E. coli* count on MI agar into the following categories:

Low (# 10 *E. coli* / 100 mL, samples 1 and 2),
Medium (11-30 *E. coli* / 100 mL, samples 3 and 4), and
High (> 30 *E. coli* / 100 mL, samples 5 and 6).

[3] These values are based on triplicate analyses by each laboratory. The reference laboratory analyzed three sets of samples: the initial and final samples prepared and a sample shipped along with the other 18 lab samples.

[4] These values were obtained after removing outliers by the AOAC procedure (Reference 16.5).

[5] S_r, Single Operator Standard Deviation, a measure of repeatability.

[6] RSD_r, Single Operator Relative Standard Deviation (Coefficient of Variance), a measure of repeatability.

[7] $\bar{\chi}$, The mean of the replicate analyses for all laboratories.

[8] S_R, Overall Standard Deviation, a measure of reproducibility.

[9] RSD_R, Overall Relative Standard Deviation (Coefficient of Variation), a measure of reproducibility.

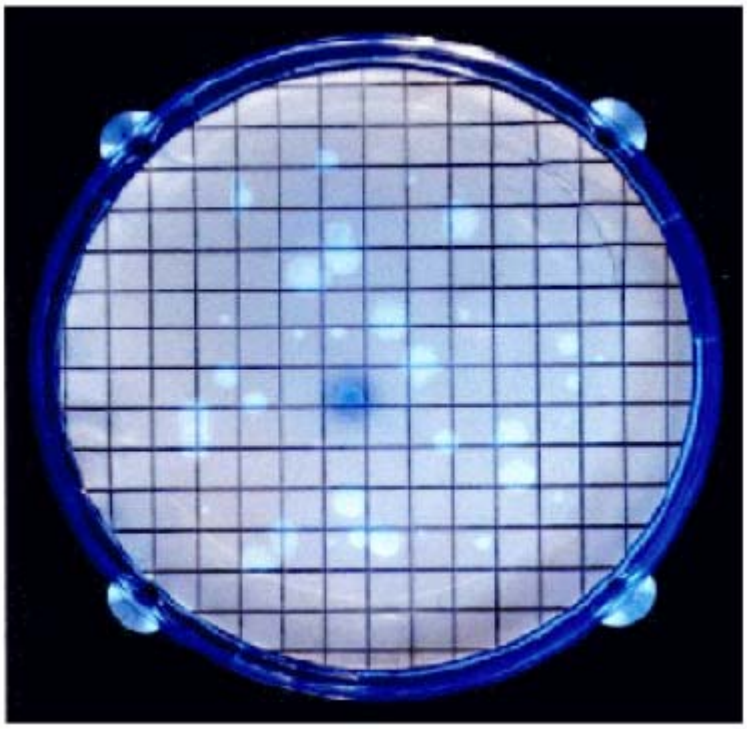


Figure 1. This photograph shows *Escherichia coli* (blue/green fluorescence) and total coliforms other than *E. coli* (blue/white fluorescence) on MI agar under long wave UV light (366 nm). The sample used was a wastewater-spiked Cincinnati, Ohio tap water.

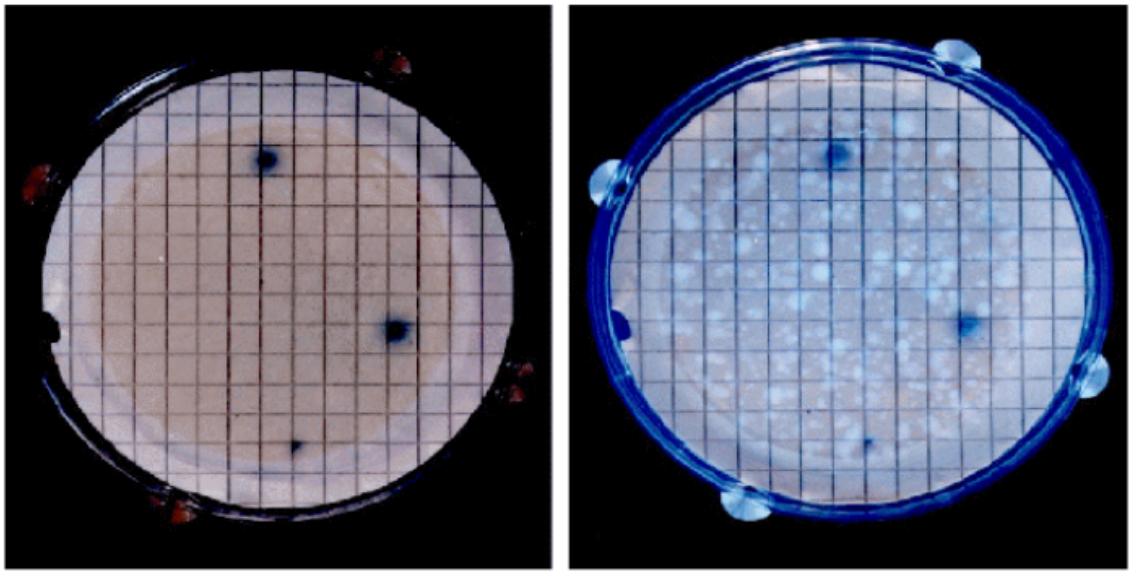


Figure 2. These photographs show *Escherichia coli* and total coliforms from cistern water on MI agar. The confluent plate was photographed under different lighting: ambient light on the left, and long wave UV light (366 nm) on the right. Under ambient light, *E. coli* are blue, and total coliforms other than *E. coli* and non-coliforms are their natural color. Under long wave UV light, all total coliforms, including *E. coli*, are fluorescent, and noncoliforms are non-fluorescent (*i.e.*, they are not visible).

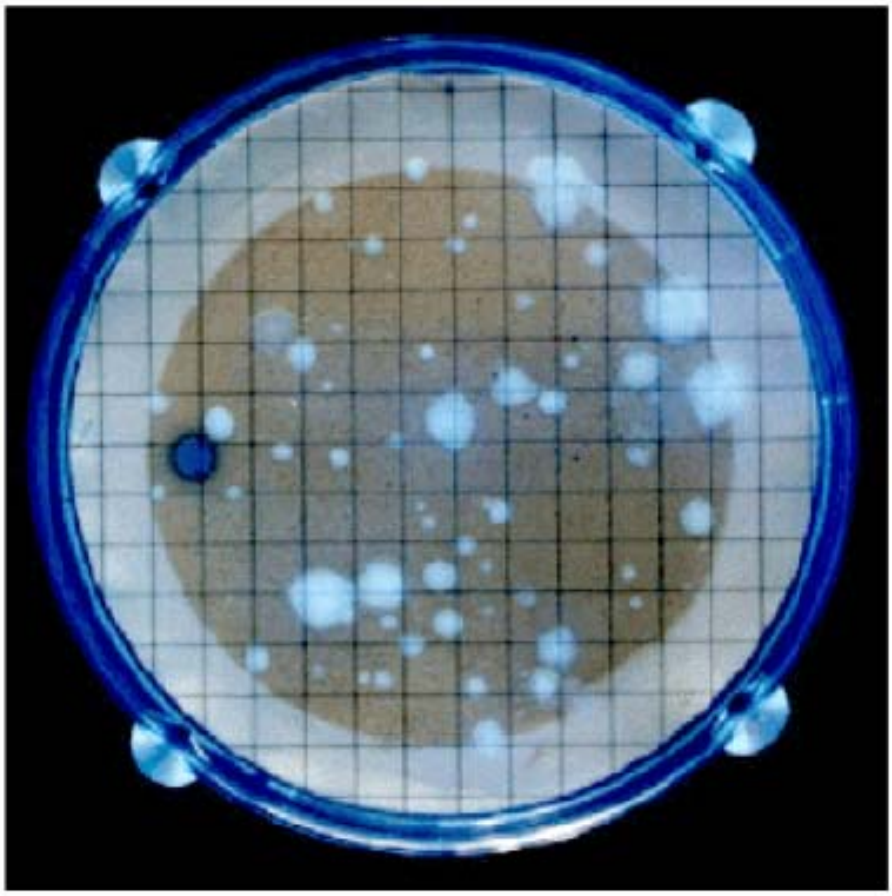


Figure 3. This photograph shows that *Escherichia coli* (blue/green fluorescence) and total coliforms other than *E. coli* (blue/white fluorescence) can easily be detected on MI agar plates from samples with high turbidity levels. The sample used was surface water-spiked Cincinnati, Ohio tap water.

Method 1605: *Aeromonas* in Finished Water by Membrane Filtration using Ampicillin-Dextrin Agar with Vancomycin (ADA-V)

Disclaimer

This method has been validated by the U.S. Environmental Protection Agency through an interlaboratory validation study, and will be proposed for use in drinking water monitoring in the *Federal Register*. This method is not an EPA-approved method until it is promulgated as an approved method in the *Federal Register*.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Introduction

Aeromonas is a common genus of bacteria indigenous to surface waters, and may be found in non-chlorinated or low-flow parts of chlorinated water distribution systems. Monitoring their presence in distribution systems is desirable because some aeromonads may be pathogenic and pose a potential human health risk. Method 1605 describes a membrane filtration technique for the detection and enumeration of *Aeromonas* species. This method uses a selective medium that partially inhibits the growth of non-target bacterial species while allowing most species of *Aeromonas* to grow. *Aeromonas* is presumptively identified by the production of acid from dextrin fermentation and the presence of yellow colonies on ampicillin-dextrin agar medium with vancomycin (ADA-V). Yellow colonies are counted and confirmed by testing for the presence of cytochrome *c* (oxidase test), and the ability to ferment trehalose, and produce indole.

Laboratories are not permitted to modify ADA-V media or procedures associated with filtration (Sections10.1 through 10.10). However, the laboratory is permitted to modify method procedures related to the confirmation of colonies (Section 10.11) to improve performance or lower the costs of measurements provided that 1) presumptively identified yellow colonies submitted to confirmation are tested for the presence of cytochrome c (oxidase test), and the ability to ferment trehalose, and the ability produce indole, and 2) all quality control (QC) tests cited in Section 9.2.12 are performed acceptably and QC acceptance criteria are met. For example, laboratories may prefer to streak colonies that are submitted to confirmation on tryptic soy agar (TSA), instead of nutrient agar. The laboratory may not omit any quality control analyses.

This method is for use in the Environmental Protection Agency's (EPA's) data gathering and monitoring programs under the Safe Drinking Water Act.

Questions concerning this method or its application should be addressed to:

Mary Ann Feige
U.S. EPA Office of Water
Office of Ground Water and Drinking Water
Technical Support Center
26 West Martin Luther King Drive
Cincinnati, OH 45268-1320

Method 1605: *Aeromonas* in Finished Water by Membrane Filtration using Ampicillin-Dextrin Agar with Vancomycin (ADA-V)

1.0 Scope and Application

1.1 This method describes a membrane filter (MF) procedure for the detection and enumeration of *Aeromonas* species in finished water samples. *Aeromonas* is a common genus of bacteria indigenous to surface waters. Its numbers are more likely to be greater during periods of warmer weather and when increased concentrations of organic nutrients are present. It is also more likely to be found in non-chlorinated water distribution systems or low-flow parts of chlorinated systems. Some *Aeromonas* species are opportunistic pathogens.

1.2 This method is adapted from Havelaar et al. (1987) for the enumeration of *Aeromonas* species in finished water by membrane filtration (Reference 15.1). It is a quantitative assay that uses a selective medium which partially inhibits the growth of non-target bacterial species while allowing *Aeromonas* to grow. *Aeromonas* is presumptively identified by the production of acid from dextrin fermentation producing yellow colonies. Presumptively positive colonies are counted and confirmed by testing for the presence of cytochrome *c* (oxidase test), and the ability to ferment trehalose, and produce indole.

1.3 This method is designed to meet the finished water monitoring requirements of the U.S. Environmental Protection Agency. *Aeromonas* was included on the Contaminant Candidate List (CCL) (Mar. 2, 1998, 63 *FR* 10274) and in the Revisions to the Unregulated Contaminant Monitoring Proposed Rule (UCMR) (September 17, 1999, 64 FR 50556). Contaminants listed in the UCMR are candidates for future regulation and may be included in a monitoring program for unregulated contaminants. Unregulated contaminant monitoring would be required for large systems and a representative sample of small and medium sized water distribution systems.

1.4 This method was subjected to an interlaboratory validation study involving 11 laboratories and 11 finished drinking water matrices. This method was not validated for other water types. Use of this method and appropriate validation for other water types is the responsibility of the user.

2.0 Summary of Method

2.1 The method provides a direct count of *Aeromonas* species in water based on the growth of yellow colonies on the surface of the membrane filter using a selective medium. A water sample is filtered through 0.45-Fm-pore-size membrane filter. The filter is placed on ampicillin-dextrin agar with vancomycin (ADA-V) and incubated at 35EC ± 0.5EC for 24 ± 2 hours. This medium uses ampicillin and vancomycin to inhibit non-*Aeromonas* species, while allowing most *Aeromonas* species to grow. The medium uses dextrin as a fermentable carbohydrate, and bromothymol blue as an indicator of acidity produced by the fermentation of dextrin. Presumptively identified yellow colonies are counted and confirmed by testing for the presence of cytochrome c (oxidase test), and the ability to ferment trehalose and produce indole.

2.2 The membrane filtration procedure provides a direct count of culturable Aeromonas in water samples that is based on the growth of bacterial colonies on the surface of the membrane filter placed on a selective medium.

2.3 Aeromonas isolates may be archived for further analysis to determine species or hybridization group by inoculating a nutrient agar slant for short term use or shipping, or nutrient broth for freezing.

3.0 Definitions

3.1 Aeromonas are bacteria that are facultative anaerobes, Gram-negative, oxidase-positive, polarly flagellated, and rod shaped. They are classified as members of the family Aeromonadaceae. Demarta et al. (1999) reported 15 Aeromonas species based on 16S rDNA sequences though not all are officially recognized. Some species have been associated with human disease. In this method, Aeromonas are those bacteria that grow on ampicillin-dextrin agar with vancomycin (ADA-V), produce yellow colonies, are oxidase-positive, and have the ability to ferment trehalose and produce indole.

3.2 Definitions for other terms are provided in the glossary at the end of the method (Section 17.3).

4.0 Interferences and Contamination

4.1 Water samples containing colloidal or suspended particulate material may clog the membrane filter and prevent filtration or cause spreading of bacterial colonies which could interfere with identification of target colonies.

4.2 Other ampicillin/vancomycin resistant bacteria that are not aeromonads may be able to grow on this medium. Some of these bacteria may also produce yellow colonies if they are able to produce acid byproducts from the fermentation of dextrin or some other media component, or if they produce a yellow pigment. Enterococcus are reported to produce pinpoint-size yellow colonies on ADA. Confirmation of presumptive Aeromonas colonies is necessary to mitigate false positives.

5.0 Safety

5.1 Some strains of Aeromonas are opportunistic pathogens. Sample containers and waste materials should be autoclaved prior to cleaning or disposal.

5.2 The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents, and other materials.

5.3 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

6.0 Equipment and Supplies

Note: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

6.1 Equipment for collection and transport of samples to laboratory

- **6.1.1** Autoclavable sample container—Use sterile, non-toxic, glass or plastic containers with a leak-proof lid. Ensure that the sample container is capable of holding a 1-L sample with ample headspace to facilitate mixing of sample by shaking prior to analysis.
- **6.1.2** Ice chest
- **6.1.3** Ice packs

6.2 Autoclavable dilution bottles—125-mL marked at 99 mL or 90 mL; commercially produced dilution bottles may be used.

6.3 Rinse water bottles

6.4 Sterile plastic or autoclavable glass pipettes with a 2.5% tolerance—to deliver (TD), 1- and 10-mL

6.5 Pipette bulbs or automatic pipetter.
6.6 Autoclavable pipette container (if using glass pipettes).
6.7 Thermometer—with 0.5EC gradations checked against a National Institute of Standards and Technology (NIST) certified thermometer, or one that meets the requirements of NIST Monograph SP 250-23.
6.8 Inoculating loop—Sterile metal, plastic, or wooden applicator sticks.
6.9 Burner—Flame or electric incinerator for sterilizing metal inoculating loops and forceps.
6.10 Colony counting device—Mechanical, electric or hand tally.
6.11 Hotplate stirrer
6.12 Magnetic stir bar
6.13 Graduated cylinders—100 mL, 500 mL and 1 L, sterile, polypropylene or glass
6.14 Balance—Capable of weighing samples up to 200 g, with a readability of 0.1 g
6.15 Weigh boats
6.16 pH meter
6.17 Turbidimeter (optional)
6.18 Equipment for membrane filter procedure

6.18.1 Incubator—Hot air or water-jacketed microbiological type to maintain a temperature of 35EC ± 0.5EC
6.18.2 Petri dishes—sterile, 50 × 9 mm or other appropriate size
6.18.3 Membrane filtration units (filter base and funnel made of glass, plastic, or stainless steel), wrapped with aluminum foil or Kraft paper, and sterilized by autoclaving.
6.18.4 Vacuum source
6.18.5 Flasks—1-L vacuum filter with appropriate tubing; a filter manifold to hold a number of units is optional
6.18.6 Side-arm flask to place between vacuum source and filtration devices or filter manifold
6.18.7 Membrane filters—Sterile, cellulose ester, white, gridded, 47-mm-diameter with 0.45-Fm pore size (Gelman E04WG04700 or equivalent)
6.18.8 Forceps—Sterile, straight or curved, with smooth tips to handle filters without causing damage
6.18.9 Ethanol or other alcohol in a container to sterilize forceps
6.18.10 Test tubes—125 × 16 mm sterile, screw-cap tube

6.19 Dissecting microscope—Low power (10X to 15X), binocular, illuminated
6.20 Autoclave—Capable of 121EC at 15 psi. Must meet requirements set forth in the Manual for the Certification of Laboratories Analyzing Drinking Water, 4th Edition. (Reference 15.5)
6.21 Membrane filters (for sterilization purposes)—Sterile with 0.22-Fm pore size (Gelman Acrodisc No. 4192 or equivalent)

7.0 Reagents and Standards
7.1 Purity of reagents and culture media—Reagent-grade chemicals shall be used in all tests. Unless otherwise indicated, reagents and culture media shall conform to the specifications in Standard Methods for the Examination of Water and Wastewater (latest edition approved by EPA in 40 CFR Part 141), Section 9050 (Reference 15.2). The agar used in preparation of culture media must be of microbiological grade.
7.2 Purity of water—Reagent-grade water conforming to specifications in Manual for the Certification of Laboratories Analyzing Drinking Water, 4th Edition (Reference 15.5) or

Standard Methods for the Examination of Water and Wastewater (latest edition approved by EPA in 40 CFR Part 141), Section 9020 (Reference 15.2).

7.3 Phosphate buffered dilution water

7.3.1 Concentrated stock phosphate buffer solution—Dissolve 34.0 g potassium dihydrogen phosphate (KH_2PO_4) in 500 mL reagent-grade water. Adjust the pH to 7.2 ± 0.5 with 1N sodium hydroxide (NaOH) and dilute to 1 L with reagent-grade water. Autoclave or filter sterilize through a filter with 0.22-Fm-pore-size.

7.3.2 Magnesium chloride solution—Dissolve 81.1 g magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_20$) in reagent-grade water and dilute to 1 L. Autoclave or filter sterilize through a 0.22-Fm-pore-size filter.

7.3.3 Prepare phosphate buffered dilution water by adding 1.25 mL of concentrated stock phosphate buffer solution (Section 7.3.1) and 5.0 mL of magnesium chloride solution (Section 7.3.2) to a 1-L graduated cylinder and adjust final volume to 1 L with reagent-grade water. Prepare a portion of buffered dilution water in 1-L bottles for rinse water. Autoclave or filter sterilize through a filter with 0.22-Fm-pore-size.

7.3.4 Stored phosphate buffered dilution water should be free from turbidity.

7.4 Ampicillin-dextrin agar with vancomycin (ADA-V)

7.4.1 Preparation of dextrin agar—EPA highly recommends the use of commercial ADA (m-Aeromonas Selective Agar Base [Havelaar]), Section **7.4.1.1**. However, ADA may be prepared by the laboratory (Section 7.4.1.2)

7.4.1.1 Commercial dextrin agar—Tech Pac (distributor, tech@fuse.net), Cincinnati, Ohio; Biolife (www.biolifeit.com) Italiana Srl, 272 Viale Monza, Milan, Italy, Cat. No. 401019 or equivalent. Prepare 1-L of media, according to manufacturer's instructions. Cool to room temperature, and adjust pH to 8.0 using 1N NaOH or 1N HCl. Autoclave for 15 min, cool to 50EC.

7.4.1.2 Laboratory-prepared dextrin agar.

7.4.1.2.1 5.0 g tryptose—Difco cat. no. 0124-17, or equivalent

7.4.1.2.2 11.4 g dextrin—Difco cat. no. 0161-17, or equivalent

7.4.1.2.3 2.0 g yeast extract—Difco cat. no. 0127-17, or equivalent

7.4.1.2.4 3.0 g sodium chloride (NaCl)—Baker cat. no. 3624, or equivalent

7.4.1.2.5 2.0 g potassium chloride (KCl)—Fisher cat. no. P217-500, or equivalent

7.4.1.2.6 0.1 g magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)—Fishercat. no. M63-500, or equivalent

7.4.1.2.7 0.06 g ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$)—Sigma cat. no. F-2877, or equivalent

7.4.1.2.8 0.08 g bromothymol blue—Baker cat. no. D470, or equivalent

7.4.1.2.9 Sodium deoxycholate—Sigma cat. no. D-6750, or equivalent. Add 100 mg of sodium deoxycholate to 10 mL of reagent water.

7.4.1.2.10 13.0 g agar, bacteriological grade—Fisher cat. no. BP1423-500, or equivalent.

7.4.1.2.11 Add reagents in Sections 7.4.1.2.1 through 7.4.1.2.8 to 1-L of reagent-grade water, stir to dissolve and adjust pH to 8.0 using1N NaOH or 1N HCl. After the pH has been adjusted, add sodium deoxycholate (Section 7.4.1.2.9) and agar (Section7.4.1.2.10) and heat to dissolve. Autoclave for 15 min, cool to 50^{o}C.

7.4.2 Ampicillin, sodium salt—Sigma cat. no. A0166, or equivalent. Add 10 mg of ampicillin, sodium salt to 10 mL reagent water. Prepare on the same day that medium is prepared and filter sterilize through a 0.22-Fm-pore-size filter. Alternatively, use Biolife cat. no. 4240012 prepared according to manufacturer's instructions, taking care to use an appropriate amount of ampicillin for the volume of media being prepared (for example, use two vials for a 1-L batch of ADA-V). Follow manufacturer's instructions for appropriate storage temperature and shelf-life. Wear suitable protective clothing, gloves, and eye/face protection and prepare stock solutions in a chemical fume hood.

7.4.3 Vancomycin hydrochloride—Sigma cat. no. V2002, or equivalent. Add 2 mg of vancomycin hydrochloride to 10 mL of reagent water. Filter sterilize through a 0.22-Fm-pore-size filter. Follow manufacturer's instructions for appropriate storage temperature and time. Wear suitable protective clothing, gloves, and eye/face protection and prepare stock solutions in a chemical fume hood.

7.4.4 After dextrin agar (Section 7.4.1) has been autoclaved and cooled to 50EC, add the sterile ampicillin (Section 7.4.2) and sterile vancomycin hydrochloride solutions (Section 7.4.3).

7.4.5 Add approximately 5 mL of ADA-V per 50 × 9 mm Petri dish and allow to solidify. For larger plates, adjust volume appropriately. ADA-V plates should be stored in a tight fitting container (i.e. sealed plastic bag) at a temperature of 1EC to 5EC for no longer than 14 days.

7.5 Pentahydrate ACS Reagent grade sodium thiosulfate—Fisher cat. no. S445-500, or equivalent. Prepare a 3% stock solution by adding 3 g sodium thiosulfate to 100 mL reagent-grade water.

7.6 Disodium salt of ethylenediaminetetraacetic acid (EDTA)—Sigma cat. no. E 4884, or equivalent. EDTA should only be added to samples if metals in water samples exceed 1.0 mg/L. To prepare stock solution, add 12.4 g EDTA to 80 mL of reagent-grade water. Adjust pH to 8.0 using 10N NaOH. After the pH has been adjusted, bring the volume up to 100 mL with reagent-grade water.

7.7 Positive control culture—Aeromonas hydrophila ATCC #7966; obtained from the American Type Culture Collection (ATCC, 10801 University Blvd, Manassas, VA, 20110-2209; http://www.atcc.org).

7.8 Negative culture control—Negative culture controls serve two purposes: to ensure the laboratories are familiar with the color and morphology of non-Aeromonas bacteria that may grow on ADA-V and to ensure that confirmation test results are appropriate. E. coli (ATCC #25922) is the negative culture control for oxidase, Pseudomonas aeruginosa (ATCC #27853) is the negative culture control for trehalose fermentation, and Bacillus cereus (ATCC #11778) is the negative culture control for indole.

7.9 Nutrient agar—Difco cat. no. 0001-17 or equivalent. Prepare according to manufacturers instructions.

7.10 Oxidase reagents—Dry Slide BBL cat. no. 231746 or equivalent.

7.11 0.5% Trehalose confirmation reagent

7.11.1 Add 5 g trehalose (Sigma cat. no. T0167, or equivalent) to 100 mL water and filter sterilize solution through a filter with 0.22-Fm-pore-size.

7.11.2 Prepare 900 mL purple broth base (Difco cat. no. 0222-17, or equivalent) according to manufacturer's instructions and autoclave.

7.11.3 Aseptically add 100 mL trehalose solution to the cooled 900 mL of purple broth base.

7.11.4 Dispense into 6 mL or larger size tubes and fill approximately half full. Store in refrigerator.

Note: *Alternatively, prepare purple broth base according to manufacturers instructions, add 5 g trehalose per liter, and filter sterilize through a filter with 0.22-*F*m-pore-size.*

7.12 Tryptone broth—Oxoid cat. no. CM0087B, or equivalent. Alternatively, the laboratory may prepare tryptone broth by adding 10 g of tryptone (Difco cat. no. 0123-17 or equivalent) and 5 g of NaCl to 1 L of reagent water. Autoclave or filter sterilize through a filter with 0.22-Fm-pore-size.

7.13 Kovac's reagent—Biomeriuex cat. no. V7050, or equivalent

8.0 Sample Collection, Preservation, and Storage

8.1 Adherence to sample preservation procedures and holding time limits specified in *Standard Methods for the examination of Water and Wastewater* (Reference 15.2) is critical to the production of valid data. Sample results will be considered invalid if those conditions are not met.

8.2 Preparation of sample bottles and sample collection—Samples must be representative of the drinking water distribution system. Water taps used for sampling should be free of aerators, strainers, hose attachments, mixing type faucets, and purification devices. Cold water taps should be used. The service line should be cleared before sampling by maintaining a steady water flow for at least two minutes (until the water changes temperature).

8.2.1 Use sterile, non-toxic, glass or plastic container (Section 6.1.1) with a leak-proof lid. Ensure that the sample container is capable of holding a 1-L sample with ample headspace to facilitate mixing of sample by shaking prior to analysis. Sampling procedures are described in detail in *Standard Methods for the Examination of Water and Wastewater*, Section 9060 (Reference 15.2).

8.2.2 Add 1 mL of 3% sodium thiosulfate stock (Section 7.5) per L of sample to sample bottles prior to autoclave sterilization. Alternatively, if using presterilized sample bottles, sodium thiosulfate should be autoclaved for 15 minutes or filter sterilized through a filter with 0.22-Fm-pore-size before adding to the sample bottles.

8.2.3 If metals in the sample exceed 1.0 mg/L, add 3 mL of EDTA stock solution (Section 7.6) per L of sample to sample bottles prior to autoclave sterilization. If using presterilized sample bottles, EDTA should be autoclaved for 15 minutes or filter sterilized through a filter with 0.22-Fm-pore-size.

8.2.4 Collect a minimum of 1-L of sample.

8.3 Sample preservation and handling

8.3.1 Immediately following sample collection, tighten the sample container lid(s) and place the sample container(s) upright in an insulated, plastic-lined storage cooler with ice packs or in a refrigerator to chill prior to packing the cooler for shipment. Do not freeze the sample.

8.3.2 Use enough solidly frozen ice packs to ensure that the samples will arrive at a temperature of 1°C to 10°C. Use a minimum of two ice packs per shipment and add extra ice packs for multiple samples. Place one or more ice packs on each side of the container to stabilize samples.

8.3.3 Samples must be maintained at a temperature of 1°C to 10°C during shipment. Samples must not be frozen.

Note: Sample temperature during shipment is critical. Ice packs must be frozen solid immediately prior to shipment.

8.4 Verify and record sample arrival temperature when received in the laboratory. Refrigerate samples at 1°C to 5°C upon receipt at the laboratory and analyze as soon as possible after collection. Samples must be analyzed within 30 hours of sample collection.

9.0 Quality Control

9.1 Each laboratory that uses Method 1605 is required to operate a formal quality assurance (QA) program. The minimum QA requirements consist of the initial demonstration of capability (IDC) test (Section 9.4), ongoing analysis of spiked reagent water (ODC test, Section 9.8) and spiked finished drinking water samples (MS/MSD, Section 9.7), and analysis of negative culture controls (Section 9.6), dilution/rinse water blanks (Section 9.5), and media sterility checks (Section 9.2.6) as tests of continued acceptable performance. Spiked sample results are compared to acceptance criteria for precision, which are based on data generated during the interlaboratory validation of Method 1605 involving 11 laboratories and 11 finished water matrices. The more stringent QA requirements in this method, relative to other, currently used methods for bacterial determination, are an effort to improve overall microbiological QA. The specifications contained in this method can be met if the analytical system is maintained under control. Laboratories are not permitted to modify ADA-V media or procedures associated with filtration (Sections 10.1 through 10.10). However, the laboratory is permitted to modify method procedures related to the confirmation of colonies (Section 10.11) to improve performance or lower the costs of measurements provided that 1) presumptively identified yellow colonies submitted to confirmation are tested for the presence of cytochrome c (oxidase test), and the ability to ferment trehalose, and the ability produce indole, and 2) all quality control (QC) tests cited in Section 9.2.12 are performed acceptably and QC acceptance criteria are met. For example, laboratories may prefer to streak colonies that are submitted to confirmation on tryptic soy agar (TSA), instead of nutrient agar. The laboratory may not omit any quality control analyses.

9.2 General QC requirements—Specific quality control (QC) requirements for Method 1605 are provided below. QA and QC criteria for facilities, personnel, and laboratory equipment, instrumentation, and supplies used in microbiological analyses must be followed according to Standard Methods for the Examination of Water and Wastewater (latest edition approved by EPA in 40 CFR Part 141, Reference 15.2) and the U.S. EPA Manual for the Certification of Laboratories Analyzing Drinking Water, Fourth Edition (March 1997) (Reference 15.5).

9.2.1 Initial demonstration of capability (IDC). The laboratory shall demonstrate the ability to generate acceptable performance with this method by performing an IDC test before analyzing any field samples. The procedure for performing the IDC is described in Section 9.4. IDC tests must be accompanied by a dilution/rinse water blank(s) (Section 9.2.2), negative culture controls (Section 9.2.3), and media sterility checks (Section 9.2.6).

9.2.2 Dilution/rinse water blanks. The laboratory shall analyze dilution/rinse water blanks to demonstrate freedom from contamination. The procedures for analysis of dilution/rinse water blanks are described in Section 9.5. At a minimum, dilution/rinse water blanks must be processed at the beginning and end of each filtration series to check for possible cross-contamination. A filtration series ends when 30 minutes or more elapse between sample filtrations. An additional dilution/rinse water blank is also required for every 20 samples, if more than 20 samples are processed during a filtration series.

9.2.3 Negative culture controls. The laboratory shall analyze negative culture controls (Section 9.6) to ensure that ADA-V and the confirmation procedures are performing properly. Negative culture controls should be run whenever a new batch of media or reagents is used. On an ongoing basis, the laboratory must perform, at a minimum, one negative culture control per week during weeks the laboratory analyzes field samples.
9.2.4 Matrix spike/matix spike duplicate (MS/MSD). The laboratory shall analyze one set of MS/MSD samples when samples are first received from a finished drinking water source for which the laboratory has never before analyzed samples (Section 9.7). Subsequently, 5% of field samples from a given source must include an MS/MSD test. Additional MS/MSD tests are also recommended when drinking water treatment is adjusted or when other events take place, for example, when scrubbing or replacing lines. When possible, MS/MSD analyses should be conducted on the same day as ODC samples, using the same spiking procedure and volume.
9.2.4.1 Precision. MS/MSD sample results should meet the precision criteria set forth in Section 12, Table 1.
9.2.4.2 Recovery. QC acceptance criteria for Aeromonas recovery are not included in this method because the number of Aeromonas in the spike is unknown. However, each laboratory should control chart the mean number of Aeromonas per MS/MSD set (adjusted for background) and maintain a record of spike preparation procedures and spike volume. The laboratory should compare number of Aeromonas in MS/MSD samples to results of ODC samples (Section 9.2.5 and 9.8) spiked on the same day. This comparison should help the laboratory recognize when a matrix is interfering with method recovery. If the laboratory observes consistent ODC results from week to week, control charting the MS/MSD results by source may also help to recognize fluctuations in recovery from a particular source.

9.2.5 Ongoing demonstration of capability (ODC). The laboratory shall demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples (positive control/positive control duplicate, Section 9.8).
9.2.5.1 Frequency. The laboratory shall analyze one set of ODC samples after every 20 field and MS samples or one set per week that samples are analyzed, whichever occurs more frequently. No more than one set of ODC samples is required per day, provided that the same equipment (i.e., incubators) are being used for all the samples.
9.2.5.2 Precision. ODC sample results must meet the precision criteria set forth in Section 12, Table 1.
9.2.5.3 Recovery. QC acceptance criteria for Aeromonas recovery are not included in this method because the initial spike dose for ODC samples is unknown.
As a result, each laboratory should control chart the mean number of Aeromonas per ODC sample set and maintain a record of spike preparation procedures and ODC spike volume. Maintaining this information will enable the laboratory to recognize when problems arise. Example: A laboratory that prepares spiking suspensions according to Section 9.3, spikes QC samples with 5 mL of dilution D2, and typically recovers approximately 50 Aeromonas per sample, and maintains a control chart of these counts. If the laboratory continues to prepare spiking suspensions the same way, but the number of Aeromonas counted declines noticeably (e.g. 20 Aeromonas per sample), then there may be a problem with the media, reagents, or the spiking suspension.
9.2.6 Media sterility checks. The laboratory shall test media sterility by incubating one unit (tube or plate) from each batch of medium (ADA-V, nutrient agar slant, nutrient agar, streak plate, trehalose, and tryptone) at 35°C ± 0.5°C for 24 ± 2 hours and observing for growth.

9.2.7 Analyst colony counting variability. If the laboratory has two or more analysts, each are required to count target colonies on the same membrane from one ODC sample per month (Section 9.9), at a minimum.

9.2.8 Record maintenance. The laboratory shall maintain records to define the quality of data that are generated. The laboratory shall maintain a record of the date and results of all QC sample analyses described in Section 9.2. A record of media sterility check, dilution/rinse water blank, analyst counting variability, IDC, ODC, and MS/MSD sample results must be maintained. Laboratories shall maintain reagent and material lot numbers along with samples analyzed using each of the lots. Laboratories shall also maintain media preparation records.

9.2.9 Performance studies. The laboratory should periodically analyze external QC samples, such as performance evaluation (PE) samples, when available. The laboratory should also participate in available interlaboratory performance studies conducted by local, state, and federal agencies or commercial organizations. The laboratory should review results, correct unsatisfactory performance, and record corrective actions.

9.2.10 Autoclave sterilization verification. At a minimum, the laboratory shall verify autoclave sterilization according to the procedure in Section 9.10 on a monthly basis.

9.2.11 Culture maintenance. The laboratory should use 24 ± 2 hour-old nutrient agar slant cultures for preparation of IDC, ODC, and MS/MSD spiking suspension dilutions. The laboratory should use 22 to 72 hour-old nutrient agar slant cultures to inoculate ADA-V streak plates for analysis of negative culture controls. With regard to the preparation of subcultures, it is recommended that a maximum of three passages be prepared to help avoid contamination. After three passages, start a new subculture from the frozen stock.

9.2.12 Method modification.

9.2.12.1 Membrane filtration. Because recovery criteria are not available for this method, laboratories are not permitted to modify the membrane filtration procedures (Section 10.1 through Section 10.10.) or ADA-V media.

9.2.12.2 Confirmation procedures. The confirmation procedures in Section 10.11 may be modified, provided that the laboratory demonstrates the ability to generate acceptable performance by performing an IDC test (Section 9.2.1) and the appropriate negative culture control test(s) (Section 9.2.3) before analyzing any field samples using the modified confirmation. 100% of the colonies submitted to confirmation from IDC and negative culture control samples must give the appropriate confirmation response. These tests must be accompanied by a dilution/rinse water blank(s) (Section 9.2.2) and media sterility checks (Section 9.2.6).

9.3 Preparation of Aeromonas spiking suspension for use in spiking IDC, ODC, and MS/MSD samples—This dilution scheme is adapted from Standard Methods for the Examination of Water and Wastewater, 19th Edition, Section 9020 B (Reference 15.9). This entire process should be performed quickly to avoid loss of viable organisms. See Section 16, Flowchart 1, for an example of this dilution scheme. Please note: Provided that all QC acceptance criteria are met and the recommended target range of 20 - 60 CFU per plate are typically observed, laboratories may prepare QC spiking suspensions using commercial products or other procedures such as growing bacteria in a broth, measuring optical density, and spiking each test sample with an equivalent volume.

9.3.1 Inoculate Aeromonas hydrophila (ATCC #7966) onto the entire surface of several nutrient agar slants with a slope approximately 6.3 cm long in a 125 × 16 mm screw-cap tube. Incubate for 24 ± 2 hours at 35°C ± 0.5°C.

9.3.2 From the slant that has the best growth, prepare serial dilutions using four dilution bottles with 99 mL of sterile buffered dilution water (A, B, C and D below in Sections 9.3.3 and 9.3.4) and one dilution bottle containing 90-mL of sterile buffered dilution water (D2 below in Section 9.3.5).

9.3.3 Pipette 1 mL of buffered dilution water from bottle "A" to one of the slants. Emulsify the growth on the slant by gently rubbing the bacterial film with the pipette, being careful not to tear the agar. Pipette the suspension back into dilution bottle "A." Repeat this procedure a second time to remove any remaining growth on the agar slant, without disturbing the agar.

9.3.4 Make serial dilutions as follows:

9.3.4.1 Shake bottle "A" vigorously and pipette 1 mL to bottle "B"

9.3.4.2 Shake bottle "B" vigorously and pipette 1 mL to bottle "C"

9.3.4.3 Shake bottle "C" vigorously and pipette 1 mL to bottle "D"

9.3.4.4 Shake bottle "D" vigorously and pipette 10 mL to bottle "D2"; this should result in a final dilution of approximately 10 CFU / mL.

9.3.5 Filter 1- to 5-mL portions in triplicate from bottles "D" and "D2" according to the procedure in Section 10 to determine the number of CFU in the dilutions. The recommended target dilution and spike volume is one that produces 20 to 60 colonies per ADA-V plate. (It may be difficult to count plates with more than 60 colonies due to crowding.) Dilutions should be stored at 1EC to 5EC and may be used throughout the day they are prepared. However, it should be noted that the QC acceptance criteria were established using dilutions that were prepared immediately prior to spiking samples.

9.3.6 Analysts may practice the dilution scheme by placing filters on nutrient agar plates instead of ADA-V plates. After a growth pattern is determined and the analyst can accurately determine the target concentrations, dilutions from Section 9.3.5 may be used for spiking IDC, ODC, and MS/MSD samples. However, multiple dilutions should be analyzed in replicate when new cultures are received from an outside source to ensure that the analyst can accurately spike target concentrations.

Note: If it is more convenient for your laboratory, an acceptable alternative to the dilution scheme presented in Section 9.3, is to pipette 11 mL of dilution D into a dilution bottle D2, which contains 99 mL of dilution water. There should be approximately 10^{10} Aeromonas hydrophila CFU per slant. Therefore, dilution bottles "A" through "D2" should contain approximately 10^{10}, 10^{8}, 10^{6}, 10^{4}, and 10^{3} CFU per dilution bottle, respectively. Depending on the growing conditions, these numbers may vary. As a result, until experience has been gained, more dilutions may need to be filtered to determine the appropriate dilution.

9.4 Initial demonstration of capability (IDC)—The IDC test is performed to demonstrate acceptable performance with the method prior to analysis of field samples. IDC tests must be accompanied by a dilution/rinse water blank(s) (Section 9.2.2), negative culture controls (Section 9.2.3), and media sterility checks (Section 9.2.6).

9.4.1 Prepare an Aeromonas QC spiking suspension according to the procedure in Section 9.3.1 through 9.3.4.

9.4.2 For each of the four IDC test samples, spike enough volume of the appropriate dilution into 500 mL of sterile reagent water to obtain the recommended target range of 20-60 CFU per filter. (It may be difficult to count plates with more than 60 colonies due to crowding.) Filter immediately after spiking.

9.4.3 Process IDC test samples according to the procedure in Section 10.1 through 10.10 and record the number of presumptive positives for each sample. Submit 2 colonies per IDC test sample to the confirmation procedures in Section 10.11.

9.4.4 Using all four IDC sample results, compute the relative standard deviation (RSD) of Aeromonas CFU per 100 mL. (See glossary for definition of RSD.) Compare the RSD with the corresponding limits for IDC (Section 12). If the RSD meets the acceptance criteria, the system performance is acceptable and analysis of samples may begin. If the RSD falls outside the range, system performance is unacceptable. In this event, identify and correct the problem and repeat the test.

9.5 Dilution/rinse water blanks—On an ongoing basis, dilution/rinse water blanks must be processed at the beginning and end of each filtration series to check for possible cross-contamination. A filtration series ends when 30 minutes or more elapse between sample filtrations. An additional dilution/rinse water blank is also required for every 20 samples, if more than 20 samples are processed during a filtration series. For example, if a laboratory plans to run 30 samples during a filtration series, a dilution/rinse water blank should be processed at the beginning, middle, and end of the filtration series.

9.5.1 Process 100-mL dilution/rinse water blanks according to the procedures in Section 10, as appropriate.

9.5.2 No growth should appear in dilution/rinse water blanks. If growth appears, prepare new dilution/rinse water and reanalyze a 100-mL dilution/rinse water blank. If colonies are present after analyzing the new dilution/rinse water, assess laboratory technique and reagents. If growth in dilution/rinse water blank(s) is presumptively positive, all associated sample results should be discarded and sources re-sampled immediately.

9.6 Negative culture controls— Negative controls should be run whenever a new batch of medium or reagents is used. On an ongoing basis, the laboratory must perform, at a minimum, one negative culture control per week during weeks the laboratory analyzes field samples. Negative culture controls serve two purposes: to ensure the laboratories are familiar with the color and morphology of non-*Aeromonas* bacteria on ADA-V and to ensure that confirmation test results are appropriate. *E. coli* is (ATCC #25922) the negative culture control for oxidase, *Pseudomonas aeruginosa* (ATCC #27853) is the negative culture control for trehalose fermentation, and *Bacillus cereus* (ATCC #11778) is the negative culture control for indole.

9.6.1 Using pure cultures obtained from a qualified outside source (Sections 7.7 and 7.8), inoculate negative culture controls onto nutrient agar slants and incubate at 35^{o}C ± 0.5^{o}C for 24 ± 2 hours. Alternatively, nutrient agar slants may be inoculated up to 72 hours in advance. If nutrient agar slants will be incubated for more than 24 ± 2 hours, consider incubation at room temperature to ensure that the slants do not dry out prior to use.

9.6.2 For each negative culture control, place a membrane filter on an ADA-V plate, streak onto the filter, taking care not to break the filter, and incubate at 35°C ± 0.5°C for 24 ± 2 hours. Streaking on a filter will give the laboratory a more realistic example of the appearance of these organisms in field samples. Although not recommended, laboratories may streak directly onto the ADA-V (without the filter).

9.6.3 For each ADA-V negative culture control plate, pick a single colony, streak the colony onto a plate of nutrient agar medium (Section 7.9), and incubate at 35°C ± 0.5°C overnight to obtain isolated colonies. *Please note: Bacillus cereus typically grows only at the point of inoculation on ADA-V or not at all. If Bacillus cereus did not grow on the ADA-V plate, inoculate the streak plate from the nutrient agar slant that was originally used to inoculate the ADA-V plate.*

9.6.4 Negative culture control confirmation procedures

9.6.4.1 Oxidase negative culture control—From the streak plate, submit a single *E. coli* colony to the oxidase confirmation procedure described in Section 10.11.

9.6.4.2 Trehalose negative culture control—From the streak plate, submit a single *Pseudomonas aeruginosa* colony to the trehalose confirmation procedure described in Section 10.11.

9.6.4.3 Indole negative culture control—From the streak plate, submit a single *Bacillus cereus* colony to the indole confirmation procedure described in Section 10.11.

9.6.5 If any of the negative culture controls result in a positive confirmation, prepare, check and/or replace the associated media, reagents, and/or respective control organism and reanalyze the appropriate negative culture control(s). All presumptively positive colonies that have been archived from field samples (10 per sample) should be confirmed using media/reagents that exhibit the appropriate negative culture control response.

9.7 Matrix spike/matrix spike duplicate (MS/MSD)—The laboratory shall analyze MS/MSD samples when samples are first received from a finished drinking water source for which the laboratory has never before analyzed samples. Subsequently, 5% of field samples from a given source must include an MS/MSD test. Additional MS/MSD tests are also recommended when drinking water treatment is adjusted or when other events take place, for example, when scrubbing or replacing lines.

9.7.1 Prepare an Aeromonas QC spiking suspension according to the procedure in Sections 9.3.1 through 9.3.4.

9.7.2 For each of the 500-mL MS and MSD test samples, spike enough volume of the appropriate dilution to obtain the recommended target range of 20-60 CFU per filter. (It may be difficult to count plates with more than 60 colonies due to crowding.) Filter immediately after spiking.

9.7.3 Process MS/MSD test samples and an unspiked finished drinking water sample according to the procedure in Section 10.1 through 10.10 and record the number of presumptive positives for each sample. (If the filter clogs during filtration, follow the instructions in Section 10, making sure to filter the same volume for both the MS and MSD. The same QC acceptance criteria apply.) Submit 10 colonies per IDC test sample to the confirmation procedures in Section 10.11.

Note: If results exceed the optimum range because of "background" target colonies (as indicated by the results of the unspiked matrix sample), the MS/MSD should be repeated and a smaller volume of sample, for example 200-mL, should be spiked.

9.7.4 For the MS and MSD test samples, calculate the number of confirmed Aeromonas CFU per 100 mL according to Section 11 and adjust based on any background Aeromonas observed in the unspiked sample.

9.7.5 Calculate the relative percent difference (RPD) using the following equation:

$$RPD = 100 \frac{| X_{MS} - X_{MSD} |}{X_{mean}}$$

where **RPD** is the relative percent difference
$\mathbf{X_{MS}}$ is the number of confirmed Aeromonas per 100 mL in the MS sample (minus the count of any background Aeromonas colonies observed in the unspiked finished water sample)
$\mathbf{X_{MSD}}$ is the number of confirmed Aeromonas per 100 mL in the MSD sample (minus the count of any background Aeromonas colonies observed in the unspiked finished water sample)
$\mathbf{X_{mean}}$ is the mean number of confirmed Aeromonas per 100 mL in the MS and MSD

9.7.6 Compare the RPD with the corresponding limits in Table 1 in Section 12. If the RPD meets the acceptance criteria, the system performance is acceptable and analysis of finished water samples from this source may continue. If the MS/MSD results are unacceptable and the ODC sample results associated with this batch of samples are acceptable, a matrix interference may be causing the poor results. If the MS/MSD results are unacceptable, all associated field data should be flagged.

9.8 Ongoing demonstration of capability (ODC)—The laboratory shall demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples (positive control/positive control duplicate). The laboratory shall analyze one set of ODC samples after every 20 field and MS samples or one set per week that samples are analyzed, whichever occurs more frequently.

9.8.1 Prepare an Aeromonas QC spiking suspension according to the procedure in Section 9.3.1 through 9.3.4.

9.8.2 For each of the 500-mL positive control (PC) and positive control duplicate (PC/PCD) test samples, spike enough volume of the appropriate dilution into 500 mL of sterile reagent water to obtain the recommended target range of 20-60 CFU per filter. (It may be difficult to count plates with more than 60 colonies due to crowding.) Filter immediately after spiking.

9.8.3 Process PC/PCD test samples according to the procedure in Section 10.1 through 10.10 and record the number of presumptive positives for each sample. Submit 2 colonies per PC/PCD test sample to the confirmation procedures in Section 10.11.

9.8.4 Calculate the relative percent difference (RPD) using the following equation:

$$PRD = 100 \frac{| X\text{-}X_{PCD} |}{Xmean}$$

where
RPD is the relative percent difference
X_{PC} is the number of confirmed Aeromonas per 100 mL in the PC sample
X_{PCD} is the number of confirmed Aeromonas per 100 mL in the PCD sampleX_{mean} is the mean number of confirmed Aeromonas per 100 mL in the PC and PCD samples

9.8.5 Compare the RPD with the corresponding limits in Table 1 in Section 12. If the RPD meets the acceptance criteria, the system performance is acceptable and analysis of samples may continue. If RPD falls outside the range, system performance is unacceptable. Identify and correct the problem and perform another ODC test before continuing with the analysis of field samples.
9.8.6 As part of the QA program for the laboratory, method precision for ODC samples should be charted and records retained.

9.9 Analyst colony counting variability—If the laboratory has two or more analysts, each are required to count target colonies on the same membrane from one positive field sample per month. Compare each analyst's count of the target colonies. Counts should fall within 10% between analysts. If counts fail to fall within 10% of each other, analysts should perform additional sets of counts, until the number of target colonies counted fall within 10% between analysts for at least three consecutive samples. If there are no positive samples, an MS, MSD, or ODC sample can be used for this determination (MS or MSD are preferable to ODC samples, since they may have other background growth).

9.10 Autoclave sterilization verification—Verify autoclave sterilization monthly by placing Bacillus stearothermophilus spore suspensions or strips inside glassware. Sterilize at 121°C for 15 minutes. Place in trypticase soy broth tubes and incubate at 55°C for 48 hours. Check for growth to verify that sterilization was adequate. If sterilization was inadequate, determine appropriate time for autoclave sterilization. Filter sterilization may be used provided that these same QC steps are instituted for the filtrate.

10.0 Procedure
10.1 The membrane filter (MF) procedure with ampicillin-dextrin agar with vancomycin (ADA-V) is used to enumerate Aeromonas in finished waters.
10.2 Label each Petri dish with sample identification, preparation date, and analysis start date/time.
10.3 Use a sterile MF unit assembly (Section 6.18.3) at the beginning of each filtration series. The laboratory must sanitize each MF unit between filtrations by using a UV sanitizer, flowing steam, or boiling water for 2 min. A filtration series ends when 30 minutes or more elapse between sample filtrations.

10.4 Sterilize forceps with alcohol. Flame off excess alcohol. Using sterile forceps, place the MF (grid side up) over the sterilized funnel. Carefully place the top half of the filtration unit over the funnel and lock it in place.
10.5 Shake the sample bottle vigorously approximately 25 times to distribute the bacteria uniformly. Using aseptic technique, transfer one, 500-mL aliquot of sample to a single funnel. Use a graduated cylinder with a "to deliver" tolerance of approximately 2.5%.
Note: Laboratories must filter the entire 500-mL sample volume unless the filter clogs. If the filter clogs, a minimum of 100 mL of sample must be filtered, which may require multiple filtrations. If less than 500 mL are filtered and analyzed due to filter clogging, measure the residual, unfiltered volume to determine the volume filtered, and adjust the reporting limit accordingly.
10.6 Filter each sample under partial vacuum through a sterile membrane filter. Rinse the funnel after each sample filtration by filtering three, 30-mL portions of sterile buffered dilution water, being sure to thoroughly rinse the sides of the funnel.
10.7 Upon completion of the final rinse, disengage the vacuum and remove the funnel.
10.8 Using sterile forceps, immediately remove the MF and place it grid-side-up on the ADA-V medium with a rolling motion to avoid trapping air under the filter. Reseat the membrane filter if bubbles occur. Place the inverted Petri dishes in the 35^{o}C ± 0.5^{o}C incubator within 30 minutes of preparation. Sterilize forceps and sanitize the MF unit between the analysis of each sample.
10.9 After 24 ± 2 hours of incubation at 35^{o}C ± 0.5^{o}C, count and record yellow colonies under magnification using a dissecting microscope.
10.10 Isolation of a yellow colony on ampicillin-dextrin agar with vancomycin (ADA-V) should be considered presumptively positive for Aeromonas.
10.11 Confirmation—All presumptive colonies, up to ten per sample, must be submitted to confirmation. In this method, any presumptive colony that is positive for oxidase (Section 10.11.2), ferments trehalose (Section 10.11.3), and produces indole (Section 10.11.4) is considered to be Aeromonas. If the result for any confirmation procedure is negative, no further confirmation steps are necessary. Slight variations in color and morphology may be present between different Aeromonas species grown on ADA-V medium. The colonies selected for confirmation should be representative of all yellow (presumptively positive) colony morphology types on ADA-V plate. For example, if 30 bright yellow colonies and 20 dull yellow colonies are observed, then 6 bright yellow and 4 dull yellow colonies should be submitted to confirmation.
Note: It is important to record the number of colonies of each presumptively positive morphological type so that the final density of Aeromonas can be reported based on percent confirmation of each morphological type. Also, the laboratory may submit more than ten presumptively positive colonies to the confirmation step.
10.11.1 Nutrient agar streak plate. To confirm as Aeromonas, pick a colony and streak the colony onto a plate of nutrient agar medium (Section 7.9) and incubate at 35^{o}C ± 0.5^{o}C overnight to obtain isolated colonies.
10.11.2 Oxidase confirmation. Apply a very small amount of a discreet colony from the nutrient agar to the oxidase dry slide using a wooden or plastic applicator. Do not use iron or other reactive wire because it may cause false positive reactions. Also, do not transfer any medium with the culture material, as this could lead to inconsistent results. A blue/purple color reaction within 10

seconds is considered a positive oxidase test. For commercially-prepared reagent, adhere to manufacturer's expiration date. Freshly-made solutions should be used within one week. Please note: This method was validated using nutrient agar, if the oxidase reagent is to be dropped directly on colonies, use tryptic soy agar plates because nutrient agar plates give inconsistent results. The use of tryptic soy agar plates for streaking (Section 10.11.1) has not been validated and is considered a method modification and, as a result, the laboratory must demonstrate acceptable performance for the QC analyses described in Section 9.2.12.

Note: Timing of the color reaction is critical, as some Gram-positive bacteria may give false positives after 10 seconds. Also, it is important to put just a small amount of the colony on the oxidase dry slide or saturated pad, as too much bacteria can also cause a false positive oxidase test.

10.11.3 Trehalose confirmation. If the oxidase test is positive, then test for trehalose fermentation. Trehalose fermentation is determined by inoculating a tube containing 3-10 mL (depending on the size of the tube used - fill about half full) of 0.5% trehalose in purple broth base (Section 7.11) with a colony from the nutrient agar and incubating at 35^{o}C ± 0.5^{o}C for 24 ± 2 hours. A change in color of the medium from purple to yellow is considered a positive for trehalose fermentation.

10.11.4 Indole confirmation. If the oxidase and trehalose tests are positive, then test for indole production. (If the laboratory prefers, the indole confirmation procedure may be started on the same day as the trehalose confirmation.) Indole production is determined by inoculating a tube containing 3-10 mL (depending on the size of the tube used - fill about half full) of tryptone broth (Section 7.12) with a colony from the nutrient agar and incubating at 35^{o}C ± 0.5^{o}C for 24 ± 2 hours. After incubation, add 0.2 to 0.3 mL (4 to 6 drops) of Kovac's test reagent (Section 7.13) to each tube, let stand for approximately 10 minutes and observe results. A pink to red color in the surface layer constitutes a positive indole test. The original color of the Kovac's reagent indicates a negative indole test. An orange color probably indicates the presence of skatole, a breakdown product of indole, and is considered a positive result.

10.11.5 If a colony is oxidase, trehalose, and indole positive, report as a confirmed Aeromonas and archive the colony for further identification.

Note: If samples are to be archived for further analysis to determine species or hybridization group, from the nutrient agar plate (Section 10.11.1), inoculate a nutrient agar slant for short term use or shipment to another laboratory.

11.0 Data Analysis and Calculations

11.1 See Standard Methods for the Examination of Water and Wastewater (Reference 15.2) for general counting rules. The density of Aeromonas determined by the membrane filter (MF) procedure is calculated by direct identification and enumeration of yellow colonies by a dissecting microscope (Section 6.19) followed by oxidase, trehalose, and indole confirmation. Bacterial density is recorded as presumptive Aeromonas colony forming units (CFU) per 100 mL of sample and confirmed Aeromonas CFU per 100 mL.

11.2 Counting colonies on ADA-V

11.2.1 Record the number of presumptive Aeromonas CFU/100mL. If there is more than one morphological type that is considered to be presumptively positive, record the number of presumptive positives for each morphological type, as well as the total number of presumptive positives.

11.2.2 If there are more than 200 colonies, including background colonies, report results as too numerous to count (TNTC) and resample. If the filter is TNTC with more background colonies than presumptive aeromonads, split the 500 mL resample between 3 or 4 filters in order to better differentiate the colony morphology types. If the filter is TNTC with mostly aeromonads, a minimum of three dilutions (e.g. 100 mL, 10 mL and 1 mL) should be analyzed.
11.2.3 If the colonies are not discrete and appear to be growing together, report results as confluent growth (CG) and resample.

11.3 Confirmation and calculation of Aeromonas density
11.3.1 In this method, any presumptive colony that is positive for oxidase (Section 10.11.2), ferments trehalose (Section 10.11.3), and produces indole (Section 10.11.4) is considered to be Aeromonas. For the final density of confirmed Aeromonas, adjust the initial, presumptive count based on the positive confirmation percentage for each presumptively positive morphological type and report as confirmed CFU per 100 mL.
11.3.2 Calculate the number of positive confirmations for each presumptively positive morphological type from all filters of a given sample using the following equation:

$$\left(\frac{\text{Number positively confirmed}}{\text{Number submitted to confirmation}} \text{ X Number of presumptive positives}\right) = A$$

$$A \text{ X } \frac{100}{\text{mL filtered}} = \text{Confirmed Aeromonas /100 mL}$$

11.3.3 Record the number of confirmed Aeromonas per 100 mL for each colony morphology.
11.3.4 Sum the number of confirmed Aeromonas per 100 mL for all presumptively positive colony types (Section 11.3.2) and report as the density of confirmed Aeromonas per 100 mL.
11.3.5 Example 1: In this example, 500 mL of sample was filtered and two different morphological types of presumptively positive colonies were observed.

Example 1 Morphological Description	Number of presumptively positive colonies per volume filtered	Number submitted to confirmation steps	Number positively confirmed	Number of confirmed *Aeromonas* per 100 mL
Type A: Bright yellow, round, opaque	30	6	6	6
Type B: Dull yellow, oval, translucent	20	4	3	3
			Total number of confirmed *Aeromonas* per sample:	**9 per 100 mL**

Example 1 results in 9 confirmed *Aeromonas* / 100 mL.
11.3.6 Example 2: In this example, ***200 mL*** of sample was filtered and two different morphological types of presumptively positive colonies were observed.

Example 2 Morphological Description	Number of presumptively positive colonies per volume filtered	Number submitted to confirmation steps	Number positively confirmed	Number of confirmed *Aeromonas* per 100 mL
Type A: Dull yellow, round, opaque	40	5	5	20
Type B: Dull yellow, round, translucent	40	5	3	12
			Total number of confirmed *Aeromonas* per sample:	**32 per 100 mL**

Example 2 results in 32 confirmed *Aeromonas* / 100 mL.
11.3.7 If there were no presumptively positive colonies or if none of the presumptive colonies are confirmed, then report the results as less than the detection limit (DL) in CFU per 100 mL based on sample volume filtered. If less than 500 mL are filtered, then adjust the reporting limit per 100 mL accordingly. The DL may be calculated as follows:

DL per 100 mL = 100 / volume filtered CFU per 100mL

11.3.7.1 Example 3: If 500 mL of sample was filtered and there were no confirmed colonies, then report as <0.2 CFU/100 mL.
11.3.7.2 Example 4: If 100 mL of sample was filtered and there were no confirmed colonies, then report as <1.0 CFU/100 mL.

12.0 Method Performance

12.1 Specificity of media **12.1.1** Please refer to Section 16, Table 2, for results of *Aeromonas* growth after 24 hours on ADA at 30EC and 35EC and ADA-V at 35EC .

12.1.2 ADA-V was able to support the growth of the *Aeromonas* species (*hydrophila, caviae,* and *veronii/sobria*) most often associated with human disease.

12.1.3 Efforts continue to identify colonies which give a presumptive positive on the ADA-V media but do not confirm.

12.2 The QC acceptance criteria listed in Table 1, below are based on data generated through the interlaboratory validation of Method 1605 involving 11 laboratories and 11 finished drinking water matrices. Detailed method QC procedures applicable to these criteria are discussed in Section 9.

Table 1. QC Acceptance Criteria for Method 1605

QC specification	Maximum acceptable precision
Initial demonstration of capability (IDC): This test will require the analysis of 4 spiked reagent water samples	RSD = 22%
Ongoing demonstration of capability (ODC): This test will require the analysis of 2 spiked reagent water samples	RPD = 37%
Matrix spike/matrix spike duplicate (MS/MSD) precision: This test will require the analysis of 2 spiked finished water (matrix) samples	RPD = 48%

13.0 Pollution Prevention

13.1 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.

13.2 Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

14.0 Waste Management

14.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the biohazard waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

14.2 Samples, reference materials, and equipment known or suspected of having bacterial contamination from this work must be sterilized prior to disposal.

14.3 For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" and "Less is Better: Laboratory Chemical Management for Waste Reduction," both of which are available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

15.0 References

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15.9 Standard Methods for the Examination of Water and Wastewater. 1995. 19th Edition. Eds. A.D. Eaton, L.S. Clesceri, and A. Greenberg. American Public Health Association, American Water Works Association, and Water Environment Federation. American Public Health Association, Washington, D.C., publisher.

15.10 Janda, J.M. and S.L. Abbott. 1998. Evolving concepts regarding the genus *Aeromonas*: an expanding panorama of species, disease presentations, and unanswered questions. Journal of Clinical Infectious diseases. 27:332-344.

16.0 Tables and Flowcharts

Collection #	Hybridization group	*Aeromonas* species	ADA at 30°C	ADA at 35°C	ADA-V at 35°C
ATCC 7966	Group 1	*hydrophila*	+	+	+
ATCC 35654	Group 1	*hydrophila*	+	+	+
AMC 12723-W	Group 1	*hydrophila*	+	+	+
ATCC 51108	Group 2	*bestiarum*	+	+	+
AMC 14228-V	Group 2	*bestiarum*	+	+	+
ATCC 33658[1]	Group 3	*salmonicida/salmonicida*	-	-	NA
AMC 15228-V	Group 3	*salmonicida*	+	+	+
ATCC 15468	Group 4	*caviae*	+	+	+
MML 1685-E	Group 4	*caviae*	+	+	+
ATCC 33907	Group 5	*media*	-	-	NA
AMC Leftwich	Group 5	*media*	-	-	NA
ATCC 23309[1]	Group 6	*eucrenophila*	+	-	NA
ATCC 35993	Group 7	*sobria*	+	+	+
Muldoon SMHC	Group 7	*sobria*	+	+	+
ATCC 9071	Group 8	*veronii/sobria*	+	+	+
AMC 1123-W	Group 8	*veronii/sobria*	+	+	+
ATCC 43700	Group 12	*schubertii*	+[2]	+[5]	+[5]
AMC 1108-W	Group 12	*schubertii*	+	-	NA
ATCC 49657[3]	unknown	*trota*	-	-	NA
NMRI 206	unknown	*trota*	-	-	NA
ATCC 51208	unknown	*allosaccharophila*	+	+	+
ATCC 49568	Group 9	*jandaei*	+	+	+
AS 14	Group 9	*jandaei*	+	+	+
ATCC 35622	Group 10	*veronii/veronii*	+	+	+
WR 4659	Group 10	*veronii/veronii*	+	+	+
CECT 4342	Group 11	*encheleia*	+	-	NA
LMG 17541[4]	unknown	*popoffii*	+	+	+
AMC (ATCC 35941)	unknown	ornithine positive	-	-	NA
AMC (ATCC 43946)	unknown	Group 501	+	+	+
CDC 0434-84	Group 3	Motile Group 3	+	+	+

(1) Respective *Aeromonas* cultures grew on ADA medium when streaked, but not when filtered.

(2) Respective Aeromonas cultures grew when streaked on ADA medium at 30°C, however filtration was not performed with these cultures.(3) Respective Aeromonas cultures did not grow on ADA medium when streaked.(4) Respective Aeromonas cultures grew poorly on ADA medium at both temperatures and on ADA-V at 35°C. The same pattern of poor growth was also observed on non-selective media.(5) Respective Aeromonas cultures grew poorly on ADA and ADA-V medium at 35°C. The same pattern of poor growth was also observed on non-selective media.

Results: A Based on ADA results, it was assumed that the culture would not grow on ADA-V at 35°C.

\+ positive growth

-No growth

ATCC = American Type Culture Collection, Manassas, VA Other cultures were obtained from Amy Carnahan, University of Maryland. Serial dilutions representing approximately 10-200 CFU were filtered and the membrane placed on ADA or ADA-V medium as described in Section 10. Additional membranes representing the same dilution for each of the respective cultures were placed on brain heart infusion agar as a control.

17.3 Definitions

Confirmed colonies—Presumptively positive colonies that test positive for oxidase, ferment trehalose, and produce indole

Dilution/rinse water blank—A 100-mL aliquot of dilution/rinse water that is treated exactly as a sample and carried through all portions of the procedure until determined to be negative or positive. The Dilution/rinse water blank is used to determine if the sample has become contaminated by the introduction of a foreign microorganism through poor technique.

Initial demonstration of capability (IDC)—The IDC test is performed to demonstrate acceptable performance with the method prior to analysis of field samples.

Must—This action, activity, or procedural step is required.

Negative culture control—A non-*Aeromonas* bacteria processed to ensure the laboratories are familiar with the color and morphology of non-*Aeromonas* bacteria on ADA-V and to ensure that confirmation test results are appropriate.

Ongoing demonstration of capability (ODC)—The laboratory shall demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples (positive control/positive control duplicate).

Positive control—A 500-mL reagent water spiked with 20 - 80 CFU of Aeromonas. The positive control is analyzed exactly like a sample. Its purpose is to ensure that the results produced by the laboratory remain within the limits specified in this method for precision and recovery.

Presumptive positive colonies—Colonies that are yellow on ADA-V.

Relative Standard Deviation (RSD)—The standard deviation times 100 divided by the mean.

Selective medium—A culture medium designed to suppress the growth of unwanted microorganisms and encourage the growth of the target bacteria.

Should—This action, activity, or procedural step is suggested but not required.

Chlorine Section



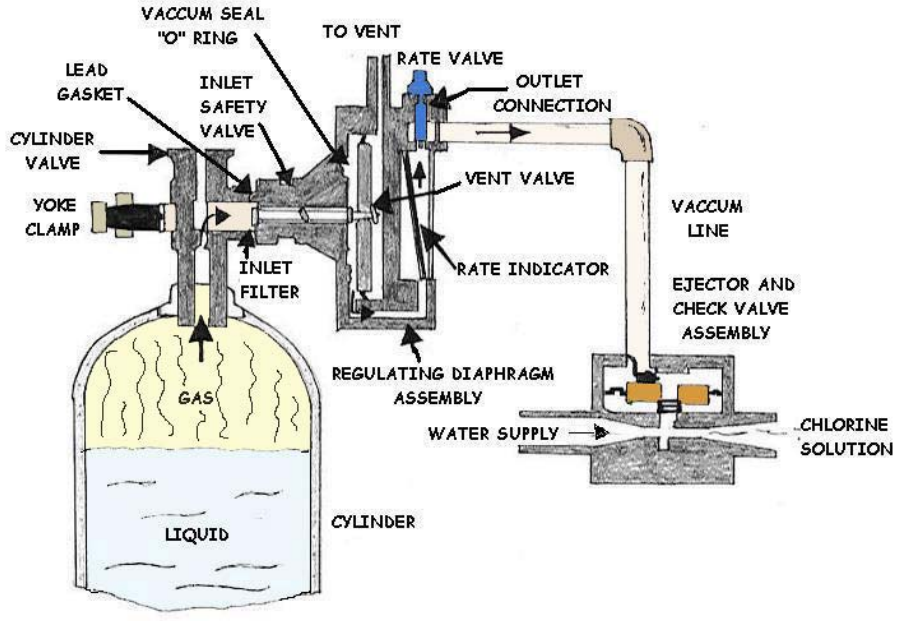
1 Ton Containers

The top line or valve is for extracting the gas, and the bottom line is for extracting the Cl2 liquid. Never place water on a leaking metal cylinder. The water will help create acid which will make the leak larger.





150 Pound Chlorine Cylinder



Chlorine Exposure Limits and Related Information

This information is necessary to pass your pre-test.

*** OSHA PEL 1 PPM - IDLH 10 PPM and Fatal Exposure Limit 1,000 PPM**

The current Occupational Safety and Health Administration (**OSHA**) permissible exposure limit (**PEL**) for chlorine is 1 ppm (3 milligrams per cubic meter ($mg/m^{(3)}$)) as a ceiling limit. A worker's exposure to chlorine shall at no time exceed this ceiling level. *** IDLH 10 PPM**

Physical and chemical properties of chlorine: A yellowish green, nonflammable and liquefied gas with an unpleasant and irritating smell. Can be readily compressed into a clear, amber-colored liquid, a noncombustible gas, and a strong oxidizer. Solid chlorine is about 1.5 times heavier than water and gaseous chlorine is about 2.5 times heavier than air. Atomic number of chlorine is 17. Cl is the elemental symbol and Cl2 is the chemical formula.

Monochloramine, dichloramine, and trichloramine are also known as Combined Available Chlorine. $Cl_2 + NH_4$.

HOCl and OCl-; The **OCL-** is the hypochlorite ion and both of these species are known as free available chlorine. These are the two main chemical species formed by chlorine in water and they are known collectively as hypochlorous acid and the hypochlorite ion. When chlorine gas is added to water, it rapidly hydrolyzes. The chemical equation that best describes this reaction is **$Cl2 + H_2O$ --> H+ + Cl- + HOCl.** Hypochlorous acid is the most germicidal of the chlorine compounds with the possible exception of chlorine dioxide.

Yoke-type connectors should be used on a chlorine cylinder's valve, assuming that the threads on the valve may be worn.

The connection from a chlorine cylinder to a chlorinator should be replaced by using a new, approved gasket on the connector. Always follow your manufacturer's instructions.

On 1 ton Chlorine gas containers, the chlorine pressure reducing valve should be located downstream of the evaporator when using an evaporator. This is the liquid chlorine supply line and it is going to be made into Chlorine gas.

In water treatment, chlorine is added to the effluent before the contact chamber (before the clear well) for complete mixing. One reason for not adding it directly to the chamber is that the chamber has very little mixing due to low velocities.

Here are several safety precautions when using chlorine gas. In addition to protective clothing and goggles, chlorine gas should be used only in a well-ventilated area so that any leaking gas cannot concentrate. Emergency procedures in the case of a large uncontrolled chlorine leak are as follows: Notify local emergency response team, warn and evacuate people in adjacent areas, and be sure that no one enters the leak area without adequate self-contained breathing equipment.

Here are several symptoms of chlorine exposure. Burning of eyes, nose, and mouth, coughing, sneezing, choking, nausea and vomiting, headaches and dizziness, fatal pulmonary edema, pneumonia, and skin blisters. A little Cl_2 will corrode the teeth and then progress to throat cancer.

Approved method for storing a 150 - 200 pound chlorine cylinder: Secure each cylinder in an upright position, attach the protective bonnet over the valve and firmly secure each cylinder. Never store near heat. Always store the empty in an upright, secure position with proper signage.



ABC Repair Kit



Rail Car Tanker

Chlorine Timeline

1879
- This marked the first time that chlorine was applied as a disinfectant. William Soper of England treated the feces of typhoid patients before disposal into the sewer. He used chlorinated lime, which was a common form of chlorine used initially. (White, 1972)

1893
- This date was the first time that chlorine was applied as a disinfectant on a plant scale basis. This application was made at Hamburg, Germany. (White, 1972)

1903
- This marked the first time chlorine gas was used as a disinfectant in drinking water. This took place in Middlekerke, Belgium. Prior to this date, chlorine was applied through the use of hydrated lime, chloride of lime, or bleaching powder. The use of chlorine gas was designed by Maurice Duyk, a chemist for the Belgian Ministry of Public Works. (Pontius, 1990)

1908
- The first full scale chlorine installation at a drinking water plant in the United States was initiated in this year. This installation took place at the Bubbly Creek Filter Plant in Chicago. This plant served the Chicago Stockyards and was designed by George A. Johnson. The raw water contained a large amount of sewage which was causing sicknesses in the livestock. Johnson implemented chlorine through chloride of lime, and the bacterial content of the water dropped drastically. (Pontius, 1990)

1910
- C. R. Darnall became the first to use compressed chlorine gas from steel cylinders, which is an approach still commonly used today. His installation was in Youngstown, Ohio. His implementation used a pressure-reducing mechanism, a metering device, and an absorption chamber. It was moderately successful, but his setup was only used once.

1912
- John Kienle, chief engineer of the Wilmington, Delaware water department, invented another way to apply chlorine to drinking water. He developed a way to push compressed chlorine from cylinders into an absorption tower in which water was flowing opposite the flow of the chlorine. Because the gas flow was opposite the water flow, the chlorine was able to disinfect the water. (Pontius, 1990)

1913
- An Ornstein chlorinator was installed at Kienle's Wilmington, Delaware water treatment plant. This marked the first time a commercial chlorination system was installed at a municipal water treatment plant. The chlorinator used the same basic premise that Kienle's previous installation did, but the Ornstein chlorinator used both a high and low pressure gauge to more accurately control the amount of chlorine added to the system. (Pontius, 1990)

1914
- On October 14, 1914, the Department of the Treasury enacted the first set of standards that required the use of disinfection for drinking water. These standards called for a maximum level of bacterial concentration of 2 coliforms per 100 milliliters. Because chlorination was the main disinfectant at the time, these standards dramatically increased the number of treatment plants using chlorine. (White, 1972)

1919
- Two important discoveries were made during this year. Wolman and Enslow discovered the concept of chlorine demand which states that the amount of chlorine needed to disinfect the water is related to the concentration of the waste and the amount of time the chlorine has to contact the water. The other important discovery of 1919 was by Alexander Houston. He discovered that chlorine can also eliminate taste and odor problems in water. (Pontius, 1990)

1925
- New drinking water standards were enacted that reduced the maximum permissible limit of coliforms from 2 to 1 coliform per 100 milliliters. This increased the amount and frequency of chlorination again. (White, 1972)

1939
- The theory of the chlorine breakpoint was discovered in this year. Chlorine breakpoint theory is discussed in the following section. (White, 1972)

1960
- A new implementation practice was discovered in this year. The compound loop principle of chlorinator control was implemented, which is the most recent major discovery in chlorine application. (White, 1972)

1972
- A report entitled "Industrial Pollution of the Lower Mississippi River in Louisiana" was published containing the first evidence of disinfection byproducts in drinking water resulting from organic pollution in source water. (Pontius, 1990)

As is evident by the dates in the timeline, most of the innovation in chlorination occurred over 70 years ago. Very few innovations or discoveries have been made recently. Most of the current research is being performed in other areas of disinfection. These areas include ozone, chlorine dioxide, and UV radiation. Chlorine is still the most widely used disinfectant in the United States, but other areas of the world are beginning to use other methods of disinfection with increasing frequency. Since chlorine is still widely used, a thorough understanding of how it disinfects and is implemented is important to those interested in water treatment.

Chlorine Supplement **Pre-Quiz**

1. How should the connection from a chlorine cylinder to a chlorinator be replaced?

2. How many turns should a chlorine gas cylinder be initially opened?

3. If the temperature of a full chlorine cylinder is increased by 50°F or 30°C, what is the most likely result?

4. What is meant by the specific gravity of a liquid?

5. Which metals are the only metals that are ***TOTALLY*** inert to moist chlorine gas?

6. What will be discharged when opening the top valve on a one-ton chlorine cylinder?

7. What are the approved methods for storing a chlorine cylinder?

8. What are normal conditions for a gas chlorination start-up?

9. Name a safety precaution when using chlorine gas?

10. What compounds are formed in water when chlorine gas is introduced?

11. Why should roller bearings not be used to rotate a one-ton chlorine cylinder?

12. What are the physical and chemical properties of chlorine?

13. What are the necessary emergency procedures in the case of a large uncontrolled chlorine leak?

14. Name several symptoms of chlorine exposure.

15. 5 lbs. of a 70% concentration sodium hypochlorite solution is added to a tank containing 650 gallons of water. What is the chlorine dosage?

16. As soon as Cl_2 gas enters the throat area, a victim will sense a sudden stricture in this area - nature's way of signaling to prevent passage of the gas to the lungs. At this point, the victim must attempt to do two things. Name them.

17. Positive pressure SCBAs and full face piece SARs can be used in oxygen deficient atmospheres containing less than what percentage of oxygen in the atmosphere?

18. Death is possible from asphyxia, shock, reflex spasm in the larynx, or massive pulmonary edema. Populations at special risk from chlorine exposure are individuals with pulmonary disease, breathing problems, bronchitis, or chronic lung conditions.
A. TRUE
B. FALSE

19. Chlorine gas reacts with water producing a strongly oxidizing solution causing damage to the moist tissue lining the respiratory tract when the tissue is exposed to chlorine. The respiratory tract is rapidly irritated by exposure to 10-20 ppm of chlorine gas in air, causing acute discomfort that warns of the presence of the toxicant.
A. TRUE
B. FALSE

20. Even brief exposure to 1,000 ppm of Cl_2 can be fatal.
A. TRUE
B. FALSE

21. What are the two main chemical species formed by chlorine in water and what name are they are known collectively as?

22. When chlorine gas is added to water, it rapidly hydrolyzes according to the reaction:

23. Which chemical reaction equation represents the dissociation of hypochlorous acid?

24. This species of chlorine is the most germicidal of all chlorine compounds with the possible exception of chlorine dioxide.



Here are both half ton container and 150 pound chlorine gas cylinders.
Answers in rear section before the final assignment.



Chlorine evaporators next to a rail tank chlorine cylinder. Bottom photograph, SCBA should always be stored on the outside of the chlorine storage room.





Chlorine C repair kit, used for the tank cars and tankers.



Chlorine Introduction



Name: Chlorine
Symbol: Cl
Atomic Number: 17
Atomic Mass: 35.4527 amu
Melting Point: -100.98 °C (172.17 K, -149.764 °F)
Boiling Point: -34.6 °C (238.55 K, -30.279997 °F)
Number of Protons/Electrons: 17
Number of Neutrons: 18
Classification: Halogen
Crystal Structure: Orthorhombic
Density @ 293 K: 3.214 g/cm^3
Color: Green
Uses: Water purification, bleaches
Obtained From: Salt
Date of Discovery: 1774
Discoverer: Carl Wilhelm Scheele
Name Origin: From the Greek word *khlôros* (green)

Chlorine Gas Information
Identifiers
1. CAS No.: 7782-50-5
2. RTECS No.: FO2100000
3. DOT UN: 1017 20
4. DOT label: Poison gas



Chlorinators

Safety Data
NIOSH IDHL: 25 ppm
NIOSH Ceiling: **0.5ppm/15 minutes**
PEL/TWA: **1 ppm**
TLV/TWA: **1 ppm**
TLV/STEL: **3 ppm**
TLV/IDLH: **25 ppm**

Physical Data
1. **Molecular weight:** 70.9
2. **Boiling point (at 760 mm Hg):** -34.6 degrees C (-30.28 degrees F)
3. **Specific gravity (liquid):** 1.41 at 20 degrees C (68 degrees F) and a pressure of 6.86 atm
4. **Vapor density**: 2.5
5. **Melting point:** -101 degrees C (-149.8 degrees F)
6. **Vapor pressure at 20 degrees C (68 degrees F):** 4,800 mm Hg
7. **Solubility**: Slightly soluble in water; soluble in alkalis, alcohols, and chlorides.
8. **Evaporation rate:** Data not available.

Chlorine's Appearance and Odor
Chlorine is a greenish-yellow gas with a characteristic pungent odor. It condenses to an amber liquid at approximately -34 degrees C (-29.2 degrees F) or at high pressures. Odor thresholds ranging from 0.08 to part per million (ppm) parts of air have been reported. Prolonged exposures may result in olfactory fatigue.

Reactivity
1. **Conditions Contributing to Instability**: Cylinders of chlorine may burst when exposed to elevated temperatures. Chlorine in solution forms a corrosive material.
2**. Incompatibilities**: Flammable gases and vapors form explosive mixtures with chlorine. Contact between chlorine and many combustible substances (such as gasoline and petroleum products, hydrocarbons, turpentine, alcohols, acetylene, hydrogen, ammonia, and sulfur), reducing agents, and finely divided metals may cause fires and explosions. Contact between chlorine and arsenic, bismuth, boron, calcium, activated carbon, carbon disulfide, glycerol, hydrazine, iodine, methane, oxomonosilane, potassium, propylene, and silicon should be avoided. Chlorine reacts with hydrogen sulfide and water to form hydrochloric acid, and it reacts with carbon monoxide and sulfur dioxide to form phosgene and sulfuryl chloride. Chlorine is also incompatible with moisture, steam, and water.
3. **Hazardous Decomposition Products:** None reported.
4**. Special Precautions:** Chlorine will attack some forms of plastics, rubber, and coatings.

Flammability
Chlorine is a non-combustible gas.
The National Fire Protection Association has assigned a flammability rating of 0 (no fire hazard) to chlorine; however, most combustible materials will burn in chlorine.
1. **Flash point:** Not applicable.
2. **Autoignition temperature**: Not applicable.
3. **Flammable limits in air:** Not applicable.
4. **Extinguishant:** For small fires use water only; do not use dry chemical or carbon dioxide. Contain and let large fires involving chlorine burn. If fire must be fought, use water spray or fog.

Fires involving chlorine should be fought upwind from the maximum distance possible.

Keep unnecessary people away; isolate the hazard area and deny entry. For a massive fire in a cargo area, use unmanned hose holders or monitor nozzles; if this is impossible, withdraw from the area and let the fire burn. Emergency personnel should stay out of low areas and ventilate closed spaces before entering.

Containers of chlorine may explode in the heat of the fire and should be moved from the fire area if it is possible to do so safely. If this is not possible, cool fire exposed containers from the sides with water until well after the fire is out. Stay away from the ends of containers. Firefighters should wear a full set of protective clothing and self-contained breathing apparatus when fighting fires involving chlorine.

Chlorine Exposure Limits

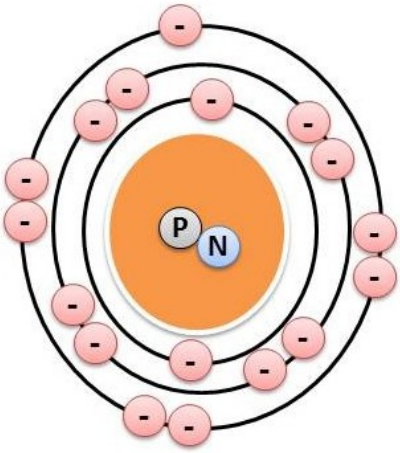
*** OSHA PEL**
The current **OSHA** permissible exposure limit (**PEL**) for chlorine is 1 ppm (3 milligrams per cubic meter ($mg/m^{(3)}$)) as a ceiling limit. A worker's exposure to chlorine shall at no time exceed this ceiling level [29 CFR 1910.1000, Table Z-1].

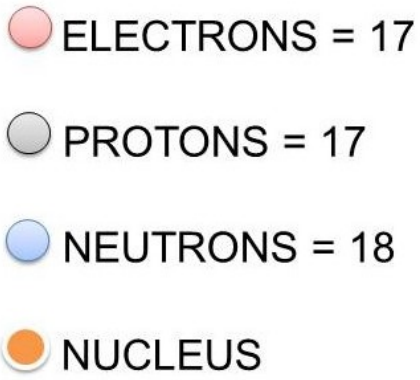
*** NIOSH REL**
The National Institute for Occupational Safety and Health (**NIOSH**) has established a recommended exposure limit (**REL**) for chlorine of 0.5 ppm $mg/m^{(3)}$) as a TWA for up to a 10-hour workday and a 40-hour workweek and a short-term exposure limit (**STEL**) of 1 ppm (3 $mg/m^{(3)}$)[NIOSH 1992].

*** ACGIH TLV**
The American Conference of Governmental Industrial Hygienists (**ACGIH**) has assigned chlorine a threshold limit value (**TLV**) of 0.5 ppm (1.5 $mg/m^{(3)}$) as a TWA for a normal 8-hour workday and a 40-hour workweek and a **STEL** of 1 ppm (2.9 $mg/m^{(3)}$) for periods not to exceed 15 minutes. Exposures at the STEL concentration should not be repeated more than four times a day and should be separated by intervals of at least 60 minutes [ACGIH 1994, p. 15].

*** Rationale for Limits**
The NIOSH limits are based on the risk of severe eye, mucous membrane and skin irritation [NIOSH 1992]. The ACGIH limits are based on the risk of eye and mucous membrane irritation [ACGIH 1991, p. 254].

Chlorine's Atomic Structure





Isotopes

Isotope	Half Life
Cl-35	Stable
Cl-36	301000.0 years
Cl-37	Stable
Cl-38	37.2 minutes



Top photograph, this blue device prevents the liquid from being pulled and freezing the lines. Bottom photograph, the application of an ammonia mist to detect a chlorine gas leak.



Chlorine Basics

Chlorine is one of 90 natural elements, the basic building blocks of our planet. To be useful, an element must be relatively abundant or have extremely desirable properties. Chlorine has both characteristics. As a result -- over the course of many decades of careful research and development -- scientists have learned to use chlorine and the products of chlorine chemistry to make drinking water safe, destroy life-threatening germs, produce life-saving drugs and medical equipment, shield police and fire fighters in the line of duty, and ensure a plentiful food supply.

In 1774, in his small experimental laboratory, Swedish pharmacist Carl Wilhem Scheele released a few drops of hydrochloric acid onto a piece of manganese dioxide. Within seconds, a greenish-yellow gas arose. Although he had no idea at the time, he had just discovered chlorine.

The fact that the greenish-yellow gas was actually an element was only recognized several decades later by English chemist Sir Humphrey Davy. Until that time, people were convinced that the gas was a compound of oxygen. Davy gave the element its name on the basis of the Greek word khloros, for greenish-yellow. In 1810 he suggested the name "*chloric gas*" or "*chlorine*."

One of the most effective and economical germ-killers, chlorine also destroys and deactivates a wide range of dangerous germs in homes, hospitals, swimming pools, hotels, restaurants, and other public places. Chlorine's powerful disinfectant qualities come from its ability to bond with and destroy the outer surfaces of bacteria and viruses. First used as a germicide to prevent the spread of "child bed fever" in the maternity wards of Vienna General Hospital in Austria in 1846, chlorine has been one of society's most potent weapons against a wide array of life-threatening infections, viruses, and bacteria for 150 years.

When the first men to set foot on the moon returned to earth (Apollo 11 mission: 24.7.69) a hypochlorite solution was chosen as one of the disinfectants for destroying any possible moon germs.

What Happens to Chlorine When it Enters the Environment?

- When released to air, chlorine will react with water to form hypochlorous acid and hydrochloric acid, which are removed from the atmosphere by rainfall.

- Chlorine is slightly soluble in water. It reacts with water to form hypochlorous acid and hydrochloric acid. The hypochlorous acid breaks down rapidly. The hydrochloric acid also breaks down; its breakdown products will lower the pH of the water (makes it more acidic).

- Since chlorine is a gas it is rarely found in soil. If released to soil, chlorine will react with moisture forming hypochlorous acid and hydrochloric acid. These compounds can react with other substances found in soil.

- Chlorine does not accumulate in the food chain.

Disinfectant Qualities

Restaurants and meat and poultry processing plants rely on chlorine bleach and other chlorine-based products to kill harmful levels of bacteria such as *Salmonella* and *E. coli* on food preparation surfaces and during food processing. Chlorine is so important in poultry processing that the US Department of Agriculture requires an almost constant chlorine rinse for much of the cutting equipment. In fact, no proven economical alternative to chlorine disinfection exists for use in meat and poultry processing facilities.

Properties

Because it is highly reactive, chlorine is usually found in nature bound with other elements like sodium, potassium, and magnesium. When chlorine is isolated as a free element, chlorine is a greenish yellow gas, which is 2.5 times heavier than air. It turns to a liquid state at -34°C (-29°F), and it becomes a yellowish crystalline solid at -103°C (-153°F). Chemists began experimenting with chlorine and chlorine compounds in the 18th century. They learned that chlorine has an extraordinary ability to extend a chemical bridge between various elements and compounds that would not otherwise react with each other. Chlorine has been especially useful in studying and synthesizing organic compounds -- compounds that have at least one atom of the element carbon in their molecular structure. All living organisms, including humans, are composed of organic compounds.

Chlorine is one of the most abundant chemical elements on Earth. It is ubiquitous in soils, minerals, plants and animals. Seawater is a huge reservoir of dissolved chlorine weathered from the continents and transported to the oceans by Earth's rivers.

Chlorine is also one of the most useful chemical elements. Each chemical element has its own set of unique properties and chlorine is known as a very reactive element--so reactive, in fact, that it is usually found combined with other elements in the form of compounds. More than 3,500 naturally occurring chlorinated organic (associated with living organisms) compounds alone have been identified.

Chlorine's chemical properties have been harnessed innovatively for good use. For example, this element plays a huge role in public health. Chlorine-based disinfectants are capable of removing a wide variety of disease-causing germs from drinking water and wastewater as well as from hospital and food production surfaces. Additionally, chlorine plays an important role in the manufacture of thousands of products we depend upon every day, including such diverse items as cars, computers, pharmaceuticals and military flak jackets. As the ninth largest chemical produced in the U.S. by volume, chlorine is truly a "workhorse chemical."

Released From the Salt of the Earth

Chlorine is produced industrially from the compound sodium chloride, one of the many salts found in geologic deposits formed from the slow evaporation of ancient seawater. When electricity is applied to a brine solution of sodium chloride, chlorine gas (Cl2), caustic soda (NaOH) and hydrogen gas (H_2) are generated according to the following reaction:

Salt	+	Water	→	Chlorine gas	+	Caustic soda	+	Hydrogen gas
2 NaCl	+	2 H_2O	→	Cl_2	+	2 NaOH	+	H_2

Co-Products
As the reaction demonstrates, chlorine gas cannot be produced without producing caustic soda, so chlorine and caustic soda are known as "co-products," and their economics are inextricably linked. Caustic soda, also called "alkali," is used to produce a wide range of organic and inorganic chemicals and soaps. In addition, the pulp and paper, alumina and textiles industries use caustic soda in their manufacturing processes. Thus, the "chlor-alkali" industry obtains two very useful chemicals by applying electrical energy to sea salt.



Definitions

Chlorine Gas Feed Room
A chlorine gas feed room, for the purposes of this document, is a room that contains the chlorinator(s) and active cylinder(s) used to apply chlorine gas at a water or wastewater facility.

Chlorine Gas Storage Room
A chlorine gas storage room, for the purposes of this document, is a room other than a chlorine gas feed room, in which full, partial, or empty chlorine gas cylinders or ton containers are stored at a water or wastewater facility.

Gas Chlorinator
A gas chlorinator is a device used to meter and control the application rate of chlorine gas into a liquid. There is the danger of the gas escaping at a water or wastewater treatment facility. The gas chlorinator should be isolated from a water or wastewater treatment plant.

Chlorine Cabinet
A chlorine cabinet is a pre-assembled or factory built unit that contains the equipment used to apply chlorine gas at a water or wastewater treatment facility. It is isolated from a water or wastewater treatment plant.



Top photograph, a view of the top of a 150 gas cylinder. Bottom, always work in pairs when working around Chlorine. Here the hoist is being used to move the container.



Chemical Equations, Oxidation States, and Balancing of Equations

Before we breakdown chlorine and other chemicals, let's start with this review of basic chemical equations.

Beginning
The common chemical equation could be A + B --> C + D. This is chemical A + chemical B, the two reacting chemicals will go to products C + D, etc.

Oxidation
The term "oxidation" originally meant a reaction in which oxygen combines chemically with another substance, but its usage has long been broadened to include any reaction in which electrons are transferred.

Oxidation and reduction always occur simultaneously (redox reactions), and the substance which gains electrons is termed the oxidizing agent. For example, cupric ion is the oxidizing agent in the reaction: Fe (metal) + Cu++ --> Fe++ + Cu (metal); here, two electrons (negative charges) are transferred from the iron atom to the copper atom; thus the iron becomes positively charged (is oxidized) by loss of two electrons, while the copper receives the two electrons and becomes neutral (is reduced).

Electrons may also be displaced within the molecule without being completely transferred away from it. Such partial loss of electrons likewise constitutes oxidation in its broader sense and leads to the application of the term to a large number of processes, which at first sight might not be considered to be oxidation. Reaction of a hydrocarbon with a halogen, for example, CH_4 + 2 Cl --> CH_3Cl + HCl, involves partial oxidation of the methane; halogen addition to a double bond is regarded as an oxidation.

Dehydrogenation is also a form of oxidation; when two hydrogen atoms, each having one electron, are removed from a hydrogen-containing organic compound by a catalytic reaction with air or oxygen, as in oxidation of alcohol to aldehyde.

Oxidation Number
The number of electrons that must be added to or subtracted from an atom in a combined state to convert it to the elemental form; i.e., in barium chloride ($BaCl_2$) the oxidation number of barium is +2 and of chlorine is -1. Many elements can exist in more than one oxidation state.

Now, let us look at some common ions. An ion is the reactive state of the chemical, and is dependent on its place within the periodic table.

Have a look at the "periodic table of the elements". It is arranged in columns of elements, there are 18 columns. You can see column one, H, Li, Na, K, etc. These all become ions as H^+, Li^+, K^+, etc. The next column, column 2, Be, Mg, Ca etc. become ions Be^{2+}, Mg^{2+}, Ca^{2+}, etc. Column 18, He, Ne, Ar, Kr are inert gases. Column 17, F, Cl, Br, I, ionize to a negative F^-, Cl^-, Br^-, I^-, etc.

What you now need to do is memorize the table of common ions, both positive ions and negative ions.

Table of Common Ions

Positive Ions

Valency 1		Valency 2		Valency 3	
lithium	Li^+	magnesium	Mg_2^+	aluminum	Al_3^+
sodium	Na^+	calcium	Ca_2^+	iron III	Fe_3^+
potassium	K^+	strontium	Sr_2^+	chromium	Cr_3^+
silver	Ag^+	barium	Ba_2^+		
hydronium	H_3O^+	copper II	Cu_2^+		
(or hydrogen)	H^+	lead II	Pb_2^+		
ammonium	NH_4^+	zinc	Zn_2^+		
copper I	Cu^+	manganese II	Mn_2^+		
mercury I	Hg^+	iron II	Fe_2^+		
		tin II	Sn_2^+		

Negative Ions

Valency 1		Valency 2		Valency 3	
fluoride	F^-	oxide	O_2^-	phosphate	PO_4^{3-}
chloride	Cl^-	sulfide	S_2^-		
bromide	Br^-	carbonate	CO_3^{2-}		
iodide	I^-	sulfate	SO_4^{2-}		
hydroxide	OH^-	sulfite	SO_3^{2-}		
nitrate	NO_3^-	dichromate	$Cr_2O_7^-$		
bicarbonate	HCO_3^-	chromate	CrO_4^{2-}		
bisulphate	HSO_4^-	oxalate	$C_2O_4^{2-}$		
nitrite	NO_2^-	thiosulfate	$S_2O_3^{2-}$		
chlorate	ClO_3^-	tetrathionate	$S_4O_6^{2-}$		
permanganate	MnO_4^-	monohydrogen phosphate	HPO_4^{2-}		
hypochlorite	OCl^-				
dihydrogen phosphate	$H_2PO_4^-$				

Positive ions will react with negative ions, and vice versa. This is the start of our chemical reactions. For example:

$Na^+ + OH^- \rightarrow NaOH$ (sodium hydroxide)

$Na^+ + Cl^- \rightarrow NaCl$ (salt)

$3H^+ + PO_4^{3-} \rightarrow H_3PO_4$ (phosphoric acid)

$2Na^+ + S_2O_3^{2-} \rightarrow Na_2S_2O_3$

You will see from these examples, that if an ion of one (+), reacts with an ion of one (-) then the equation is balanced. However, an ion like PO_4^{3-} (phosphate) will require an ion of 3+ or an ion of one (+) (but needs three of these) to neutralize the 3- charge on the phosphate. So, what you are doing is balancing the charges (+) or (-) to make them zero, or cancel each other out.

For example, since aluminum exists in its ionic state as Al^{3+}, it will react with many negatively charged ions; for example: Cl^-, OH^-, SO_4^{2-}, PO_4^{3-}.

Let us do these examples and balance them.
$Al^{3+} + Cl^- \rightarrow AlCl$ **(incorrect)**
$Al^{3+} + 3Cl^- \rightarrow AlCl_3$ **(correct)**

How did we work this out?
Al^{3+} has three positives (3+)
Cl^- has one negative (-)
It will require **3 negative charges** to cancel out the **3 positive charges** on the aluminum (Al^{3+}).

When the **left hand side** of the equation is written, to balance the number of chlorine's (Cl^-) required, the number 3 is placed in front of the ion concerned, in this case Cl^-, becomes $3Cl^-$.

On the **right hand side** of the equation, where the ions have become a compound (a chemical compound), the number is transferred to after the relevant ion, Cl_3.

Another example:
$Al^{3+} + SO_4^{2-} \rightarrow AlSO_4$ (**incorrect**)
$2Al^{3+} + 3SO_4^{2-} \rightarrow Al_2(SO_4)_3$ (**correct**)

Let me give you an easy way of balancing:
Al is 3+
SO_4 is 2-
Simply transpose the number of positives (or negatives) for each ion, to the other ion, by placing this value of one ion, in front of the other ion. That is, Al^{3+} the 3 goes in front of the SO_4^{2-} as $3SO_4^{2-}$, and SO_4^{2-}, the 2 goes in front of the Al^{3+} to become $2Al^{3+}$. Then on the **right hand side** of the equation, this same number (now in front of each ion on the **left side** of the equation), is placed after each "ion" entity.

Let us again look at:
$Al^{3+} + SO_4^{2-} \rightarrow AlSO_4$ **(incorrect)**
$Al^{3+} + SO_4^{2-} \rightarrow Al_2(SO_4)_3$ **(correct)**
Put the three from the Al in front of the SO_4^{2-} and the 2 from the SO_4^{2-} in front of the Al^{3+}.
Equation becomes:
$2Al^{3+} + 3SO_4^{2-} \rightarrow Al_2(SO_4)_3$. You simply place the valency of one ion, as a whole number, in front of the other ion, and vice versa.

Remember to encase the SO_4 in brackets. **Why?** Because we are dealing with the sulfate ion, SO_4^{2-}, and it is this ion that is 2- charged (not just the O_4), so we have to ensure that the "ion" is bracketed. Now to check, the 2 times $3^+ = 6^+$, and 3 times $2^- = 6^-$. We have equal amounts of positive ions, and equal amounts of negative ions.

Another example:
$NaOH + HCl$ --> ?
Na is Na^+, OH is OH^-, so this gave us NaOH. Originally, the one positive canceled the one negative.

HCl is $H^+ + Cl^-$, this gave us HCl.

Reaction is going to be the Na^+ reacting with a negatively charged ion. This will have to be the chlorine, Cl^-, because at the moment the Na^+ is tied to the OH^-. **So:** $Na^+ + Cl^-$ --> NaCl
The H+ from the HCl will react with a negative (-) ion this will be the OH^- from the NaOH.
So: $H^+ + OH^-$ --> H_2O (water).

The complete reaction can be written:
$NaOH + HCl$ --> $NaCl + H_2O$. We have **equal amounts** of all atoms **each side** of the equation, so the equation is **balanced**.
or
$Na^+OH^- + H^+Cl^-$ --> $Na^+Cl^- + H^+OH^-$

Something More Difficult:
$Mg(OH)_2 + H_3PO_4$ --> ? (equation on left **not** balanced)
$Mg^{2+}\ 2OH^- + 3H^+PO_4^{3-}$ --> ? (equation on left **not** balanced), so let us rewrite the equation in **ionic form**.

The Mg^{2+} **needs to react with a negatively charged ion**, this will be the PO_4^{3-},
so: $3Mg^{2+} + 2PO_4^{3-}$ --> $Mg_3(PO_4)_2$
(**Remember** the **swapping** of the positive or negative charges on the ions in the **left side** of the equation, and placing it in front of each ion, and then placing this number after each ion on the **right side** of the equation)

What is left is the H^+ from the H_3PO_4 and this will react with a negative ion, we only have the OH^- from the $Mg(OH)_2$ left for it to react with.
$6H^+ + 6OH^-$ --> $6H_2O$

Where did I get the 6 from? When I balanced the Mg^{2+} with the PO_4^{3-}, the equation became $3Mg^{2+} + 2PO_4^{3-}$ --> $Mg_3(PO_4)_2$

Therefore, I must have required $3Mg(OH)_2$ to begin with, and $2H_3PO_4$, (because we originally had $(OH)_2$ attached to the Mg, and H_3 attached to the PO_4. I therefore have $2H_3$ reacting with $3(OH)_2$. We have to write this, on the **left side** of the equation, as $6H^+ + 6OH^-$ because we need it in ionic form.
The equation becomes:
$6H^+ + 6OH^-$ --> $6H_2O$

The full equation is now balanced and is:
$3Mg(OH)_2 + 2H_3PO_4$ --> $Mg_3(PO_4)_2 + 6H_2O$
I have purposely split the equation into segments of reactions. This is showing you which ions are reacting with each other. Once you get the idea of equations you will not need this step.

The balancing of equations is simple. You need to learn the valency of the common ions (see tables). The rest is pure mathematics; you are balancing valency charges, positives versus

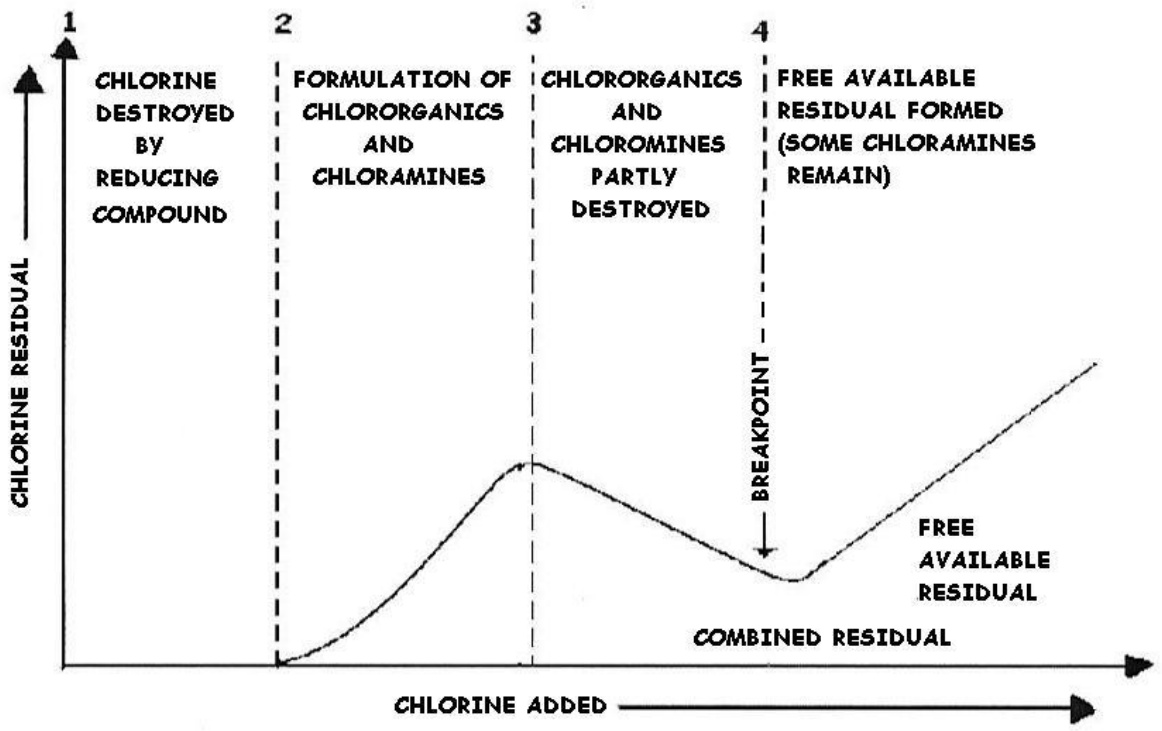
negatives. You have to have the **same number** of **negatives**, or **positives**, each side of the equation, and the **same number** of **ions** or **atoms** each side of the equation.

If one ion, example Al^{3+}, (3 positive charges) reacts with another ion, example OH^- (one negative ion) then we require 2 more negatively charged ions (in this case OH^-) to counteract the 3 positive charges the Al^{3+} contains.

Take my earlier hint, place the 3 from the Al^{3+} in front of the OH^-, now reads $3OH^-$, place the 1 from the hydroxyl OH^- in front of the Al^{3+}, now stays the same, Al^{3+} (the 1 is **never** written in chemistry equations).

$Al^{3+} + 3OH^- \rightarrow Al(OH)_3$

The 3 is simply written in front of the OH^-, a recognized ion, there are no brackets placed around the OH^-. On the right hand side of the equation, all numbers in front of each ion on the left hand side of the equation are placed after each same ion on the right side of the equation. Brackets are used in the right side of the equation because the result is a compound. Brackets are also used for compounds (reactants) in the left side of equations, as in $3Mg(OH)_2 + 2H_3PO_4 \rightarrow ?$





Hard to tell, but these are one ton chlorine gas containers. Notice the five gallon bucket of motor oil in the bottom photograph. Also notice that this photograph is the only eye wash station that we found during our inspection of 10 different facilities. Do you have an eye wash and emergency shower?



Chemistry of Chlorination

Chlorine can be added as sodium hypochlorite, calcium hypochlorite or chlorine gas. When any of these is added to water, chemical reactions occur as these equations show:

Cl 2 + H 2 O → HOCl + HCl
(chlorine gas) (water) (hypochlorous acid) (hydrochloric acid)

CaOCl + H 2 O → 2HOCl + Ca(OH)
(calcium hypochlorite) (water) (hypochlorous acid) (calcium hydroxide)

NaOCl + H 2 O → HOCl + Na(OH)
(sodium hypochlorite) (water) (hypochlorous acid) (sodium hydroxide)

All three forms of chlorine produce hypochlorous acid (HOCl) when added to water. Hypochlorous acid is a weak acid but a strong disinfecting agent. The amount of hypochlorous acid depends on the pH and temperature of the water. Under normal water conditions, hypochlorous acid will also chemically react and break down into a hypochlorite. ion

(OCl -): HOCl H + + OCl – Also expressed HOCl → H + + OCl –
(hypochlorous acid) (hydrogen) (hypochlorite ion)
The hypochlorite ion is a much weaker disinfecting agent than hypochlorous acid, about 100 times less effective.

Let's now look at how pH and temperature affect the ratio of hypochlorous acid to hypochlorite ions. As the temperature is decreased, the ratio of hypochlorous acid increases. Temperature plays a small part in the acid ratio. Although the ratio of hypochlorous acid is greater at lower temperatures, pathogenic organisms are actually harder to kill. All other things being equal, higher water temperatures and a lower pH are more conducive to chlorine disinfection.

Types of Residual

If water were pure, the measured amount of chlorine in the water should be the same as the amount added. But water is not 100% pure. There are always other substances (interfering agents) such as iron, manganese, turbidity, etc., which will combine chemically with the chlorine.

This is called the ***chlorine demand.*** Naturally, once chlorine molecules are combined with these interfering agents, they are not capable of disinfection. It is free chlorine that is much more effective as a disinfecting agent.

So let's look now at how free, total and combined chlorine are related. When a chlorine residual test is taken, either a total or a free chlorine residual can be read.

Total residual is all chlorine that is available for disinfection.

Total chlorine residual = free + combined chlorine residual.

Free chlorine residual is a much stronger disinfecting agent. Therefore, most water regulating agencies will require that your daily chlorine residual readings be of free chlorine residual.

Break-point chlorination is where the chlorine demand has been satisfied, and any additional chlorine will be considered ***free chlorine*****.**

Residual Concentration/Contact Time (CT) Requirements

Disinfection to eliminate fecal and coliform bacteria may not be sufficient to adequately reduce pathogens such as Giardia or viruses to desired levels. Use of the ***"CT"*** disinfection concept is recommended to demonstrate satisfactory treatment, since monitoring for very low levels of pathogens in treated water is analytically very difficult.

The CT concept, as developed by the United States Environmental Protection Agency (Federal Register, 40 CFR, Parts 141 and 142, June 29, 1989), uses the combination of disinfectant residual concentration (mg/L) and the effective disinfection contact time (in minutes) to measure effective pathogen reduction. The residual is measured at the end of the process, and the contact time used is the T10 of the process unit (time for 10% of the water to pass).

CT = Concentration (mg/L) x Time (minutes)

The effective reduction in pathogens can be calculated by reference to standard tables of required CTs.

Required Giardia/Virus Reduction
All surface water treatment systems shall ensure a minimum reduction in pathogen levels: 3-log reduction in Giardia; and 4-log reduction in viruses.

These requirements are based on unpolluted raw water sources with Giardia levels of = 1 cyst/100 L, and a finished water goal of 1 cyst/100,000 L (equivalent to 1 in 10,000 risk of infection per person per year). Higher raw water contamination levels may require greater removals as shown on Table 4.1.

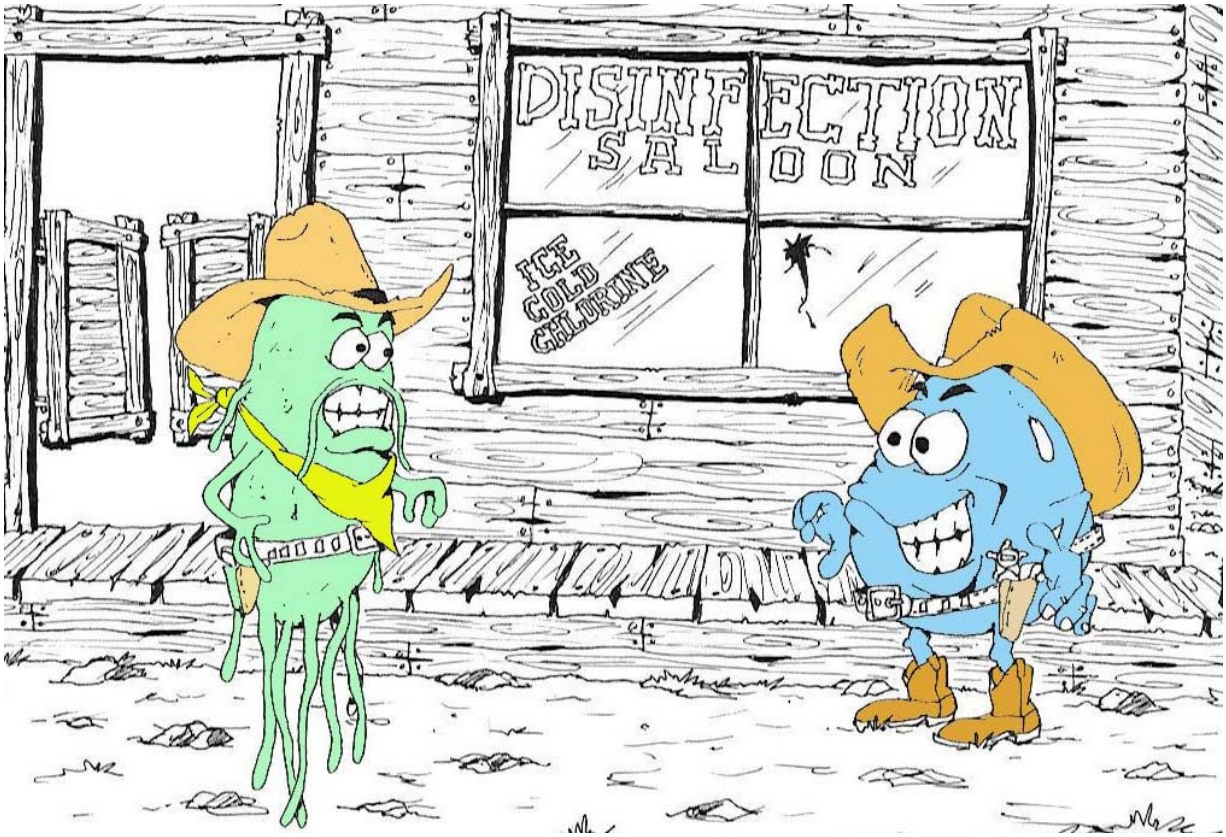
TABLE 4.1
Level of Giardia Reduction
Raw Water Giardia Levels*
Recommended Giardia Log Reduction
< 1 cyst/100 L 3-log
1 cyst/100 L - 10 cysts/100 L 3-log - 4-log
10 cysts/100 L - 100 cysts/100 L 4-log - 5-log
> 100 cysts/100 L > 5-log
*Use geometric means of data to determine raw water Giardia levels for compliance.

Required CT Value
Required CT values are dependent on pH, residual concentration, temperature, and the disinfectant used. The tables attached to Appendices A and B shall be used to determine the required CT.

Calculation and Reporting of CT Data
Disinfection CT values shall be calculated daily, using either the maximum hourly flow and the disinfectant residual at the same time, or by using the lowest CT value if it is calculated more frequently. Actual CT values are then compared to required CT values.

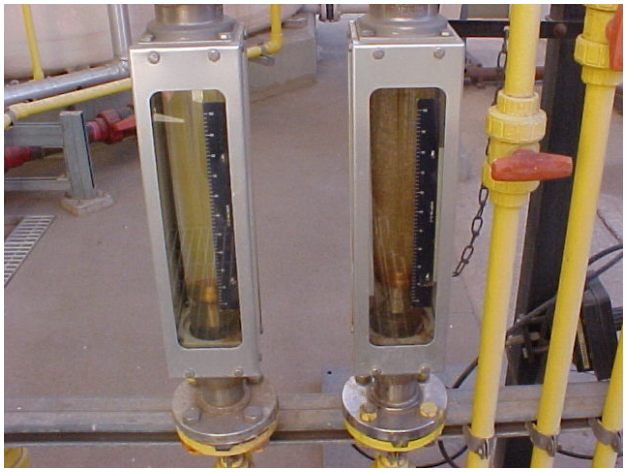
Results shall be reported as a reduction Ratio, along with the appropriate pH, temperature, and disinfectant residual. The reduction Ratio must be greater than 1.0 to be acceptable.

Users may also calculate and record actual log reductions.
Reduction Ratio = CT actual divide by CT required.



Chlorinator Components

A. **Ejector**
B. **Check Valve Assembly**
C. **Rate Valve**
D. **Diaphragm Assembly**
E. **Interconnection Manifold**
F. **Rotometer Tube and Float**
G. **Pressure Gauge**
H. **Gas Supply**



Chlorine measurement devices or Rotometers.



Chlorine Safety Information: There is a fusible plug on every chlorine tank. This metal plug will melt at 158 to 165° F. This is to prevent a build-up of excessive pressure and the possibility of cylinder rupture due to fire or high temperatures.

Chlorine Review

Chlorine Demand: The minimum amount of chlorine needed to react in a water purification system; used as a monitoring measurement by system operators.

Chlorine Residual: The concentration of chlorine in the water after the chlorine demand has been satisfied. The concentration is normally expressed in terms of total chlorine residual, which includes both the free and combined or chemically bound chlorine residuals.

Combined Chlorine Residual: The amount of chlorine used up in a water purification system; used as a monitoring measurement by system operators. Combined chlorine is defined as the residual chlorine existing in water in chemical combination with ammonia or organic amines which can be found in natural or polluted waters. Ammonia is sometimes deliberately added to chlorinated public water supplies to provide inorganic chloramines.

Free Chlorine: Free chlorine is defined as the concentration of residual chlorine in water present as dissolved gas (Cl_2), hypochlorous acid (HOCl), and/or hypochlorite ion (OCl-). The three forms of free chlorine exist together in equilibrium.

$$Cl_2 + H_2O \quad HOCl + H^+ + Cl^-$$

$$HOCl \quad H^+ + OCl^-$$

Their relative proportions are determined by the pH value and temperature. Regardless of whether pre-chloration is practiced or not, a free chlorine residual of at least 10 mg/L should be maintained in the clear well or distribution reservoir immediately downstream from the point of post-chlorination and .2 mg/L in the distribution system to guard against backflow.

Total Chlorine Residual: The total of free residual and combined residual chlorine in a water purification system; used as a monitoring measurement by system operators. Total chlorine is the sum of free and combined chlorine. When chlorinating most potable water supplies, total chlorine is essentially equal to free chlorine since the concentration of ammonia or organic nitrogen compounds (needed to form combined chlorine) will be very low. When chloramines are present in the municipal water supply, then total chlorine will be higher than free chlorine.

Pre-chlorination: The addition of chlorine at the plant headworks or prior to other water treatment or groundwater production processes and mainly used for disinfection and control of tastes, odors, and aquatic growths.

Post-chlorination: The addition of chlorine after a process or adding chlorine downstream to meet a demand in the system.

Breakpoint chlorination: Breakpoint chlorination means adding Cl_2 to the water until the Cl_2 demand is satisfied. Until all the microorganisms are killed.

What is the process of chlorination called as a treatment process and how does it differ from sterilization?
Chlorination: A method of water disinfection where gaseous, liquid, or dissolved chlorine is added to a water supply system. Water which has been treated with chlorine is effective in preventing the spread of disease. The chlorination of public drinking supplies was originally met with resistance, as people were concerned about the health effects of the practice. The use of chlorine has greatly reduced the prevalence of waterborne disease as it is effective against almost all bacteria and viruses, as well as amoeba. Sterilization kills everything.

What are the physical properties of chlorine, what hazards does it present, what advantages does it have over most other disinfectants, and how does it react with bacteria?
Physical and chemical properties of chlorine: A yellowish green, nonflammable and liquefied gas with an unpleasant and irritating smell. Can be readily compressed into a clear, amber-colored liquid, a noncombustible gas, and a strong oxidizer. Solid chlorine is about 1.5 times heavier than water and gaseous chlorine is about 2.5 times heavier than air. Atomic number of chlorine is 17. Cl is the elemental symbol and Cl2 is the chemical formula.

Chlorine reacts with bacteria as if it was very corrosive and burns the skin or covering killing the bacteria.

What is the purpose of a fusible plug, at what temperature does it melt, and where is it located on 150-lb. and 1-ton cylinders?
Fusible plug is a safety device that melts. If the temperature of a full Cl2 cylinder is increased by 50^{o} F or 30^{o} C, a rupture may occur. It will melt at 158 to 165 degrees F. It is found on the side of a 1 ton container and on top of the 150 pound cylinder and is located in the valve below the valve seat.

What is the correct procedure to follow in changing a chlorine cylinder and what item should always be replaced with a new one in doing so?
Hook up the chlorinator to the container or cylinder with the chlorine valve turned off. Use the gas side not the liquid if using a 1 ton container. Remove the cylinder valve outlet cap and check the valve face or damage. Clean with wire brush if necessary. If the valve face is smooth, clean proceed with hooking up the cylinder. Check the inlet face of the chlorinator and clean if necessary. Place a new lead gasket on the chlorinator inlet, place the chlorinator on the cylinder valve, install the yoke clamp and slowly tighten the yoke clamp until the two faces are against the lead gasket. Tighten the yoke, compressing the gasket one half to three quarters turn, do not over tighten. Replace the lead gasket with every change out.

How, when and where should chlorine residuals be taken and what information do they provide? The sample must be taken within the distribution system of your PWS. If you take it before the distribution system you will not get an accurate reading. The sample must be taken at the same tap that you take the Bac-t sample.

Chlor-Alkali Membrane Process

The electrolysis occurs in a cell containing electrodes submerged in solutions called electrolytes. One electrode is referred to as the anode and is submerged in a salt water solution. The second electrode is the cathode and is submerged in a sodium hydroxide (caustic soda) solution. A membrane is used to keep the two different solutions from mixing. This particular method of producing chlorine is called the chlor-alkali membrane process.

When a low voltage direct current (DC) power supply is applied to the electrodes in the cell, the sodium and chlorine ions in the brine are attracted in opposite directions to the polarized electrodes. The sodium ion passes across an ion selective membrane leaving the chlorine ion to combine with a second chlorine ion, which makes a chlorine gas bubble at the anode (electrode).

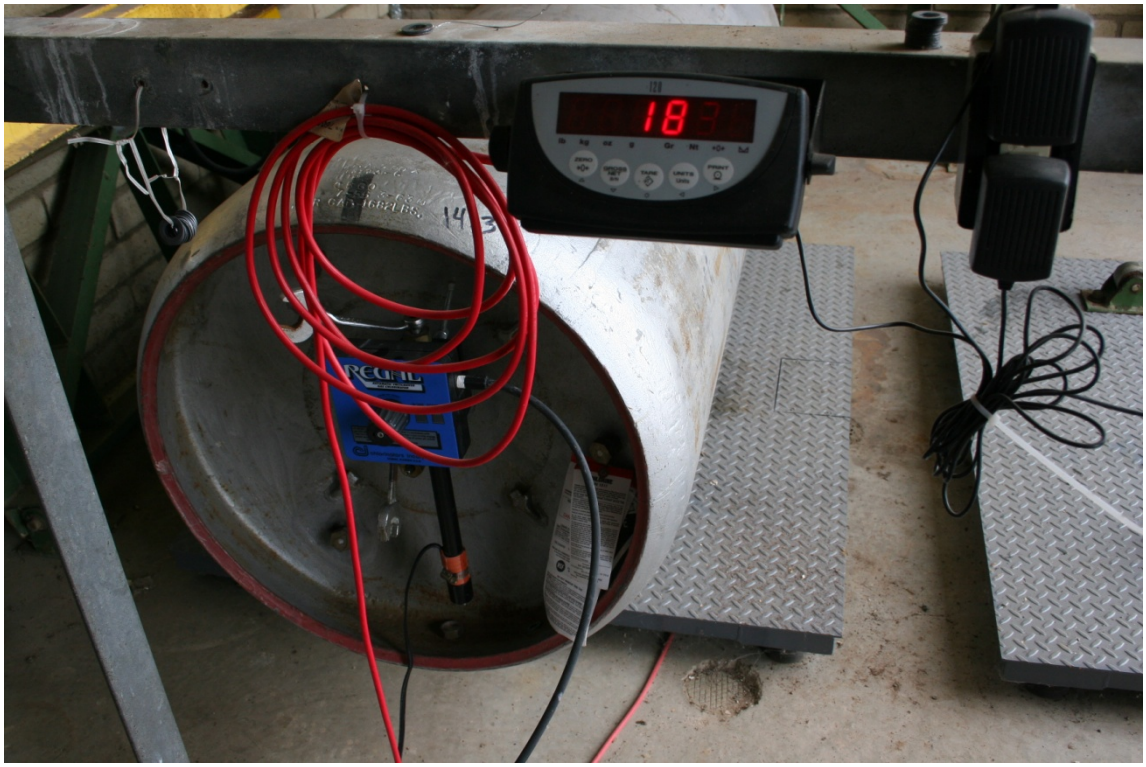
When the sodium crosses the membrane, it combines with a hydroxyl ion at the cathode (electrode) making sodium hydroxide, or caustic soda (NaOH). The hydroxyl ion originates from the dissolution of water at the cathode where hydrogen gas also develops. The membrane in the cell keeps the two solutions separate; otherwise, the chlorine gas bubble would immediately combine with the caustic soda forming sodium hypochlorite, or bleach. This process, which uses a membrane to separate the two solutions, is called the chlor-alkali process. The chemical equation for the chlor-alkali process is illustrated in the following equation:

2NaCl + 2H2O + electric current (2NaOH + Cl2- +- H2)





Top photograph, adjusting the Chlorine leak alarm sensor. Bottom photograph, Chlorine container weight scales.



Chlorine's Effectiveness

The effectiveness of chlorination depends on the chlorine demand of the water, the concentration of the chlorine solution added, the time that chlorine is in contact with the organism, and water quality. These effects can be summarized in the following manner:

- As the concentration of the chlorine increases, the required contact time to disinfect decreases.
- Chlorination is more effective as water temperature increases.
- Chlorination is less effective as the water's pH increases (becomes more alkaline).
- Chlorination is less effective in cloudy (turbid) water.
- When chlorine is added to the water supply, part of it combines with other chemicals in water (like iron, manganese, hydrogen sulfide, and ammonia) and is not available for disinfection. The amount of chlorine that reacts with the other chemicals plus the amount required to achieve disinfection is the **chlorine demand** of the water.



The safest way to be sure that the amount of chlorine added is sufficient is to add a little more than is required. This will result in a free chlorine residual that can be measured easily. This chlorine residual must be maintained for several minutes depending on chlorine level and water quality. Table 4 lists the free chlorine residual level needed for different contact times, water temperatures and pH levels.

Kits are available for measuring the chlorine residual by looking for a color change after the test chemical is added. The test is simple and easy for a homeowner to perform. If chlorination is required for the water supply, the chlorine residual should be tested regularly to make sure the system is working properly. The kit should specify that it measures the free chlorine residual and not the total chlorine. Once chlorine has combined with other chemicals it is not effective as a disinfectant. If a test kit does not distinguish between free chlorine and chlorine combined with other chemicals, the test may result in an overestimation of the chlorine residual.

Chlorine will kill bacteria in water, but it takes some time (Table 4) . The time needed depends on the concentration of chlorine. Two methods of chlorination are used to disinfect water: **simple chlorination** and **superchlorination**.

Table 4. Necessary chlorine residual to disinfect water for various contact times, water temperatures and pH			
Water Temp. 50 degrees F			
Contact time (minutes)	**Necessary chlorine residual (mg/l)**		
	pH 7	**pH 7.5**	**pH 8**
40	0.2	0.3	0.4
30	0.3	0.4	0.5
20	0.4	0.6	0.8
10	0.8	1.2	1.6
5	1.6	2.4	3.2
2	4.0	6.0	8.0
1	8.0	12.0	16.0
Water Temp. 32 - 40 degrees F			
Contact time (minutes)	**Necessary chlorine residual (mg/l)**		
	pH 7	**pH 7.5**	**pH 8**
40	0.3	0.5	0.6
30	0.4	0.6	0.8
20	0.6	0.9	1.2
10	1.2	1.8	2.4
5	2.4	3.6	4.8
2	6.0	9.0	12.0
1	12.0	18.0	24.0

Example: ***What is the necessary chlorine residual for well water with pH 7.5?***

The well water is 38 degrees F when it enters the house. The pump delivers 7 gallons per minute and after the chlorine is added it is held in a 100 gallon holding tank.

1. Contact time (from Table 5) - gallons per minute for 50 gallon tank = 5 minutes
2. Multiply by 2 for a 100 gallon tank = 10 minutes.
3. Necessary chlorine residual (from Table 4)- for water at 38 degrees F and pH 7.5 = **1.8 mg/l.**

Simple chlorination involves maintaining a low level of free residual chlorine at a concentration between 0.30 to .5 mg/l for at least 30 minutes. The residual is measured at the faucet most distant from the where chlorine is added to the water supply.

To ensure the proper contact time of at least 30 minutes, a holding tank can be installed (Table 5). Pressure tanks, while often thought to be sufficient, are usually too small to always provide 30 minutes of contact time.

Table 5. Available contact time from a 50-gallon holding tank	
Water flow rate (gallons per minute)	**Holding time (minutes)**
5	7
7	5
10	3.5

Another way to maintain necessary contact time is to run the chlorinated water through a coil of pipe (Table 6).

Table 6. Available contact time from 1000 feet of 1-1/4 inch pipe	
Water flow rate (gallons per minute)	**Holding time (minutes)**
5	9.2
7	6.6
10	4.6

When the water cannot be held for at least 30 minutes before it is used, super chlorination is an alternative. For **superchlorination**, a chlorine solution is added to the water to produce a chlorine residual of between 3.0 and 5.0 mg/l, which is about ten times stronger than for simple chlorination.

The necessary contact time for this concentration is reduced to less than five minutes (Table 4). The water will have a very strong chlorine smell. If this is not desirable, the chlorine can be removed just before it is used with a carbon filter (Note: may not be currently allowed under your Department of Health for private water supplies).

Oxidation Chemistry

Oxidation chemistry has long been an accepted and effective part of many water treatment programs. Oxidizing chemicals used in today's water treatment programs include: chlorine, chlorine dioxide, bromine, bromine/chlorine releasing compounds, ozone and hydrogen peroxide.

Oxidizing microbiocides are often found at the forefront of many cooling water treatment programs. In large volume or once-through cooling systems they are usually the primary biocide and often are the most cost-effective programs available to a plant. When selecting these economical and versatile chemicals, several factors should be considered before a technically sound program is implemented. Environmental and regulatory impact, system pH, process contamination, and equipment capital and maintenance expense all play a role in the decision-making process.

The primary killing mechanism these types of microbiocides use is oxidizing protein groups within a microorganism. Proteins are the basic components of essential cellular enzymes that are necessary for life-sustaining cellular processes such as respiration. The destruction of these proteins deprives the cell of its ability to carry out fundamental life functions and quickly kills it. One oxidant is chlorine dioxide, which appears to provide an additional killing mechanism. Chlorine dioxide is able to diffuse readily through hydrophobic lipid layers of an organism, allowing it to react with cellular amino acids, which directly inhibits protein synthesis. Since amino acids are the basic building blocks of all cellular proteins, destruction of these molecules has a devastating effect on the microorganism.



Staff shall be familiar with the locations of the chemical feed building as indicated by a posted site plan. Self-contained breathing apparatus (SCBA) and personal protective equipment should be facing the chemical feed building. Emergency repair kits “B” and “C” should be stored on site close to the chemical feed building.



Chlorine scrubber

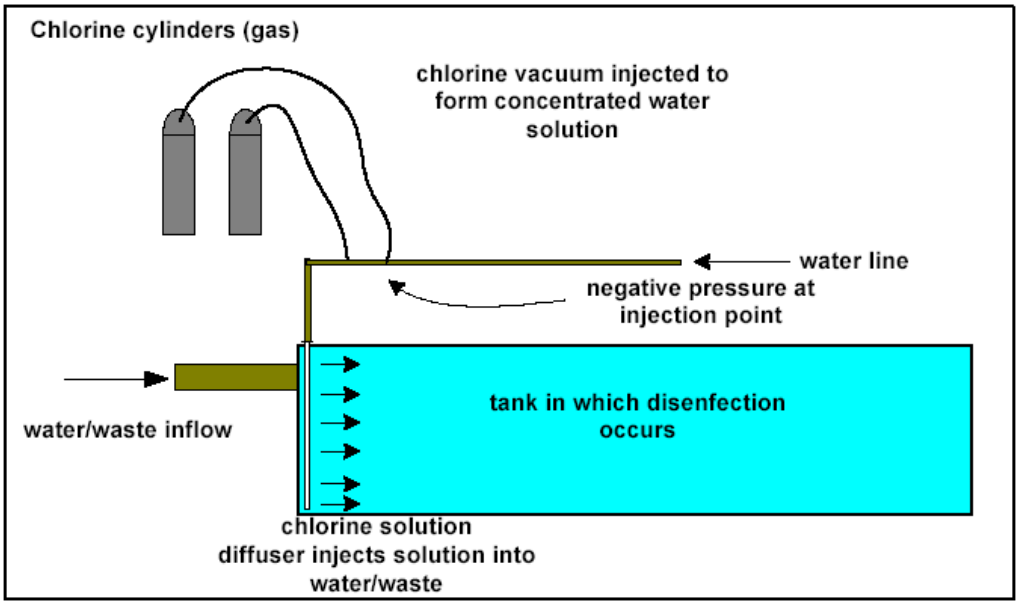
Chlorine Gas Section

Chlorine Gas



Background: Chlorine gas is a pulmonary irritant with intermediate water solubility that causes acute damage in the upper and lower respiratory tract. Chlorine gas was first used as a chemical weapon at Ypres, France in 1915. Of the 70,552 American soldiers poisoned with various gasses in World War I, 1843 were exposed to chlorine gas. Approximately 10.5 million tons and over 1 million containers of chlorine are shipped in the U.S. each year.

Chlorine is the most common chemical used to disenfect water and wastewater. It is added as a gas to most large installations. At very small facilities sodium hypochlorite (bleach) is added as a powder (tablets).



Chlorine is a yellowish-green gas at standard temperature and pressure. It is extremely reactive with most elements. Because its density is greater than that of air, the gas settles low to the ground. It is a respiratory irritant, and it burns the skin. Just a few breaths of it are fatal. Cl2 gas does not occur naturally, although Chlorine can be found in a number of compounds.

Chlorine gas is likely the most widely used oxidizing microbiocide. It has traditionally been the biocide of choice in many cooling water treatment systems. It is a strong oxidizer that is relatively easy to feed and is quite inexpensive. Upon introduction into the water stream, chlorine hydrolyzes into hypochlorous acid (HOCl) and hydrochloric acid (HCl).

This hydrolization provides the active toxicant, HOCl, which is pH-dependent. In alkaline cooling systems, it readily dissociates to form the hypochlorite ion (OCl-). This dissociation phenomenon is important to remember when working with systems that will operate at a higher pH. In alkaline conditions, OCl- becomes the predominant species and lacks the biocidal efficacy of the non-dissociated form. Considerably more HOCl is present at a pH of 7.0 than at pH 8.5.

It is also widely known that chlorine is non-selective, making it very sensitive to contamination from either cooling water makeup or from in-plant process leaks. Ammonia, organic acids and organic compounds, sulfides, iron and manganese all easily react with HOCl. The amount of chlorine needed to react with these contamination species is referred to as chlorine demand and it must be satisfied before active HOCl is available to provide a free chlorine residual.

The combination of high chlorine demand in process-contaminated systems and the dissociation process in alkaline systems creates the need for greater chlorine feed to obtain the same microbial efficacy. This results in a higher concentration of HCl in the cooling system. Since HCl removes alkalinity, pH depression and system corrosion could occur. In low pH water the passive metal oxide layers protecting the metal may resolubulize, exposing the surface to corrosion. At free mineral acidity (pH <4.3), many passivating inhibitors become ineffective, and corrosion will proceed rapidly. Increased chloride may also have a negative impact on system corrosion. The chloride ion (Cl^-) can damage or penetrate the passive oxide layer, leading to localized damage of the metal surface.

High chlorine concentrations have also been shown to directly attack traditional organic-based corrosion inhibitors. When these inhibitors are "deactivated," the metal surface would then be susceptible to corrosion. Process Safety Management (PSM) guidelines dictated by the U.S. Occupational Safety and Health Administration (OSHA), discharge problems related to chlorinated organic compounds such as trihalomethane (THM), dezincification of admiralty brass and delignification of cooling tower wood are other significant concerns associated with the use of chlorine.

Pathophysiology
Chlorine is a greenish-yellow, noncombustible gas at room temperature and atmospheric pressure. The intermediate water solubility of chlorine accounts for its effect on the upper airway and the lower respiratory tract.

Exposure to chlorine gas may be prolonged because its moderate water solubility may not cause upper airway symptoms for several minutes. In addition, the density of the gas is greater than that of air, causing it to remain near ground level and increasing exposure time.



The odor threshold for chlorine is approximately 0.3-0.5 parts per million (ppm); however, distinguishing toxic air levels from permissible air levels may be difficult until irritative symptoms are present.

Mechanism of Activity
The mechanisms of the above biological activity are poorly understood and the predominant anatomic site of injury may vary, depending on the chemical species produced. Cellular injury is believed to result from the oxidation of functional groups in cell components, from reactions with tissue water to form hypochlorous and hydrochloric acid, and from the generation of free oxygen radicals.

Although the idea that chlorine causes direct tissue damage by generating free oxygen radicals was once accepted, this idea is now controversial. The cylinders on the right contain chlorine gas.

The gas comes out of the cylinder through a gas regulator. The cylinders are on a scale that operators use to measure the amount used each day. The chains are used to prevent the tanks from falling over. Chlorine gas is stored in vented rooms that have panic bar equipped doors. Operators have the equipment necessary to reduce the impact of a gas leak, but rely on trained emergency response teams to contain leaks.

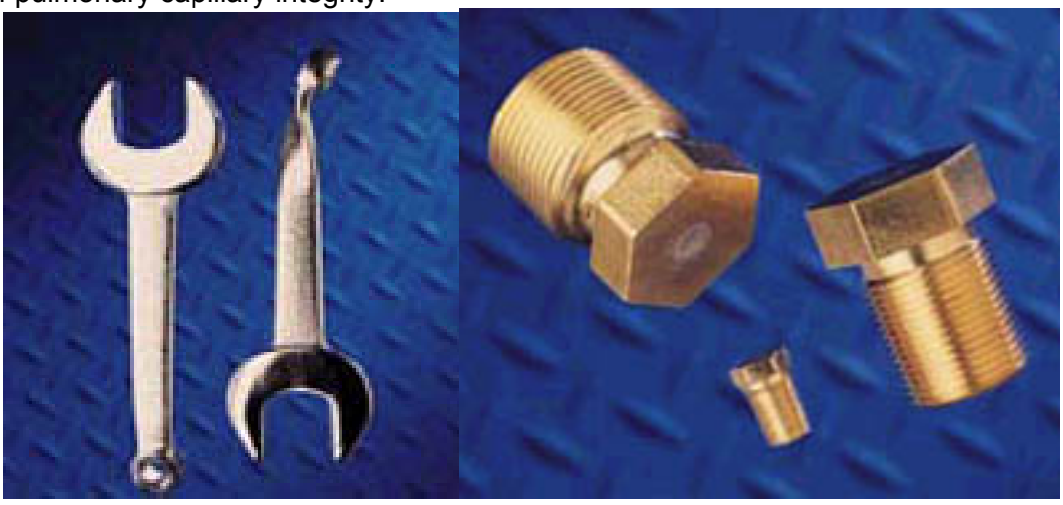
Solubility Effects
Hydrochloric acid is highly soluble in water. The predominant targets of the acid are the epithelia of the ocular conjunctivae and upper respiratory mucus membranes. Hypochlorous acid is also highly water soluble with an injury pattern similar to hydrochloric acid. Hypochlorous acid may account for the toxicity of elemental chlorine and hydrochloric acid to the human body.

Early Response to Chlorine Gas
Chlorine gas, when mixed with ammonia, reacts to form chloramine gas. In the presence of water, chloramines decompose to ammonia and hypochlorous acid or hydrochloric acid. The early response to chlorine exposure depends on the (1) concentration of chlorine gas, (2) duration of exposure, (3) water content of the tissues exposed, and (4) individual susceptibility.

Immediate Effects
The immediate effects of chlorine gas toxicity include acute inflammation of the conjunctivae, nose, pharynx, larynx, trachea, and bronchi. Irritation of the airway mucosa leads to local edema secondary to active arterial and capillary hyperemia. Plasma exudation results in filling the alveoli with edema fluid, resulting in pulmonary congestion.

Pathological Findings
Pathologic findings are nonspecific. They include severe pulmonary edema, pneumonia, hyaline membrane formation, multiple pulmonary thromboses, and ulcerative tracheobronchitis.

The hallmark of pulmonary injury associated with chlorine toxicity is pulmonary edema, manifested as hypoxia. Noncardiogenic pulmonary edema is thought to occur when there is a loss of pulmonary capillary integrity.



Chlorine Gas Cylinder Wrenches and Fusible Plugs.



Simple DPD Chlorine Field Test Kit.



Using DPD Method for Chlorine Residuals

N, N – diethyl-p-phenylenediame



Small portable chlorine measuring kit. The redder the mixture the "hotter" or stronger the chlorine in solution.

Measuring Chlorine Residual

Chlorine residual is the amount of chlorine remaining in water that can be used for disinfection. A convenient, simple and inexpensive way to measure chlorine residual is to use a small portable kit with pre-measured packets of chemicals that are added to water.

(Make sure you buy a test kit using the ***DPD method***, and not the outdated orthotolodine method.)

Chlorine test kits are very useful in adjusting the chlorine dose you apply. You can measure what chlorine levels are being found in your system (especially at the far ends).

Free chlorine residuals need to be checked and recorded daily. These results should be kept on file for a health or regulatory agency inspection during a regular field visit.

The most accurate method for determining chlorine residuals to use the laboratory amperometric titration method.

Amperometric Titration

The chlorination of water supplies and polluted waters serves primarily to destroy or deactivate disease-producing microorganisms. A secondary benefit, particularly in treating drinking water, is the overall improvement in water quality resulting from the reaction of chlorine with ammonia, iron, manganese, sulfide, and some organic substances.

Chlorination may produce adverse effects. Taste and odor characteristics of phenols and other organic compounds present in a water supply may be intensified. Potentially carcinogenic chloro-organic compounds such as chloroform may be formed.

Combined chlorine formed on chlorination of ammonia- or amine-bearing waters adversely affects some aquatic life. To fulfill the primary purpose of chlorination and to minimize any adverse effects, it is essential that proper testing procedures be used with a foreknowledge of the limitations of the analytical determination.

Chlorine applied to water in its molecular or hypochlorite form initially undergoes hydrolysis to form free chlorine consisting of aqueous molecular chlorine, hypochlorous acid, and hypochlorite ion. The relative proportion of these free chlorine forms is pH- and temperature-dependent. At the pH of most waters, hypochlorous acid and hypochlorite ion will predominate.



Free chlorine reacts readily with ammonia and certain nitrogenous compounds to form combined chlorine. With ammonia, chlorine reacts to form the chloramines: monochloramine, dichloramine, and nitrogen trichloride.

The presence and concentrations of these combined forms depend chiefly on pH, temperature, initial chlorine-to-nitrogen ratio, absolute chlorine demand, and reaction time. Both free and combined chlorine may be present simultaneously. Combined chlorine in water supplies may be formed in the treatment of raw waters containing ammonia or by the addition of ammonia or ammonium salts.

Chlorinated wastewater effluents, as well as certain chlorinated industrial effluents, normally contain only combined chlorine. Historically the principal analytical problem has been to distinguish between free and combined forms of chlorine.

Hach's AutoCAT 9000™ Automatic Titrator is the newest solution to hit the disinfection industry – a comprehensive, benchtop chlorine-measurement system that does it all: calibration, titration, calculation, real-time graphs, graphic print output, even electrode cleaning. More a laboratory assistant than an instrument, the AutoCAT 9000 gives you:

- High throughput, performs the titration and calculates concentration, all automatically.
- Forward titration, USEPA-accepted methods for free and total chlorine and chlorine dioxide with chlorite.
- Back titration, USEPA-accepted method for total chlorine in wastewater.
- Accurate, yet convenient: the easiest way to complete ppb-level amperometric titration.

Sodium Hypochlorite Section

Physical Properties - Sodium Hypochlorite
Description: Clear greenish yellow liquid.
Warning properties: Chlorine odor; inadequate warning of hazardous concentrations.
Molecular weight: 74.44 daltons
Boiling point (760 mm Hg): Decomposes above 40ºC (HSDB 2001)
Freezing point: 6ºC (21ºF)
Specific gravity: 1.21 (14% NAOCl solution) (water=1)
Water solubility: 29.3 g/100 g at 32ºF (0ºC)
Flammability: Not flammable

Alternative Names
Bleach; Clorox; Carrel-Dakin solution

Incompatibilities
Calcium or sodium hypochlorite react explosively or form explosive compounds with many common substances such as ammonia, amines, charcoal, or organic sulfides

Introduction
The world's most universal and reliable means of water and wastewater disinfection is chlorination. Two fundamental methods include gas chlorination (Cl2) and liquid chlorination (NaOCl) otherwise known as Sodium Hypochlorite. Sodium hypochlorite (NaOCl) is a solution made from reacting chlorine with a sodium hydroxide solution. These two reactants are the major co-products from most chlor-alkali cells. Sodium hypochlorite has a variety of uses and is an excellent disinfectant/antimicrobial agent. Sodium hypochlorite also significantly increases the pH of the water. When sodium hypochlorite is used, it must be counterbalanced by a strong acid like sodium bisulfate or muriatic acid to keep the pH within the ideal range.

The hypochlorite form of chlorine has been used since 1850. The most widely used form of hypochlorite is the liquid, sodium hypochlorite (NaOCl), with more than 150 tons per day consumed in the United States. Sodium hypochlorite application in cooling water is essentially the same as with gas chlorine; HOCl is produced as the active toxicant. The HOCl is equally susceptible to process contamination, has the same chlorine demand as gas chlorine and displays the same tendency to dissociate.

Sodium hypochlorite differs from chlorine gas in two respects: method of feed and hydrolization properties. Sodium hypochlorite can either be gravity-fed or applied with a metering pump. The latter is generally recognized as a consistently more accurate method. The second difference, in hydrolysis, lies in the end products. The NaOCl reaction with water liberates sodium hydroxide (NaOH).

The addition of NaOH differs in that it tends to add alkalinity to the water. In large concentrations it may artificially elevate pH, leading to precipitation of calcium carbonate. While NaOCl eliminates low pH corrosion as a concern, the use of large quantities in contaminated systems still introduces a high concentration of the chloride ion, which can be very aggressive to cooling system metals. Many of the other problems associated with chlorine remain present with sodium hypochlorite.

When was Sodium Hypochlorite Discovered?
Sodium hypochlorite has a long history. Around 1785 the Frenchman Berthollet developed liquid bleaching agents based on sodium hypochlorite. The Javel company introduced this product and called it 'liqueur de Javel'. At first, it was used to bleach cotton. Because of its specific characteristics it soon became a popular compound. Hypochlorite can remove stains from clothes at room temperature. In France, sodium hypochlorite is still known as 'eau de Javel'.

Characteristics of Sodium hypochlorite
Sodium hypochlorite is a clear, slightly yellowish solution with a characteristic odor.

Sodium hypochlorite has a relative density of is 1.1 (5.5% watery solution).

As a bleaching agent for domestic use it usually contains 5% sodium hypochlorite (with a pH of around 11, it is irritating). If it is more concentrated, it contains a concentration 10-15% sodium hypochlorite (with a pH of around 13, it burns and is corrosive).

Sodium hypochlorite is unstable. Chlorine evaporates at a rate of 0,75 gram active chlorine per day from the solution. Then heated sodium hypochlorite disintegrates. This also happens when sodium hypochlorite comes in contact with acids, sunlight, certain metals and poisonous and corrosive gasses, including chlorine gas. Sodium hypochlorite is a strong oxidator and reacts with flammable compounds and reductors. Sodium hypochlorite solution is a weak base that is inflammable. These characteristics must be kept in mind during transport, storage and use of sodium hypochlorite.

pH value When Sodium Hypochlorite is Added to Water
Due to the presence of caustic soda in sodium hypochlorite, the pH of the water is increased. When sodium hypochlorite dissolves in water, two substances form, which play a role in oxidation and disinfection. These are hypochlorous acid (HOCl) and the less active hypochlorite ion (OCl^-). The pH of the water determines how much hypochlorous acid is formed. While sodium hypochlorite is used, hydrochloric (HCl) is used to lower the pH. Sulfuric acid (H_2SO_4) can be used as an alternative for acetic acid. Less harmful gasses are produced when sulfuric acid is used. Sulfuric acid is a strong acid that strongly reacts with bases and is very corrosive.

How Can Sodium Hypochlorite be produced?
Sodium hypochlorite can be produced in two ways:
- By dissolving salt in softened water, which results in a concentrated brine solution. The solution is electrolyzed and forms a sodium hypochlorite solution in water. This solution contains 150 g active chlorine (Cl_2) per liter. During this reaction the explosive hydrogen gas is also formed.
- By adding chlorine gas (Cl_2) to caustic soda (NaOH). When this is done, sodium hypochlorite, water (H_2O) and salt (NaCl) are produced according to the following reaction:
$Cl_2 + 2NaOH \rightarrow NaOCl + NaCl + H_2O$

Applications of Sodium Hypochlorite
Sodium hypochlorite is used on a large scale; for example agriculture, chemical industries, paint- and lime industries, food industries, glass industries, paper industries, pharmaceutical industries, synthetics industries and waste disposal industries all use it. In the textile industry sodium hypochlorite is used to bleach textile. It is sometimes added to industrial waste water--this is done to reduce odors.

Hypochlorite neutralizes sulphur hydrogen gas (SH) and ammonia (NH3). It is also used to detoxify cyanide baths in metal industries. Hypochlorite can be used to prevent algae and shellfish growth in cooling towers. In water treatment, hypochlorite is used to disinfect water. In households, hypochlorite is used frequently for the purification and disinfection of the house.

How does Sodium Hypochlorite Disinfection Work?
By adding hypochlorite to water, hypochlorous acid (HOCl) is formed:
$NaOCl + H_2O \rightarrow HOCl + NaOH$

Hypochlorous acid is divided into hydrochloric acid (HCl) and oxygen (O). The oxygen atom is a very strong oxidant.

Sodium hypochlorite is effective against bacteria, viruses and fungi. Sodium hypochlorite disinfects the same way as chlorine does.

There are various ways to use sodium hypochlorite. For on-site salt electrolysis, a solution of salt (NaCl) in water is applied. Sodium (Na^+) and chloride (Cl^-) ions are produced.
$4NaCl \rightarrow 4Na^+ + 4Cl^-$

Subsequently, chlorine and hydroxide react to form hypochlorite:
$OH^- + Cl_2 \rightarrow HOCl + Cl^-$

You can work these problems in this section.

Salt Electrolysis System
The advantage of the salt electrolysis system is that no transport or storage of sodium hypochlorite is required. When sodium hypochlorite is stored for a long time, it becomes inactive. Another advantage of the onsite process is that chlorine lowers the pH and no other acid is required to lower pH.

The hydrogen gas that is produced is explosive and as a result ventilation is required for explosion prevention. This system is slow and a buffer of extra hypochlorous acid needs to be used. The maintenance and purchase of the electrolysis system is much more expensive than sodium hypochlorite.

When sodium hypochlorite is used, acetic or sulfuric acid are added to the water. An overdose can produce poisonous gasses. If the dosage is too low, the pH becomes too high and can irritate the eyes.

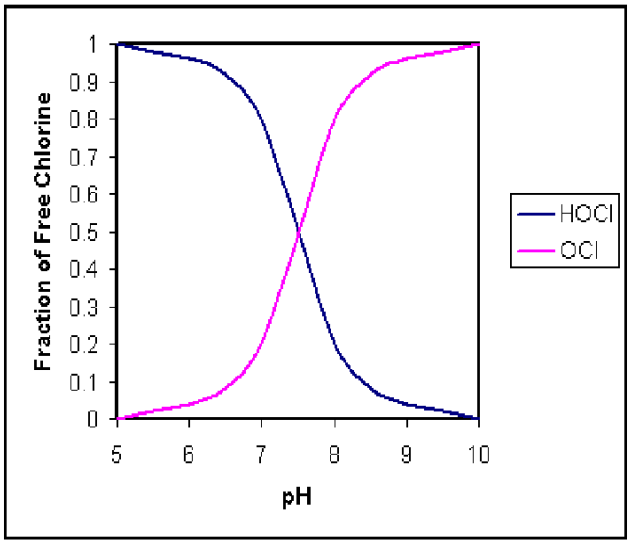
Because sodium hypochlorite is used both to oxidize pollutants (urine, sweat, cosmetics) and to remove pathogenic microorganisms, the required concentration of sodium hypochlorite depends on the concentrations of these pollutions. Especially the amount of organic pollutants helps determine the required concentration. If the water is filtered before sodium hypochlorite is applied, less sodium hypochlorite is needed.

Theory

Disinfection with chlorine is very popular in water and wastewater treatment because of its low cost, ability to form a residual, and its effectiveness at low concentrations. Although it is used as a disinfectant, it is a dangerous and potentially fatal chemical if used improperly.

Despite the fact the disinfection process may seem simple, it is actually a quite complicated process. Chlorination in wastewater treatment systems is a fairly complex science which requires knowledge of the plant's effluent characteristics.

When free chlorine is added to the wastewater, it takes on various forms depending on the pH of the wastewater. It is important to understand the forms of chlorine which are present because each has a different disinfecting capability. The acid form, HOCL, is a much stronger disinfectant than the hypochlorite ion, OCL-. The graph below depicts the chlorine fractions at different pH values (Drawing by Erik Johnston).



Ammonia present in the effluent can also cause problems as chloramines are formed, which have very little disinfecting power. Some methods to overcome the types of chlorine formed are to adjust the pH of the wastewater prior to chlorination or to simply add a larger amount of chlorine. An adjustment in the pH would allow the operators to form the most desired form of chlorine, hypochlorus acid, which has the greatest disinfecting power.

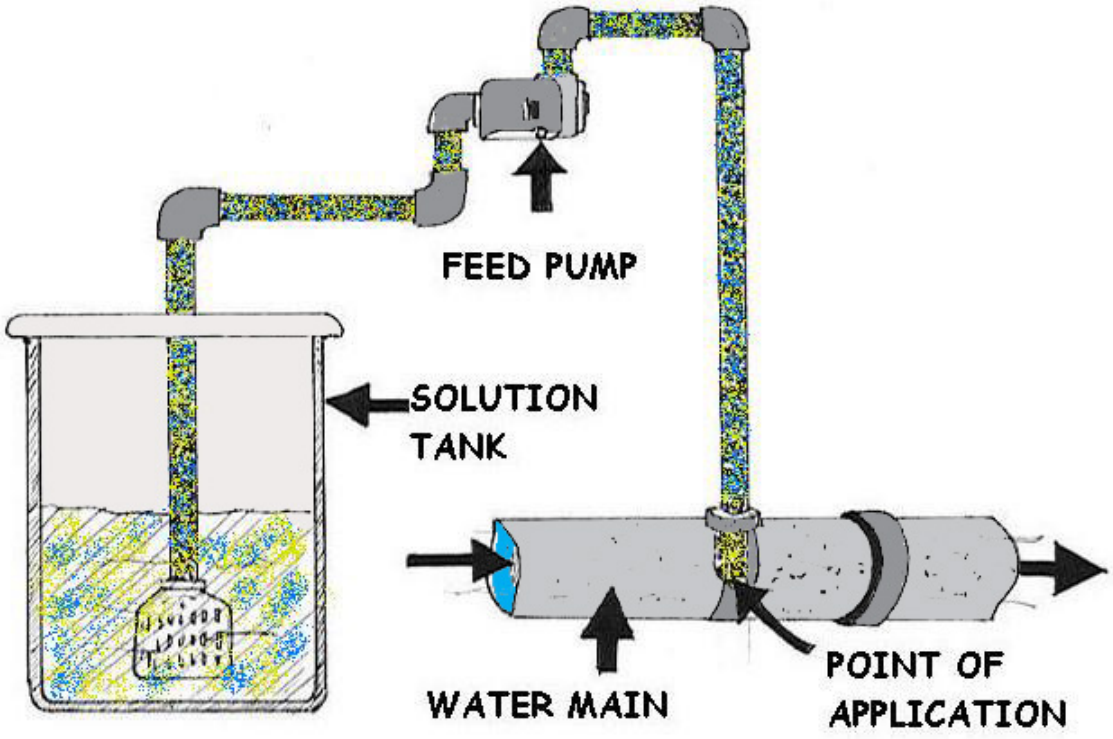
Adding larger amounts of chlorine would be an excellent method to combat the chloramines because the ammonia present would bond to the chlorine but further addition of chlorine would stay in the hypochlorus acid or hypochlorite ion state.

a) Chlorine gas, when exposed to water reacts readily to form hypochlorus acid, HOCl, and hydrochloric acid. Cl2 + H_2O -> HOCl + HCl

b) If the pH of the wastewater is greater than 8, the hypochlorus acid will dissociate to yield hypochlorite ion. HOCl <-> H^+ + OCl^-- If however, the pH is much less than 7, and then HOCl will not dissociate.

c) If ammonia is present in the wastewater effluent, then the hypochlorus acid will react to form one three types of chloramines depending on the pH, temperature, and reaction time.

Recommendations for Preparing/Handling/Feeding Sodium Hypochlorite Solutions



As a result of the pressures brought to bear by Health and Safety requirements, some users of gas have chosen to seek alternative forms of disinfectants for their water and wastewater treatment plants. One of these alternative forms is sodium hypochlorite (**NaOCl**)). This is often purchased commercially at 10 to 15% strength.

The handling and storage of NaOCl presents the plant with a new and sometimes unfamiliar, set of equipment installation configurations and operating conditions.

Product Stability The oxidizing nature of this substance means that it should be handled with extreme care. As NaOCl is relatively unstable, it degrades over time.

There are Three Ways in Which NaOCl Solutions Degrade

- Chlorate-forming reaction due to age, temperature, light and minor reduction in pH.
- Oxygen-producing reaction that occurs when metals, such as iron, copper or nickel, or metal oxides are brought into contact with the solution.
- Chlorine-producing reaction when solution pH falls below 6.

There are Many Factors that Affect the Stability of a NaOCl Solution

- Initial solution strength.
- pH solution.
- Temperature of the solution.
- Exposure of the solution to sunlight.

Exposure

There is no threshold value for to sodium hypochlorite exposure. Various health effects occur after exposure to sodium hypochlorite. People are exposed to sodium hypochlorite by inhalation of aerosols. This causes coughing and a sore throat. After swallowing sodium hypochlorite the effects are stomach ache, a burning sensation, coughing, diarrhea, a sore throat and vomiting. Sodium hypochlorite on skin or eyes causes redness and pain. After prolonged exposure, the skin can become sensitive. Sodium hypochlorite is poisonous for water organisms. It is mutagenic and very toxic when it comes in contact with ammonium salts.

Routes of Exposure
Inhalation
Hypochlorite solutions can liberate toxic gases such as chlorine. Chlorine's odor or irritant properties generally provide adequate warning of hazardous concentrations. However, prolonged, low-level exposures, such as those that occur in the workplace, can lead to olfactory fatigue and tolerance of chlorine's irritant effects. Chlorine is heavier than air and may cause asphyxiation in poorly ventilated, enclosed, or low-lying areas.

Children exposed to the same levels of gases as adults may receive a larger dose because they have greater lung surface area/body weight ratios and higher minute volumes/weight ratios. Children may be more vulnerable to corrosive agents than adults because of the smaller diameter of their airways. In addition, they may be exposed to higher levels than adults in the same location because of their short stature and the higher levels of chlorine found nearer to the ground.

Skin/Eye Contact
Direct contact with hypochlorite solutions, powder, or concentrated vapor causes severe chemical burns, leading to cell death and ulceration. Because of their relatively larger surface area/weight ratio, children are more vulnerable to toxicants affecting the skin.

Ingestion
Ingestion of hypochlorite solutions causes vomiting and corrosive injury to the gastrointestinal tract. Household bleaches (3 to 6% sodium hypochlorite) usually cause esophageal irritation, but rarely cause strictures or serious injury such as perforation. Commercial bleaches may contain higher concentrations of sodium hypochlorite and are more likely to cause serious injury. Metabolic acidosis is rare, but has been reported following the ingestion of household bleach. Pulmonary complications resulting from aspiration may also be seen after ingestion.

Sources/Uses
Sodium and calcium hypochlorite are manufactured by the chlorination of sodium hydroxide or lime. Sodium and calcium hypochlorite are used primarily as oxidizing and bleaching agents or disinfectants. They are components of commercial bleaches, cleaning solutions, and disinfectants for drinking water and waste water purification systems and swimming pools.

Sodium Hypochlorite as a Disinfectant has the Following Advantages:
It can be easily stored and transported when it is produced on-site. Dosage is simple; transport and storage of sodium hypochlorite are safe. Sodium hypochlorite is as effective as chlorine gas for disinfection. Sodium hypochlorite produces residual disinfectant.

Disadvantages
Sodium hypochlorite is a dangerous and corrosive substance. While working with sodium hypochlorite, safety measures have to be taken to protect workers and the environment.

Sodium hypochlorite should not come in contact with air, because that will cause it to disintegrate. Both sodium hypochlorite and chlorine do not deactivate Giardia Lambia and Cryptosporidium. The regulation for sodium hypochlorite is the same as the regulation considering chlorine.

Household bleaches usually contain sodium hypochlorite in a 3% to 6% solution. Some sodium hydroxide (lye) is added to keep the pH high to avoid decomposition. If the solution is made more acidic, sodium hypochlorite will dissociate, producing chlorine gas and oxygen. It is made by bubbling chlorine gas through a solution of sodium hydroxide. In the environment, it breaks down into water, oxygen, and table salt.

Conditions that tend to increase gassing in Sodium Hypochlorite Solutions are:
* Elevated temperatures
* High concentration solution
* Exposure to sunlight or UV rays
* Reduction in pressure
* Cavitation
* Poor piping conditions
* Contact with metallic impurities
* Contact with organic impurities
* Age of solution
* Quality of solution

Reciprocating Piston Metering Pumps
When handling sodium hypochlorite and acids, be certain to wear gloves and a face shield for protection. Sodium hypochlorite is introduced to treated water by a chemical feeder (pump.) Chemical feeders tend to clog often, so it's important to clean the feeder regularly. Sodium Hypochlorite is subject to degradation within the piping and pump systems as it releases oxygen gas and results in crystallization of the residual. If the oxygen gas or vapor is allowed to build up within the piping and reagent head in sufficient volume, a typical reciprocating piston metering pump, used for accurately feeding chlorine to the process, will not function properly as gas in the pump head is compressed, minimizing the discharge check valve to open upon discharge stroke of the pump. Consequently, this effect could require that the pump be re-primed for operation. Reciprocating piston metering pumps or diaphragm metering pumps have been historically preferred in the dispensing of Sodium Hypochlorite because of their superior ability to accurately dose chemicals into a process stream with great precision and repeatability at a constant pressure. Additionally, the diaphragm metering pump is sealless and leak proof by design with negligible maintenance and simple commissioning.

Traditionally, the diaphragm metering pump industry has promoted the use of degas valves on the discharge port of the pump which diverts gas back to the suction supply source of the bleach. This method has been widely accepted and successful in many applications. However, the small diameter ports in the valve system tend to plug and require continuous flushing or cleaning through human intervention since the system is open to atmosphere on the discharge side of the orifice. Additionally, an external bypass piping system and degas valve assembly require additional costs and maintenance while presenting more opportunities for undesired chlorine leak paths.



A ten minute emergency air escape pack. We suggest that any one that utilizes chlorine gas purchase several of these type devices and station these around the facility and in your work vehicle.

Troubleshooting Hypochlorination Problems

Problem
1. Chemical feed pump won't run.
2. Low chlorine residual at POE.
2. Low chlorine residual at POE.
3. Chemical feed pump won't prime.
4. Loss of prime

Possible Causes
1A. No power.
1B. Electrical problem with signal from well pump or flow sensor.
1C. Motor failure.
2A. Improper procedure for running chlorine residual test or expired chemical reagents.
2B. Pump not feeding an adequate quantity of chlorine.
2C. Change in raw water quality.
2D. Pump **air bound**.
2E. Chlorine supply tank empty.
2F. Reduced effectiveness of chlorine solution.
2G. Damaged suction or discharge lines. (cracks or crimps)
2H. Connection at point of **injection** clogged or leaking.
3A. Speed and stroke setting inadequate.
3B. Suction lift too high due to feed pump relocation.
3C. Discharge pressure too high.
3D. Suction fitting clogged.
3E. Trapped air in suction line.
3F. Suction line not submerged in solution.
4A. Solution tank empty.
4B. Air leaks in suction fittings.
4C. Foot valve not in vertical position.
4D. Air trapped in suction tubing.

Possible Solutions
1A. Check to see if plug is securely in place.
Insure that there is power to the outlet and control systems.
1B. Check pump motor starter. Bypass flow sensor to determine if pump will operate manually.
1C. Check manufacturer's information.
2A Check expiration date on **chemical reagents**. Check test procedure as described in test kit manual. Speed or stroke setting too low.
2B. Damaged **diaphragm** or suction leak.
2C. Test raw water for constituents that may cause increased chlorine demand. (i.e. iron, manganese, etc.)
2D. Check foot valve.
2E. Fill supply tank.
2F. Check date that chlorine was received. Sodium hypochlorite solution may lose effectiveness after 30 days. If that is the case, the feed rate must be increased to obtain the desired residual.
2G. Clean or repair lines with problems.

2H. Flush line and connection with mild acid such as **Acetic** or **Muriatic**. Replace any damaged parts that may be leaking.
3A. Check manufacturers' recommendations for proper settings to prime pump.
3B. Check maximum suction lift for pump and relocate as necessary.
3C. Check well pump discharge pressure.
Check pressure rating on chemical feed pump.
3D. Clean or replace screen.
3E. Insure all fittings are tight.
3F. Add chlorine solution to supply tank.
4A. Fill tank.
4B. Check for cracked fittings.
4C. Adjust foot valve to proper position.
4D. Check connections and fittings.



Small Chlorine solution tank.

Shock Chlorination — Well Maintenance

Shock chlorination is a relatively inexpensive and straightforward procedure used to control bacteria in water wells. Many types of bacteria can contaminate wells, but the most common are iron and sulfate-reducing bacteria.

Although not a cause of health problems in humans, bacteria growth will coat the inside of the well casing, water piping and pumping equipment, creating problems such as:

- Reduced well yield
- Restricted water flow in distribution lines
- Staining of plumbing fixtures and laundry
- Plugging of water treatment equipment
- ***"Rotten egg"*** odor.

Bacteria may be introduced during drilling of a well or when pumps are removed for repair and laid on the ground. However, iron and sulfate-reducing bacteria (as well as other bacteria) can exist naturally in groundwater.

A well creates a direct path for oxygen to travel into the ground where it would not normally exist. When a well is pumped, the water flowing in will also bring in nutrients that enhance bacterial growth.

Note: All iron staining problems are not necessarily caused by iron bacteria. The iron naturally present in the water can be the cause.

Ideal Conditions for Iron Bacteria
Water wells provide ideal conditions for iron bacteria. To thrive, iron bacteria require 0.5-4 mg/L of dissolved oxygen, as little as 0.01 mg/L dissolved iron and a temperature range of 5 to 15°C. Some iron bacteria use dissolved iron in the water as a food source.



Signs of Iron and Sulfate-Reducing Bacteria
There are a number of signs that indicate the presence of iron and sulfate-reducing bacteria. They include:

- Slime growth
- Rotten egg odor
- Increased staining.

Slime Growth
The easiest way to check a well and water system for iron bacteria is to examine the inside surface of the toilet flush tank. If you see a greasy slime or growth, iron bacteria are probably present. Iron bacteria leave this slimy by-product on almost every surface the water is in contact with.

Rotten Egg Odor
Sulfate-reducing bacteria can cause a rotten egg odor in water. Iron bacteria aggravate the problem by creating an environment that encourages the growth of sulfate-reducing bacteria in the well. Sulfate-reducing bacteria prefer to live underneath the slime layer that the iron bacteria form.

Some of these bacteria produce hydrogen sulfide as a by-product, resulting in a "***rotten egg***" or sulfur odor in the water. Others produce small amounts of sulfuric acid which can corrode the well casing and pumping equipment.

Increased Staining Problems
Iron bacteria can concentrate iron in water sources with low iron content. It can create a staining problem where one never existed before or make an iron staining problem worse as time goes by.

Use the following checklist to determine if you have an iron or sulfate-reducing bacteria problem. The first three are very specific problems related to these bacteria. The last two problems can be signs of other problems as well.

Checklist to Determine an Iron or Sulfate-Reducing Bacteria Problem
- Greasy slime on inside surface of toilet flush tank
- Increased red staining of plumbing fixtures and laundry
- Sulfur odor
- Reduced well yield
- Restricted water flow

Mixing a Chlorine Solution
Add a half gallon of bleach to a clean pail with about 3 gallons of water. This is generally sufficient to disinfect a 4 inch diameter well 100 feet deep or less. For wells greater than 100 feet deep or with a larger casing diameter, increase the amount of bleach proportionately.

If you have a dug well with a diameter greater than 18 inches, use 2 to 4 gallons of bleach added directly to the well. Please note that many dug wells are difficult or impossible to disinfect due to their unsanitary construction.

Shock Chlorination — Well Maintenance

Shock Chlorination Method
Shock chlorination is used to control iron and sulfate-reducing bacteria and to eliminate fecal coliform bacteria in a water system. To be effective, shock chlorination must disinfect the following:
- The entire well depth
- The formation around the bottom of the well
- The pressure system
- Some water treatment equipment
- The distribution system.

To accomplish this, a large volume of super chlorinated water is siphoned down the well to displace all the water in the well and some of the water in the formation around the well.

Effectiveness of Shock Chlorination
With shock chlorination, the entire system (from the water-bearing formation, through the well-bore and the distribution system) is exposed to water which has a concentration of chlorine strong enough to kill iron and sulfate reducing bacteria. Bacteria collect in the pore spaces of the formation and on the casing or screened surface of the well.

To be effective, you must use enough chlorine to disinfect the entire cased section of the well and adjacent water-bearing formation.

The procedure described below does not completely eliminate iron bacteria from the water system, but it will hold it in check.

To control the iron bacteria, you may have to repeat the procedure each spring and fall as a regular maintenance procedure. If your well has never been shock chlorinated or has not been done for some time, it may be necessary to use a stronger chlorine solution, applied two or three times, before you notice a significant improvement in the water.

You might also consider hiring a drilling contractor to thoroughly clean and flush the well before chlorinating in order to remove any buildup on the casing.

In more severe cases, the pump may have to be removed and chemical solutions added to the well and vigorous agitation carried out using special equipment. This is to dislodge and remove the bacterial slime. This should be done by a drilling contractor.

Shock Chlorination Procedure for Small Drilled Wells

A modified procedure is also provided for large diameter wells.

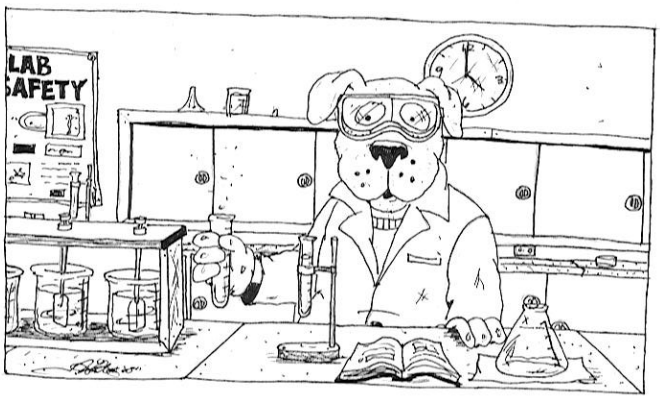
Caution: If your well is low yielding or tends to pump any silt or sand, you must be very careful using the following procedure because over pumping may damage the well.

When pumping out the chlorinated solution, monitor the water discharge for sediment.

Follow these steps to shock chlorinate your well.

Store sufficient water to meet farm and family needs for 8 to 48 hours.

Pump the recommended amount of water (see Amount of Chlorine Required to Obtain a Chlorine Concentration of 1000 PPM) into clean storage. A clean galvanized stock tank or pickup truck box lined with a 4 mil thick plastic sheet is suitable. The recommended amount of water to use is twice the volume of water present in the well casing. To measure how much water is in the casing, subtract the non-pumping water level from the total depth of the well.



12% industrial sodium hypochlorite and 70% high test hypochlorite are available from:
• Water treatment suppliers
• Drilling contractor
• Swimming pool maintenance suppliers
• Dairy equipment suppliers
• Some hardware stores.

Amount of Chlorine required to obtain a Chlorine Concentration of 1000 PPM. Since a dry chemical is being used, it should be mixed with water to form a chlorine solution before placing it in the well.

Calculate the amount of chlorine that is required. Mix the chlorine with the previously measured water to obtain a 1000 ppm chlorine solution.

Calculating Amount of Chlorine Example
If your casing is 6 in. and you are using 12% industrial sodium hypochlorite, you will require .091 L per ft. of water in the casing. If you have 100 ft. of water in the casing, you will use 0.091 L x 100 ft. = 9.1 L of 12% chlorine.
Calculate the amount of chlorine you will need for your well.

Casing diameter_____ Chlorine strength_____

L needed per 1 ft. of water_____ x _____ ft. of water in casing = _____ L of chlorine.

Caution: Chlorine is corrosive and can even be deadly.
If your well is located in a pit, you must make sure there is proper ventilation during the chlorination procedure. Well pits are no longer legal to construct. Use a drilling contractor who has the proper equipment and experience to do the job safely.

Shock Chlorination — Well Maintenance
Siphon this solution into the well.
Open each hydrant and faucet in the distribution system (including all appliances that use water such as dishwasher, washing machine, furnace humidifier) until the water coming out has a chlorine odor. This will ensure all the plumbing fixtures are chlorinated.

Allow the hot water tank to fill completely. Consult your water treatment equipment supplier to find out if any part of your water treatment system should be bypassed to prevent damage.

Leave the chlorine solution in the well and distribution system for 8 to 48 hours. The longer the contact time, the better the results.

- Open an outside tap and allow the water to run until the chlorine odor is greatly reduced. Make sure to direct the water away from sensitive plants or landscaping.
- Flush the chlorine solution from the hot water heater and household distribution system. The small amount of chlorine in the distribution system will not harm the septic tank.

Backwash and regenerate any water treatment equipment.

If you have an old well that has not been routinely chlorinated, consider hiring a drilling contractor to thoroughly clean the well prior to chlorinating. Any floating debris should be removed from the well and the casing should be scrubbed or hosed to disturb the sludge buildup.

Modified Procedure for Large Diameter Wells
Due to the large volume of water in many bored wells the above procedure can be impractical. A more practical way to shock chlorinate a bored well is to mix the recommended amount of chlorine right in the well. The chlorinated water is used to force some of the chlorine solution into the formation around the well. Follow these steps to shock chlorinate a large diameter bored well.

Pump 200 gal. (1000 L) of water into a clean storage tank at the well head.

Mix 20 L of 5 1/4% domestic chlorine bleach (or 8 L of 12% bleach or 1.4 kg of 70% calcium hypochlorite) into the 200 gal. of stored water.

Calculate the amount of chlorine you require per foot of water in the casing and add directly into the well. (Note that the 70% hypochlorite powder should be dissolved in water to form a solution before placing in the well.)

Circulate chlorine added to the water in the well by hooking a garden hose up to an outside faucet and placing the other end back down the well. This circulates the chlorinated water through the pressure system and back down the well. Continue for at least 15 minutes.

Siphon the 200 gal. bleach and water solution prepared in Steps 1 and 2 into the well. Complete the procedure as described in Steps 5 to 9 for drilled wells.

Don't mix acids with chlorine. This is dangerous.

Calculating Water and Chlorine Requirements for Shock Chlorination
Complete the following table using your own figures to determine how much water and chlorine you need to shock chlorinate your well.

Casing Volume of 5 1/4% 12% Industrial **1**70%
Diameter Water Needed Domestic Sodium High Test
Chlorine Bleach Hypochlorite
Imperial gal. needed per Dry weight
1 ft. of water L per 1 ft. (30 cm) L per 1 ft. (30 cm) per 1 ft. (30 cm)
(in) (mm) in the casing of water of water of water
4 (100) _____ft. x 1.1 gal. =_____ _____ft. x 0.095 L =_____ _____ft. x 0.042 L =_____ _____ft. x 7.2 g =_____
6 (150) _____ft. x 2.4 gal. =_____ _____ft. x 0.21 L =_____ _____ft. x 0.091 L =_____ _____ft. x 15.6 g =_____
8 (200) _____ft. x 4.2 gal. =_____ _____ft. x 0.36 L =_____ _____ft. x 0.16 L =_____ _____ft. x 27.3 g =_____
24 (600)**2** extra 200 gal. _____ft. x 1.7 L =_____ _____ft. x 0.74 L =_____ _____ft. x 127 g =_____
36 (900)**2** extra 200 gal. _____ft. x 3.8 L =_____ _____ft. x 1.7 L =_____ _____ft. x 286 g =_____

What Should I do if My Well Water is Contaminated with Bacteria?

First, don't panic'! Bacterial contamination is very common. Studies have found that over 40 percent of private water supplies are contaminated with coliform bacteria. Spring water supplies are the most frequently contaminated, with over 70 percent containing coliform bacteria.

Improving protection of a well or spring from the inflow of surface water is an important option to consider if the supply is contaminated with bacteria. It is important to remember that the groundwater is not necessarily contaminated in these cases; rather the well is acting to funnel contaminants down into the groundwater. Although well pits were the common method of construction several years ago, they are no longer considered sanitary construction.

A properly protected well is evidenced by the well casing extending above the surface of the ground and the ground sloping away from the well to prevent water from collecting around the casing. A properly protected spring is developed underground and the water channeled to a sealed spring box. At no time should the water be exposed to the ground surface.

Keeping the plumbing system clean is an important part of maintaining a sanitary water supply. Anytime work is performed on the plumbing or pump, the entire water system should be disinfected with chlorine. Simply pulling the pump out of the well, setting it on the grass to work on it, and returning it to the well is enough to contaminate the well with bacteria.

The procedure for cleaning and sanitizing a well or spring with chlorine is called **shock chlorination**. Concentrations of chlorine ranging from 50 to 200 mg/1 are used in the shock chlorination process. This is 100 to 400 times the amount of chlorine found in "***city water***." The highly chlorinated water is held in the pipes for 12 to 24 hours before it is flushed out and the system is ready to be used again.

Periodic shock chlorination also may be effective in reducing an **iron bacteria** problem. The amount of chlorine needed to shock chlorinate a water system is determined by the amount of water standing in the well.

Table 3 lists the amount of chlorine laundry bleach or powdered high-test hypochlorite (**HTH**) that is needed for wells. If in doubt, it is better to use more chlorine than less.

Calcium Hypochlorite Section ($CaCl_2O_2$)



Physical Properties - Calcium Hypochlorite
Description: White powder, pellets or flat plates
Warning properties: Chlorine odor; inadequate warning of hazardous concentrations
Molecular weight: 142.98 daltons
Boiling point (760 mm Hg): Decomposes at 100ºC (HSDB 2001)
Freezing point: Not applicable
Specific gravity: 2.35 (water = 1)
Water solubility: 21.4% at 76ºF (25ºC)
Flammability: Not flammable

There are two forms of calcium hypochlorite: powder and tablets. Tablets range in size from 5 mg about the size of an Aspirin to 3 inch tablets. Synonyms of calcium hypochlorite include Losantin, hypochlorous acid, calcium salt, BK powder, Hy-Chlor, chlorinated lime, lime chloride, chloride of lime, calcium oxychloride, HTH, mildew remover X-14, perchloron, and pittchlor.

Calcium hypochlorite is generally available as a white powder, pellets, or flat plates; sodium hypochlorite is usually a greenish yellow, aqueous solution. Although not flammable, they may react explosively.

Calcium hypochlorite decomposes in water to release chlorine and oxygen; sodium hypochlorite solutions can react with acids or ammonia to release chlorine or chloramine. Odor may not provide an adequate warning of hazardous concentrations.

Toxic
Both hypochlorites are toxic by the oral and dermal routes and can react to release chlorine or chloramine which can be inhaled. The toxic effects of sodium and calcium hypochlorite are primarily due to the corrosive properties of the hypochlorite moiety. Systemic toxicity is rare, but metabolic acidosis may occur after ingestion.

Description
Solid chlorine stands alone as the safest form of chlorine disinfection. Requiring only minimal safety equipment for handling, users can breathe easy knowing our tablets are safe for both people and the environment. The elimination of costly scrubbers, containment, or hazard response capability, guarantees lower initial costs and reduced operating expense. Calcium hypochlorite is generally available as a white powder, pellets, or flat plates. It decomposes readily in water or when heated, releasing oxygen and chlorine. It has a strong chlorine odor, but odor may not provide an adequate warning of hazardous concentrations. Calcium hypochlorite is not flammable, but it acts as an oxidizer with combustible material and may react explosively with ammonia, amines, or organic sulfides. Calcium hypochlorite should be stored in a dry, well-ventilated area at a temperature below 120ºF (50ºC) separated from acids, ammonia, amines, and other chlorinating or oxidizing agents.

Chlorine Tablet Feeder
These feed systems are low maintenance and an extremely effective means to treat water or wastewater. Dry tablet feeder may or may not have mechanical components and most require no electricity. The dry tablet feeding system is a good alternative to liquid bleach and potential gas hazards. With no chlorine gas cylinders to handle, chlorine releases are non existent. Process safety Management and Risk Management Program compliance worries disappear.

Chlorine Tablet Feeder Capacities: range - 1,500 to 200,000 (GPD)

Chlorine tablets are stable for 3 years or more.

If a tablet produces 1000 PPM in a liter of water when first off the press, the tablet will produce 1000 PPM plus. This guarantees the activity will be at least 100% 3 years later and probably for much longer than that. In fact, tablets have been stored for 6 years at 6% C and 42% C and still contained the specified levels of available chlorine. Sodium hypochlorite liquid, on the other hand, is inherently unstable and degrades with age until all the active strength disappears. This degradation accelerates in conditions of high temperature or strong sunlight.





These two different tablet chlorinator feeding systems are installed as a sidestream (see the clear plastic line) to the mainstream water flow or directly in the well casing. Using a flow meter or timed device, a chlorine tablet is dropped or delivered inside the well casing or to another location in the distribution system. Sometimes, the chlorinated balance is piped to an integrated solution tank. Then the resulting concentrated chlorine solution is pumped into a pressurized line or holding tank. By mixing chlorinated water from the solution tank with unchlorinated water from the main stream, a controllable level of available chlorine is achieved.



Accuracy
Because of their stability, chlorine tablets are an accurate dose, always yielding the stated level of available chlorine in water or very slightly over, never under. Liquid chlorine strengths vary so widely and are mostly unknown (the container usually says "less than 5%") that it is impossible to make up accurate in-use solutions without access to laboratory equipment.

Storage and Distribution
In recent years, concern regarding the safety hazards associated with liquid chlorine has grown to such an extent that several major cities now restrict transportation of chlorine within their boundaries. Tablets, on the other hand, are easy and convenient to store and transport. One pallet containing 600 jars each of 200 tablets is equivalent to 120,000 x 1 liter in use bleach solutions of 1,000 PPM active chlorine concentration.

Liquid chlorine is bulky, heavy and prone to leakage and spillage. Chlorine tablets are compact, economical and safe to ship and can even be sent by airfreight.

Effectiveness
Both chlorine tablets and liquid Sodium hypochlorite produce Hypochlorous Acid (HOCl) and Hypochlorite ion (OCl-) in solution. It has been postulated by Ortenzio and Stuart in 1959 and again by Trueman in 1971 that Hypochlorous Acid is the predominantly active species whilst Hypochlorite ion has little activity due to its negative charge impeding penetration of the cell wall and membrane. The ratio of Hypochlorous Acid to Hypochlorite ion increases with acidity. Chlorine tablets have a pH of 6.7 and liquid hypochlorite a pH of between 9 and 12. Ergo; tablets have a greater disinfection capacity and are less prone to inactivation due to soiling.

Safety
Chlorine tablets in dry form will not leak or splash and do not damage clothing. Liquid chlorine can affect eyes, skin and mucous membranes; it is easily splashed and rots clothing.

Corrosion
Chlorine tablets are much less corrosive than liquid chlorine, which is highly corrosive to most metals

Comparison
The final very important comparison to be made between Sodium hypochlorite (NaOCL) and Sodium dichloroisocyanurate (NaDCC) is their neutralization by organic matter. They are both prone to this but by using horse serum, it has been shown (Coates 1988) that the degree of neutralization is directly proportional to the concentration of serum present. However, the degree of neutralization of Naocl disinfectant is much greater than that of NaDCC disinfectant and the disparity increases with the concentration of serum. Hence, where there is a high concentration of organic material present, NaDCC will be very much more effective than NaOCL.

The degree of inactivation of NaOCL and NaDCC solutions by different concentrations of horse serum demonstrates that NaDCC solutions are less prone to inactivation by serum than are NaOCL solutions. For example, in 30% serum it required only 4000 PPM av. CI of NaDCC as opposed to 17000 PPM av CI of NaOCL to exhibit similar bactericidal activity.

System Sizing
To determine the correct system for your application, some specific information is required:

What form of chlorination is used now? ___ Gas ___ Liquid Bleach ___ Granular

Source water temperature _____ ___ Other (specify: _____)

Is chlorination performed ___ intermittently or ___ continuously?

What is the current consumption? _____ LBS or GALS/DAY

What is the system pressure to be treated?_____ PSI

What is the system flow rate to be treated?_____ GPM

What is the final desired chlorine concentration?_____ PPM

Dose Rate Math

_____ System Flow Rate (GPM)

x _____ Tablets Demand (PPM)

x 0.0005 Conversion Factor

Total _____ = LBS/HR Tablets Required

System Selection
Compare the LBS/HR of chlorine requirement above with the following system capacity and delivery characteristics. An additional factor that needs to be considered is how frequently the unit must be refilled in light of the volume of tablets that the feeder holds.

Health Effects
* Hypochlorite powder, solutions, and vapor are irritating and corrosive to the eyes, skin, and respiratory tract. Ingestion and skin contact produces injury to any exposed tissues. Exposure to gases released from hypochlorite may cause burning of the eyes, nose, and throat; cough as well as constriction and edema of the airway and lungs can occur.
* Hypochlorite produces tissue injury by liquefaction necrosis. Systemic toxicity is rare, but metabolic acidosis may occur after ingestion.

Acute Exposure
The toxic effects of sodium and calcium hypochlorite are primarily due to the corrosive properties of the hypochlorite moiety. Hypochlorite causes tissue damage by liquefaction necrosis. Fats and proteins are saponified, resulting in deep tissue destruction. Further injury is caused by thrombosis of blood vessels. Injury increases with hypochlorite concentration and pH. Symptoms may be apparent immediately or delayed for a few hours. Calcium hypochlorite decomposes in water releasing chlorine gas.

Sodium Hypochlorite Solutions
Sodium hypochlorite solutions liberate the toxic gases chlorine or chloramine if mixed with acid or ammonia (this can occur when bleach is mixed with another cleaning product). Thus, exposure to hypochlorite may involve exposure to these gases.

Children do not always respond to chemicals in the same way that adults do. Different protocols for managing their care may be needed.

Gastrointestinal
Pharyngeal pain is the most common symptom after ingestion of hypochlorite, but in some cases (particularly in children), significant esophagogastric injury may not have oral involvement. Additional symptoms include dysphagia, stridor, drooling, odynophagia, and vomiting. Pain in the chest or abdomen generally indicates more severe tissue damage. Respiratory distress and shock may be present if severe tissue damage has already occurred. In children, refusal to take food or drink liquid may represent odynophagia.

Ingestion of hypochlorite solutions or powder can also cause severe corrosive injury to the mouth, throat, esophagus, and stomach, with bleeding, perforation, scarring, or stricture formation as potential sequelae.

Dermal
Hypochlorite irritates the skin and can cause burning pain, inflammation, and blisters. Damage may be more severe than is apparent on initial observation and can continue to develop over time. Because of their relatively larger surface area/body weight ratio, children are more vulnerable to toxins affecting the skin.

Ocular
Contact with low concentrations of household bleach causes mild and transitory irritation if the eyes are rinsed, but effects are more severe and recovery is delayed if the eyes are not rinsed. Exposure to solid hypochlorite or concentrated solutions can produce severe eye injuries with necrosis and chemosis of the cornea, clouding of the cornea, iritis, cataract formation, or severe retinitis.

Respiratory
Ingestion of hypochlorite solutions may lead to pulmonary complications when the liquid is aspirated. Inhalation of gases released from hypochlorite solutions may cause eye and nasal irritation, sore throat, and coughing at low concentrations. Inhalation of higher concentrations can lead to respiratory distress with airway constriction and accumulation of fluid in the lungs (pulmonary edema). Patients may exhibit immediate onset of rapid breathing, cyanosis, wheezing, rales, or hemoptysis. Pulmonary injury may occur after a latent period of 5 minutes to 15 hours and can lead to reactive airways dysfunction syndrome (RADS), a chemical irritant-induced type of asthma.

Children may be more vulnerable to corrosive agents than adults because of the smaller diameter of their airways. Children may also be more vulnerable to gas exposure because of increased minute ventilation per kg and failure to evacuate an area promptly when exposed.

Metabolic
Metabolic acidosis has been reported in some cases after ingestion of household bleach.

Potential Sequelae
Exposure to toxic gases generated from hypochlorite solutions can lead to reactive airways dysfunction syndrome (RADS), a chemical irritant-induced type of asthma. Chronic complications following ingestion of hypochlorite include esophageal obstruction, pyloric stenosis, squamous cell carcinoma of the esophagus, and vocal cord paralysis with consequent airway obstruction.

Chronic Exposure
Chronic dermal exposure to hypochlorite can cause dermal irritation.

Carcinogenicity
The International Agency for Research on Cancer has determined that hypochlorite salts are not classifiable as to their carcinogenicity to humans.

Reproductive and Developmental Effects
No information was located regarding reproductive or developmental effects of calcium or sodium hypochlorite in experimental animals or humans. Calcium and sodium hypochlorite are not included in Reproductive and Developmental Toxicants, a 1991 report published by the U.S. General Accounting Office (GAO) that lists 30 chemicals of concern because of widely acknowledged reproductive and developmental consequences.

Sources/Uses
Sodium and calcium hypochlorite are manufactured by the chlorination of sodium hydroxide or lime. Sodium and calcium hypochlorite are used primarily as oxidizing and bleaching agents or disinfectants. They are components of commercial bleaches, cleaning solutions, and disinfectants for drinking water and waste water purification systems and swimming pools.



Chlorine-Based Disinfectants Chloramines

This process involves the addition of ammonia and chlorine compounds to a water filtration plant. When properly controlled, the mixture forms chloramines. They are commonly used to maintain a residual in the distribution system following treatment with a stronger disinfectant, such as free chlorine.

Chloramine Advantages

- Persistent residual.
- Taste and odor minimization.
- Lower levels of trihalomethane (THM) and haloacetic acid (HAA) formation.
- Effective disinfection of biofilms in the distribution system.

Chloramine Disadvantages

- Produces disinfection by-products (DBPs), including nitrogen-based compounds and chloral hydrate, which may be regulated as a DBP in the future. There is limited information on the toxicity of chloramine DBPs. In an analysis of the health effects of alternatives, Bull states that "there is little information on which to base an estimate of the health hazard that chloramine poses."
- Presents problems to individuals on dialysis machines. Chloramine residuals in tap water can pass through membranes in dialysis machines and directly induce oxidant damage to red blood cells.
- Causes eye irritation. Exposure to high levels of chloramine may result in eye irritation.
- Requires increased dosage and contact time (higher CT values, *i.e.,* concentration X time).
- Has questionable value as viral and parasitic biocide.
- Can promote growth of algae in reservoirs and an increase in distribution system bacteria due to residual ammonia.
- Can produce HAAs.
- Provides weaker oxidation and disinfection capabilities than free chlorine.

Chloramine Section

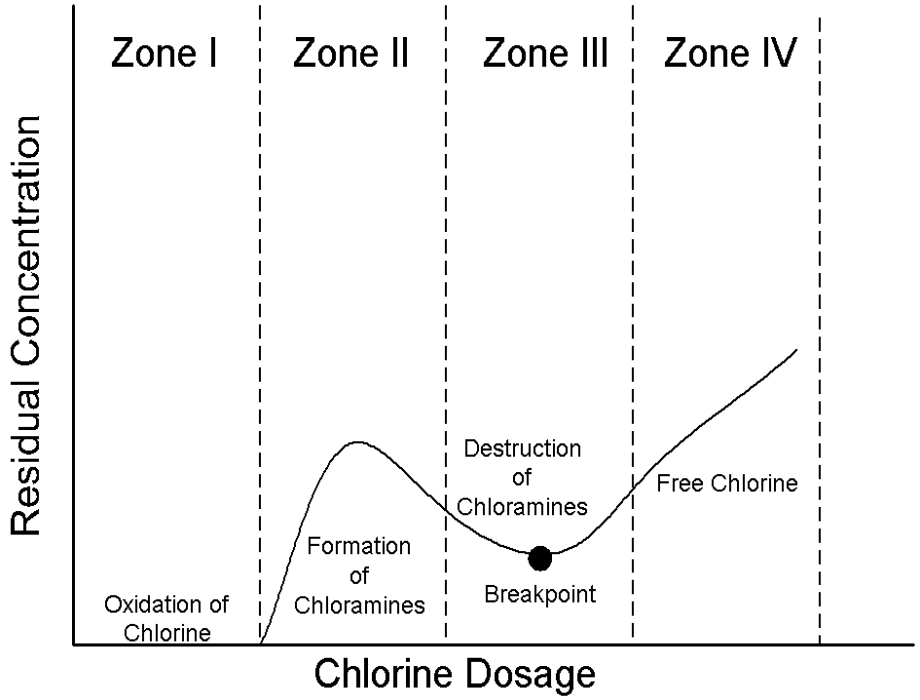
Monochloramine and dichloramine are formed in the pH range of 4.5 to 8.5, however, monochloramine is most common when the pH is above 8. When the pH of the wastewater is below 4.5, the most common form of chloramine is trichloramine which produces a very foul odor. The equations for the formation of the different chloramines are as follows: (Reynolds & Richards, 1996)

Monochloramine: $NH_3 + HOCl \rightarrow NH_2Cl + H_2O$
Dichloramine: $NH_2Cl + 2HOCl \rightarrow NHCl2 + 2H_2O$
Trichloramine: $NHCl2 + 3HOCl \rightarrow NHCl_3 + 3H_2O$

Chloramines are an effective disinfectant against bacteria but not against viruses. As a result, it is necessary to add more chlorine to the wastewater to prevent the formation of chloramines and form other stronger forms of disinfectants.
d) The final step is that additional free chlorine reacts with the chloramine to produce hydrogen ion, water, and nitrogen gas which will come out of solution. In the case of the monochloramine, the following reaction occurs:
$2NH_2Cl + HOCl \rightarrow N_2 + 6HCl + H_2O$

Thus, added free chlorine reduces the concentration of chloramines in the disinfection process. Instead the chlorine that is added is allowed to form the stronger disinfectant, hypochlorus acid.

Perhaps the most important stage of the water or wastewater treatment process is the disinfection stage. This stage is most critical because it has the greatest effect on public health as well as the health of the world's aquatic systems. It is important to realize that wastewater treatment is not a cut and dry process but requires in depth knowledge about the type of wastewater being treated and its characteristics to obtain optimum results.



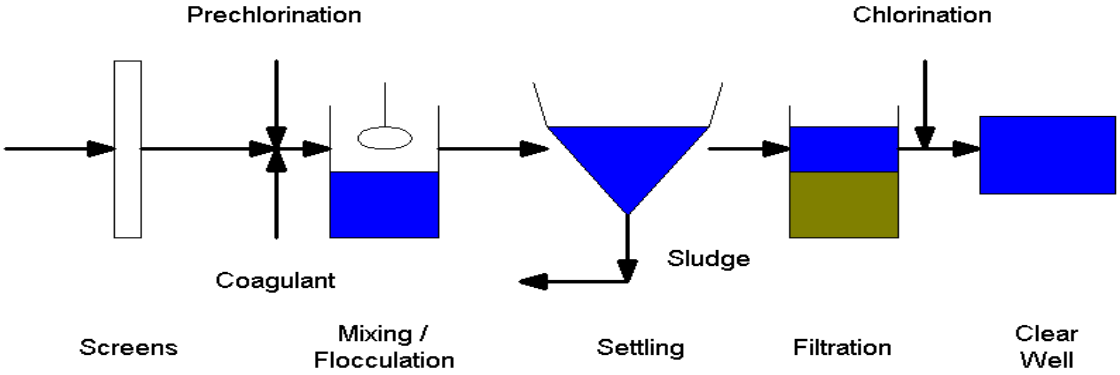
The graph shown on the last page depicts the chlorine residual as a function of increasing chlorine dosage with descriptions of each zone given below (Drawing by Erik Johnston, adapted from Reynolds and Richards, 1996).

· Zone I: Chlorine is reduced to chlorides.
· Zone II: Chloramines are formed.
· Zone III: Chloramines are broken down and converted to nitrogen gas which leaves the system (Breakpoint).
· Zone IV: Free residual.

Therefore, it is very important to understand the amount and type of chlorine that must be added to overcome the difficulties in the strength of the disinfectant which results from the water or wastewater's characteristics.

Water Treatment

The following is a schematic of a water treatment plant (Drawing by Matt Curtis).



In water treatment, pre-chlorination is utilized mainly in situations where the inflow is taken from a surface water source such as a river, lake, or reservoir. Chlorine is usually added in the rapid mixing chamber and effectively prevents the majority of algal growth.

Algae is a problem in water treatment plants because it builds up on the filter media and increases the head which means that the filters need to be backwashed more frequently. In addition, the algal growth on the filter media causes taste and odor problems in the treated water.

Post Chlorination

Post chlorination is almost always done in water treatment, but can be replaced with chlorine dioxide or chloramines. In this stage chlorine is fed to the drinking water stream which is then sent to the chlorine contact basin to allow the chlorine a long enough detention time to kill all viruses, bacteria, and protozoa that were not removed and rendered inactive in the prior stages of treatment.

Drinking water requires a large addition of chlorine because there must be a residual amount of chlorine in the water that will carry through the system until it reaches the tap of the user. After post chlorination, the water is retained in a clear well prior to distribution.

Chlorine Production Section

Faraday's laws states that one coulomb of electricity deposits 0.00111801 grams (g) of silver, or 1 ampere of electric current deposits 0.00111801 g of silver in one second.

Faraday's second law states that the quantity of electricity that liberates one gram equivalent weight (e.w.) of an element is the same for all elements. Since the equivalent weight of silver is 107.880, it takes one faraday, or 96,493 coulombs (107.88 e.w. / 0.00111801 g), to liberate 1 gram equivalent weight of any element.

The 1986 recommended value for one faraday is 96,485.309 coulomb. Being consistent with Faraday's laws, one faraday (96,485.3 coulomb) of electricity liberates 1.008 gram of hydrogen and 35.457 grams of chlorine in the chlor-alkali process. Since there are 86,400 seconds in each day (60 sec/min x 60 min/hour x 24 hour/day) and 454 grams in each pound of element, 14.3 amperes of current are required to liberate one pound of chlorine gas during a 24 hour period:

454 g/lb x 96,485.3 coulomb = 14.3 amperes
35.457 g Cl 86,400 sec/day

Coulomb (C)

> The SI unit of electric charge. One coulomb is the amount of charge accumulated in one second by a current of one ampere. Electricity is actually a flow of charged particles, such as electrons, protons, or ions. The charge on one of these particles is a whole-number multiple of the charge *e* on a single electron, and one coulomb represents a charge of approximately 6.241 506 x 10^{18} *e*. The coulomb is named for a French physicist, Charles-Augustin de Coulomb (1736-1806), who was the first to measure accurately the forces exerted between electric charges.

Considering typical current efficiencies of 95 percent, the actual electrochemical reaction requires roughly 15 amperes of direct current to produce one pound of chlorine gas during a 24-hour period: 14.3 amperes / 0.95 (efficiency factor) = 15 amperes

The chlorine gas is vacuum swept from the chlorine cell by a Venturi ejector. The chlorine mixes with the ejector water to form hypochlorous acid and/or hypochlorite ion depending on the water pH.

Industrial Uses

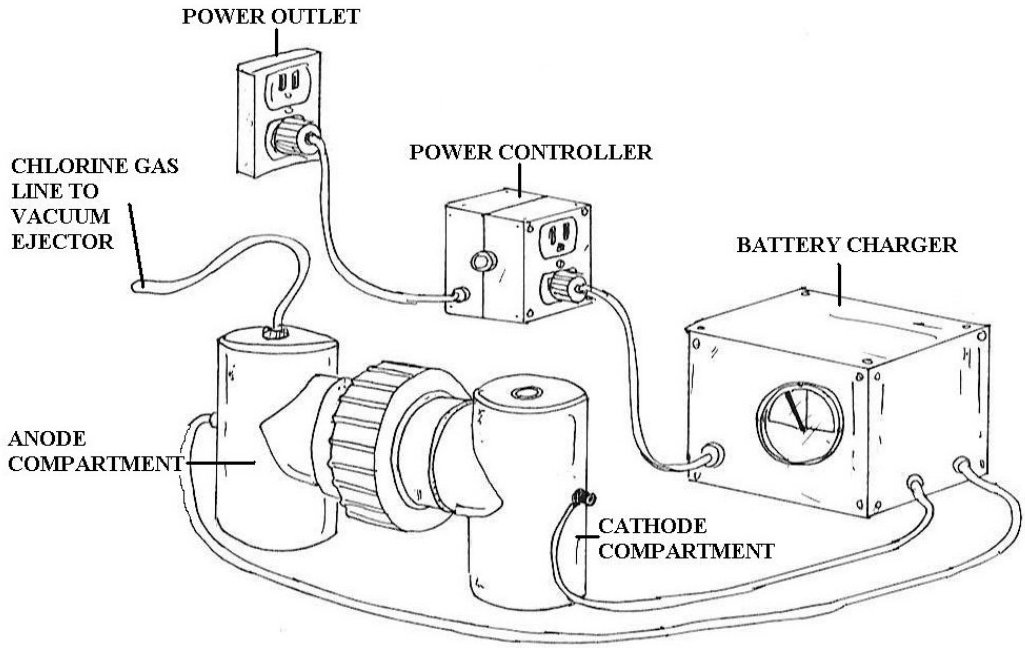
In addition to water treatment chemicals, chlorine is used to make plastics such as PVC (polyvinyl chloride) and polyurethanes, pulp and paper treatment chemicals, solvents and a large number of chemicals.

The most common industrial use of chlorine is the manufacture of a versatile plastic known as polyvinyl chloride, or PVC. PVC is a polymer, meaning on a microscopic level many small units of the same types of atoms are bound together to form long chains, similar to the linking of multiple paper clips. One use of PVC is as lightweight, durable pipes for safe drinking water delivery. PVC pipes resist the pitting and corrosion common in metal pipes which often develop a slimy build-up of disease-causing microbes known as "biofilm." Biofilms can be destroyed by elevated levels of chlorine disinfectant. With so many varied and valuable uses, chlorine chemistry is truly an indispensable asset to modern life.

Chlorine Generator Process
The chlorine generator is as simple as a battery. There are no moving parts; however, the chlorine generator does require operation and maintenance. On this page you will discover:

- Process Components
- Process Installation
- System Operation
- Dose Control
- Salt Addition
- Sodium Hydroxide Dilution
- System Maintenance

Process Components
The chlorine generator requires a cell, DC power supply (battery charger, battery, solar power), power controller, energy source (power outlet, generator), and a pressurized water supply to operate the Venturi ejector.



Cell: The cell includes the anode and cathode compartments that are hydraulically isolated by an ion selective membrane located between the two cell compartments. The anode compartment contains the anode (electrode), salt, saltwater electrolyte, and chlorine.
Chlorine gas generated from the anode compartment is swept under vacuum by the Venturi ejector into the water supply. The cathode compartment contains the cathode (electrode), sodium hydroxide (caustic soda) electrolyte, and hydrogen.

The hydrogen produced from the cathode compartment is vented to the outside atmosphere. The two cell compartments are joined together by a union pipe fitting that also holds the ion selective membrane between the union flanges. Please note that the use of a union pipe fitting in the cell configuration is patented and subject to royalty fees.

DC Power Supply
The DC power supply can be any DC battery charger of adequate size to handle the needed chlorine demand. See our power supply sizing page for determining the size of power supply needed.

Power Controller
The power controller is simply a common dimmer switch (used to dim lights) that the power supply is plugged into to adjust the voltage input to the power supply. Like dimming your lights, the power controller will "dim" your chlorine production to the desired chlorine level.

Energy Source
The energy source can basically be a 120 VAC power outlet you plug the Power Controller into.

Pressurized Water Supply
The water passes through a Venturi creating a vacuum that is applied to the anode compartment of the cell. The Venturi ejector also includes a flow switch connected to a relay that operates the Power Controller. This safety feature ensures that flow is going through the Venturi ejector before chlorine is generated. The discharge from the vacuum ejectors is highly chlorinated water in the form of hypochlorous acid and/or hypochlorite ion.

Process Installation
Provided the plumbing for the system is complete (existing vacuum ejector, or simply using a garden hose connected to the ejector); it should not take longer than 30 minutes to an hour to have your chlorine generator completely operational. The installation includes the following steps:

- Remove components from the box, check contents for any missing or broken parts
- Soak the membrane in warm water
- Install the membrane on the cell flange
- Add salt and water to the anode compartment
- Add water and dry sodium hydroxide (i.e. Draino) to the cathode compartment
- Connect water supply to Venturi ejector
- Connect the vacuum tubing from the anode compartment to the Venturi ejector
- Clamp red (positive) power clamp from power supply to anode
- Clamp black (negative) power clamp from power supply to cathode
- Turn on power supply switch
- Plug power supply into power controller
- Plug power controller into power circuit
- Operate Venturi ejector and energize power circuit, adjust power controller to desired chlorine level.

System Operation

The system operation includes the control of the system, addition of salt and water to the anode compartment, periodic dilution of the sodium hydroxide in the cathode compartment, and occasional cleaning of the cell membrane. There are several ways the chlorine generator can be operated. The simplest way is to plug the power controller into a power outlet that is only energized at times when the generator is needed for chlorine production. This on/off operation procedure can be accomplished by installing a power control relay on the power outlet circuit. Nearly all municipal well installations include this type of circuit typically used for a hypochlorination pump.

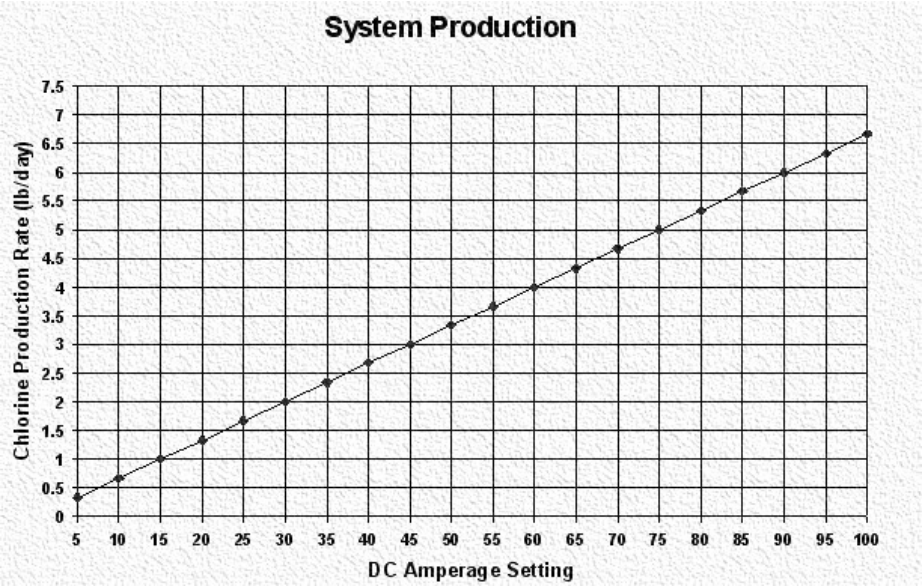
The power controller includes a flow switch that ensures operation of the chlorine generator only when there is flow through the Venturi ejector. Having the pool filter and water supply fill line on the vacuum ejector will allow the chlorine generator to operate at any moment when water is moving into the pool.

At a booster pump station having multiple pumps, a chlorine generator for each pump circuit will supply the step chlorine dosage needed depending on the number of pumps operating. This operational procedure eliminates the need for an electronic logic controlled loop and/or pacing valve systems.

The chlorine generator can be controlled in an automatic mode associated with a chlorine demand change. The automatic mode requires an electronic input signal (4-20 mA) associated with the demand change that controls an optional EP1 Series - SCR Power.

Dose Control

Chlorine output is adjusted by the power input of the process. Every 15 amps of direct current (DC) provide a chlorine production rate of 1 pound per day (24 hours). The graph below illustrates the equivalent chlorine production at the desired amperage setting.



Note: With time, the membrane accumulates calcium and other mineral deposits that increase the resistance between the electrodes. The increased resistance causes a reduced amperage output and a corresponding reduced chlorine output. The system needs periodic membrane cleaning to recover the desired amperage output. A water softener system can be added to the system water supply to reduce the amount of calcium, thus increasing the service life of the membrane.

Connecting your power supply (battery charger) into the power controller will allow you to manually adjust the voltage supply to your battery charger, thus controlling the DC amperage output to the cell. The power controller provided with each cell includes a 600 watt dimmer switch to manually adjust the input voltage to the battery charger. Adjustment of the dimmer switch will increase or decrease the voltage output of your battery charger to the desired amperage setting. For example, a chlorine output of 0.5 lbs per day is desired for a 50 gpm well. Based on amperage conversions, approximately 8 amperes of DC power is needed for the well. The operator would adjust the dimmer switch to achieve a power output of 8 DC amperes for the cell.

Salt Addition

For every 50 lb bag of water softener salt, approximately 30 lbs of chlorine is made. The amount of salt that can be added to the cell depends on the shape of the salt pellets; however, a typical amount of salt added in each cycle is roughly 12 lbs. Twelve pounds of salt in the anode compartment will generate 7 lbs of chlorine considering that not all the salt is used in each cycle. The frequency of salt addition depends on the operating cycle. For example:

A 150 gpm municipal well operating a total of 6 hours per day (54,000 gpd) using a 1.5 lb/day (22 amperes) chlorine dosage rate (0.5 mg/l chlorine residual w/ a 0.35 mg/l chlorine demand) will need to have salt added every 18 days (7 lbs/cell / [1.5 lb/day * 6 hr / 24 hr]).

Salt replenishment in the anode compartment requires the drainage of the brine, flushing the anode compartment with water, and addition of new salt and water to the cell. Adding of salt to the cell without flushing and cleaning is not recommended for several reasons. First, the anode compartment contains residual chlorine gas that will be displaced when salt is added. The amount of chlorine gas in this space is small (0.02 lbs); however, this amount of chlorine gas is irritating, especially if in a confined space. Second, the brine contains concentrated mineral impurities that will foul the membrane at a more rapid rate if it is not removed.

Replenishing the Salt Consists of:

- Turn off the power supply to the cell.
- Add roughly 1 cup of sodium hydroxide to the anode compartment. The sodium hydroxide will neutralize the residual chlorine in the brine and make a salt saturated hypochlorite solution.
- Drain the brine solution to waste and flush out the cell removing all the residual matter in the bottom of the cell.
- Add new salt and water to the anode compartment (it is desirable to use softened water to reduce the mineral fouling of the membrane).
- Place system back into service.

Sodium Hydroxide Dilution

Safety: Please note that sodium hydroxide is corrosive and irritating to the skin. If sodium hydroxide touches the skin, wash with water immediately to prevent chemical burn. Wear protective clothing such as rubber gloves and goggles when handling sodium hydroxide.

For every 50 lb bag of water softener salt, approximately 36 gallons of 18 percent sodium hydroxide solution is made. The actual amount of sodium hydroxide produced is dependent upon the level and frequency of dilution. Assuming a 7 lb chlorine cycle per cell, the amount of sodium hydroxide produced from the cell is approximately 8.5 gallons. Using the same 18-day operational cycle as discussed above, approximately one-half gallon of sodium hydroxide solution is produced every day of operation.

Dilution of sodium hydroxide in the cathode compartment requires the removal of approximately one-half gallon of sodium hydroxide and the addition of dilution water to 4-inches from the top of the cathode compartment (Note: more sodium hydroxide is produced than water added for dilution). It is desirable to use softened water for the dilution to reduce the mineral fouling of the membrane.

Maintenance of the sodium hydroxide solution within the optimum range (10-18 percent) provides extended life of the membrane. Daily testing of the sodium hydroxide solution with a typical battery hydrometer will verify the need to dilute the sodium hydroxide. The following table illustrates the specific gravity and concentration of sodium hydroxide at a temperature of 60 degrees F (15.5 degrees C):

% NaOH	Specific Gravity
2	1.023
4	1.045
6	1.067
10	1.090
12	1.112
14	1.134
16	1.156
18	1.178
20	1.201
22	1.223

Dilution of the Sodium Hydroxide Consists of:

- Turn off the power supply to the cell.
- Removal of 1/2 gallon of solution (or more if operating the cell at higher rates or longer periods of time), and dispose/store as desired (this could be disposal down a sanitary drain if solution is not needed; however, sodium hydroxide is needed for pH adjustment in the pool, CIP cleaning for diaries, lift station cleaning in sewer systems, and/or pH adjustment of water for lead and copper corrosion control in municipal drinking water).

- Add dilution water (preferably softened water) to a level of 4-inches from the top of the cathode compartment.
- place system back into service.
- check small amount of solution daily with a hydrometer.

System Maintenance
The chlorine generator has no moving parts and requires minimal maintenance. The system maintenance involves the periodic cleaning of the membrane. The salt and water added to the chlorine generator contain calcium and other minerals that accumulate on the surface of the membrane. These mineral deposits increase the electrical resistance across the membrane eventually reducing the amperage to the cell, thus reducing the chlorine production. Using the same 18-day operational cycle as discussed above, you may achieve two to four months of cell operation before needing to clean the cell membrane (depends on the dilution water quality) Operating the cell at 1 lb/day for 24 hours/day may require membrane cleaning every month (again, depending on the dilution water quality).

Cleaning the Membrane Involves the Following:
- Turn off the power supply to the cell.
- Remove brine and salt from anode compartment as described above.
- Draining and storage of sodium hydroxide from the cathode compartment.
- Flushing the interior of both compartments with water to remove all loose deposits.
- Encrusted mineral deposits on the membrane can be removed by one of two methods.
    - The membrane removal and replacement method requires the removal of the membrane from the union fitting and replacement with a cleaned membrane. The membrane removed from the cell is then cleaned in a weak hydrochloric acid solution (muriatic or pool acid) to dissolve the mineral deposits. After cleaning, observe the condition of the membrane and discard if pin-holes are observed in the membrane. Otherwise, store membrane in a water solution for the next cleaning cycle.
    - The insitu cleaning method involves the addition of water to top of the horizontal pipe connecting the anode and cathode compartments. Addition of 1 cup of muriatic acid to each cell compartment and agitate cell. After five minutes, drain the cell and flush with water.
- Restore sodium hydroxide to the cathode chamber and salt and water to the anode chamber.
- Reconnect power to cell and resume operation.

Replacement Items
Items that wear and need eventual replacement include the vacuum tubing, rubber gasket/o-ring, membrane, and the anode. The anode has an expected life of five years based on a chlorine rate of 1 lb/day under moderate usage. The membrane has an anticipated life of one year depending on the frequency and dilution of the sodium hydroxide (see above). The rubber gasket in the cathode compartment may also need to be replaced every few years as needed.

The vacuum tubing should be checked annually and replace when cracks are observed. Use chlorine compatible tubing such as polyethylene tubing when replacing.

Conclusions

- Chlorine is one of nature's most common chemical elements.
- Electricity applied to salt solutions enables the chlor-alkali industry to harness chlorine captured in salt deposits of ancient oceans.
- Chlorine's chemical properties make it an extremely effective disinfectant and essential component in the chemical manufacture of literally thousands of vital products used every day.
- Pairs of substances that chlorine will react explosively or form explosive compounds with are acetylene and ether, turpentine and ammonia and hydrogen and finely divided metals.
- Monochloramine, dichloramine, and trichloramine are known as combined available chlorine.
- The chlorine pressure reducing valve should be located downstream of the evaporator when using an evaporator.
- Chlorine is added to the effluent before the contact chamber for complete mixing. What is the reason for not adding it directly to the chamber? The chamber has very little mixing due to low velocities.
- The two main chemical species formed by chlorine in water and the name that are they are known collectively are HOCl and OCl-; free available chlorine.
- When chlorine gas is added to water, it rapidly hydrolyzes. The chemical equation that best describes this reaction is $C_{l2} + H_2O$ --> H+ + Cl- + HOCl.
- Yoke-type connectors should be used on a chlorine cylinder's valve; always assume the threads on the valve may be worn.
- Excessive chlorine can kill the aerobic organisms in the secondary treatment plant. Take precaution when applying chlorine in the sewer line near a wastewater treatment plant to control hydrogen sulfide production and anaerobic bacteria.
- When replacing the connection from a chlorine cylinder to a chlorinator always use a new, approved gasket on the connector and follow the manufacturer’s instructions.
- Safety precautions when using chlorine gas: In addition to protective clothing and goggles, chlorine gas should be used only in a well-ventilated area so that any leaking gas cannot concentrate.
- Several symptoms of chlorine exposure: Burning of eyes, nose, and mouth, coughing, sneezing, choking, nausea and vomiting; headaches and dizziness; fatal pulmonary edema, pneumonia, and skin blisters.
- Approved method for storing a chlorine cylinder: Secure each cylinder in an upright position, attach the protective bonnet over the valve and firmly secure each cylinder.
- Emergency procedures in the case of a large uncontrolled chlorine leak: Notify local emergency response team, warn and evacuate people in adjacent areas, be sure that no one enters the leak area without adequate self-contained breathing equipment.

Disinfection Supplement

Chlorine

Upon adding chlorine to water, two chemical species, known together as free chlorine, are formed. These species, hypochlorous acid (HOCl, electrically neutral) and hypochlorite ion (OCl-, electrically negative), behave very differently. Hypochlorous acid is not only more reactive than the hypochlorite ion, but is also a stronger disinfectant and oxidant.

The ratio of hypochlorous acid to hypochlorite ion in water is determined by the pH. At low pH (higher acidity), hypochlorous acid dominates while at high pH hypochlorite ion dominates. Thus, the speed and efficacy of chlorine disinfection against pathogens may be affected by the pH of the water being treated. Fortunately, bacteria and viruses are relatively easy targets of chlorination over a wide range of pH. However, treatment operators of surface water systems treating raw water contaminated by the parasitic protozoan Giardia may take advantage of the pH-hypochlorous acid relationship and adjust the pH to be effective against Giardia, which is much more resistant to chlorination than either viruses or bacteria.

Another reason for maintaining a predominance of hypochlorous acid during treatment has to do with the fact that pathogen surfaces carry a natural negative electrical charge. These surfaces are more readily penetrated by the uncharged, electrically neutral hypochlorous acid than the negatively charged hypochlorite ion. Moving through slime coatings, cell walls and resistant shells of waterborne microorganisms, hypochlorous acid effectively destroys these pathogens. Water is made microbiologically safe as pathogens either die or are rendered incapable of reproducing. A typical bacterium has a negatively charged slime coating on its exterior cell wall, which is effectively penetrated by electrically neutral hypochlorous acid, favored by lower pH's.

Factors in Chlorine Disinfection: Concentration and Contact Time

In an attempt to establish more structured operating criteria for water treatment disinfection, the CXT concept came into use in 1980. Based on the work of several researchers, CXT values [final free chlorine concentration (mg/L) multiplied by minimum contact time (minutes)], offer water operators guidance in computing an effective combination of chlorine concentration and chlorine contact time required to achieve disinfection of water at a given temperature. The CXT formula demonstrates that if an operator chooses to decrease the chlorine concentration, the required contact time must be lengthened. Similarly, as higher strength chlorine solutions are used, contact times may be reduced (Connell, 1996).

Chloramines

Chloramines are chemical compounds formed by combining a specific ratio of chlorine and ammonia in water. Because chloramines are relatively weak as a disinfectant, they are almost never used as a primary disinfectant. Chloramines provide a durable residual, and are often used as a secondary disinfectant for long distribution lines and where free chlorine demand is high. Chloramines may also be used instead of chlorine in order to reduce chlorinated byproduct formation and to remove some taste and odor problems.

Advantages

- Reduced formation of THMs, HAAs
- Will not oxidize bromide to bromine forming brominated byproducts
- More stable residual than free chlorine
- Excellent secondary disinfectant, has been found to be better than free chlorine at controlling coliform bacteria and biofilm growth
- Lower taste and odor than free chlorine

Limitations

- Weak disinfectant and oxidant
- Requires shipment and handling of ammonia or ammonia compounds as well as chlorinating chemicals
- Ammonia is toxic to fish, and may pose problems for aquarium owners
- Will cause problems for kidney dialysis if not removed from water

Chlorine Dioxide

Chlorine dioxide (ClO_2) is generated on-site at water treatment facilities. In most generators sodium chlorite and elemental chlorine are mixed in solution, which almost instantaneously forms chlorine dioxide. Chlorine dioxide characteristics are quite different from chlorine. In solution it is a dissolved gas, which makes it largely unaffected by pH but volatile and relatively easily stripped from solution. Chlorine dioxide is also a strong disinfectant and a selective oxidant. While chlorine dioxide does produce a residual it is only rarely used for this purpose.

Advantages

- ✓ Effective against Cryptosporidium
- ✓ Up to five times faster than chlorine at inactivating Giardia
- ✓ Disinfection is only moderately affected by pH
- ✓ Will not form chlorinated byproducts (THMs, HAAs)
- ✓ Does not oxidize bromide to bromine (can form bromate in sunlight)
- ✓ More effective than chlorine in treating some taste and odor problems
- ✓ Selective oxidant used for manganese oxidation and targeting some chlorine resistant organics

Limitations

- ✓ Inorganic byproduct formation (chlorite, chlorate)
- ✓ Highly volatile residuals
- ✓ Requires on-site generation equipment and handling of chemicals (chlorine and sodium chlorite)
- ✓ Requires a high level of technical competence to operate and monitoring equipment, product and residuals
- ✓ Occasionally poses unique odor and taste problems
- ✓ High operating cost (chlorite chemical cost is high)

Understanding Chlorine Basics

Chlorine is applied to water in one of three forms: elemental chlorine (chlorine gas), hypochlorite solution (bleach), or dry calcium hypochlorite. All three forms produce free chlorine in water.

Advantages

- ✓ Highly effective against most pathogens
- ✓ Provides a residual to protect against recontamination and to reduce bio-film growth in the distribution system
- ✓ Easily applied, controlled, and monitored
- ✓ Strong oxidant meeting most preoxidation objectives
- ✓ Operationally the most reliable
- ✓ The most cost-effective disinfectant

Limitations

- ✓ Byproduct formation (THMs, HAAs)
- ✓ Will oxidize bromide to bromine, forming brominated organic byproducts
- ✓ Not effective against Cryptosporidium
- ✓ Requires transport and storage of chemicals

Elemental Chlorine

Elemental chlorine is the most commonly used form of chlorine. It is transported and stored as a liquefied gas under pressure. Water treatment facilities typically use chlorine in 100 and 150-lb cylinders or one-ton containers. Some large systems use railroad tank cars or tanker trucks.

Advantages

- ✓ Lowest cost of chlorine forms
- ✓ Unlimited shelf-life

Limitations

- ✓ Hazardous gas requires special handling and operator training
- ✓ Additional regulatory requirements, including EPA's Risk Management Program and the Occupational Safety and Health Administration's Process Safety Management program

Factors in Chlorine Disinfection: Concentration and Contact Time

In an attempt to establish more structured operating criteria for water treatment disinfection, the CXT concept came into use in 1980. Based on the work of several researchers, CXT values [final free chlorine concentration (mg/L) multiplied by minimum contact time (minutes)], offer water operators guidance in computing an effective combination of chlorine concentration and chlorine contact time required to achieve disinfection of water at a given temperature. The CXT formula demonstrates that if an operator chooses to decrease the chlorine concentration, the required contact time must be lengthened. Similarly, as higher strength chlorine solutions are used, contact times may be reduced (Connell, 1996).

Disinfection and Bioterrorism
Disinfection is crucial to water system security, providing the 'front line' of defense against biological contamination. Normal filtration and disinfection processes would dampen or remove the threats posed by a number of potential bioterrorism agents. In addition, water systems should maintain an ability to increase disinfection doses in response to a particular threat.
However, conventional treatment barriers in no way guarantee safety from biological attacks. For many potential bioterrorism agents, there is little scientific information about what levels of reduction can be achieved with chlorine or other disinfectants. In addition, contamination of water after it is treated could overwhelm the residual disinfectant levels in distribution systems. Furthermore, typical water quality monitoring does not provide real-time data to warn of potential problems (Rose 2002). Additional research and funding are needed to improve prevention, detection, and responses to potential threats.

Protecting Chlorine and Other Treatment Chemicals
As part of its vulnerability assessment, each water system must consider its transportation, storage and use of treatment chemicals. These chemicals are both critical assets (necessary for delivering safe water) and potential vulnerabilities (may pose significant hazards, if released). For example, a release of chlorine gas would pose an immediate threat to system operators, and a large release may pose a danger to the surrounding community. As part of its vulnerability assessment, a water system using chlorine must determine if existing layers of protection are adequate. If not, a system should consider additional measures to reduce the likelihood of an attack or to mitigate the potential consequences.

Possible measures to address chlorine security include: enhanced physical barriers (e.g., constructing secure chemical storage facilities), policy changes (e.g., tightening procedures for receiving chemical shipments), reducing quantities stored on site, or adopting alternative disinfection methods. These options must be weighed and prioritized, considering the unique characteristics and resources of each system. Water system officials must evaluate the risk-tradeoffs associated with each option. For example, reducing the chemical quantities on-site may reduce a system's ability to cope with an interruption of chemical supplies. Furthermore, changing disinfection technologies will not necessarily improve overall safety and security.

Understanding Calculation and Reporting of CT Data
Basically, log inactivation is a measurement of how effective a disinfection process is at killing microorganisms in a specific environment. Operationally, directly measuring log inactivation is not practical, but determining the microbial inactivation for an individual water treatment plant (WTP) can be achieved using the log inactivation calculations. The log inactivation calculation adjusts the WTP's CT value to account for the disinfection chemical reaction process variables that influence the disinfection process efficiency.

Log Inactivation
"Log inactivation" is a convenient way to express the number or percent of microorganisms inactivated (killed or unable to replicate) through the disinfection process. For example, a 3 log inactivation value means that 99.9% of microorganisms of interest have been inactivated. Log inactivation measures the effectiveness of the disinfection process, which is influenced by variables including disinfectant concentration, temperature, pH and disinfectant type (e.g., lower temperature results in less inactivation since the reactions slow down as temperature decreases).

CT and Log Inactivation Calculation Overview

This reference takes you step by step through the CT and log inactivation calculation procedure, through an example calculation, and presents the disinfection segment concept.

"CT" (minutes•mg/L) in the context of water treatment is defined as the product of: C, for "residual disinfectant concentration" in mg/L (determined before or at the first customer) and T, for the corresponding "disinfectant contact time" in minutes. CT is a measure of the disinfection process reaction time, but CT is only one of several variables that control the effectiveness of the disinfection
process.

CTCALC = Concentration Time, Calculated Value (minutes•mg/L)

C = Residual disinfectant concentration measured during peak flow (mg/L)

T = Actual Detention Time (minutes)

CTCALC = C × T

TDT = Theoretical Detention Time (minutes)

V = Volume, based on low water level (gallons)

Q = Peak hourly flow (gpm)

TDT = V/Q

Volume Equations:
Cylindrical: π x r2 x d Pipeline: π x r2 x l
Rectangular: l x w x d
d = minimum water depth
π = 3.1416

Disinfection Segments
Total inactivation = Σ log inactivation from each disinfection segment
Disinfection Profile
Almost all community and non-transient, non-community public water systems that use Surface Water or Ground Water Under the Direct Influence of Surface Water sources are required to develop a disinfection profile. Systems are required to retain the disinfection profile in graphic form and it must be available for review by the state as part of a sanitary survey.

Disinfection Profile and Benchmark
• A disinfection profile is a graphical representation of a system's level of *Giardia lamblia* or virus inactivation measured, at least weekly, during the course of a year.
• A benchmark is the lowest monthly average microbial inactivation during the disinfection profile time period.

The EPA has developed a disinfection profile spreadsheet calculator that calculates and graphs the disinfection profile for *Giardia* and viruses. The spreadsheet can be downloaded from: http://www.epa.gov/safewater/mdbp/lt1eswtr.html.

Understanding Chlorine Demand
The amount of chlorine used by reactions with substances that oxidize in the water before chlorine residual can be measured. It is the difference between the amount of chlorine added to wastewater and the amount of chlorine residual remaining after a given contact time. Chlorine demand may change with dosage, time, temperature, pH, and the type and amount of pollutants in the water.

The presence of chlorine residual in drinking water indicates that: 1) a sufficient amount of chlorine was added initially to the water to inactivate the bacteria and some viruses that cause diarrheal disease; and, 2) the water is protected from recontamination during storage. The presence of free residual chlorine in drinking water is correlated with the absence of disease-causing organisms, and thus is a measure of the potability of water.

While chlorine's most important attributes are its broad-spectrum germicidal potency and persistence in water distribution systems, its ability to efficiently and economically address many other water treatment concerns has also supported its wide use. Chlorine-based compounds are the only major disinfectants exhibiting lasting residual properties. Residual protection guards against microbial regrowth and prevents contamination of the water as it moves from the treatment plant to household taps.

Definitions
When chlorine is added to water, some of the chlorine reacts first with organic materials and metals in the water and is not available for disinfection (this is called the chlorine demand of the water). The remaining chlorine concentration after the chlorine demand is accounted for is called total chlorine. Total chlorine is further divided into: 1) the amount of chlorine that has reacted with nitrates and is unavailable for disinfection which is called combined chlorine and, 2) the free chlorine, which is the chlorine available to inactivate disease-causing organisms, and thus a measure to determine the potability of water.

For example, if using completing clean water the chlorine demand will be zero, and there will be no nitrates present, so no combined chlorine will be present. Thus, the free chlorine concentration will be equal to the concentration of chlorine initially added. In natural waters, especially surface water supplies such as rivers, organic material will exert a chlorine demand, and nitrates will form combined chlorine. Thus, the free chlorine concentration will be less than the concentration of chlorine initially added.

Chlorine Dose, Demand, and Residual
Most water treatment plants are required to disinfect the water, a process used to kill harmful bacteria. The most frequently used method of disinfection is the addition of chlorine. Here, we will briefly introduce three terms used during chlorination - chlorine dose, chlorine demand, and chlorine residual. These three characteristics are related to each other using the following equation:

(Chlorine demand) = (Chlorine dose) - (Chlorine residual)

The amount of chlorine added to the water is known as the chlorine dose. This is a measured quantity chosen by the operator and introduced into the water using a chlorinator or hypochlorinator.

As the chlorine reacts with bacteria and chemicals in the water, some of the chlorine is used up. The amount of chlorine used up by reacting with substances in the water is known as the chlorine demand. If nothing reacts with the chlorine (as would be the case in distilled water), then the chlorine demand is zero. However, in most cases the operator should count on some of the chlorine dose being used up when it reacts with substances in the water.

The amount of chlorine remaining in the water after some of the chlorine reacts with substances in the water is known as the chlorine residual. This lab introduces a test which can be used to calculate the chlorine residual. The chlorine residual is the most important of these three values - dose, demand, and residual - because it represents the actual amount of chlorine remaining in the water to act as a disinfectant.

The test for chlorine residual is performed frequently at most water treatment plants. Since regulations require a certain level of chlorine in water at the far ends of the distribution system, operators should be sure to test the chlorine residual in the distribution system as well as in the clear well.

Combined residual chlorination involves the addition of chlorine to water to produce, with natural ammonia present or with ammonia added, a combined available chlorine residual. Combined available chlorine forms have lower oxidation potentials than free available chlorine forms and are less effective as oxidants. They are also less effective as disinfectants. In fact, 25 times more combined available residual chlorine must be obtained to meet the same disinfectant level as a free available residual. The contact time has to be up to 100 times greater to obtain the same level of bacterial kill at the same pH and temperature conditions.

When a combined available chlorine residual is desired, the character of the water determines how it can be accomplished. These conditions may have to be considered:
1. If the water contains sufficient ammonia to produce the desired level of combined residual, the application of sufficient chlorine alone is all that is needed.
2. If the water contains too little or no ammonia, then addition of both chlorine and ammonia is required.
3. If the water has a free available chlorine, the addition of ammonia alone is all that is required. A combined chlorine residual should contain little or no free available chlorine.

The practice of combined residual chlorination is the most effective way of maintaining a stable residual throughout the distribution system to the point of consumer use. Combined residuals in the distribution system are generally longer-lasting and will carry farther into the system, but they are not as effective as free residuals are at disinfecting. The levels required by the regulatory agencies, when using combined residuals, is 1.0 ppm to 2.0 ppm.

Understanding Chlorine Residual
The amount of available chlorine present in wastewater after a given contact time (20 minutes at peak flow; 30 minutes at average flow), and under specific conditions including pH and temperature.

For effective water treatment, the water supply industry has recognized the need for adequate exposure to the disinfectant and sufficient disinfectant dosage for a certain amount of time. In the 1980s, the two functions were combined with the development of the CT values for various disinfectants.

CT represents the combination of the disinfectant dosage and the length of time water has been exposed to a minimum amount of the disinfectant residual.

Mathematically it is represented as CT = concentration x time
concentration = final disinfectant concentration in mg/l
time = minimum exposure time in minutes

In an assessment of disinfection effectiveness, two types of organisms have been chosen as disinfection surrogates – the protozoan Giardia and viruses. CT values established for disinfection of surface waters require treatment plants to achieve a three-log or 99.9% reduction in Giardia and a four-log or 99.99% virus reduction. It is important to recognize that the use of chlorine as the disinfectant is only one part of the treatment process. Equally important is the

need for improved filtration to remove organisms. A combination of proper disinfection and filtration is most effective in providing safe drinking water. Recent experiments in controlling Cryptosporidium also suggest the effectiveness of filtration in the water treatment process.

Free residual chlorination involves the application of chlorine to water to produce--either directly or by first destroying any naturally present ammonia--a free available chlorine residual and to maintain this residual through part or all of the water treatment plant and distribution system. Free available residual forms have higher oxidation potentials than combined available chlorine forms and are more effective as disinfectants.

When free available chlorine residuals are desired, the characteristics of the water will determine how this will be accomplished. This may have to be considered:
1. If the water contains no ammonia or other nitrogen compounds, any application of chlorine will yield a free residual once it has reacted with any bacteria, virus and other microorganisms present in the water.
2. If the water contains ammonia, it results in the formation of a combined residual, which must be destroyed by applying an excess of chlorine.

Breakpoint Chlorination
Breakpoint chlorination is the name of the process of adding chlorine to water until the chlorine demand has been satisfied. Chlorine demand equals the amount of chlorine used up before a free available chlorine residual is produced. Further additions of chlorine will result in a chlorine residual that is directly proportional to the amount of chlorine added beyond the breakpoint. Public water supplies normally chlorinate past the breakpoint.

When chlorine is initially added to water, the following may happen:
1. If the water contains some iron, manganese, organic matter, and ammonia, the chlorine reacts with these materials and no residual is formed, meaning that no disinfection has taken place.
2. If additional chlorine is added at this point, it will react with the organics and ammonia to form chloramines. The chloramines produce a combined chlorine residual. As the chlorine is combined with other substances, it loses some of the disinfection strength. Combined residuals have poor disinfection power and may be the cause of taste and odor problems.
3. With a little more chlorine added, the chloramines and some of the chlororganics are destroyed.
4. With still more chlorine added, a free chlorine residual is formed, free in the sense that it can react quickly.

Free available chlorine is the best residual for disinfection. It disinfects faster and without the swimming-pool odor of combined residual chlorine. The free available residual forms at the breakpoint; therefore, the process is called breakpoint chlorination. The common practice today is to go just beyond the breakpoint to a residual of about .2 to .5 ppm.

A variety of reactions take place during chlorination. When chlorine is added to a water containing ammonia (NH_3), the ammonia reacts with hypochlorous acid (HOCL) to form monochloramine, dichloramine, and trichloramine. The formation of these chloramines depends on the pH of the water and the initial chlorine-ammonia ratio.

Ammonia + Hypochlorous acid ----> Chloramine + Water
NH3 + HOC1----------> NH2C1 + H20 Monochloramine
NH2C1 + HOC1---------- > NHC12 + H20 Dichloramine
NHC12 + HOC1---------- > NC13 + H20 Trichloramine

At the pH of most natural water (pH 6.5 to 7.5), monochloramine and dichloramine exist together. At pH levels below 5.5, dichloramine exists by itself. Below pH 4.0, trichloramine is the only compound found. The monochloramine and dichloramine forms have a definite disinfection power. Dichloramine is a more effective disinfecting agent than monochloramine.

However, dichloramine is not recommended as a disinfectant due to the possibility of the formation of taste and odor compounds. Chlorine reacts with phenol and salicylic acid to form chlorophenol, which has an intense medicinal odor. This reaction is much slower in the presence of monochloramines.

Both the chlorine residual and the contact time are essential for effective disinfection. It is important to have complete mixing. The operator also needs to be aware that changes in the pH may affect the ability of the chlorine to disinfect the water. The operator must examine the application and select the best point of feed and the best contact time to achieve the results desired. The operator needs to consider:
1. Whether the injection point and the method of mixing is designed so that the disinfectant is able to get into contact with all of the water to be disinfected. This also depends on whether pre- and/or post-chlorination is being used.
2. Contact time. In situations of good initial mixing, the longer the contact time, the more effective the disinfection.
3. Effectiveness of upstream treatment processes. The lower the turbidity of the water, the more effective the disinfection.
4. Temperature. At higher temperatures the rate of disinfection is more rapid.
5. Dosage and type of chemical. Usually the higher the dose, the quicker the disinfection rate. The form of disinfectant (chloramine or free chlorine) and the type of chemical used influence the disinfection rate.
6. pH. The lower the pH, the better the disinfection.

Emergency Disinfection of Drinking Water
USE ONLY WATER THAT HAS BEEN PROPERLY DISINFECTED FOR DRINKING, COOKING, MAKING ANY PREPARED DRINK, OR FOR BRUSHING TEETH
1. Use bottled water that has not been exposed to flood waters if it is available.
2. If you don't have bottled water, you should boil water to make it safe. Boiling water will kill most types of disease-causing organisms that may be present. If the water is cloudy, filter it through clean cloths or allow it to settle, and draw off the clear water for boiling. Boil the water for one minute, let it cool, and store it in clean containers with covers.
3. If you can't boil water, you can disinfect it using household bleach. Bleach will kill some, but not all, types of disease-causing organisms that may be in the water. If the water is cloudy, filter it through clean cloths or allow it to settle, and draw off the clear water for disinfection. Add 1/8 teaspoon (or 8 drops) of regular, unscented, liquid household bleach for each gallon of water, stir it well and let it stand for 30 minutes before you use it. Store disinfected water in clean containers with covers.
4. If you have a well that has been flooded, the water should be tested and disinfected after flood waters recede. If you suspect that your well may be contaminated, contact your local or state health department or agriculture extension agent for specific advice.
(U.S. federal agencies and the Red Cross recommend these same four steps to disinfect drinking water in an emergency. Please, read the text below for important details about disinfection.
More information about disinfection

- ✓ In times of crisis, follow advice from local officials. Local health departments or public water systems may urge consumers to use more caution or to follow additional measures than the information provided here.

- ✓ Look for other sources of potable water in and around your home.

- ✓ When your home water supply is interrupted by natural or other forms of disaster, you can obtain limited amounts of water by draining your hot water tank or melting ice cubes. In most cases, well water is the preferred source of drinking water. If it is not available and river or lake water must be used, avoid sources containing floating material and water with a dark color or an odor. Generally, flowing water is better quality than stagnant water.

Examine the physical condition of the water.
When emergency disinfection is necessary, disinfectants are less effective in cloudy, murky or colored water. Filter murky or colored water through clean cloths or allow it to settle. It is better to both settle and filter. After filtering until it is clear, or allowing all dirt and other particles to settle, draw off the clean and clear water for disinfection. Water prepared for disinfection should be stored only in clean, tightly covered, containers, not subject to corrosion.

- ✓ Choose a disinfection method.

- ✓ Boiling and chemical treatment are two general methods used to effectively disinfect small quantities of filtered and settled water.

Boiling

Boiling is the surest method to make water safe to drink and kill disease-causing microorganisms like Giardia lamblia and Cryptosporidium, which are frequently found in rivers and lakes. These disease-causing organisms are less likely to occur in well water (as long as it has not been affected by flood waters). If not treated properly and neutralized, Giardia may cause diarrhea, fatigue, and cramps after ingestion. Cryptosporidium is highly resistant to disinfection. It may cause diarrhea, nausea and/or stomach cramps. People with severely weakened immune systems are likely to have more severe and more persistent symptoms than healthy individuals.

Boil filtered and settled water vigorously for one minute (at altitudes above one mile, boil for three minutes). To improve the flat taste of boiled water, aerate it by pouring it back and forth from one container to another and allow it to stand for a few hours, or add a pinch of salt for each quart or liter of water boiled.

If boiling is not possible, chemical disinfection of filtered and settled water collected from a well, spring, river, or other surface water body will still provide some health benefits and is better than no treatment at all.

Chemical Treatment
When boiling is not practical, certain chemicals will kill most harmful or disease-causing organisms. For chemical disinfection to be effective, the water must be filtered and settled first. Chlorine and iodine are the two chemicals commonly used to treat water. They are somewhat effective in protecting against exposure to Giardia, but may not be effective in controlling more resistant organisms like Cryptosporidium. Chlorine is generally more effective than iodine in controlling Giardia, and both disinfectants work much better in warm water. You can use a non-scented, household chlorine bleach that contains a chlorine compound to disinfect water. Do not use non-chlorine bleach to disinfect water. Typically, household chlorine bleaches will be 5.25% available chlorine. Follow the procedure written on the label. When the necessary procedure is not given, find the percentage of available chlorine on the label and use the information in the following table as a guide. (Remember, 1/8 teaspoon and 8 drops are about the same quantity.)

Available Chlorine

Drops per Quart/Gallon of Clear Water Drops per Liter of Clear Water
1% 10 per Quart - 40 per Gallon 10 per Liter
4-6% 2 per Quart - 8 per Gallon (1/8 teaspoon) 2 per Liter
7-10% 1 per Quart - 4 per Gallon 1 per Liter

(If the strength of the bleach is unknown, add ten drops per quart or liter of filtered and settled water. Double the amount of chlorine for cloudy, murky or colored water or water that is extremely cold.)

Mix the treated water thoroughly and allow it to stand, preferably covered, for 30 minutes. The water should have a slight chlorine odor. If not, repeat the dosage and allow the water to stand for an additional 15 minutes. If the treated water has too strong a chlorine taste, allow the water to

stand exposed to the air for a few hours or pour it from one clean container to another several times.

You can use granular calcium hypochlorite to disinfect water.
Add and dissolve one heaping teaspoon of high-test granular calcium hypochlorite (approximately ¼ ounce) for each two gallons of water, or 5 milliliters (approximately 7 grams) per 7.5 liters of water. The mixture will produce a stock chlorine solution of approximately 500 milligrams per liter, since the calcium hypochlorite has available chlorine equal to 70 percent of its weight. To disinfect water, add the chlorine solution in the ratio of one part of chlorine solution to each 100 parts of water to be treated. This is roughly equal to adding 1 pint (16 ounces) of stock chlorine to each 12.5 gallons of water or (approximately ½ liter to 50 liters of water) to be disinfected. To remove any objectionable chlorine odor, aerate the disinfected water by pouring it back and forth from one clean container to another.

You can use chlorine tablets to disinfect filtered and settled water.

Chlorine tablets containing the necessary dosage for drinking water disinfection can be purchased in a commercially prepared form. These tablets are available from drug and sporting goods stores and should be used as stated in the instructions. When instructions are not available, use one tablet for each quart or liter of water to be purified.

You can use tincture of iodine to disinfect filtered and settled water.

Common household iodine from the medicine chest or first aid kit may be used to disinfect water. Add five drops of 2 percent U.S. or your country's approved Pharmacopeia tincture of iodine to each quart or liter of clear water. For cloudy water add ten drops and let the solution stand for at least 30 minutes.

You can use iodine tablets to disinfect filtered and settled water.

Purchase commercially prepared iodine tablets containing the necessary dosage for drinking water disinfection at drug and sporting goods stores. Use as stated in instructions. When instructions are not available, use one tablet for each quart or liter of filtered and settled water to be purified.

ONLY USE WATER THAT HAS BEEN PROPERLY DISINFECTED FOR DRINKING, COOKING, MAKING ANY PREPARED DRINK, OR FOR BRUSHING TEETH.

Summary and Illustration of Key Points

- ✓ Filter murky or colored water through clean cloths or allow it to settle. It is better to both settle and filter.

- ✓ Boiling is the surest method to make water safe to drink and kill disease-causing microorganisms like Giardia lamblia and Cryptosporidium, which are frequently found in rivers and lakes.

- ✓ To improve the flat taste of boiled water, aerate it by pouring it back and forth from one container to another and allow it to stand for a few hours, or add a pinch of salt for each quart or liter of water boiled.

- ✓ When boiling is not practical, certain chemicals will kill most harmful or disease-causing organisms. Chlorine (in the form of unscented bleach) and iodine are the two chemicals commonly used to treat water.

- ✓ You can use a non-scented, household chlorine bleach that contains a chlorine compound to disinfect water. (Remember, 1/8 teaspoon and 8 drops are about the same quantity.)
- ✓ You can use tincture of iodine to disinfect filtered and settled water. Common household iodine from the medicine chest or first aid kit may be used to disinfect water.
- ✓ Tincture of iodine. For cloudy water add ten drops and let the solution stand for at least 30 minutes.

Understanding Combined Chlorine Residual

The residual consisting of chlorine that is combined with ammonia, nitrogen, or nitrogenous compounds (chloramines).

Understanding Free Available Chlorine
The residual consisting of hypochlorite ions (OCl-), hypochlorous acid (HOCl) or a combination of the two. These are the most effective in killing bacteria.

Total Combined Chlorine Residual
The total amount of chlorine present in a sample. This is the sum of the free chlorine residual and the combined available chlorine residual.

Understanding Pre-Chlorination
Chlorination is the application of chlorine to water to accomplish some definite purpose. In this lesson, we will be concerned with the application of chlorine for the purpose of disinfection, but you should be aware that chlorination can also be used for taste and odor control, iron and manganese removal, and to remove some gases such as ammonia and hydrogen sulfide. Chlorination is currently the most frequently used form of disinfection in the water treatment field. However, other disinfection processes have been developed. These alternatives will be discussed at the end of this lesson.

Pre-Chlorination and Post-Chlorination
Like several other water treatment processes, chlorination can be used as a pretreatment process (prechlorination) or as part of the primary treatment of water (postchlorination). Treatment usually involves either postchlorination only or a combination of prechlorination and postchlorination.

Pre-chlorination is the act of adding chlorine to the raw water. The residual chlorine is useful in several stages of the treatment process - aiding in coagulation, controlling algae problems in basins, reducing odor problems, and controlling mudball formation. In addition, the chlorine has a much longer contact time when added at the beginning of the treatment process, so prechlorination increases safety in disinfecting heavily contaminated water.

Post-chlorination is the application of chlorine after water has been treated but before the water reaches the distribution system. At this stage, chlorination is meant to kill pathogens and to provide a chlorine residual in the distribution system. Post-chlorination is nearly always part of the treatment process, either used in combination with prechlorination or used as the sole disinfection process.

Until the middle of the 1970s, water treatment plants typically used both prechlorination and post-chlorination. However, the longer contact time provided by prechlorination allows the chlorine to react with the organics in the water and produce carcinogenic substances known as trihalomethanes. As a result of concerns over trihalomethanes, prechlorination has become much less common in the United States. Currently, prechlorination is only used in plants where trihalomethane formation is not a problem.

Understanding Breakpoint Chlorination
Addition of chlorine to water until the chlorine demand has been satisfied. Since ammonia is present in all domestic wastewaters, the reaction of ammonia with chlorine is a great significance. When chlorine is added to waters containing ammonia, the ammonia reacts with hypochlorous acid (HOCl) to form monochloramine, dichloramine and trichloramine. The formation of these chloramines depends on the pH of the solution and the initial chlorine-ammonia ratio.

Chlor-Alkali Membrane Process
The chloralkali process (also chlor-alkali and chlor alkali) is an industrial process for the electrolysis of sodium chloride solution (brine). Depending on the method, several products besides hydrogen can be produced. If the products are separated, chlorine and sodium hydroxide (caustic soda) are the products; by mixing, sodium hypochlorite or sodium chlorate are produced, depending on the temperature. Higher temperatures are needed for the production of sodium chlorate instead of sodium hypochlorite. Industrial scale production began in 1892. When using calcium chloride or potassium chloride, the products contain calcium or potassium instead of sodium.

The process has a high energy consumption, for example over 4 billion kWh per year in West Germany in 1985, and produces equal (molar) amounts of chlorine and sodium hydroxide, which makes it necessary to find a use for the product for which there is less demand, usually the chlorine. There are three production methods in use. While the mercury cell method produces chlorine-free sodium hydroxide, the use of several tons of mercury leads to serious environmental problems. In a normal production cycle a few hundred pounds of mercury per year are emitted, which accumulate in the environment. Additionally, the chlorine and sodium hydroxide produced via the mercury-cell chloralkali process are themselves contaminated with trace amounts of mercury. The membrane and diaphragm method use no mercury, but the sodium hydroxide contains chlorine, which must be removed.

Understanding Chlorine's Effectiveness
In 1881, German bacteriologist Robert Koch demonstrated under controlled laboratory conditions that pure cultures of bacteria could be destroyed by hypochlorite (bleach). The bulk of chlorine disinfection research, which was conducted from the 1940s to the 1970s with a focus on bacteria, provided observations as to how chlorine kills the microorganism. The observations that (1) bacterial cells dosed with chlorine release nucleic acids, proteins and potassium and (2) membrane functions such as respiration and active transport are affected more by chlorine than are cytoplasmic processes, directed researchers' attention to the surface of the bacterial cell. The hypothesis was that the bacterial cell wall, under environmental stress, could interact with chlorine.

Chlorine exposure appears to cause physical, chemical, and biochemical alterations to the cell wall, thus destroying the cell's protective barrier, terminating vital functions, resulting in death of the microorganism. A possible sequence of events during chlorination would be: (1) disruption of the cell wall barrier by reactions of chlorine with target sites at the cell surface, (2) release of vital cellular constituents from the cell, (3) termination of membrane-associated functions, and (4) termination of cellular functions within the cell. During the course of this sequence of events, the microorganism dies, meaning it is no longer capable of growing or causing disease.

Understanding Chlorine Solubility Effects
Chlorine is only slightly soluble in water; its maximum solubility is approximately one percent at 49° C. At temperatures below this point it combines with water to form chlorine ice, a crystalline substance. When the water supply to a gas chlorinator is below normal room temperature, it may cool the chlorine gas to the point at which chlorine ice is formed and accumulates on the needle valve and gas outlet tube, resulting in erratic feed results. Because the vapor pressure of chlorine increases with rising temperatures, its solubility also decreases. At 212° F. chlorine is insoluble in water.

Chlorine dissolved in water forms a weak corrosive mixture of hydrochloric and hypochlorous acid. The corrosivity of chlorine solutions in water creates problems in handling chlorine spills and chlorine containers. Chlorine reacts with many compounds. Because of its great affinity for hydrogen, it removes hydrogen from some compounds, such as hydrogen sulfide. It also reacts with ammonia or other nitrogen-containing compounds to form various mixtures of chloramines. It reacts with organic materials, sometimes with explosive violence.

Chemicals like chlorine, bromine, and ozone are examples of oxidizers. It is their ability to oxidize or steal electrons from other substances that makes them good water sanitizers. As soon as the oxidizing agent is added to the water, it begins to combine with microorganisms like bacteria, algae, and whatever else the water may contain.

Now the free and available oxidizer is combining with contaminants and its effectiveness is reduced according to how much combining took place. Although the hydrogen ion does not play a direct reduction role on copper surfaces, pH can influence copper corrosion by altering the equilibrium potential of the oxygen reduction half-reaction and by changing the speciation of copper in solution (Reiber, 1989). Copper corrosion increases rapidly as the pH drops below 6; in addition, uniform corrosion rates can be high at low pH values (below about pH 7), causing metal thinning. At higher pH values (above about pH 8), copper corrosion problems are almost always associated with non-uniform or pitting corrosion processes (Edwards et al., 1994a; Ferguson et al., 1996). Edwards et al. (1994b) found that for new copper surfaces exposed to simple solutions that contained bicarbonate, chloride, nitrate, perchlorate or sulphate, increasing the pH from 5.5 to 7.0 roughly halved corrosion rates, but further increases in pH yielded only subtle changes.

The prediction of copper levels in drinking water relies on the solubility and physical properties of the cupric oxide, hydroxide and basic carbonate solids that comprise most scales in copper water systems (Schock et al., 1995). In the cupric hydroxide model of Schock et al. (1995), a decrease in copper solubility with higher pH is evident. Above a pH of approximately 9.5, an upturn in solubility is predicted, caused by carbonate and hydroxide complexes increasing the solubility of cupric hydroxide. Examination of experience from 361 utilities reporting copper levels under the U.S. EPA Lead and Copper Rule revealed that the average 90th-percentile copper levels were highest in waters with pH below 7.4 and that no utilities with pH above 7.8 exceeded the U.S. EPA's action level for copper of 1.3 mg/L (Dodrill and Edwards, 1995). However, problems associated with copper solubility were also found to persist up to about pH 7.9 in cold, high-alkalinity and high-sulphate groundwater (Edwards et al., 1994a).

In the pH range of 7-9, both the corrosion rate and the degree of tuberculation of iron distribution systems generally increase with increasing pH (Larson and Skold, 1958; Stumm, 1960; Hatch, 1969; Pisigan and Singley, 1987). Iron levels, however, were usually reported to decrease with increasing pH (Karalekas et al., 1983; Kashinkunti et al., 1999; Broo et al., 2001; Sarin et al., 2003). In a pipe loop system constructed from 90- to100-year-old unlined cast iron pipes taken from a Boston distribution system, iron concentrations were found to steadily decrease when the pH was raised from 7.6 to 9.5 (Sarin et al., 2003). Similarly, when iron was measured in the distribution system following a pH increase from 6.7 to 8.5, a consistent downward trend in iron concentrations was found over 2 years (Karalekas et al., 1983). These observations are consistent with the fact that the solubility of iron-based corrosion by-products decreases with increasing pH.

Water with low pH, low alkalinity and low calcium is particularly aggressive towards cement materials. The water quality problems that may occur are linked to the chemistry of the cement. Lime from the cement releases calcium ions and hydroxyl ions into the drinking water. This, in turn, may result in a substantial pH increase, depending on the buffering capacity of the water (Leroy et al., 1996). Pilot-scale tests were conducted to simulate low-flow conditions of newly lined cement mortar pipes carrying low-alkalinity water (Douglas et al., 1996). In the water with an initial pH of 7.2, alkalinity of 14 mg/L as calcium carbonate and calcium at 13 mg/L as calcium carbonate, measures of pH as high as 12.5 were found.

Similarly, in the water with an initial pH of 7.8, alkalinity of 71 mg/L as calcium carbonate and calcium at 39 mg/L as calcium carbonate, measures of pH as high as 12 were found. The most significant pH increases were found during the 1st week of the experiment, and pH decreased slowly with aging of the lining. In a series of field and test rig trials to determine the impact of in situ cement mortar lining on water quality, Conroy et al. (1994) observed that in low-flow and low-

alkalinity water (around 10 mg/L as calcium carbonate), pH increases exceeding 9.5 could occur for over 2 years following the lining.

Understanding Amperometric Titration

It appears that DPD colorimetric determination and amperometric titration as described in Standard Methods are the procedures most commonly used for routine measurement of total chlorine. Few studies have been conducted to evaluate these or other total residual chlorine measurement techniques. Bender5 studied approximately 10 test procedures and found that results using the DPD colorimetric procedure were consistently higher than those using amperometric titration. Brooks and Seegert6 described an amperometric titration procedure employing a recording polargraph and microburette, which was reported to be accurate and free from interference. The reliability of the DPD colorimetric method for free chlorine has been increasingly questioned in recent years. The suitability of that procedure for accurate total chlorine determinations appears to the authors to be questionable, as well. Amperometric titration as described in Standard Methods cannot be used to measure total chlorine concentrations less than about 0.05 mg/L, which is at least an order of magnitude greater than levels of concern in natural waters for potential toxicity to aquatic organisms. A reliable, simple procedure for low-level total chlorine determinations is clearly needed.

Analytical Procedure
Section 409C of Standard Methods includes a General Discussion section on amperometric titration for the determination of chlorine in aqueous solutions. That discussion is applicable to the procedure used by the authors. Also included in Standard Methods is a section concerning the titration apparatus. Basically, the titration equipment consists of a buret capable of accurately delivering 0.01 mL of titrant, a sample cup, and a stirring device in which is housed a platinum electrode and a KCl reference electrode. Several companies manufacture amperometric titrators that fit this general description. The experience of the senior author is that some of the commercial titrators are less suitable than others, primarily because of the small surface area of some of the electrodes employed. A Wallace and Tiernan amperometric titrator was used by the authors in developing and applying the procedure described below.

Reagents
a. Chlorine-free water. Only distilled or demineralized water that is free of chlorine should be used in preparing reagents. Chlorine-free water may be prepared by passing distilled or demineralized water through a suitable activated carbon filter adsorption column. The water may be tested for the presence of chlorine by titrating a sample as described in the Procedure section. Any deflection in the meter upon the addition of PAO titrant indicates the presence of chlorine or other oxidants that would interfere in the titration procedure.
b. Standard phenylarsine oxide (PAO), 0.00564 N. See Standard Methods Section 409B, paragraph 3a.
Standardization – Dilute 50.00 mL of freshly prepared 0.0002256 N potassium biniodate to 200 mL in chlorine-free water. Add approximately 1.5 g KI and stir to dissolve. Add 1 mL acetate buffer and allow to stand in the dark for 6 minutes. Titrate using the amperometric titrator and determine the equivalence point as detailed in the Procedure section. If the standard PAO is 0.00564 N, exactly 2.00 mL of PAO will be required to reach the equivalence point.
c. Phenylarsine oxide titrant, 0.000564 N. Dilute 10.00 mL of 0.00564 N PAO to 100.0 mL in chlorine-free water.
Standardization – Dilute 5.00 mL of 0.0002256 N potassium biniodate to 200 mL with chlorine-free water. Add approximately 1.5 g KI and stir to dissolve. Add 1 mL acetate buffer and allow to stand in the dark for 6 minutes. Titrate using the amperometric titrator and determine the equivalence point as detailed in the Procedure section below. If the PAO titrant is 0.000564 N, exactly 2.00 mL of PAO will be required to reach the equivalence point.
d. Potassium biniodate, 0.0002256 N. Dissolve 0.7332 g reagent grade $KH(IO_3)2$ in 500 mL chlorine-free water and dilute to 1.00 L. Dilute 10.00 mL of that solution to 100.0 mL with chlorine-free water. That solution is used for the standardization of the PAO and should be freshly prepared.
e. Acetate buffer solution, pH 4. See Standard Methods1 Section 409B, paragraph 3e.

f. Potassium iodide, (KI), reagent grade crystals.

Procedure

a. Titrant selection. Normally a 200-mL sample is used in titration. Each 0.1 mL of 0.000564 N PAO corresponds to 0.01 mg/L in a 200-mL sample. The titrant normality should be selected such that no more than about 4 mL of titrant will be required to reach the equivalence point. Thus, if the chlorine concentration in the majority of the samples to be titrated is less than about 0.4 mg/L, use 0.000564 N PAO as the titrant. If only samples containing chlorine concentrations in excess of 0.4 mg/L are to be analyzed, use 0.00564 N PAO as the titrant. If samples containing concentrations of chlorine in excess of about 0.4 mg/L are to be titrated only occasionally and the volume of 0.000564 N PAO required for titration is found to be excessive, a suitable subsample may be used and diluted to 200 mL with chlorine-free water.

b. Titration procedure (total residual chlorine). Prior to beginning the titration, rinse the buret with PAO titrant by filling it completely and allowing the titrant to run into an empty sample cup. Repeating this operation three or four times will ensure that the correct titrant concentration reaches the sample cup. Remove the sample cup and rinse with distilled water and with the sample to be titrated. Add 200 mL of the sample to the sample cup. Add approximately 1.5 g (± 0.2 g) crystalline KI and allow to dissolve, using the agitator on the titrator for mixing.

The exact amount of KI added is not critical, but the analyst should weigh 1.5 g of this reagent periodically to become familiar with the approximate amount required. Add 1 mL of acetate buffer and allow the microammeter on the titrator to reach a stable reading; the titration should be started within about 30 seconds following the addition of the KI to the sample.

Full-scale deflection on the microammeter is 100 units. The meter should be initially adjusted to read between 90 and 100 units. Record the initial reading prior to the addition of titrant. Titrate by adding suitable volumes of titrant and recording the titrant volume added and the resultant current reading. At least three (and preferably five to ten) readings of current and titrant volume added should be obtained prior to passing the equivalence point; then add excess titrant to ensure that there is no further meter deflection. Record the final meter reading. If, during the titration, the meter reading falls to near or below 10 units, record the low reading, re-adjust the meter to read between 90 and 100 units, record the high reading, and continue the titration. This approach allows calculation of the total meter deflection, which is used in determining the equivalence point.

The equivalence point is determined by plotting the total meter deflection as a function of titrant volume added. It is important that the total meter deflection be used in preparing this plot. A straight line is drawn through the first few points in the plot and a second straight line is drawn parallel to the abscissa and corresponding to the final total deflection in the meter reading. The equivalence point is determined by the intersection of those two lines. When 0.000564 N PAO is used as the titrant, the chlorine concentration is 0.1-times the titrant volume at the equivalence point. This plotting procedure is also outlined in the ASTM Water Manual8 under procedures ASTM D1253 (Tests for Residual Chlorine in Water) and ASTM D1427 (Tests for Residual Chlorine in Waste Water).

c. Sample storage and handling. Chlorine measurements should be made as soon after sample collection as possible. Samples to be analyzed for chlorine should be stored in the dark and packed on ice if they must be held for more than a few minutes before analysis. Chlorine compounds are highly reactive and may be rapidly lost from samples due to the effects of volatilization, phototransformation, and chlorine demand. Storage of samples on ice and in the dark between sampling and analysis will help minimize the rate of dissipation. It is important to estimate the changes that occur in chlorine content in the subject water between sample collection and analysis.

This can be accomplished by performing a “time-lag” test. To perform a time-lag test, a single large (approximately 2-L) sample of the water being analyzed is collected. The chlorine

concentration in that sample is determined six to ten times over a period of one to three hours, depending on the normal sample holding time. The measured concentrations are then plotted as a function of time, normally on semilog paper. In most cases, the decrease in chlorine concentration over time can be described by first-order reaction kinetics.

The original chlorine content in any sample can be computed given the measured concentration and the holding time. A time-lag study should be performed on a regular basis for each type of water being analyzed because of variability in water compositions. The sample set used for the study should be handled in the same way as other samples (i.e., the samples should be kept cold and in the dark). Even when time-lag studies are made a part of the routine analytical procedure, it is important that the delay between sample collection and chlorine analysis be held to a minimum.

Sodium Hypochlorite
Sodium Hypochlorite, or bleach, is produced by adding elemental chlorine to sodium hydroxide. Typically, hypochlorite solutions contain from 5 to 15% chlorine, and are shipped by truck in one- to 5,000- gallon containers.

Advantages
- ✓ Solution is less hazardous and easier to handle than elemental chlorine
- ✓ Fewer training requirements and regulations than elemental chlorine

Limitations
- ✓ Limited shelf-life
- ✓ Potential to add inorganic byproducts (chlorate, chlorite and bromate) to water
- ✓ Corrosive to some materials and more difficult to store than most solution chemicals
- ✓ Higher chemical costs than elemental chlorine

Calcium Hypochlorite
- ✓ Calcium hypochlorite is another chlorinating chemical used primarily in smaller applications. It is a white, dry solid containing approximately 65% chlorine, and is commercially available in granular and tablet forms.

Advantages
- ✓ More stable than sodium hypochlorite, allowing longer storage
- ✓ Fewer training requirements and regulations than elemental chlorine

Limitations
- ✓ Dry chemical requires more handling than sodium hypochlorite
- ✓ Precipitated solids formed in solution complicate chemical feeding
- ✓ Higher chemical costs than elemental chlorine
- ✓ Fire or explosive hazard if handled improperly
- ✓ Potential to add inorganic byproducts (chlorate, chlorite and bromate) to water

Onsite Hypochlorite Generation

In recent years some municipalities have installed on-site hypochlorite generators that produce weak hypochlorite solutions (~0.8%) using an electrolytic cell and a solution of salt water.

Advantages

- ✓ Minimal chemical storage and transport

Limitations

- ✓ More complex and requires a higher level of maintenance and technical expertise
- ✓ High capital cost
- ✓ Operating costs are often higher than for commercial hypochlorite
- ✓ Requires careful control of salt quality
- ✓ Weak solution requires high volume chemical feed and control
- ✓ Byproducts in generated hypochlorite may be difficult to monitor and control
- ✓ System backup may be more difficult and costly

Ozone

Ozone (O_3) is generated on-site at water treatment facilities by passing dry oxygen or air through a system of high voltage electrodes. Ozone is one of the strongest oxidants and disinfectants available. Its high reactivity and low solubility, however, make it difficult to apply and control. Contact chambers are fully contained and non-absorbed ozone must be destroyed prior to release to avoid corrosive and toxic conditions. Ozone is more often applied for oxidation rather than disinfection purposes.

Advantages

- ✓ Strongest oxidant/disinfectant available
- ✓ Produces no chlorinated THMs, HAAs
- ✓ Effective against Cryptosporidium at higher concentrations
- ✓ Used with Advanced Oxidation processes to oxidize refractory organic compounds

Limitations

- ✓ Process operation and maintenance requires a high level of technical competence
- ✓ Provides no protective residual
- ✓ Forms brominated byproducts (bromate, brominated organics)
- ✓ Forms nonhalogenated byproducts (ketenes, organic acids, aldehydes)
- ✓ Breaks down more complex organic matter; smaller compounds can enhance microbial re-growth in distribution systems and increase DBP formation during secondary disinfection processes.
- ✓ Higher operating and capital costs than chlorination
- ✓ Difficult to control and monitor particularly under variable load conditions

Ultraviolet Radiation

Ultraviolet (UV) radiation, generated by mercury arc lamps, is a non-chemical disinfectant. When UV radiation penetrates the cell wall of an organism, it damages genetic material, and prevents the cell from reproducing. Although it has a limited track record in drinking water applications, UV has been shown to effectively inactivate many pathogens while forming limited disinfection byproducts.

Advantages

- ✓ Effective at inactivating most viruses, spores and cysts
- ✓ No chemical generation, storage, or handling
- ✓ Effective against Cryptosporidium
- ✓ No known byproducts at levels of concern

Limitations

- ✓ No residual protection
- ✓ Low inactivation of some viruses (reoviruses and rotaviruses)

- ✓ Difficult to monitor efficacy
- ✓ Irradiated organisms can sometimes repair and reverse the destructive effects of UV through a process known as photo-reactivation
- ✓ May require additional treatment steps to maintain high-clarity water
- ✓ Does not provide oxidation, or taste and odor control
- ✓ High cost of adding backup/emergency capacity
- ✓ Mercury lamps may pose a potable water and environmental toxicity risk

Alternative Disinfectants

Up until the late 1970s, chlorine was virtually the only disinfectant used to treat drinking water. Chlorine was considered an almost ideal disinfectant, based on its proven characteristics:

- ✓ Effective against most known pathogens
- ✓ Provides a residual to prevent microbial re-growth and protect treated water throughout the distribution system
- ✓ Suitable for a broad range of water quality conditions
- ✓ Easily monitored and controlled

Test Methods Available for Residual Chlorine

Residual Chlorine can be measured using different methods. Iodometric and DPD colorimetric methods are the most common methods. Each method has its own set of reagents and concentration range.

Iodometric Method
Residual Chlorine by Iodometric has a minimum detectable concentration of 40ppb if 0.01N sodium thiosulfate is used. Prepare the sample for titration by adding 5mL of acetic acid and 1g of potassium iodide to the sample. Titrate the sample with 0.01N sodium thiosulfate. Concentrations below 1 mg/L should be measured by using either 0.00564N sodium thiosulfate or 0.00564N phenylarsine oxide.

DPD Colorimetric Method
Residual Chlorine can also be measured by the DPD Colorimetric method. This method has a minimum detectable concentration of 10ppb. In this method, the calibration is either made up from a chlorine solution or a potassium permanganate solution. The typical calibration range for this method is 0.05 to 4mg/L. The reagents used in this method are a phosphate buffer and N,N-diethyl-p-phenylenediamine indicator solution. The samples are mixed with the reagents and then read on a spectrophotometer at a wavelength of 515nm.

Chlorine in water solutions is not stable. As a result, its concentration in samples decreases rapidly. Exposure to sunlight or other strong light, air, or agitation will further reduce the quantity of chlorine present in solutions. Samples to be analyzed for chlorine cannot be stored or preserved. Tests must be started immediately after sampling. Therefore, samples taken for the chlorine residual test must be grab samples only and excessive agitation must be avoided.

It is not necessary to use special sampling devices or containers for the chlorine residual test. However, the sampling container should be capable of collecting samples from a representative sampling point following chlorine contact, and should be made of resistant materials that will not rust or corrode, and which can be easily cleaned.

NOTE: A long handled aluminum dipper attached to a wooden handle, or an equivalent device, is acceptable for collecting samples. Do not use coffee cans, bleach bottles, etc.

Preparation of Chemicals
At a minimum, hand and eye protection should be used when handling any of the chemicals mentioned in this section. Before working with any chemical, consult the appropriate Material Safety Data Sheet (MSDS) to determine if other safety precautions are necessary.

Chlorine Residual Reagents
Iodometric and Amperometric Methods:
I. Standard Phenylarsine Oxide (PAO) Solution, 0.00564 N

A. Prepare 0.3 N sodium hydroxide solution (NaOH) by dissolving 12.0 g NaOH in 800 mL distilled water and diluting to 1 liter.

B. Prepare a 6.0 N hydrochloric acid solution (HCl) by adding 108 mL concentrated HCl to 800 mL distilled water and diluting to 1 liter. (Caution: Concentrated HCl fumes can burn eyes and lungs—do not breathe fumes!)

C. Prepare an approximately 0.00564 N solution of PAO using the following procedures:

1. Dissolve approximately 0.8 g PAO powder in 150 mL of 0.3 N NaOH solution, and allow to settle.

2. Decant 110 mL into 800 mL distilled water and mix thoroughly.

3. Bring to pH 6 to 7 with 6N HCl and dilute to 950 mL with distilled water.
(Caution: PAO is poisonous. Wash thoroughly after use and do not ingest.)

D. Standardization

1. Accurately measure 5 to 10 mL freshly standardized 0.0282 N iodine solution into a flask and add 1 mL potassium iodide solution (50g KI dissolved and diluted to 1 L with freshly boiled and cooled distilled water.

2. Titrate with PAO solution, using starch solution as an indicator, until blue disappears.

3. Normality (N) of PAO = (mL iodine solution x 0.0282)/mL PAO titrated.

4. Adjust PAO to 0.00564 N and recheck.

II. Standard Sodium Thiosulfate Solution, 0.00564 N

A. Prepare a 0.1 N sodium thiosulfate solution by dissolving 25 g $Na_2S_2O_3$ $5H_2O$ in 1000 mL of freshly boiled distilled water. Store reagent for at least 2 weeks to allow oxidation of any bisulfite ion present. Add a few mL of chloroform ($CHCl_3$) to minimize bacterial decomposition.

Standardize by one of the following methods:

1. Iodate Method

a. Dissolve 3.249 g anhydrous primary standard quality potassium bi-iodate ($KH(IO_3)2$) or 3.567 g potassium iodate (KIO_3) dried at 103 +/-2°C for 1 hour in distilled water and dilute to 1000 mL to yield a 0.1000 N iodate solution. Store in a glass stoppered bottle.

b. Add, with constant stirring, 1 mL concentrated sulfuric acid (H_2SO_4), 10 mL 0.1000 N iodate solution, and 1 g potassium iodide (KI) to 80 mL distilled water. Titrate immediately with 0.1 N sodium thiosulfate ($Na_2S_2O_3$) until the yellow color of the liberated iodine is almost discharged. Add 1 mL starch indicator solution and continue titration until the blue color disappears.

c. The normality (N) of the sodium thiosulfate is calculated as follows: N of $Na_2S_2O_3$ = 1/mL $Na_2S_2O_3$ for titration

2. Dichromate Method

A. Dissolve 4.904 g anhydrous primary standard grade potassium dichromate ($K_2Cr_2O_7$) in distilled water and dilute to 1000 mL to yield a 0.1000 N dichromate solution. Store in a glass stoppered bottle.

B. For maximum stability of the standard 0.00564 N sodium thiosulfate solution, prepare by diluting an aged 0.1N $Na_2S_2O_3$ standard solution with freshly boiled distilled water. Add 10 mg Mercuric iodide and 4 g of sodium borate per liter of solution. Standardize daily using 0.00564 N potassium dichromate or iodate solution.

III. Standard Iodine Solution (I_2), 0.1 N

A. Dissolve 40 g potassium iodide (KI) in 25 mL chlorine-demand-free water.

B. Add 13 g resublimed iodine (I_2) and stir until dissolved.

C. Transfer to a 1 liter volumetric flask and dilute to the mark.

D. Standardization

1. Volumetrically measure 40 to 50 mL 0.1 N arsenite solution into a flask.

2. Titrate with 0.1 N iodine solution using starch solution as an indicator.

3. Just before end-point is reached, add a few drops of hydrochloric acid solution to liberate sufficient carbon dioxide (CO_2) to saturate the solution.

4. Titrate until blue color first appears and remains.

5. Normality (N) of iodine = (mL of arsenite solution used x 0.1)/mL of iodine titrated

IV. Standard Iodine Titrant (I_2), 0.0282 N

A. Dissolve 25 g KI in a bottle of distilled water in a 1L volumetric flask.

B. Add the correct amount of the exactly standardized 0.1 N iodine solution to yield a 0.0282 N solution.

C. Dilute to one liter with chlorine-demand-free water.

D. Store iodine solutions in amber bottles or in the dark, and protect from exposure to direct sunlight. Do not use rubber stoppers; keep iodine from all contact with rubber.

E. Check titrant normality daily against 0.00564 N PAO or sodium thiosulfate solution. A procedure for calculating a correction factor for this titrant is given in Appendix C.

V. Standard Potassium Iodate Titrant (KIO3), 0.00564 N

A. Dissolve 201.2 mg primary standard grade potassium iodate (KIO_3), dried for 1 hour at 103°C, or 183.3 mg primary standard grade anhydrous potassium bi-iodate ($KH(IO_3)2V$) in distilled water.

B. Dilute to 1 liter volumetrically.

C. Store in glass bottles in the dark and protect from exposure to direct sunlight.

VI. Potassium Iodide Solution (KI), 5% W/V

A. Dissolve 50 g KI in freshly boiled and cooled distilled water and dilute to 1 liter.

B. Store in a brown glass-stoppered bottle in the dark, preferably at 4°C.

C. Discard when solution becomes yellow.

VII. Acetate Buffer Solution, pH 4.0

A. Dissolve 146 g anhydrous sodium acetate ($NaC_2H_3O_2$ $3H_2O$) in 400 mL distilled water.

B. CAREFULLY add 458 mL concentrated (glacial) acetic acid.

C. Dilute to 1 liter with chlorine-demand-free water.

VIII. Standard Arsenite Solution (As_2O_3), 0.1N

A. Accurately weigh a dried, cooled stoppered weighing bottle.

NOTE: Use forceps or tongs—do not handle weighing bottle with fingers.

B. In weighing bottle, weigh out approximately 4.95 g arsenic trioxide (As_2O_3).

C. Transfer without loss to a 1 liter volumetric flask

NOTE: Do not attempt to brush out remaining arsenic trioxide).

D. Reweigh bottle and record weight of arsenic trioxide transferred.

E. Add enough distilled water to moisten the arsenic trioxide.

F. Add 15 g sodium hydroxide (NaOH) and 100 mL distilled water.

G. Swirl flask gently until As_2O_3 is dissolved.

H. Dilute to 250 mL and saturate the solution with carbon dioxide (CO_2) by bubbling CO_2 gas through the solution for a few minutes.

NOTE: This converts the sodium hydroxide (NaOH) to sodium bicarbonate ($NaHCO_3$).

I. Dilute to the 1 liter mark, stopper, and mix thoroughly.

J. This solution has an almost indefinite shelf life.

CAUTION: This solution is highly poisonous and is a suspected cancer causing agent: handle carefully!

IX. Starch Indicator

A. Weigh out 5 g soluble or potato starch.

B. Add enough distilled water to make a thin paste.

C. Pour into 1 liter boiling distilled water, stir and let settle overnight.

D. Transfer clear supernatant into a storage container and preserve by adding 1.25 g salicylic acid, 4 g zinc chloride, or a combination of 4 g sodium propionate and 2 g sodium azide per liter of starch solution.

E. Some commercial starch substitutes or powder indicators are acceptable.

X. Phosphoric Acid solution (H_3PO_4), 1 + 9

A. Carefully add 100 mL of phosphoric acid (H_3PO_4), 85%, to 900 mL of freshly boiled distilled water.

B. Caution should be used when handling this solution, as it can be corrosive.

XI. Phosphoric Acid—Sulfamic Acid Solution

A. Dissolve 20 g sulfamic acid (NH_2SO_3H) in 1 liter of 1 + 9 phosphoric acid (H_3PO_4).

DPD Titrimetric Method

I. Phosphate Buffer Solution

A. Dissolve 24 g anhydrous disodium hydrogen phosphate (Na_2HPO_4) in 400 to 500 mL distilled water.

B. Add 46 g anhydrous potassium dihydrogen phosphate (KH_2PO_4).

C. Dissolve 800 mg disodium ethylenediaminetetraacetate dihydrate (EDTA) in a separate container.

NOTE: This chemical is also known as (ethylenediamine) tetraacetic acid sodium salt.

D. Combine the 2 solutions and dilute to 1 liter.

E. Add 20 mg mercuric chloride to prevent mold growth.

F. Caution: Mercuric chloride is toxic. Take care to avoid ingestion.

II. DPD Indicator Solution

A. Add 8 mL of a 1 + 3 sulfuric acid solution (H_2SO_4) into 500 mL distilled water. Prepare by mixing one part concentrated H_2SO_4 to 3 parts distilled water. (For example, 5 mL H_2SO_4 to 15 mL distilled water.)

B. Add 200 mg EDTA (disodium ethylenediaminetetraacetate dihydrate).

C. Add 1 g DPD Oxalate (N, N-Diethyl-p-phenylenediamine oxalate).

D. Dilute to 1 liter and store in a brown glass-stoppered bottle and discard when discolored.

CAUTION: The DPD oxalate is poisonous, handle carefully!

III. Standard Ferrous Ammonium Sulfate (FAS) Titrant, 0.00282 N

A. Add 1 mL of 1 + 3 sulfuric acid solution (H2SO4) to 500 mL of freshly boiled and cooled distilled water. Prepare by adding one part concentrated H2SO4 to 3 parts distilled water.

B. Dissolve 1.106 g ferrous ammonium sulfate ($Fe(NH_4)2(SO_4)2\ 6H_2O$)

C. Dilute to 1 liter.

D. This standard can be used for 1 month before replacement.

E. Standardize weekly using the following procedure:

1. Measure 100 mL of FAS standard solution into an Erlenmeyer flask.

2. Add 10 mL of 1 + 5 sulfuric acid. Prepare by adding one part concentrated H_2SO_4 to 5 parts distilled water.

3. Add 5 mL concentrated phosphoric acid.

4. Add 2 mL 0.1% barium diphenylamine sulfonate indicator. Prepare by dissolving 0.1 g ($C_6H_5NHC_6H_4$-4-SO_3) Ba in 100 mL distilled water.

5. Titrate with 0.100N potassium dichromate (see iodometric and amperometric section for preparation directions) to a violet end-point that persists for 30 seconds.

DPD Colorimetric Method

I. Phosphate Buffer Solution

(see DPD Titrimetric Method chemicals)

II. DPD Indicator Solution

(see DPD Titrimetric Method chemicals)

III. Potassium Permanganate Stack Solution

A. Dissolve 891 mg potassium permanganate ($KMnO_4$) in distilled water and dilute to 1000 mL.

IV. Potassium Permanganate Standard Solution

A. Dilute 10 mL of stock solution to 100 mL in a volumetric flask.

B. 1 mL of the standard solution diluted to 100 mL with distilled water will be equivalent to 1.0 mg/L chlorine residual in a DPD reaction.

C. Prepare standard solutions by diluting appropriate volumes to 100 mL with distilled water.

If a direct concentration readout colorimeter is used, the DPD and buffer reagents should be prepared or ordered in accordance with the instrument manufacturer's instructions. If the Hach DR100 colorimeter is used, the prepared DPD powder pillows used with the Hach direct reading colorimeters may be purchased from the Hach Company at the following address:

Hach Company
P.O. Box 389
Loveland, Colorado 80539

Orion Model 97-70 Electrode Method

With the exception of the 1 ppm potassium iodate standard and the chlorine water (100 ppm), all of the reagents required for this method can be purchased from Orion Research at the following address:

Orion Research Incorporated
840 Memorial Drive
Cambridge, Massachusetts 02139

I. Prepare a 1 mg/L iodate standard by volumetrically diluting 1 mL of the 100 ppm iodate standard to 100 mL with distilled water.

II. Prepare the chlorine water (approximately 100 ppm) by diluting 1 mL hypochlorite solution (household chlorine bleach) to 500 mL with distilled water.

Hach Model CN-66 Test Kit Method
The DPD indicator powder pillows used in the Hach Model CN-66 Test Kit may be purchased from the Hach Company at the following address:

Hach Company
P.O. Box 389
Loveland, Colorado 80539

More recently, drinking water providers have faced an array of new challenges, including:

- ✓ Treating resistant pathogens such as Giardia and Cryptosporidium
- ✓ Minimizing disinfection byproducts
- ✓ New environmental and safety regulations
- ✓ Strengthening security at treatment facilities

To meet these new challenges, water system managers must design unique disinfection approaches to match each system's characteristics and source water quality. While chlorination remains the most commonly used disinfection method by far, water systems may use alternative disinfectants, including chloramines, chlorine dioxide, ozone, and ultraviolet radiation. No single disinfection method is right for all circumstances, and in fact, water systems may use a variety of methods to meet overall disinfection goals at the treatment plant, and to provide residual protection throughout the distribution system.
In response to new regulations, emerging science on microbial contaminants, as well as safety and security concerns related to treatment chemicals, water system managers will continue to evaluate chlorine and other disinfection methods.

Despite these challenges, a number of factors indicate that drinking water chlorination will remain a corner-stone of waterborne disease prevention.

- ✓ Disinfection is unquestionably the most important step in drinking water treatment, and chlorine's wide range of benefits cannot be provided by any other single disinfectant.
- ✓ It is uncertain that alternative disinfectants reduce potential DBP risks significantly (IPCS 2000). All chemical disinfectants produce byproducts. Generally, the best approach to control disinfection byproducts is to remove natural organic precursors prior to disinfection (EPA 2001). To comply with the forthcoming Long Term 2 Enhanced Surface Water Treatment Rule, some systems with high levels of Cryptosporidium in their source water may choose to adopt alternative disinfection methods (e.g., chlorine dioxide, ozone, or UV). However, most water systems are expected to meet disinfection requirements without changing treatment technologies.
- ✓ The U.S. EPA's forthcoming Groundwater Rule, as well as efforts to strengthen Canadian drinking water standards following the E coli. outbreak in Walkerton, ON will likely increase the use of chlorination for ground water systems.
- ✓ Only chlorine-based disinfectants provide residual protection, an important part of the multi-barrier approach to preventing waterborne disease.
- ✓ World leaders increasingly recognize safe drinking water as a critical building block of sustainable development (see Sidebar). Chlorination can provide cost-effective disinfection for remote rural villages and large cities alike, helping to bring safe water to those in need.

Microscopic Waterborne Agents
It is easy to take for granted the safety of modern municipal drinking water, but prior to widespread filtration and chlorination, contaminated drinking water presented a significant public health risk. The microscopic waterborne agents of cholera, typhoid fever, dysentery and hepatitis A killed thousands of U.S. residents annually before disinfection methods were employed routinely, starting about a century ago. Although these pathogens are defeated regularly now by technologies such as chlorination, they should be thought of as ever-ready to stage a come-back given conditions of inadequate or no disinfection.

Understanding Bacteria
Bacteria are microorganisms often composed of single cells shaped like rods, spheres or spiral structures. Prior to widespread chlorination of drinking water, bacteria like Vibrio cholerae, Salmonella typhii and several species of Shigella routinely inflicted serious diseases such as cholera, typhoid fever and bacillary dysentery, respectively. As recently as 2000, a drinking water

outbreak of E. coli in Walkerton, Ontario sickened 2,300 residents and killed seven when operators failed to properly disinfect the municipal water supply.
While developed nations have largely conquered water-borne bacterial pathogens through the use of chlorine and other disinfectants, the developing world still grapples with these public health enemies

Understanding Viruses
Viruses are infectious agents that can reproduce only within living host cells. Shaped like rods, spheres or filaments, viruses are so small that they pass through filters that retain bacteria. Enteric viruses, such as hepatitis A, Norwalk virus and rotavirus are excreted in the feces of infected individuals and may contaminate water intended for drinking. Enteric viruses infect the gastrointestinal or respiratory tracts, and are capable of causing a wide range of illness, including diarrhea, fever, hepatitis, paralysis, meningitis and heart disease (American Water Works Association, 1999).

Understanding Protozoan Parasites
Protozoan parasites are single-celled microorganisms that feed on bacteria found in multicellular organisms, such as animals and humans. Several species of protozoan parasites are transmitted through water in dormant, resistant forms, known as cysts and oocysts. According to the World Health Organization, Cryptosporidium parvum oocysts and Giardia lamblia cysts are introduced to waters all over the world by fecal pollution. The same durable form that permits them to persist in surface waters makes these microorganisms resistant to normal drinking water chlorination (WHO, 2002b). Water systems that filter raw water may successfully remove protozoan parasites.

Emerging Pathogens
An emerging pathogen is one that gains attention because it is one of the following:
- a newly recognized disease-causing organism
- a known organism that starts to cause disease
- an organism whose transmission has increased

Understanding Oxidizing Agents
Oxidizing agents act by oxidizing the cell membrane of microorganisms, which results in a loss of structure and leads to cell lysis and death. A large number of disinfectants operate in this way. Chlorine and oxygen are strong oxidizers, so their compounds figure heavily here.
- ✓ Sodium hypochlorite is very commonly used. Common household bleach is a sodium hypochlorite solution and is used in the home to disinfect drains, toilets, and other surfaces. In more dilute form, it is used in swimming pools, and in still more dilute form, it is used in drinking water. When pools and drinking water are said to be chlorinated, it is actually sodium hypochlorite or a related compound—not pure chlorine—that is being used. Chlorine partly reacts with proteinaceous liquids such as blood to form non-oxidizing N-chloro compounds, and thus higher concentrations must be used if disinfecting surfaces after blood spills. Commercial solutions with higher concentrations contain substantial amounts of sodium hydroxide for stabilization of the concentrated hypochlorite, which would otherwise decompose to chlorine, but the solutions are strongly basic as a result.
- ✓ Other hypochlorites such as calcium hypochlorite are also used, especially as a swimming pool additive. Hypochlorites yield an aqueous solution of hypochlorous acid that is the true disinfectant. Hypobromite solutions are also sometimes used.
- ✓ Electrolyzed water or "Anolyte" is an oxidizing, acidic hypochlorite solution made by electrolysis of sodium chloride into sodium hypochlorite and hypochlorous acid. Anolyte has an oxidation-reduction potential of +600 to +1200 mV and a typical pH range of 3.5—8.5, but the most potent solution is produced at a controlled pH 5.0–6.3 where the predominant oxychlorine species is hypochlorous acid.
- ✓ Chloramine is often used in drinking water treatment.
- ✓ Chloramine-T is antibacterial even after the chlorine has been spent, since the parent compound is a sulfonamide antibiotic.

- ✓ Chlorine dioxide is used as an advanced disinfectant for drinking water to reduce waterborne diseases. In certain parts of the world, it has largely replaced chlorine because it forms fewer byproducts. Sodium chlorite, sodium chlorate, and potassium chlorate are used as precursors for generating chlorine dioxide.
- ✓ Hydrogen peroxide is used in hospitals to disinfect surfaces and it is used in solution alone or in combination with other chemicals as a high level disinfectant. Hydrogen peroxide is sometimes mixed with colloidal silver. It is often preferred because it causes far fewer allergic reactions than alternative disinfectants. Also used in the food packaging industry to disinfect foil containers. A 3% solution is also used as an antiseptic.
- ✓ Hydrogen peroxide vapor is used as a medical sterilant and as room disinfectant. Hydrogen peroxide has the advantage that it decomposes to form oxygen and water thus leaving no long term residues, but hydrogen peroxide as with most other strong oxidants is hazardous, and solutions are a primary irritant. The vapor is hazardous to the respiratory system and eyes and consequently the OSHA permissible exposure limit is 1 ppm (29 CFR 1910.1000 Table Z-1) calculated as an eight hour time weighted average and the NIOSH immediately dangerous to life and health limit is 75 ppm. Therefore, engineering controls, personal protective equipment, gas monitoring etc. should be employed where high concentrations of hydrogen peroxide are used in the workplace. Vaporized hydrogen peroxide is one of the chemicals approved for decontamination of anthrax spores from contaminated buildings, such as occurred during the 2001 anthrax attacks in the U.S. It has also been shown to be effective in removing exotic animal viruses, such as avian influenza and Newcastle disease from equipment and surfaces.
- ✓ The antimicrobial action of hydrogen peroxide can be enhanced by surfactants and organic acids. The resulting chemistry is known as Accelerated Hydrogen Peroxide and is produced by Virox Technologies Inc. A 2% solution, stabilized for extended use, achieves high-level disinfection in 5 minutes, and is suitable for disinfecting medical equipment made from hard plastic, such as in endoscopes.[19] The evidence available suggests that products based on Accelerated Hydrogen Peroxide, apart from being good germicides, are safer for humans and benign to the environment.
- ✓ Iodine is usually dissolved in an organic solvent or as Lugol's iodine solution. It is used in the poultry industry. It is added to the birds' drinking water. In human and veterinary medicine, iodine products are widely used to prepare incision sites prior to surgery. Although it increases both scar tissue formation and healing time, tincture of iodine is used as an antiseptic for skin cuts and scrapes, and remains among the most effective antiseptics known.
- ✓ Ozone is a gas used for disinfecting water, laundry, foods, air, and surfaces. It is chemically aggressive and destroys many organic compounds, resulting in rapid decolorization and deodorization in addition to disinfection. Ozone decomposes relatively quickly, however, so that tap water chlorination cannot be entirely replaced by ozonation, as the ozone would decompose already in the water piping. Instead, it is used to remove the bulk of oxidizable matter from the water, which would produce small amounts of organochlorides if treated with chlorine only.
- ✓ Peracetic acid is a disinfectant produced by reacting hydrogen peroxide with acetic acid. It is broadly effective against microorganisms and is not deactivated by catalase and peroxidase, the enzymes that break down hydrogen peroxide. It also breaks down to food safe and environmentally friendly residues (acetic acid and hydrogen peroxide), and therefore can be used in non-rinse applications. It can be used over a wide temperature range (0-40°C), wide pH range (3.0-7.5), in clean-in-place (CIP) processes, in hard water conditions, and is not affected by protein residues.
- ✓ Performic acid is the simplest and most powerful perorganic acid. Formed from the reaction of hydrogen peroxide and formic acid, it reacts more rapidly and powerfully than peracetic acid before breaking down to water and carbon dioxide.
- ✓ Potassium permanganate ($KMnO_4$) is a purplish-black crystalline powder that colors everything it touches, through a strong oxidizing action. This includes staining "stainless" steel, which somehow limits its use and makes it necessary to use plastic or glass containers. It is used to disinfect aquariums and is also widely used in community

swimming pools to disinfect ones feet before entering the pool. Typically, a large shallow basin of KMnO4/water solution is kept near the pool ladder. Participants are required to step in the basin and then go into the pool. Additionally, it is widely used to disinfect community water ponds and wells in tropical countries, as well as to disinfect the mouth before pulling out teeth. It can be applied to wounds in dilute solution.

Understanding Waterborne Viruses

More than 100 types of human pathogenic viruses may be present in fecal-contaminated waters (Havelaar and others, 1993). Treatment processes and watershed management strategies designed on the basis of bacteriological criteria do not necessarily protect against viral infection because viruses are generally more persistent in the environment and are not removed as completely by treatment. In addition, because of their smaller size, viruses (0.023 to 0.080 µm) are transported further in ground water than bacteria (0.5 to 3µ m) or protozoan pathogens (4 to 15 µm) (Abbaszadegan and others, 1998). Because of the importance of viruses as a major public health concern, new methods for detection of enteric viruses and the search for indicators of viral contamination continue.

The current method for culturing enteric viruses under the ICR (U.S. Environmental Protection Agency, 1996c) is recognized as being difficult to implement; therefore, the ICR does not preclude the use of additional methods for research purposes. In addition, cell-culture methods are not available or suitable
for all viruses of public health concern. One method, reverse-transcriptase-polymerase chain reaction (RT-PCR), a gene-probe method that amplifies and recognizes the nucleic acids of target viruses, has been adequately validated by the USEPA (G. Shay Fout, U.S. Environmental Protection Agency, written
commun., 1997) and is becoming widely used for environmental monitoring of enteric viruses. The RTPCR method, however, does not determine the infectivity of the virus, and it is technically demanding, time consuming, and costly for routine use.

Because monitoring of enteric viruses is recognized as being difficult and time consuming, some researchers advocate the use of coliphage as indicator viruses for fecal contamination (Sobsey and others, 1995). Coliphages are bacteriophages that infect and replicate in coliform bacteria. The two main groups of coliphages that are considered as candidates for viral indicators are somatic and F-specific coliphages.

Somatic coliphages infect coliform bacteria by attachment to the outer cell membrane or cell wall. They are widely distributed in both fecal-contaminated and uncontaminated waters; therefore, they may not be reliable indicators of fecal contamination (Sobsey and others, 1995). F-specific coliphages attach only to the F-pilus of coliforms that carry the F+ plasmid; F-pili are made only by bacteria grown at higher temperatures.

Hence, F-specific coliphages found in environmental samples presumably come from warm-blooded animals or sewage (Handzel and others 1993). Although somatic and F-specific coliphages are not consistently found in feces, they are found in high numbers in sewage and are thought to be reliable indicators of the sewage contamination of waters (International Association of Water Pollution Research and Control, 1991). Coliphage is also recognized to be representative of the survival and transport of viruses in the environment. To date, however, coliphage has not been found to correlate with the presence of pathogenic viruses.

Sampling Procedures

Streamwater Sample Collection

When designing a sampling plan, consider that the spatial and temporal distribution of microorganisms in surface water can be as variable as the distribution of suspended sediment because microorganisms are commonly associated with solid particles. The standard samplers used in by the majority of samplers can be used to collect streamwater samples for bacterial and viral indicators, Cryptosporidium, and Giardia providing that the equipment coming in contact with the water is properly cleaned and sterilized. For streamwater samples, these include the US-D77TM, US-D95, US-DH81, and weighted- and open-bottle samplers with autoclavable Teflon, glass, or polypropylene components.

• Prepare a separate set of sterile equipment (bottles nozzles, and caps) for sampling at each site.
• Follow sampling techniques given in Shelton (1994) to ensure that a sample is representative of the flow in the cross section. Use equal-width increment (EWI) or equal-discharge-increment (EDI) methods described in Edwards and Glysson (1988), unless site characteristics dictate otherwise.
• Because churn and cone splitters cannot be autoclaved, use a sterile 3-L bottle to composite subsamples for bacterial and viral indicators when using EDI and EWI methods. If possible, composite
by collecting subsamples at vertical locations in the cross section without overfilling the bottle.
• Alternatively, if the stream depth and (or) velocity is not sufficient to use depth-width integrating techniques, collect a sample by a hand-dip method (Myers and Sylvester, 1997).
• Collect approximately 1 L of streamwater for bacterial and viral indicators. Process the sample for E. coli and enterococci; send the remainder (at least 500 mL) on ice to the laboratory for C. perfringens and coliphage analysis.

Cryptosporidium and Giardia Analysis
For Cryptosporidium and Giardia analysis by Method 1623 (U.S. Environmental Protection Agency, 1999c), collect 10 L of streamwater for each protozoan pathogen using standard sampling techniques described in Myers and Sylvester (1997). Special sterilization procedures are needed for equipment used in the collection of samples for Cryptosporidium and Giardia. Autoclaving is not effective in neutralizing the epitopes on the surfaces of the oocysts and cysts that will react with the antibodies used for detection.
• Wash and scrub the equipment with soap and warm tap water to remove larger particulates and rinse with deionized water. Submerge the equipment in a vessel containing 12 percent hypochlorite solution for 30 minutes. Wash the equipment free of residual sodium hypochlorite solution with three rinses of filter-sterilized water; do not de-chlorinate the equipment using sodium thiosulfate. This procedure is best done in the office with dedicated sampling equipment for each site; however, it may be done in the field as long as the hypochlorite solution is stored and disposed of properly.
• Composite the sample in a 10-L cubitainer that is pre-sterilized by the manufacturer. The cubitainer is sent in a cardboard box to laboratory for Cryptosporidium analysis. The sample does not have to be kept on ice during transport. At this time, two methods are recommended for analysis of water samples for enteric viruses: (1) the reverse-transcriptase, polymerase chain reaction (RTPCR) method (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997) and (2) the cell-culture method (U.S. Environmental Protection Agency, 1996c). Sampling and equipment cleaning procedures are more thoroughly described elsewhere (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997; U.S. Environmental Protection Agency, 1996c). Briefly, 100 L of streamwater is pumped by means of a specially designed sampling apparatus and passed through a Virosorb1 1MDS filter (Cuno, Meriden, Conn.).The 1MDS filters, which remove viruses present in the water by charge interactions, are kept on ice and sent to a central laboratory for virus elution, concentration, and detection.

Ground-Water Sample Collection
Collecting
Ground-water samples by use of sterile techniques requires knowledge of the type of well, its use, its construction, and its condition.
• Swab the electronic tape used for water-level measurements with isopropyl or ethyl alcohol.
• In sampling subunit survey wells, once purging criteria have been met as described in Koterba and others (1995), collect the sample directly from the tap into a sterile container.
• Remove screens, filters, other devices from the tap before collecting the sample, and do not sample from leaking taps.

Because we are interested in the microbial population in the ground water and not in the distribution system, it is best to sample directly from the wellhead using a pump with sterile tubing, if possible.

Disinfection of Water and Wastewater
The disinfection of potable water and wastewater provides a degree of protection from contact with pathogenic organisms including those causing cholera, polio, typhoid, hepatitis and a number of other bacterial, viral and parasitic diseases. Disinfection is a process where a significant percentage of pathogenic organisms are killed or controlled. As an individual pathogenic organism can be difficult to detect in a large volume of water or wastewater, disinfection efficacy is most often measured using "indicator organisms" that coexist in high quantities where pathogens are present. The most common indicator organism used in the evaluation of drinking water is Total Coliform (TC), unless there is a reason to focus on a specific pathogen.

The most common indicator organism for wastewater evaluation is fecal coliform but there has been discussion regarding the use of Escherichia coli (E. coli) or Total Coliform. As domestic wastewater contains approximately 1,000 times more indicator organisms than typical surface water, understanding wastewater disinfection will make it easier to understand water disinfection.

Chlorine gas is primarily a respiratory irritant and concentrations in air above one ppm can usually be detected by most persons. Chlorine causes varying degrees of irritation of the skin, mucus membranes, and the respiratory system, depending on the concentration and the duration of exposure. Severe exposure can cause death, but the severe irritating effect makes it unlikely that anyone would remain in the chlorine-containing atmosphere unless trapped or unconscious.

Liquid chlorine may cause skin and eye burns upon contact with these tissues. Chlorine produces no known cumulative or chronic effect, and complete recovery usually can be expected to occur shortly following mild, short term exposure. An eight-hour time-weighted exposure of one ppm and a one-hour weighted exposure are the current federal Occupational Safety and Health Administration (OSHA) standards.

Understanding Bacteriophage
Bacteriophages may have a lytic cycle or a lysogenic cycle, and a few viruses are capable of carrying out both. With lytic phages such as the T4 phage, bacterial cells are broken open (lysed) and destroyed after immediate replication of the virion. As soon as the cell is destroyed, the phage progeny can find new hosts to infect. Lytic phages are more suitable for phage therapy. Some lytic phages undergo a phenomenon known as lysis inhibition, where completed phage progeny will not immediately lyse out of the cell if extracellular phage concentrations are high. This mechanism is not identical to that of temperate phage going dormant and is usually temporary.

In contrast, the lysogenic cycle does not result in immediate lysing of the host cell. Those phages able to undergo lysogeny are known as temperate phages. Their viral genome will integrate with host DNA and replicate along with it fairly harmlessly, or may even become established as a plasmid. The virus remains dormant until host conditions deteriorate, perhaps due to depletion of nutrients; then, the endogenous phages (known as prophages) become active. At this point they initiate the reproductive cycle, resulting in lysis of the host cell. As the lysogenic cycle allows the host cell to continue to survive and reproduce, the virus is reproduced in all of the cell's offspring. An example of a bacteriophage known to follow the lysogenic cycle and the lytic cycle is the phage lambda of E. coli.

Sometimes prophages may provide benefits to the host bacterium while they are dormant by adding new functions to the bacterial genome in a phenomenon called lysogenic conversion. An eminent example is the conversion of a harmless strain of Vibrio cholerae by a phage into a highly virulent one, which causes cholera.

Attachment and Penetration
To enter a host cell, bacteriophages attach to specific receptors on the surface of bacteria, including lipopolysaccharides, teichoic acids, proteins, or even flagella. This specificity means a bacteriophage can infect only certain bacteria bearing receptors to which they can bind, which in turn determines the phage's host range. Host growth conditions also influence the ability of the phage to attach and invade them. As phage virions do not move independently, they must rely on random encounters with the right receptors when in solution (blood, lymphatic circulation, irrigation, soil water, etc.).

Myovirus bacteriophages use a hypodermic syringe-like motion to inject their genetic material into the cell. After making contact with the appropriate receptor, the tail fibers flex to bring the base plate closer to the surface of the cell; this is known as reversible binding. Once attached completely, irreversible binding is initiated and the tail contracts, possibly with the help of ATP present in the tail, injecting genetic material through the bacterial membrane. Podoviruses lack an elongated tail sheath similar to that of a myovirus, so they instead use their small, tooth-like tail fibers to enzymatically degrade a portion of the cell membrane before inserting their genetic material.

Virions
A virion is a complete functional virus that has the capacity to infect living tissue. This means that it includes the genetic material, the capsid, the enveloppe and the membrane proteins that allow the virus to bind to its host and enter it. A virus will not have an enveloppe within a cell. If the cell was burst artificially, then these virus particles cannot be called virion because they will lack certain proteins that will make them infectious even though the genetic material is present. Not all viruses have enveloppes, but most viruses have certain proteins that are necessary to permit them to enter the host cell.

Biomolecules found in virions: genetic material, either DNA or RNA, single or double stranded, nucleoprotein capsid, maybe an enveloppe usually receptor proteins or enzymes that permit binding or entry into the host. A viroid is a plant pathogen consisting of a circular piece of RNA without a protein coat.

Understanding Disinfection

Wastewater Disinfection
There are a number of chemicals and processes that will disinfect wastewater, but none are universally applicable. Most septic tanks discharge into various types of subsurface wastewater infiltration systems (SWIS), such as tile fields or leach fields. These applications rely on the formation of a biomat at the gravel-soil interface where "biodegradation and filtration combine to limit the travel of pathogens." Aerobic treatment processes reduce pathogens, but not enough to qualify as a disinfection process. "Chlorination/dechlorination has been the most widely used disinfection technology in the U.S.; ozonation and UV light are emerging technologies." Each of these three methods have different considerations for the disinfection of wastewater.

Water Disinfection
Disinfection is usually the final stage in the water treatment process in order to limit the effects of organic material, suspended solids and other contaminants. Like the disinfection of wastewater, the primary methods used for the disinfection of water in very small (25-500 people) and small (501-3,300 people) treatment systems are ozone, ultraviolet irradiation (UV) and chlorine. There are numerous alternative disinfection processes that have been less widely used in small and very small water treatment systems, including chlorine dioxide, potassium permanganate, chloramines and peroxone (ozone/hydrogen peroxide).

Surface waters have been the focal point of water disinfection regulations since their inception, as groundwaters (like wells) have been historically considered to be free of microbiological contamination. Current data indicates this to not be true. Amendments to the Safe Drinking Water Act in 1996 mandate the development of regulations to require disinfection of groundwater "as necessary." While these regulations will apply to very small systems serving twenty-five people at least 60 days out of the year, the rules will not apply to private wells. However, the EPA recommends that wells be tested at least once per year and disinfected as necessary. While these proposed regulations have not yet been finalized, they will likely include; testing by each state, identification of contaminated water supplies, corrective action requiring disinfection and compliance monitoring. The rules are currently scheduled to be implemented in July 2003.

Residual Disinfection
The EPA requires a residual level of disinfection of water in pipelines to prevent microbial re-growth and help protect treated water throughout the distribution system. EPA”s maximum residual disinfection levels (MRDLs) are 4 mg/l for chlorine, 4 mg/l for chloramines and 0.8 mg/l for chlorine dioxide. Although chlorine levels are usually significantly lower in tap water, EPA believes that levels as high as the MRDLs pose no risk of adverse health effects, allowing for an adequate margin of safety (U.S. EPA, 1998a).

Chlorate Ion
The chlorate anion has the formula ClO-3. In this case, the chlorine atom is in the +5 oxidation state. "Chlorate" can also refer to chemical compounds containing this anion; chlorates are the salts of chloric acid. "Chlorate", when followed by a roman numeral in parentheses, e.g. chlorate (VII), refers to a particular oxyanion of chlorine. As predicted by VSEPR, chlorate anions have trigonal pyramidal structures.

Chlorates are powerful oxidizers and should be kept away from organics or easily oxidized materials. Mixtures of chlorate salts with virtually any combustible material (sugar, sawdust, charcoal, organic solvents, metals, etc.) will readily deflagrate. Chlorates were once widely used in pyrotechnics for this reason, though their use has fallen due to their instability. Most pyrotechnic applications which formerly used chlorates in the past now use the more stable perchlorates instead

Examples of chlorates include

- ✓ potassium chlorate, $KClO_3$
- ✓ sodium chlorate, $NaClO_3$
- ✓ magnesium chlorate, $Mg(ClO_3)2$

Chloride Ion

The chloride ion is formed when the element chlorine, a halogen, gains an electron to form an anion (negatively-charged ion) Cl-. The salts of hydrochloric acid contain chloride ions and can also be called chlorides. The chloride ion, and its salts such as sodium chloride, are very soluble in water. It is an essential electrolyte located in all body fluids responsible for maintaining acid/base balance, transmitting nerve impulses and regulating fluid in and out of cells.

The word chloride can also form part of the name of chemical compounds in which one or more chlorine atoms are covalently bonded. For example, methyl chloride, more commonly called chloromethane, (CH_3Cl) is an organic covalently bonded compound, which does not contain a chloride ion.

Chloride is used to form salts that can preserve food such as sodium chloride. Other salts such as calcium chloride, magnesium chloride, potassium chloride have varied uses ranging from medical treatments to cement formation.

An example is table salt, which is sodium chloride with the chemical formula NaCl. In water, it dissociates into Na+ and Cl- ions.

Examples of inorganic covalently bonded chlorides that are used as reactants are:

- ✓ Phosphorus trichloride, phosphorus pentachloride, and thionyl chloride, all three of which reactive chlorinating reagents that have been used in a laboratory.
- ✓ Disulfur dichloride (S_2Cl_2), used for vulcanization of rubber.

A chloride ion is also the prosthetic group present in the amylase enzyme. Another example is calcium chloride with the chemical formula $CaCl_2$. Calcium chloride is a salt that is marketed in pellet form for removing dampness from rooms. Calcium chloride is also used for maintaining unpaved roads and for sanite fortifying roadbases for new construction. In addition, Calcium chloride is widely used as a deicer since it is effective in lowering the melting point when applied to ice.

In the petroleum industry, the chlorides are a closely monitored constituent of the mud system. An increase of the chlorides in the mud system may be an indication of drilling into a high-pressure saltwater formation. Its increase can also indicate the poor quality of a target sand. Chloride is also a useful and reliable chemical indicator of river / groundwater fecal contamination, as chloride is a non-reactive solute and ubiquitous to sewage & potable water. Many water regulating companies around the world utilize chloride to check the contamination levels of the rivers and potable water sources.

Chlorite Ion

The chlorite ion is ClO_2-. A chlorite (compound) is a compound that contains this group, with chlorine in oxidation state +3. Chlorites are also known as salts of chlorous acid. Chlorine can assume oxidation states of -1, +1, +3, +5, or +7 within the corresponding anions Cl-, ClO-, ClO_2-, ClO_3-, or ClO_4-, known commonly and respectively as chloride, hypochlorite, chlorite, chlorate, and perchlorate. An additional oxidation state of +4 is seen in the neutral compound chlorine dioxide ClO_2, which has a similar structure to chlorite ClO_2- (oxidation state +3) and the cation chloryl (ClO_2+) (oxidation state +5).

Chlorine Dioxide

Chlorine dioxide is a chemical compound with the formula ClO_2. This yellowish-green gas crystallizes as bright orange crystals at -59 °C. As one of several oxides of chlorine, it is a potent and useful oxidizing agent used in water treatment and in bleaching. The molecule ClO2 has an odd number of valence electrons and it is therefore a paramagnetic radical. Its electronic structure has long baffled chemists because none of the possible Lewis structures are very satisfactory. In 1933 L.O. Brockway proposed a structure that involved a three-electron bond.

Chemist Linus Pauling further developed this idea and arrived at two resonance structures involving a double bond on one side and a single bond plus three-electron bond on the other. In Pauling's view the latter combination should represent a bond that is slightly weaker than the double bond. In molecular orbital theory this idea is commonplace if the third electron is placed in an anti-bonding orbital. Later work has confirmed that the HOMO is indeed an incompletely-filled orbital.

Chlorine dioxide is a highly endothermic compound that can decompose extremely violently when separated from diluting substances. As a result, preparation methods that involve producing solutions of it without going through a gas phase stage are often preferred. Arranging handling in a safe manner is essential.

In the laboratory, ClO_2 is prepared by oxidation of sodium chlorite:

$$2\ NaClO_2 + Cl_2 - 2\ ClO_2 + 2\ NaCl$$

Over 95% of the chlorine dioxide produced in the world today is made from sodium chlorate and is used for pulp bleaching. It is produced with high efficiency by reducing sodium chlorate in a strong acid solution with a suitable reducing agent such as methanol, hydrogen peroxide, hydrochloric acid or sulfur dioxide. Modern technologies are based on methanol or hydrogen peroxide, as these chemistries allows the best economy and do not co-produce elemental chlorine. The overall reaction can be written;

Chlorate + Acid + reducing agent - Chlorine Dioxide + By-products

The reaction of sodium chlorate with hydrochloric acid in a single reactor is believed to proceed via the following pathway:

$$HClO_3 + HCl - HClO_2 + HOCl$$

$$HClO_3 + HClO_2 - 2\ ClO_2 + Cl_2 + 2\ H_2O$$

$$HOCl + HCl - Cl_2 + H_2O$$

The commercially more important production route uses methanol as the reducing agent and sulfuric acid for the acidity. Two advantages by not using the chloride-based processes are that there is no formation of elemental chlorine, and that sodium sulfate, a valuable chemical for the pulp mill, is a side-product. These methanol-based processes provide high efficiency and can be made very safe.

A much smaller, but important, market for chlorine dioxide is for use as a disinfectant. Since 1999 a growing proportion of the chlorine dioxide made globally for water treatment and other small-scale applications has been made using the chlorate, hydrogen peroxide and sulfuric acid method, which can produce a chlorine-free product at high efficiency.

Traditionally, chlorine dioxide for disinfection applications has been made by one of three methods using sodium chlorite or the sodium chlorite - hypochlorite method:

$2\ NaClO_2 + 2\ HCl + NaOCl - 2\ ClO_2 + 3\ NaCl + H_2O$

or the sodium chlorite - hydrochloric acid method:

$5\ NaClO_2 + 4\ HCl - 5\ NaCl + 4\ ClO_2 + 2\ H_2O$

All three sodium chlorite chemistries can produce chlorine dioxide with high chlorite conversion yield, but unlike the other processes the chlorite-HCl method produces completely chlorine-free chlorine dioxide but suffers from the requirement of 25% more chlorite to produce an equivalent amount of chlorine dioxide. Alternatively, hydrogen peroxide may efficiently be used also in small scale applications.

Very pure chlorine dioxide can also be produced by electrolysis of a chlorite solution:

$2\ NaClO_2 + 2\ H_2O - 2\ ClO_2 + 2\ NaOH + H_2$

High purity chlorine dioxide gas (7.7% in air or nitrogen) can be produced by the Gas: Solid method, which reacts dilute chlorine gas with solid sodium chlorite.

$2\ NaClO_2 + Cl_2 - 2\ ClO_2 + 2\ NaCl$

These processes and several slight variations have been reviewed.

Haloacetic Acids
Haloacetic acids are carboxylic acids in which a halogen atom takes the place of a hydrogen atom in acetic acid. Thus, in a monohaloacetic acid, a single halogen would replace a hydrogen atom. For example, chloroacetic acid would have the structural formula CH_2ClCO_2H. In the same manner, in dichloroacetic acid two chlorine atoms would take the place of two hydrogen atoms ($CHCl_2CO_2H$). The inductive effect caused by the electronegative halogens often result in the higher acidity of these compounds by stabilizing the negative charge of the conjugate base.

Contaminants in Drinking Water
Haloacetic acids (HAAs) are a common undesirable by-product of drinking water chlorination. Exposure to such disinfection by-products in drinking water has been associated with a number of health outcomes by epidemiological studies, although the putative agent in such studies has not been identified.

In water, HAAs are stable, with the five most common being:

- ✓ monochloroacetic acid (MCA) $ClCH_2COOH$;
- ✓ dichloroacetic acid (DCA) $Cl_2CHCOOH$;
- ✓ trichloroacetic acid (TCA) Cl_3CCOOH;
- ✓ monobromoacetic acid (MBA) $BrCH_2COOH$;
- ✓ dibromoacetic acid (DBA) $Br_2CHCOOH$.

Collectively, these are referred to as the HAA5. HAAs can be formed by chlorination, ozonation or chloramination of water with formation promoted by slightly acidic water, high organic matter content and elevated temperature. Chlorine from the water disinfection process can react with organic matter and small amounts of bromide present in water to produce various HAAs. A study published in August 2006 found that total levels of HAAs in drinking water were not affected by storage or boiling, but that filtration was effective in decreasing levels.

Hypochlorites
Hypochlorites are calcium or sodium salts of hypochlorous acid and are supplied either dry or in liquid form (as, for instance, in commercial bleach). The same residuals are obtained as with gas chlorine, but the effect on the pH of the treated water is different. Hypochlorite compounds contain an excess of alkali and tend to raise the pH of the water. Calcium hypochlorite tablets are the predominant form in use in the United States for swimming pools. Sodium hypochlorite is the only liquid hypochlorite disinfectant in current use. There are several grades and proprietary forms available. Pound-for-pound of available chlorine, hypochlorite compounds have oxidizing powers equal to gas chlorine and can be employed for the same purposes in water treatment. Gas chlorination requires a larger initial investment for feed equipment than what is needed for hypochlorite compounds.

Calcium hypochlorite materials used in the water industry are chemically different from those materials variously marketed for many years as bleaching powder, chloride of lime, or chlorinated lime. Materials now in common use are high-test calcium hypochlorites containing about 70 percent available chlorine and marketed under several trade names.

High-test calcium hypochlorites are white corrosive solids that give off a strong chlorine odor. Granular powdered or tablet forms are commercially available and all are readily soluble in water.

Sodium hypochlorite is sold only as a liquid and is normally referred to as liquid bleach. It is generally available in concentrations of 5 to 15 percent available chlorine. These solutions are clear, light yellow, strongly alkaline, and corrosive in addition to having a strong chlorine smell.

High-test hypochlorites, though highly active, are relatively stable throughout production, packaging, distribution, and storage. Storage at 86° F. for a year may reduce the available chlorine by about 10 percent. Storing at lower temperatures reduces the loss. All sodium-hypochlorite solutions are unstable to some degree and deteriorate more rapidly than the dry compounds. Most producers recommend a shelf life of 60 to 90 days. Because light and heat accelerate decomposition, containers should be stored in a dry, cool, and dark area.

Disinfection Byproducts
Disinfection byproducts are formed when disinfectants used in water treatment plants react with bromide and/or natural organic matter (i.e., decaying vegetation) present in the source water. Different disinfectants produce different types or amounts of disinfection byproducts. Disinfection byproducts for which regulations have been established have been identified in drinking water, including trihalomethanes, haloacetic acids, bromate, and chlorite.

Trihalomethanes (THM)
Trihalomethanes (THM) are a group of four chemicals that are formed along with other disinfection byproducts when chlorine or other disinfectants used to control microbial contaminants in drinking water react with naturally occurring organic and inorganic matter in water. The trihalomethanes are chloroform, bromodichloromethane, dibromochloromethane, and bromoform. EPA has published the Stage 1 Disinfectants/Disinfection Byproducts Rule to regulate total trihalomethanes (TTHM) at a maximum allowable annual average level of 80 parts per billion. This standard will replace the current standard of a maximum allowable annual average level of 100 parts per billion in December 2001 for large surface water public water systems. The standard will become effective for the first time in December 2003 for small surface water and all ground water systems.

Haloacetic Acids (HAA5)
Haloacetic Acids (HAA5) are a group of chemicals that are formed along with other disinfection byproducts when chlorine or other disinfectants used to control microbial contaminants in drinking water react with naturally occurring organic and inorganic matter in water. The regulated haloacetic acids, known as HAA5, are: monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid. EPA has published the Stage 1

Disinfectants/Disinfection Byproducts Rule to regulate HAA5 at 60 parts per billion annual average. This standard will become effective for large surface water public water systems in December 2001 and for small surface water and all ground water public water systems in December 2003.

Bromate is a chemical that is formed when ozone used to disinfect drinking water reacts with naturally occurring bromide found in source water. EPA has established the Stage 1 Disinfectants/Disinfection Byproducts Rule to regulate bromate at annual average of 10 parts per billion in drinking water. This standard will become effective for large public water systems by December 2001 and for small surface water and all ground public water systems in December 2003.

Chlorite
Chlorite is a byproduct formed when chlorine dioxide is used to disinfect water. EPA has published the Stage 1 Disinfectants/Disinfection Byproducts Rule to regulate chlorite at a monthly average level of 1 part per million in drinking water. This standard will become effective for large surface water public water systems in December 2001 and for small surface water and all ground water public water systems in December 2003.

Chloroform
Chloroform, typically the most prevalent THM measured in chlorinated water, is probably the most thoroughly studied disinfection byproduct. Toxicological studies have shown that high levels of chloroform can cause cancer in laboratory animals. Extensive research conducted since the early 1990s provides a clearer picture of what this means for humans exposed to far lower levels through drinking water.

One study (Larson et al. 1994a) conducted by the Centers for Health Research (CIIT) observed that a very large dose of chloroform, when given to mice once per day into the stomach (a procedure known as gavage), produced liver damage and eventually cancer. In a second CIIT cancer study (Larson et al., 1994b), mice were given the same daily dose of chloroform through the animals' drinking water. This time, no cancer was produced. Follow-up research showed that the daily gavage doses overwhelmed the capability of the liver to detoxify the chloroform, causing liver damage, cell death and regenerative cell growth, thereby increasing risks for cell mutation and cancer in exposed organs. When chloroform was given through drinking water, however, the liver could continually detoxify the chloroform as the mice sipped the water throughout the day. Without the initial liver toxicity, there was no cancer in the liver, kidney or other exposed organs (Butterworth et al., 1998).

In its most recent risk assessment, EPA considered the wealth of available information on chloroform, including the important work done at CIIT. EPA concludes that exposure to chloroform below the threshold level that causes cell damage is unlikely to increase the risk of cancer. While chloroform is likely to be carcinogenic at a high enough dose, exposures below a certain dose range are unlikely to pose any cancer risk to humans (US EPA, 2002a). For drinking water meeting EPA standards, chloroform is unlikely to be a health concern.

Sodium Chlorate
Sodium chlorate is a chemical compound with the chemical formula ($NaClO_3$). When pure, it is a white crystalline powder that is readily soluble in water. It is hygroscopic. It decomposes above 250 °C to release oxygen and leave sodium chloride. Industrially, sodium chlorate is synthesized from the electrolysis of a hot sodium chloride solution in a mixed electrode tank:

$$NaCl + 3H_2O - NaClO_3 + 3H_2$$

It can also be synthesized by passing chlorine gas into a hot sodium hydroxide solution. It is then purified by crystallization.

Chemical Oxygen Generation
Chemical oxygen generators, such as those in commercial aircraft, provide emergency oxygen to passengers to protect them from drops in cabin pressure by catalytic decomposition of sodium chlorate. The catalyst is normally iron powder. Barium peroxide (BaO_2) is used to absorb the chlorine which is a minor product in the decomposition. Iron powder is mixed with sodium chlorate and ignited by a charge which is activated by pulling on the emergency mask. The reaction produces more oxygen than is required for combustion. Similarly, the Solidox welding system used pellets of sodium chlorate mixed with combustible fibers to generate oxygen.

Toxicity in Humans
Due to its oxidative nature, sodium chlorate can be very toxic if ingested. The oxidative effect on hemoglobin leads to methaemoglobin formation, which is followed by denaturation of the globin protein and a cross-linking of erythrocyte membrane proteins with resultant damage to the membrane enzymes.

This leads to increased permeability of the membrane, and severe hemolysis. The denaturation of hemoglobin overwhelms the capacity of the G6PD metabolic pathway. In addition, this enzyme is directly denatured by chlorate reducing its activity. Therapy with ascorbic acid and methylene blue are frequently used in the treatment of methemoglobinemia. However, since methylene blue requires the presence of NADPH that requires normal functioning of G6PD system, it is less effective than in other conditions characterized by hemoglobin oxidation.

Acute severe hemolysis results, with multi-organ failure, including DIC and renal failure. In addition there is a direct toxicity to the proximal renal tubule. The treatment will consist of exchange transfusion, peritoneal dialysis or hemodialysis.

Developmental and Reproductive Effects
Several epidemiology studies have reported a possible association between disinfection byproducts and adverse reproductive outcomes, including spontaneous abortion (miscarriage). One study of women in several California communities (Waller et al. 1998) found a stronger association with bromodichloromethane (BDCM) than with other byproducts. Because the available studies have significant limitations, EPA and the American Water Works Association Research Foundation are sponsoring a new epidemiology study to replicate the 1998 Waller study.

When the Waller study was published, the available toxicology data on reproductive and developmental effects of some DBPs was quite limited. It was recognized that BDCM, in particular, should be thoroughly studied for a potential causal relationship to reproductive and developmental toxicity. The Research Foundation for Health and Environmental Effects , a tax-exempt foundation established by the Chlorine Chemistry Division of the American Chemistry Council, sponsored a set of animal studies (Christian et al. 2001, 2002) including two developmental toxicity studies on BDCM, a reproductive toxicity study on BDCM, and a reproductive toxicity study on dibromoacetic acid (DBA). The studies, published in the International Journal of Toxicology, found no adverse effects from BDCM and DBA at dose levels thousands of times higher than what humans are exposed to through drinking water. The studies were designed to comply with stringent EPA guidelines, and each study was independently monitored and peer reviewed.

Formulations
Sodium chlorate comes in dust, spray and granule formulations. There is a risk of fire and explosion in dry mixtures with other substances, especially organic materials, and other herbicides, sulfur, phosphorus, powdered metals, strong acids. In particular, when mixed with sugar, it has explosive properties. If accidentally mixed with one of these substances it should not be stored in human dwellings. Marketed formulations contain a fire retardant, but this has little effect if deliberately ignited. Most commercially available chlorate weedkillers contain

approximately 53% sodium chlorate with the balance being a fire depressant such as sodium metaborate or ammonium phosphates.

Sodium Chlorite
Sodium chlorite, like many oxidizing agents, should be protected from inadvertent contamination by organic materials to avoid the formation of an explosive mixture. The chemical explodes on percussive impact, and will ignite if combined with a strong reducing agent.

Toxicity
Sodium chlorite is a strong oxidant and can therefore be expected to cause clinical symptoms similar to the well-known sodium chlorate: methemoglobinemia, hemolysis, renal failure.[14] A dose of 10-15 grams of sodium chlorate can be lethal. Methemoglobemia had been demonstrated in rats and cats, and recent studies by the EMEA have confirmed that the clinical symptomatology is very similar to the one caused by sodium chlorate in the rat, mouse, rabbit, and the green monkey. There is only one human case in the medical literature of chlorite poisoning. It seems to confirm that the toxicity is equal to sodium chlorate. From the analogy with sodium chlorate, even small amounts of about 1 gram can be expected to cause nausea, vomiting and even life-threatening hemolysis in Glucose-6-Phosphate Dehydrogenase deficient persons. The EPA has set a maximum contaminant level of 1 milligram of chlorite per liter (1 mg/L) in drinking water.

Manufacture
The free acid, chlorous acid, $HClO_2$, is only stable at low concentrations. Since it cannot be concentrated, it is not a commercial product. However, the corresponding sodium salt, sodium chlorite, $NaClO_2$ is stable and inexpensive enough to be commercially available. The corresponding salts of heavy metals (Ag+, Hg+, Tl+, Pb2+, and also Cu2+ and NH_4+) decompose explosively with heat or shock. Sodium chlorite is derived indirectly from sodium chlorate, $NaClO_3$. First, the explosive (only at concentrations greater than 10% in atmosphere) chlorine dioxide, ClO_2 is produced by reducing sodium chlorate in a strong acid solution with a suitable reducing agent (for example, sodium sulfite, sulfur dioxide, or hydrochloric acid). The chlorine dioxide is then absorbed into an alkaline solution and reduced with hydrogen peroxide (H_2O_2), yielding sodium chlorite.

Stachybotrys
Stachybotrys is a genus of molds, or asexually-reproducing, filamentous fungi. Closely related to the genus Memnoniella, most Stachybotrys species inhabit materials rich in cellulose. The genus has a widespread distribution, and contains about 50 species. The most infamous species, S. chartarum (also known as S. atra) and S. chlorohalonata are known as "black mold" or "toxic black mold" in the U.S. and are frequently associated with poor indoor air quality that arises after fungal growth on water-damaged building materials

Symptoms of Stachybotrys Exposure in Humans
Exposure to the mycotoxins present in Stachybotrys chartarum or Stachybotrys atra can have a wide range of effects. Depending on the length of exposure and volume of spores inhaled or ingested, symptoms can manifest as chronic fatigue or headaches, fever, irritation to the eyes, mucous membranes of the mouth, nose and throat, sneezing, rashes, and chronic coughing. In severe cases of exposure or cases exacerbated by allergic reaction, symptoms can be extreme including nausea, vomiting, and bleeding in the lungs and nose.

Understanding Commonly Used Water Disinfectants
Almost all U.S. systems that disinfect their water use some type of chlorine-based process, either alone or in combination with other disinfectants. In addition to controlling disease-causing organisms, chlorination offers a number of benefits including:

- Reduces many disagreeable tastes and odors;
- Eliminates slime bacteria, molds and algae that commonly grow in water supply reservoirs, on the walls of water mains and in storage tanks;

- Removes chemical compounds that have unpleasant tastes and hinder disinfection; and
- Helps remove iron and manganese from raw water.

As importantly, only chlorine-based chemicals provide "residual disinfectant" levels that prevent microbial re-growth and help protect treated water throughout the distribution system.

The Risks of Waterborne Disease

Where adequate water treatment is not readily available, the impact on public health can be devastating. Worldwide, about 1.2 billion people lack access to safe drinking water, and twice that many lack adequate sanitation. As a result, the World Health Organization estimates that 3.4 million people, mostly children, die every year from water-related diseases.

Even where water treatment is widely practiced, constant vigilance is required to guard against waterborne disease outbreaks. Well-known pathogens such as E. coli are easily controlled with chlorination, but can cause deadly outbreaks given conditions of inadequate or no disinfection. A striking example occurred in May 2000 in the Canadian town of Walkerton, Ontario. Seven people died and more than 2,300 became ill after E. coli and other bacteria infected the town's water supply. A report published by the Ontario Ministry of the Attorney General concludes that, even after the well was contaminated, the Walkerton disaster could have been prevented if the required chlorine residuals had been maintained.

Some emerging pathogens such as Cryptosporidium are resistant to chlorination and can appear even in high quality water supplies. Cryptosporidium was the cause of the largest reported drinking water outbreak in U.S. history, affecting over 400,000 people in Milwaukee in April 1993. More than 100 deaths are attributed to this outbreak. New regulations from the U.S. Environmental Protection Agency (EPA) will require water systems to monitor Cryptosporidium and adopt a range of treatment options based on source water Cryptosporidium concentrations. Most water systems are expected to meet EPA requirements while continuing to use chlorination.

The Benefits of Chlorine

Potent Germicide

Chlorine disinfectants can reduce the level of many disease-causing microorganisms in drinking water to almost immeasurable levels. Chlorine is added to drinking water to destroy pathogenic (disease-causing) organisms. It can be applied in several forms: elemental chlorine (chlorine gas), sodium hypochlorite solution (bleach) and dry calcium hypochlorite.

When applied to water, each of these forms "free chlorine" (see Sidebar: How Chlorine Kills Pathogens). One pound of elemental chlorine provides approximately as much free available chlorine as one gallon of sodium hypochlorite (12.5% solution) or approximately 1.5 pounds of calcium hypochlorite (65% strength). While any of these forms of chlorine can effectively disinfect drinking water, each has distinct advantages and limitations for particular applications. Almost all water systems that disinfect their water use some type of chlorine-based process, either alone or in combination with other disinfectants.

Taste and Odor Control

Chlorine disinfectants reduce many disagreeable tastes and odors. Chlorine oxidizes many naturally occurring substances such as foul-smelling algae secretions, sulfides and odors from decaying vegetation.

Biological Growth Control

Chlorine disinfectants eliminate slime bacteria, molds and algae that commonly grow in water supply reservoirs, on the walls of water mains and in storage tanks.

Chemical Control
Chlorine disinfectants destroy hydrogen sulfide (which has a rotten egg odor) and remove ammonia and other nitrogenous compounds that have unpleasant tastes and hinder disinfection. They also help to remove iron and manganese from raw water.

Water Treatment
Every day, approximately 170,000 (U.S. EPA, 2002) public water systems treat and convey billions of gallons of water through approximately 880,000 miles (Kirmeyer, 1994) of distribution system piping to U.S. homes, farms and businesses. Broadly speaking, water is treated to render it suitable for human use and consumption. While the primary goal is to produce a biologically (disinfected) and chemically safe product, other objectives also must be met, including: no objectionable taste or odor; low levels of color and turbidity (cloudiness); and chemical stability (non-corrosive and non-scaling). Individual facilities customize treatment to address the particular natural and manmade contamination characteristic of their raw water.

Surface water usually presents a greater treatment challenge than groundwater, which is naturally filtered as it percolates through sediments. Surface water is laden with organic and mineral particulate matter, and may harbor protozoan parasites such as Cryptosporidium parvum and Giardia lamblia.

Water Distribution
In storage and distribution, drinking water must be kept safe from microbial contamination. Frequently, slippery films of bacteria, known as biofilms, develop on the inside walls of pipes and storage containers. Among disinfection techniques, chlorination is unique in that a pre-determined chlorine concentration may be designed to remain in treated water as a measure of protection against harmful microbes encountered after leaving the treatment facility. In the event of a significant intrusion of pathogens resulting, for example, from a broken water main, the level of the average “chlorine residual” will be insufficient to disinfect contaminated water. In such cases, it is the monitoring of the sudden drop in the chlorine residual that provides the critical indication to water system operators that there is a source of contamination in the system.

The Challenge of Disinfection Byproducts
While protecting against microbial contamination is the top priority, water systems must also control disinfection byproducts (DBPs), chemical compounds formed unintentionally when chlorine and other disinfectants react with natural organic matter in water. In the early 1970s, EPA scientists first determined that drinking water chlorination could form a group of byproducts known as trihalomethanes (THMs), including chloroform. EPA set the first regulatory limits for THMs in 1979. While the available evidence does not prove that DBPs in drinking water cause adverse health effects in humans, high levels of these chemicals are certainly undesirable. Cost-effective methods to reduce DBP formation are available and should be adopted where possible.

Chemical Safety (IPCS 2000) Strongly Cautions:
The health risks from these byproducts at the levels at which they occur in drinking water are extremely small in comparison with the risks associated with inadequate disinfection. Thus, it is important that disinfection not be compromised in attempting to control such byproducts. Recent EPA regulations have further limited THMs and other DBPs in drinking water. Most water systems are meeting these new standards by controlling the amount of natural organic material prior to disinfection.

Chlorine and Water System Security
The prospect of a terrorist attack has forced all water systems, large and small, to re-evaluate and upgrade existing security measures. Since September 11th, 2001, water system managers have taken unprecedented steps to protect against possible attacks such as chemical or biological contamination of the water supply, disruption of water treatment or distribution, and intentional release of treatment chemicals.

With passage of the Public Health Security and Bioterrorism Response Act of 2002, Congress required community water systems to assess their vulnerability to a terrorist attack and other intentional acts. As part of these vulnerability assessments, systems assess the transportation, storage and use of treatment chemicals. These chemicals are both critical assets (necessary for delivering safe water) and potential vulnerabilities (may pose significant hazards, if released). Water systems using elemental chlorine, in particular, must determine whether existing protection systems are adequate. If not, they must consider additional measures to reduce the likelihood of an attack or to mitigate the potential consequences.

Disinfection is crucial to water system security, providing the "front line" of defense against biological contamination. However, conventional treatment barriers in no way guarantee safety from biological attacks. Additional research and funding are needed to improve prevention, detection and responses to potential threats.

The Future of Chlorine Disinfection

Despite a range of new challenges, drinking water chlorination will remain a cornerstone of waterborne disease prevention. Chlorine's wide array of benefits cannot be provided by any other single disinfectant. While alternative disinfectants (including chlorine dioxide, ozone, and ultraviolet radiation) are available, all disinfection methods have unique benefits, limitations, and costs. Water system managers must consider these factors, and design a disinfection approach to match each system's characteristics and source water quality.

Understanding Disinfection Byproducts (DBPS)

Chlorine and other chemical disinfectants have been widely used by public water systems (along with filtration) to protect the public from microbial pathogens in drinking water. DBPs are formed when certain disinfectants react with DBP precursors (organic and inorganic materials) in source waters. In most cases, natural organic matter (NOM) is an important factor that affects the levels of DBPs that form (NOM is usually measured as TOC). The levels of DBPs in drinking water can vary significantly from one point in a distribution system to another, as many continue to form in the distribution system. DBP levels are generally higher in surface water systems because surface water usually contains higher DBP precursor levels and requires stronger disinfection.

Updating the Safe Drinking Water Act Regulations
EPA has regulated DBPs in drinking water since 1979. The first DBP standards limited THM levels to 100 parts per billion (ppb) for systems serving more the 10,000 people. In the 1996 Safe Drinking Water Act (SDWA) reauthorization, Congress called for EPA to revise its standards for disinfectants and DBPs in two stages. The revised regulations are designed to reduce potential DBP risks, while ensuring that drinking water is protected from microbial contamination.

Stage 1 DBP Rule
In December 1998 USEPA issued the Stage 1 Disinfectants and Disinfection Byproducts (Stage 1 DBP) rule. The regulations are based on an agreement between members of a Federal Advisory Committee that included representatives from water utilities, the Chlorine Chemistry Division of the American Chemistry Council, public health officials, environmentalists and other stakeholder groups. This diverse group of experts developed a consensus set of recommendations to cost-effectively reduce DBP levels, without compromising protection from microbial contaminants.

The Stage 1 DBP rule mandates a process called enhanced coagulation to remove natural organic matter, reducing the potential for DBPs to form. The rule also sets enforceable Maximum Contaminant Levels (MCLs) for total trihalomethanes at 80 ppb and the sum of five Haloacetic Acids (HAAs) at 60 ppb. These MCLs are based on system-wide running annual averages, meaning that concentrations may be higher at certain times and at certain points in the system, as long as the system-wide average for the year is below the MCL. In developing the Stage 1 DBP rule, EPA was very cautious about encouraging the use of alternative disinfectants. The Agency recognized that alternative disinfectants might reduce THMs and HAAs, but produce other, less understood, byproducts. The Agency also avoided making recommendations that would encourage utilities to reduce the level of disinfection currently being practiced.

Large water systems (those serving more than 10,000 persons) were required to comply with the Stage 1 DBP rule by December 2001. Systems serving fewer than 10,000 persons must comply by December 2003.

Stage 2 DBP Rule
As the Stage 1 rule is coming into full force, EPA is completing work on its Stage 2 DBP rule. The Stage 2 rule is being developed simultaneously with the Long Term 2 Enhanced Surface Water Treatment Rule (LT2) in order to address the risk trade-offs between pathogen control and exposure to DBPs. The LT2 rule deals primarily with controlling Cryptosporidium and other resistant pathogens discussed in Chapter 3. Again, the EPA sought recommendations from an advisory group, the Stage 2 Microbial and Disinfection Byproducts Federal Advisory Committee.

As outlined in the advisory committee's September 2000 Agreement in Principle, the MCLs for THMs and five HAAs will remain 80 ppb and 60 ppb respectively, based on each utility's system-wide running annual averages. However, the Stage 2 rule will also limit DPB levels at specific locations within distribution systems. When fully implemented, these locational running annual average limits will mean that no part of the distribution system will be allowed to exceed the MCLs for these substances.

Total Trihalomethanes
Trihalomethanes (THMs) are chemical compounds in which three of the four hydrogen atoms of methane (CH4) are replaced by halogen atoms. Many trihalomethanes find uses in industry as solvents or refrigerants. THMs are also environmental pollutants, and many are considered carcinogenic. Trihalomethanes with all the same halogen atoms are called haloforms. Trihalomethanes are formed as a by-product predominantly when chlorine is used to disinfect water for drinking. They represent one group of chemicals generally referred to as disinfection by-products. They result from the reaction of chlorine and/or bromine with organic matter present in the water being treated. The THMs produced have been associated through epidemiological studies with some adverse health effects. Many governments set limits on the amount permissible in drinking water. However, trihalomethanes are only one group of many hundreds of possible disinfection by-products—the vast majority of which are not monitored—and it has not yet been clearly demonstrated which of these are the most plausible candidate for causation of these health effects. In the United States, the EPA limits the total concentration of the four chief constituents (chloroform, bromoform, bromodichloromethane, and dibromochloromethane), referred to as total trihalomethanes (TTHM), to 80 parts per billion in treated water.

THM Treatment
THM levels tend to increase with pH, temperature, time, and the level of "precursors" present. Precursors are organic material which reacts with chlorine to form THM's. One way to decrease THM's is to eliminate or reduce chlorination before the filters and to reduce precursors. There are more precursors present before filtration, so we want to reduce or eliminate the time chlorine is in contact with this water. If some oxidation before filtration is required, an alternative disinfectant like potassium permanganate or peroxide could be considered. Note that this may not be an option if prechlorination is necessary to achieve required CT values.

The EPA has indicated that the best available technology for THM control at treatment plants is removal of precursors through "enhanced coagulation". Enhanced coagulation refers to the process of optimizing the filtration process to maximize removal of precursors. Removal is improved by decreasing pH (to levels as low as 4 or 5), increasing the feed rate of coagulants, and possibly using ferric coagulants instead of alum.

Understanding Cryptosporidiosis
Cryptosporidium is an emerging parasitic protozoan pathogen because its transmission has increased dramatically over the past two decades. Evidence suggests it is newly spread in increasingly popular day-care centers and possibly in widely distributed water supplies, public pools and institutions such as hospitals and extended-care facilities for the elderly. Recognized in humans largely since 1982 and the start of the AIDS epidemic, Cryptosporidium is able to cause potentially life-threatening disease in the growing number of immunocompromised patients. Cryptosporidium was the cause of the largest reported drinking water outbreak in U.S. history, affecting over 400,000 people in Milwaukee in April, 1993. More than 100 deaths are attributed to this outbreak. Cryptosporidium remains a major threat to the U.S. water supply (Ibid.).

The EPA is developing new drinking water regulations to reduce Cryptosporidium and other resistant parasitic pathogens. Key provisions of the Long Term 2 Enhanced Surface Water Treatment Rule include source water monitoring for Cryptosporidium; inactivation by all unfiltered systems; and additional treatment for filtered systems based on source water Cryptosporidium concentrations. EPA will provide a range of treatment options to achieve the inactivation requirements. Systems with high concentrations of Cryptosporidium in their source water may adopt alternative disinfection methods (e.g., ozone, UV, or chlorine dioxide). However, most water systems are expected to meet EPA requirements while continuing to use chlorination. Regardless of the primary disinfection method used, water systems must continue to maintain residual levels of chlorine-based disinfectants in their distribution systems.

Understanding Giardia lamblia
Giardia lamblia, discovered approximately 20 years ago, is another emerging waterborne pathogen. This parasitic microorganism can be transmitted to humans through drinking water that might otherwise be considered pristine. In the past, remote water sources that were not affected by human activity were thought to be pure, warranting minimal treatment. However, it is known now that all warm-blooded animals may carry Giardia and that beaver are prime vectors for its transmission to water supplies.

There is a distinct pattern to the emergence of new pathogens. First, there is a general recognition of the effects of the pathogen in highly susceptible populations such as children, cancer patients and the immunocompromised. Next, practitioners begin to recognize the disease and its causative agent in their own patients, with varied accuracy. At this point, some may doubt the proposed agent is the causative agent, or insist that the disease is restricted to certain types of patients. Finally, a single or series of large outbreaks result in improved attention to preventive efforts. From the 1960's to the 1980's this sequence of events culminated in the recognition of Giardia lamblia as a cause of gastroenteritis (Lindquist, 1999).

Understanding Waterborne Diseases
Detection and investigation of waterborne disease outbreaks is the primary responsibility of local, state and territorial public health departments, with voluntary reporting to the CDC. The CDC and the U.S. Environmental Protection Agency (EPA) collaborate to track waterborne disease outbreaks of both microbial and chemical origins. Data on drinking water and recreational water outbreaks and contamination events have been collected and summarized since 1971.

While useful, statistics derived from surveillance systems do not reflect the true incidence of waterborne disease outbreaks because many people who fall ill from such diseases do not consult medical professionals. For those who do seek medical attention, attending physicians and laboratory and hospital personnel are required to report diagnosed cases of waterborne illness to state health departments. Further reporting of these illness cases by state health departments to the CDC is voluntary, and statistically more likely to occur for large outbreaks than small ones.

Despite these limitations, surveillance data may be used to evaluate the relative degrees of risk associated with different types of source water and systems, problems in current technologies and operating conditions, and the adequacy of current regulations. (Craun, Nwachuku, Calderon, and Craun, 2002).

Understanding Bacteriological Monitoring

26 waterborne-disease outbreaks have been documented each year in the United States over the past 25 years (Kramer and others, 1996). The persistence of outbreaks over time indicates that more progress is needed to meet the “drinkable and swimmable” goals of Federal water-quality legislation. Although significant improvements in drinking water and wastewater treatment have been achieved, waterborne disease outbreaks indicate that certain types and sources of waterborne pathogens (disease-causing organisms) are still a threat to human health in the United States (Craun, 1992). In particular, waterborne disease outbreaks caused by Escherichia coli O157.:H7 were reported more frequently in 1995-96 than in previous years, and during that same period, Cryptosporidium and Giardia caused large outbreaks associated with recreational water quality (Levy and others, 1998).

Microbiological examination of water is used to determine the sanitary quality of water and the public health risk from waterborne disease. Although microbiological monitoring of finished waters is well established, microbiological monitoring of source waters and recreational waters is considered by some to be fragmented, incomplete, or virtually nonexistent in many parts of the Nation (Rose and others, 1999). Data to characterize the microbiological quality of source waters are usually collected for local purposes, most often to judge compliance with standards for protection of public health in swimmable or drinkable waters. For example, monitoring programs vary widely at the local level for recreational waters, and the result is the inconsistent use of indicator organisms across the United States (U.S. Environmental Protection Agency, 1999a).

There is a need to identify human and animal factors associated with contamination of different source and recreational waters and to understand the processes that affect microbiological water quality. Concepts about the relation between the occurrence and distribution of microbiological contaminants and a range of environmental factors such as climate, hydrology, land use, and human and animal population densities need to be tested in areas that represent the national water-use patterns for public and domestic supply and for recreational uses.

Understanding Bacteriological Monitoring
Understanding Bacteria Sampling
Waterborne bacterial pathogens in the United States include species in the genera Salmonella, Shigella, Vibrio, Campylobacter, Yersinia, and pathogenic strains of E. coli. Because bacterial pathogens generally appear intermittently in low concentrations in the environment and because methods of culturing are difficult, fecal-indicator bacteria are used to indicate the possible presence of pathogens. The most widely used bacterial indicators include total coliforms, fecal coliforms, E. coli, fecal streptococci, enterococci, and Clostridium perfringens (C. perfringens). A good indicator organism should be applicable in all types of water; unable to reproduce in ambient waters; be harmless to man and other animals; lend itself to easy, quantitative testing procedures; be of warm-blooded animal origin; correlate with fecal contamination; and be present in waters in greater numbers than and survive as long as or longer than pathogens.

The historical definition of the total-coliform group has been based on the method used for detection (lactose fermentation) rather than on systematic bacteriology (American Public Health Association and others, 1998). Total coliforms are defined as aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas formation at $35^{O}C$ within 48 hours (Britton and Greeson,1989). Elevated temperature tests identify those genera of total coliform bacteria that belong in the more specific fecal-coliform group. Fecal coliforms are total coliforms capable of producing gas from lactose at $44.5^{O}C$.

Escherichia coli is a species of the fecal-coliform group. Total coliforms include several genera that are found in the human intestine; however, some genera are also found in soils, on vegetation, and in industrial wastes. This multiplicity of sources makes the sanitary significance of total coliforms difficult to establish (Palmer and others, 1984).

They are used as a rough measure of source-water quality and as a screen for fecal contamination. In addition, speciation of the total-coliform group may provide information on treatment effectiveness and the source of colonization of a distribution system or well (American Public Health Association, 1998, p. 9-1). The fecal-coliform indicator used to assess fecal contamination of water has been faulted because of nonfecal sources of at least one member of the fecal coliform group. For example, thermotolerant Klebsiella species have been observed in pulp- and papermill effluents, textile-processing-plant effluent, cotton-mill wastewaters, and sugar-beet wastes, in the absence of fecal contamination (U.S. Environmental Protection Agency, 1986a).

Alternatively, E. coli is a natural inhabitant of the gastrointestinal tract of warm-blooded animals and is direct evidence of fecal contamination from warm-blooded animals. The fecal streptococci are a group of fecal-indicator bacteria that include a variety of species and strains that are all gram positive cocci. Although the normal habitat of fecal streptococci is the gastrointestinal tract of warm-blooded animals, some species are not exclusive to animals (American Public Health Association, 1998, p. 9-74). In fact, studies on the distribution of fecal streptococci in water indicate that at least one strain commonly found in environmental samples is ubiquitous and can exist for extended periods in soil and water (Geldreich, 1976). Fecal streptococci, therefore, have limited value as an indicator of fecal contamination in environmental samples. The enterococci group is a subgroup of the fecal streptococci, and it is considered a more specific indicator of fecal contamination.

The enterococci are differentiated from other streptococci by their ability to grow in 6.5 percent chloride, at pH 9.6, and at elevated temperatures. The enterococci method is valuable for determining the extent of fecal contamination of recreational surface waters, especially marine waters (American Public Health Association, 1998, p. 9-75).

In addition, because enterococccci cells are a different shape and have different survival rates than members of the coliform group, enterococci may be useful in assessing transport of fecal contamination in ground water. Clostridium perfringens is present in large numbers in human and animals wastes, and its spores are more resistant to disinfection and environmental stresses than is E. coli. Clostridium perfringens has been suggested as a conservative tracer of past fecal contamination and as an indicator for chlorinated water in distribution systems (Bisson and Cabelli, 1980).

Clostridium perfringens, however, is probably not an appropriate indicator for most recreational waters because spores in the sediment are resuspended into the water column from swimmer or wave disturbances (Bisson and Cabelli, 1980). One exception is that C. perfringens may be a reliable indicator of streamwater quality in tropical climates, where warm water temperatures support the growth and reproduction of E. coli and aerobic conditions preclude the growth and sporulation of C. perfringens (Fujioka and Shizumura, 1985).

Clostridium perfringens has also been found to be a sensitive indicator of microorganisms entering streams from point sources but not a reliable indicator of nonpoint sources (Sorenson and others, 1989). Detection of C. perfringens in water has been proposed as an indicator of the presence and density of pathogenic viruses and possibly other stress resistant microorganisms (U.S. Environmental Protection Agency, 1996c).

Protozoan Pathogens

The principal protozoan pathogens that affect the public health acceptability of waters in the United States are Giardia lamblia (Giardia) and Cryptosporidium parvum (Cryptosporidium). These organisms are widely distributed in the aquatic environment and have been implicated in several recent outbreaks of waterborne disease, including a well-publicized outbreak of cryptosporidiosis in Milwaukee, Wisconsin (Rose and others, 1997). Both Giardia and Cryptosporidium produce environmentally resistant forms (called cysts and oocysts), which allow for the extended survival of the parasites in water and treated water.

Because cysts and oocysts are more resistant to disinfection and survive longer in the environment than bacterial indicators, fecal-indicator bacteria are not adequate indicators for Giardia and Cryptosporidium in source waters. The presence of protozoan pathogens in water, therefore, must be verified by identification of the pathogens themselves. The USEPA-required method for detection of Giardia and Cryptosporidium in source and drinking water under the ICR involves nominal porosity filtration and indirect fluorescent antibody procedures (U.S. Environmental Protection Agency, 1996c). The ICR method has been criticized for being difficult to implement, being characterized by poor recovery of target organisms, and yielding highly variable results (U.S. Environmental Protection Agency, 1996b). As a result, the USEPA supported the development of Method 1622 for Cryptosporidium (U.S. Environmental Protection Agency, 1998b), and Method 1623 for Giardia and Cryptosporidium (U.S. Environmental Protection Agency, 1999c). Method 1622 was validated through an interlaboratory study and revised as a final, valid method in January 1999.

Understanding Routine Coliform Sampling
Streamwater sample collection
When designing a sampling plan, consider that the spatial and temporal distribution of microorganisms in surface water can be as variable as the distribution of suspended sediment because microorganisms are commonly associated with solid particles. The standard samplers can be used to collect streamwater samples for bacterial and viral indicators, *Cryptosporidium*, and *Giardia* providing that the equipment coming in contact with the water is properly cleaned and sterilized. For streamwater samples, these include the US-D77TM, US-D95, US-DH81, and weighted- and open-bottle samplers with autoclavable Teflon, glass, or polypropylene components.

• Prepare a separate set of sterile equipment (bottles nozzles, and caps) for sampling at each site.

• Follow sampling techniques given in Shelton (1994) to ensure that a sample is representative of the flow in the cross section. Use equal-width increment (EWI) or equal-discharge-increment (EDI) methods described in Edwards and Glysson (1988), unless site characteristics dictate otherwise.

• Because churn and cone splitters cannot be autoclaved, use a sterile 3-L bottle to composite subsamples for bacterial and viral indicators when using EDI and EWI methods. If possible, composite by collecting subsamples at vertical locations in the cross section without overfilling the bottle.

• Alternatively, if the stream depth and (or) velocity is not sufficient to use depth-width integrating techniques, collect a sample by a hand-dip method (Myers and Sylvester, 1997).

• Collect approximately 1 L of streamwater for bacterial and viral indicators. Process the sample for *E. coli* and enterococci; send the remainder (at least 500 mL) on ice to the laboratory for *C. perfringens* and coliphage analysis.

Method 1623
For *Cryptosporidium* and *Giardia* analysis by Method 1623 (U.S. Environmental Protection Agency, 1999c), collect 10 L of streamwater for each protozoan pathogen using standard sampling techniques described in Myers and Sylvester (1997). Special sterilization procedures are needed for equipment used in the collection of samples for *Cryptosporidium* and *Giardia*.

Autoclaving is not effective in neutralizing the epitopes on the surfaces of the oocysts and cysts that will react with the antibodies used for detection.

• Wash and scrub the equipment with soap and warm tap water to remove larger particulates and rinse with deionized water. Submerge the equipment in a vessel containing 12 percent hypochlorite solution for 30 minutes. Wash the equipment free of residual sodium hypochlorite solution with three rinses of filter-sterilized water; do not de-chlorinate the equipment using sodium thiosulfate. This procedure is best done in the office with dedicated sampling equipment for each site; however, it may be done in the field as long as the hypochlorite solution is stored and disposed of properly.
• Composite the sample in a 10-L cubitainer that is pre-sterilized by the manufacturer. The cubitainer is sent in a cardboard box to laboratory for *Cryptosporidium* analysis. The sample does not have to be kept on ice during transport. At this time, two methods are recommended for analysis of water samples for enteric viruses: (1) the reverse-transcriptase, polymerase chain reaction (RTPCR) method (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997) and (2) the cell-culture method (U.S. Environmental Protection Agency, 1996c). Sampling and equipment cleaning procedures are more thoroughly described elsewhere (G. Shay Fout, U.S. Environmental Protection Agency, 1997; U.S. Environmental Protection Agency, 1996c). Briefly, 100 L of streamwater is pumped by means of a specially designed sampling apparatus and passed through a Virosorb1 1MDS filter (Cuno, Meriden, Conn.). The sampling equipment is obtained from the analyzing laboratory; for example, the USGS Ohio District Laboratory has modified the sampling apparatus (G. Shay Fout, U.S. Environmental Protection Agency, 1997) into a self-contained box with easy-to-use control valves. The 1MDS filters, which remove viruses present in the water by charge interactions, are kept on ice and sent to a central laboratory for virus elution, concentration, and detection.

Groundwater Sample Collection
Collecting ground-water samples by use of sterile techniques requires knowledge of the type of well, its use, its construction, and its condition.
• Swab the electronic tape used for water-level measurements with isopropyl or ethyl alcohol.
• In sampling subunit survey wells, once purging criteria have been met as described in Koterba and others (1995), collect the sample directly from the tap into a sterile container.
• Remove screens, filters, other devices from the tap before collecting the sample, and do not sample from leaking taps. Because we are interested in the microbial population in the ground water and not in the distribution system, it is best to sample directly from the wellhead using a pump with sterile tubing, if possible. Because this is operationally prohibitive for private domestic wells, a tap that yields water directly from the well and before entering the holding tank is preferred. Water collected after treatment is unsuitable for microbiological analysis.
• Document the stage of the distribution system from which water was collected and details about the distribution system, including the type of tank and condition of the tank and pipes. In addition, if the well can easily be opened for inspection, document the condition of the well, including the sanitary seal (if any) and the amount of debris in the well. Any information on the location of the well, including proximity to septic systems or feedlots, should also be documented in the field at the time of sampling.

For wells without in-place pumps, samples should be obtained by use of the following methods
(in descending order from most to least desirable):
(1) a peristaltic or vacuum pump with autoclavable silicon tubing, (2) a sterile bailer, (3) a chlorine-disinfected pump and tubing, or (4) a detergent-cleaned pump and tubing. Pre-sampling activities, such as purging, must be carried out in such a way as to avoid contaminating the well. All equipment must be properly cleaned and sterilized between sites, using a Liquinox wash and a thorough tap water or deionized-water rinse. If using this last method, collect additional field blanks to evaluate the effectiveness of the cleaning procedure. Refer to Myers and Sylvester (1997) for a detailed discussion of ground-water sampling for microbiological analysis.

Because ground water is less prone to microbiological contamination than surface water, larger volumes of ground water are needed than of surface water.

• For regular sampling, collect 3 L of ground water for bacterial and viral indicators.

• Process the sample for total coliforms, *E. coli*, and enterococci using 200-mL sample volumes for each analysis; send the remainder (at least 2.5 L) to the laboratory for coliphage analysis. In the laboratory, coliphage analysis is done using 1 L for somatic and 1 L for F-specific coliphage.

• For enteric virus analysis by RT-PCR and cell culture, use the same sampler for ground-water samples as for streamwater samples; pump 2,000 L of ground water through the sampling apparatus and 1MDS filter.

Sample Preservation and Storage

Holding times for samples before processing are 6 hours for total coliforms, *E. coli*, and enterococci and 24 hours for *C. perfringens*, coliphage, *Cryptosporidium*, *Giardia*, and the 1MDS filters for enteric viruses by RTPCR and cell culture.

• After collection, immediately store the sample on ice.

• Be sure to keep the sample out of direct sunlight, because ultraviolet rays kill microorganisms.

• Add sodium thiosulfate to sample bottles for bacterial and viral indicators if the water collected contains residual chlorine. (Samples may have residual chlorine if the sampling site is downstream from a wastewater-treatment plant that chlorinates its effluents). Add ethylene diaminetetracetic acid to sample bottles when water is suspected to contain trace elements such as copper, nickel, and zinc at concentrations greater than 1 mg/L (Britton and Greeson, 1989, p. 5-6; U.S. Environmental Protection Agency, 1978, p. 6; American Public Health Association and others, 1998, p. 9-19). (Sodium thiosulfate or ethylene diaminetetracetic acid are not added to containers for *Cryptosporidium* and *Giardia)*.

Analytical Methods

Field Analysis

Analysis of water samples for total coliforms, *E. coli,* and enterococci, are done by use of membrane filtration (MF) or most-probable number (MPN) methods. Because membrane filtration is easier to use and provides a more precise quantification of bacteria than MPN, MF is recommended for most analyses. Refer to Myers and Sylvester (1997) for complete MF procedures.

Different MF methods are used for quantification of bacteria in ground-water and streamwater samples.

• For examining streamwater samples for *E. coli*, use the USEPA-recommended mTEC agar method (Environmental Protection Agency, 1986b).

• For examining ground-water samples for total coliforms and *E. coli*, use the MI method (Brenner and others, 1993).

• For enterococci, use the mEI method (U.S. Environmental Protection Agency, 1997).

• For streamwater, plate sufficient sample volumes in order to obtain at least one plate in the ideal count range. For ground water, a 200-mL sample volume is usually sufficient.

Testing of new microbiological monitoring methods and comparing the recoveries of new methods to the USEPA-approved method can be done by use of the NAWQA network. For ground-water samples, for example, one may include a commercially available MPN kit, Colilert (Idexx Laboratories, Westbrook, Maine), for simultaneous detection of total coliforms and *Escherichia coli*. For streamwater sampling, one may include a single-step modified Mtec medium with 5-bromo-6-chloro-3-indolyl'β-d-glucuronide (Bennett Smith, USEPA, Cincinnati, Ohio, oral commun., 1997); this method was developed to replace the mTEC method. Other new methods can be added to the monitoring program for field testing as they are developed.

Laboratory Analysis

Samples need to be kept on ice and shipped to a central laboratory for analysis of coliphage, *C. perfringens*, *Cryptosporidium, Giardia*, and enteric viruses by the current analytical methods. The single-agar layer (SAL), direct plating method with induction of β-galactosidase (Ijzerman and Hagedorn, 1992) is recommended for detection of somatic and F-specific coliphage in streamwater samples. In this method, 100-mL sample volumes are mixed with an agar medium, *E. coli* host culture, chemicals that induce the β-galactosidase enzyme, and appropriate antibiotics. The mixtures are poured into four 150- x 15-mm plates and incubated at 35°C.

Upon infection by coliphage in the water sample, the *E. coli* host cells are lysed and stable indolyl product that is dark blue is visible within each plaque. Viral plaques are easily identified and enumerated by the distinct blue circle. Because of contamination by naturally occurring bacteria in streamwater samples, antibiotic- resistant host-culture strains, *E. coli* CN-13 (resistant to nalidixic acid) and *E. coli* F-amp (resistant to streptomycin and ampicillin) are used as hosts for somatic and F-specific coliphage, respectively. Large sample volumes, such as 1-L volumes or greater, are recommended for detection of coliphage in ground water. Because the SAL method is impractical for sample volumes above 100 mL, an alternative method should be used for ground-water sample analysis.

One example, currently being tested by USEPA, is a two-step enrichment presence-absence method (U.S. Environmental Protection Agency, 1999e). Samples for enumeration of *C. perfringens* are analyzed by use of the mCP agar method (U.S. Environmental Protection Agency, 1996c). Standard MF techniques are used, and the plates are incubated anaerobically for 24 hours at 44.5°C. After incubation, the plates are exposed to ammonium hydroxide, and all straw-colored colonies that turn dark pink to magenta are counted as *C. perfringens*. In the laboratory, *C. perfringens* analyses are done on 100-, 30-,and 10-mL volumes of streamwater. In the case of a high-flow or high-turbidity streamwater sample, lower sample volumes may be plated.

Method 1623 (U.S. Environmental Protection Agency, 1999c) is recommended for detection of *Cryptosporidium* oocysts and *Giardia* cysts in water. The oocysts are concentrated on a capsule filter from a 10-L water sample, eluted from the capsule filter with buffer, and concentrated by centrifugation. Immunomagnetic separation (IMS) is used to separate the oocysts from other particulates in the sample. In IMS, the oocysts are magnetized by attachment of magnetic beads conjugated to an antibody and then are separated from sediment and debris by means of a magnet.

Fluorescently labeled antibodies and vital dye are used to make the final microscopic identification of oocysts and cysts. The reverse-transcriptase, polymerase chain reaction (RT-PCR) and cell-culture methods are recommended for detection of enteric viruses in water samples (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997; U.S. Environmental Protection Agency, 1996c). To prepare samples for RT-PCR and cell culture, attached viruses are eluted from a 1MDS filter with beef extract (pH 9.5), concentrated using celite (pH 4.0), and eluted with sodium phosphate (pH 9.5).

For RT-PCR analysis, viruses are isolated from the eluate by ultracentrifugation through a sucrose gradient, and trace contaminants are removed by extraction with a solvent mixture. During these steps, the 10-L streamwater sample (or 2,000-L ground-water sample) is concentrated down to 40 µL. An aliquot of the concentrate is used for RT-PCR, wherein any target viral RNA is converted to DNA and amplified by use of an enzymatic process. The RT-PCR products are analyzed by agarose gel electrophoresis and confirmed by hybridization. The enteric viruses detected by use of this method include enterovirus, hepatitis-A, rotavirus, reovirus, and calicivirus.

For cell-culture analysis, the sample eluate is added to a monlayer of a continuous cell line derived from African green monkey kidney cells (U.S. Environmental Protection Agency, 1996c). Each cell culture is examined microscopically for the appearance of cytopathic effects (CPE) for a total of 14 days; if CPE
is not observed in 14 days, a second passage is done. Results are reported as most probable number of infectious units per volume of water.

QA/QC Activities and Measures

QA/QC activities and measures to take to reduce contamination.

• Use a sterilization indicator, such as autoclave tape, in preparing sample bottles and other equipment
for collection of microbiological samples to determine whether adequate temperatures and pressures have been attained during autoclaving.

• Prepare a separate set of sterile equipment for microbiological sampling at each site.

• Before processing samples in the field vehicle, wipe down the area with a disinfectant (such as isopropyl alcohol) to ensure a sterile working surface.

• Monitor the incubators daily to ensure temperatures are appropriate for the methods used.

For bacteria samples, membrane-filtration (MF) equipment and MF procedure blanks are used to estimate analytical bias.

Field personnel should do the following:

• Prepare an MF equipment blank, a 50- to 100-mL aliquot of sterile buffered water plated before the
sample—for every sample by field personnel for total coliform, *E. coli*, and enterococci analyses to
determine the sterility of equipment and supplies.

• Prepare a MF procedure blank, a 50- to 100-mL aliquot of sterile buffered water plated after the sample— for every fourth sample to measure the effectiveness of the analyst's rinsing technique or
presence of incidental contamination of the buffered water.

If contamination from a MF equipment or procedure blank is found, results are suspect and are qualified
or not reported. Proper and consistent procedures for counting and identifying target colonies will be followed, as described in Myers and Sylvester (1997).

• After counting, turn the plate 180° and ensure the second count is within 5 percent of the first count.

Have a second analyst check calculations of bacterial concentrations in water for errors.

For coliphage, *Cryptosporidium, Giardia*, and enteric virus samples, equipment and field blanks are
used to determine sampling and analytical bias. Equipment blanks for these analyses are different from the MF equipment blanks for bacterial analysis. An equipment blank is a blank solution (sterile buffered water) subjected to the same aspects of sample collection, processing, storage, transportation, and laboratory handling as an environmental sample, but it is processed in an office or laboratory. Field blanks are the same as equipment blanks except that they are generated under actual field conditions.

• For enteric virus analysis, collect one equipment blank after collection of the first sample to ensure
that equipment cleaning and sterilization techniques are adequate.

• For coliphage, *Cryptosporidium, Giardia*, and enteric virus analyses, collect field blanks periodically.

At a minimum, the number of field blanks should equal 5 percent of the total number of samples collected. Five percent of samples collected for bacterial and viral indicators (total coliforms, *E. coli*, enterococci, *C. perfringens*, and coliphage) should be nested replicate samples to estimate

sampling and analytical variability. For streamwater samples, concurrent replicates to estimate sampling variability are collected by alternating subsamples in each vertical between two collection bottles. For ground-water samples, sequential replicates are collected one after another into separate sterile bottles. Concurrent and sequential replicates are then analyzed in duplicate (split replicates) to estimate analytical variability.
• Because of the expense associated with collection and analysis of samples for pathogens (*Cryptosporidium* and enteric viruses), collect only one replicate sample per year at a site wherein detection of pathogens was found in an earlier sample.
To assess analytical bias of the sampling and analytical method, 2 to 5 percent of the samples collected
for enteric virus should be field matrix spikes.
• Run all but 10 L of ground water through the 1 MDS filter and collect the remaining 10 L in a carboy. In the laboratory, the poliovirus vaccine will be added to the 10 L and then passed through the same 1MDS filter. Analysis will be done by use of the cell-culture and RT-PCR methods. • All cell-culture positive samples are serotyped to identify or discount laboratory contamination. Because of the variability in the performance of Method 1623 for recovery of *Cryptosporidium* and *Giardia*, each sample will be collected in duplicate—one will be a regular sample and the other a matrix spike. The laboratory will add a known quantity of cysts and oocysts to the matrix spike to determine recovery efficiency, as described in USEPA (1999c).

Quality Assurance and Quality Control in the Laboratory
The following criteria may be used to evaluate each production analytical laboratory: (1) appropriate, approved, and published methods, (2) documented standard operating procedures, (3) approved quality-assurance plan, (4) types and amount of quality-control data fully documented and technical defensible, (5) participation in the standard reference sample project (6) scientific capability of personnel, and (7) appropriate laboratory equipment.

The microbiology laboratories must follow good laboratory practices—cleanliness, safety practices, procedures for media preparation, specifications for reagent water quality—as set forth by American Public Health Association (1998) and Britton and Greeson (1989). Some specific guidelines are listed in the following paragraphs.

Reference cultures are used by the central laboratory to evaluate the performance of the test procedures, including media and reagents. Pure cultures of *E. coli, Enterobacter aerogenes,* and *Streptococcus faecalis* (American Type Culture Collection, Rockville, Md.) are used to ensure that MF culture media and buffered water are performing adequately. A pure culture of *C. perfringens*, isolated from a sewage sample and verified by standard procedures, is used to evaluate the test procedure and each lot of media and reagents.

Because contamination of samples from coliphage during the analytical procedure is highly probable (Francy and others, 2000), a negative control of host and sterile buffered water is run concurrently with each batch of samples. In addition, to ensure that the method is being executed properly, a positive-control sewage sample is run with each batch of samples. A laminar flow safety hood is recommended for processing the samples for coliphage analysis. Alternatively, a separate coliphage room may be established to discourage laboratory contamination during the analytical process. An ultraviolet light is installed and operated for 8 hours every night in the safety hood or coliphage room to reduce contamination.

The laboratory should follow the QA/QC guidelines in Method 1623 (U.S. Environmental Protection Agency, 1999c) for *Cryptosporidium* and *Giardia* and in the cell-culture and RT-PCR analysis for enteric viruses (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997; U.S. Environmental Protection Agency, 1996c).

Understanding Coliforms

Coliforms are bacteria that are always present in the digestive tracts of animals, including humans, and are found in their wastes. They are also found in plant and soil material. A fecal coliform (sometimes faecal coliform) is a facultatively anaerobic, rod-shaped, gram-negative, non-sporulating bacterium. Fecal coliforms are capable of growth in the presence of bile salts or similar surface agents, are oxidase negative, and produce acid and gas from lactose within 48 hours at 44 ± 0.5°C.

Coliform bacteria include genera that originate in feces (e.g. Escherichia) as well as genera not of fecal origin (e.g. Enterobacter, Klebsiella, Citrobacter). The assay is intended to be an indicator of fecal contamination; more specifically of E. coli which is an indicator microorganism for other pathogens that may be present in feces. Presence of fecal coliforms in water may not be directly harmful, and does not necessarily indicate the presence of feces.

"Indicator" Organisms
Water pollution caused by fecal contamination is a serious problem due to the potential for contracting diseases from pathogens (disease causing organisms). Frequently, concentrations of pathogens from fecal contamination are small, and the number of different possible pathogens is large. As a result, it is not practical to test for pathogens in every water sample collected. Instead, the presence of pathogens is determined with indirect evidence by testing for an "indicator" organism such as coliform bacteria. Coliforms come from the same sources as pathogenic organisms. Coliforms are relatively easy to identify, are usually present in larger numbers than more dangerous pathogens, and respond to the environment, wastewater treatment, and water treatment similarly to many pathogens. As a result, testing for coliform bacteria can be a reasonable indication of whether other pathogenic bacteria are present.

Total Coliforms, Fecal Coliforms, and E. Coli
The most basic test for bacterial contamination of a water supply is the test for total coliform bacteria. Total coliform counts give a general indication of the sanitary condition of a water supply.
A. Total coliforms include bacteria that are found in the soil, in water that has been influenced by surface water, and in human or animal waste.
B. Fecal coliforms are the group of the total coliforms that are considered to be present specifically in the gut and feces of warm-blooded animals. Because the origins of fecal coliforms are more specific than the origins of the more general total coliform group of bacteria, fecal coliforms are considered a more accurate indication of animal or human waste than the total coliforms.
C. Escherichia coli (E. coli) is the major species in the fecal coliform group. Of the five general groups of bacteria that comprise the total coliforms, only E. coli is generally not found growing and reproducing in the environment. Consequently, E. coli is considered to be the species of coliform bacteria that is the best indicator of fecal pollution and the possible presence of pathogens.

Are Coliform Bacteria Harmful?
Most coliform bacteria do not cause disease. However, some rare strains of E. coli, particularly the strain 0157:H7, can cause serious illness. Recent outbreaks of disease caused by E. coli 0157:H7 have generated much public concern about this organism. E. coli 0157:H7 has been found in cattle, chickens, pigs, and sheep. Most of the reported human cases have been due to eating under cooked hamburger. Cases of E. coli 0157:H7 caused by contaminated drinking water supplies are rare.

Coliform Testing
Testing for bacteria is the only reliable way to know if your water is safe. You cannot tell by the look, taste, or smell of the water if disease-causing organisms are in it. The New York State

Department of Health recommends that well owners test their water for coliform bacteria at least once a year. If you have experienced bacteria problems in the past, it is recommended that you test your well more
frequently.

When Should I Test?
Late spring or early summer are the best times to test your well, since coliform contamination is most likely to show up during wet weather. Whether your test results are positive or negative, understand that the sample you collected is just a "snapshot" of your well's water quality. The more samples you have tested, the more confident you can be about the quality of the water you are drinking.

What do the Results Mean?
If coliform bacteria are present in your drinking water, your risk of contracting a water-borne illness is increased. Although total coliforms can come from sources other than fecal matter, a positive total coliform sample should be considered an indication of pollution in your well. Positive fecal coliform results, especially positive E. Coli results, should be considered indication of fecal pollution in your well.

What Should be done if Coliform Bacteria are Detected in a Well?
When coliforms have been detected, repairs or modifications of the water system may be required. Boiling the water is advised until disinfection and retesting can confirm that contamination has been eliminated. A defective well is often the cause when coliform bacteria are found in well water.

What Kinds of Defects can Allow Contamination?
- a missing or defective well cap - seals around wires, pipes, and where the cap meets the casing may be cracked, letting in contaminants
- contaminant seepage through the well casing - cracks or holes in the well casing allow water that has not been filtered through the soil to enter the well. This seepage is common in the wells made of concrete, clay tile, or brick
- contaminant seeping along the outside of the well casing - many older wells were not sealed with grout when they were constructed
- well flooding - a common problem for wellheads located below the ground in frost pits that frequently flood during wet weather.

Long-Term Options for Dealing with Bacterial Contamination of a Well
- Connecting to the regional public water system, if possible
- Inspecting wells for defects and repairing them if possible
- Constructing a new well
- Installing continuous disinfection equipment
- Using bottled water for drinking and food preparation

Understanding Positive or Coliform Present Results
The Total Coliform Bacteria test is the standard microbiological test of the sanitary quality of drinking water. The EPA has stated that good drinking water should not contain any Coliform bacteria.

There are primarily 18 different bacteria which make up the group known as “Coliforms”. In most cases, Coliform bacteria are not harmful. However, if these bacteria are found in your water supply, this indicates that other disease causing bacteria may enter through the same pathway and be present in your drinking water.

If Coliform bacteria are found, the water supply is considered a potential health hazard and is classified as “UNSAFE” for human consumption. This test DOES NOT indicate if the water is chemically safe to drink.

E. COLI – One of the Coliform Bacteria

E. Coli is one of the approximate 18 members of Coliform group. The standard Coliform Bacteria test tells the difference between coliforms of fecal origin, specifically E. coli (from the intestines of warm-blooded animals) and coliforms from other sources. Drinking water contaminated with E. coli is considered an “EXTREME HEALTH HAZARD”.

The test results are reported as “PRESENT” or “ABSENT”

“ABSENT” – Good news, it means that Coliform bacteria were NOT in the test sample. In other words it “PASSED” the test.

“PRESENT” – Means that Coliform Bacteria were found in the sample and it “FAILED” the test. If Coliforms were found in the sample then E.coli (as a member of the Coliform Group) is also reported as
“Present” or “Absent”.

Coliforms “Present” and E. coli “Absent” – Very Unsafe – Potential Health Hazard.

Coliforms “Present” and E. coli “Present” – “EXTREME HEALTH HAZARD”.

If your water system fails, the first step is usually to perform a “batch chlorination” on your drinking water supply. NRC will send written instructions for this procedure if your test results indicate a failure. After the chlorination procedure has been performed, use your water supply for a minimum of 5-7 days and retest. Until you have a test which shows that the bacteria are ABSENT, exercise caution with this system. It is recommended that you test for Coliform bacteria at least every six months.

Understanding Maximum Contaminant Levels (MCLs)
National Primary Drinking Water Regulations (NPDWRs or primary standards) are legally enforceable standards that apply to public water systems. Primary standards protect public health by limiting the levels of contaminants in drinking water.

Emerging Pathogens
Although many of the established waterborne pathogens have been controlled by sanitation measures and water treatment processes, new diseases continue to be identified, and new discoveries present a better understanding of existing chronic diseases. Many of these discoveries raise questions about possible waterborne transmission. The bacteria that now need to be considered in this developing area are Helicobacter pylori, Mycobacterium species, Burkholderia pseudomallei and Francisella tularensis.

Helicobacter Pylori
Although spiral-shaped organisms have been observed in the stomachs of humans for many years, it was not until 1982 that a Campylobacter-like organism was isolated from patients with gastritis and a causative relationship between a new species, Helicobacter pylori, and gastric disease realized (Warren and Marshall 1983). H. pylori is a pathogen of global proportions and is

generally accepted as the cause of most gastric and peptic ulcers. These diseases may lead to gastric adenocarcinoma.

H. pylori occurs worldwide in developing and developed countries. Where low degrees of hygiene and socioeconomic problems exist, infection rates may approach 100%. In developed countries, infection rates are probably between 30 and 60%. Transmission from person to person is not fully understood, mainly because of the difficulty in culturing the organism and identifying it outside the body. Epidemiological studies show the cluster phenomenon of H. pylori infection in families. It is suggested that infected mothers may play a key role in transmission within families (Rothenbacher et al. 1999).

H. pylori has been identified in faeces, and it is assumed that transmission is therefore oral–oral or faecal–oral. The organism has not been isolated from the environment or from drinking water, and waterborne transmission remains a possibility that should be investigated. The epidemiology, however, points to person-to-person transmission in early life.

Mycobacterium

The mycobacteria are a group of slow-growing organisms. The most important is Mycobacterium tuberculosis, the causative organism of tuberculosis, which takes about 4–6 weeks to grow in the diagnostic laboratory. M. tuberculosis is not a waterborne pathogen; there are, however, a number of Mycobacterium species that occur in the environment and can cause disease in humans. Mycobacterium avium and its related species cause an infection of cervical lymph nodes; it occurs in the environment and is most probably accompanied by ingestion or inhalation. M. avium can grow in water to which no additional nutrients have been added; although water treatment processes of coagulation and filtration appear to reduce the numbers, it is not affected by chlorine levels of 1 mg/ml. It is therefore not surprising that these organisms can regrow and colonize domestic water systems. Once ingested, M. avium can colonize the pharynx without causing any disease. The number of cases reported was very low, but patients with HIV/AIDS are very susceptible.

Another species, Mycobacterium xenopi, has been reported as the waterborne cause of spinal infections following a look-back exercise on over 3000 patients who had undergone discectomy operations some years beforehand (Astagneau et al. 2001).

Mycobacterium paratuberculosis causes Johne's disease in cattle. It is a chronic wasting disease with considerable economic consequences. The organism is extremely difficult to culture; when it does grow, it is very slow and dependent on an exogenous source of mycobactin, which is an iron chelating agent produced by all other mycobacteria. Transmission is by either direct or indirect contact with infected animals and occurs mainly through the faecal–oral route. Organisms are ingested in large numbers by young animals when they feed in troughs that have been contaminated by faeces of shedding animals
(Chiodini et al. 1984).

M. paratuberculosis has recently been suggested as a cause of Crohn's disease, a non-specific chronic transmural inflammatory disease of humans that affects the intestinal tract, commonly the ileum. The disease is chronic, debilitating and of a relapsing nature; the symptoms experienced include diarrhea with blood in the stools and abdominal pain. Complications include obstruction, fistulation and abscesses. There have been many bacteria implicated over the years, but no definite etiological agent has been found. It is thought that immunological mechanisms may play an important role.

Molecular techniques have been developed for the diagnosis of M. paratuberculosis infections and applied to human surgically resected tissues. M. paratuberculosis was detected in approximately 30% of samples, but the sets of results from different laboratories have been conflicting. Some studies were unable to detect the organism; in other studies, the organism was detected in a smaller percentage of healthy subjects. In addition, a few Crohn's disease patients

have shown clinical remission when treated with anti-tuberculosis drugs. There is therefore much more work to be done to acquire a better understanding. M. paratuberculosis may be present in surface water contaminated by cattle faeces. Routine testing for indicator organisms would detect faecal pollution, and normal water treatment processes of coagulation and filtration are likely to remove mycobacteria. It is unlikely that drinking-water is a major source of M. paratuberculosis, and its association with Crohn's disease is still under investigation.

Burkholderia Pseudomallei
Burkholderia pseudomallei is the cause of melioidosis, an acute pneumonia often followed by systemic infection with later presentations of abscesses. The organism is widespread in the environment and was originally described in Rangoon in patients compromised by severe poverty who had presumably inhaled the organism in dust when sleeping on the ground. It occurs commonly in southeast Asia and has been detected in service personnel repatriated from those areas in the past. It was also investigated as a biological weapon by several nations, to be released as an aerosol and cause pneumonia infection in those exposed. A recent study in Bologna, Italy, detected B. pseudomallei in 7% of 85 samples of drinking-water collected from public and private buildings. The mean count was 578 cfu/100 ml. The occurrence of the organism was found to correlate with the HPC at 22 and 36 °C (Zanetti et al. 2000).

Francisella Tularensis
Tularaemia is a zoonosis caused by a highly infective and virulent organism Francisella tularensis, which occurs throughout the northern hemisphere but has never been isolated within the United Kingdom. It occurs in a wide range of animal reservoir posts and can be isolated from the environment in water and mud. It is transmitted to humans who come in close contact with the animal reservoir, arthropods that feed on them or debris and dust associated with them. It can also be transmitted through the ingestion of contaminated water. Human epidemics sometimes occur and are associated with epizootics in the animal

Bacteria of potential health concern 75 populations, evidenced by die-offs. There are several presentations of tularaemia in humans, depending on the route of exposure. Ingestion usually results in
oropharyngeal tularaemia, with fever, pharyngitis and cervical lymphadenitis. Other forms include ulcero-glandular, pleuropneumonic and typhoidal. Following the recent war in Kosovo, over 900 suspected cases of tularaemia were identified and 327 cases confirmed serologically. The epidemiological investigation pointed to rodent-contaminated wells, and rodent carcasses found in some wells tested positive for F. tularensis (Reintjes et al. 2002). In a waterborne outbreak reported from Spain, 19 cases who had contact with river-caught crayfish were identified (Anda et al. 2001). Attempts to isolate F. tularensis from water were unsuccessful. Drinking-water was not involved. F. tularensis is notoriously difficult to culture, requiring a source of cysteine. F. tularensis was investigated and developed as a biological weapon; the infectious dose was found to be extremely low — 10 organisms.

Examples of classical biological warfare agents

Agent	**Disease**
Bacillus anthracis	Anthrax
Brucella species	Brucellosis
Burkholderia mallei	Glanders
Burkholderia pseudomallei	Melioidosis
Francisella tularensis	Tularaemia
Yersinia pestis	Plague

Rickettsia species	Typhus
Coxiella burnetii	Q fever
Clostridium botulinum toxin	Botulism
Staphylococcus aureus enterotoxin B	Staphylococcal food poisoning
Smallpox virus	Smallpox

The remaining bacteria of concern are either heterotrophs that might have a role in disease or emerging pathogens that do have a role in disease and could possibly be waterborne. It is important that these organisms and diseases are kept under surveillance in order to confirm or refute the suggested associations. Many of the organisms are difficult to grow, and there is no validated trigger of when to look for them.

The HPC does not measure all organisms present, of which many will be non-culturable but viable, and indeed several of the organisms of concern described above would not grow on HPC media. The HPC, however, does give an indication of change in the flora of drinking-water, and the HPC should be evaluated as a trigger for further investigation.

Many new molecular techniques for the detection of pathogens and putative pathogens in water are being described (Waage et al. 1999a,b,c; Lightfoot et al. 2001). DNA chips that have the capacity to detect up to 44 pathogens on one single chip are being developed.

These tests are very expensive when compared with the routine monitoring tests carried out in the water industry and in public health monitoring. The HPC should be evaluated as the signal of changing events in a drinking-water supply to trigger the utilization of these new molecular tests to detect the new bacteria of concern and any associated virulence genes.

Understanding Heterotrophic Plate Count
The analytical methods promulgated under the authority of Section 304(h) of the Clean Water Act are sometimes referred to as the "304(h)" or "Part 136" methods. The methods measure chemical and biological pollutants in media, such as wastewater, ambient water, sediment, and biosolids (sewage sludge). These various CWA methods are tested in a variety of labs and matrices. In addition to Part 136 methods, some approved methods are published or incorporated by reference at 40 CFR Parts 401-503, approved industry-specific methods.

In addition to general purposes methods, EPA has approved special purpose analytical methods. These methods were developed to work in samples or for pollutants specific to certain industrial categories. For example, methods specific to the Pharmaceutical Manufacturing and Pesticide Chemicals industrial categories are listed in Tables IF and IG, respectively, at 40 CFR Part 136.

Methods specific to other industrial categories are listed or incorporated by reference into the regulations at 40 CFR Parts 401-503. These include methods specific to the Pulp, Paper, and Paperboard category (40 CFR Part 430), and specific to Use or Disposal of Sewage Sludge (biosolids) (at 40 CFR Part 503.) Industry-specific methods that are approved for compliance monitoring in the industry for which they are designated may be used for general use, if the same method is listed in Tables IA to IE, or IH at 40 CFR 136.3.

Understanding Total Coliforms
The Total Coliform Rule was published in 1989 and became effective in 1990. The rule set both health goals (MCLGs) and legal limits (MCLs) for the presence of total coliform in drinking water. The rule also detailed the type and frequency of testing that water systems must undertake. In 2003, EPA announced its intent to revise the Total Coliform Rule.

2010 Proposed Revisions to the Total Coliform Rule
The Environmental Protection Agency (EPA) is proposing revisions to the 1989 Total Coliform Rule (TCR), a national primary drinking water regulation (NPDWR). The purpose of the TCR is to protect public health by ensuring the integrity of the drinking water distribution system and monitoring for the presence of microbial contamination. EPA anticipates greater public health protection under the proposed revised requirements, which are based on recommendations by a federal advisory committee.

The proposed revisions to the TCR will:

- require public water systems that are vulnerable to microbial contamination to identify and fix problems, and

- establish criteria for systems to qualify for and stay on reduced monitoring, thereby providing incentives for improved water system operation.

The proposed RTCR maintains and strengthens the objectives of the current TCR and is consistent with the recommendations in the AIP. The objectives are: (1) To evaluate the effectiveness of treatment, (2) to determine the integrity of the distribution system, and (3) to signal the possible presence of fecal contamination. The proposed revision better addresses these objectives by requiring systems that may be vulnerable to fecal contamination (as indicated by their monitoring results) to do an assessment, to identify whether any sanitary defect(s) is (are) present, and to correct the defects.

Therefore, the Agency anticipates greater public health protection under the proposed RTCR compared to the current TCR because of its more preventive approach to identifying and fixing problems that affect or may affect public health.

The following is an overview of the key provisions of the proposed RTCR:

- MCLG and MCL for E. coli and coliform treatment technique for protection against potential fecal contamination. The proposed RTCR establishes a maximum contaminant level goal (MCLG) and maximum contaminant level (MCL) for E. coli. It takes a preventive approach to protecting public health by establishing a coliform treatment technique for protection against potential fecal contamination. The treatment technique uses both total coliforms and E. coli monitoring results to start an evaluation process that, where necessary, will require the PWS to conduct follow-up corrective action that could prevent future incidences of contamination and exposure to fecal contamination and/or waterborne pathogens.

- Monitoring. As with the current TCR, PWSs will continue to monitor for total coliforms and E. coli according to a sample siting plan and schedule specific to the system.

Sample siting plans under the proposed RTCR must continue to be representative of the water throughout the distribution system. Under the proposed RTCR, systems have the flexibility to propose repeat sample locations that best verify and determine the extent of potential contamination of the distribution system rather than having to sample within five connections upstream and downstream of the total coliform-positive sample location. In lieu of proposing new repeat sample locations, the systems may stay with the default used under the current TCR of five connections upstream and downstream of the total coliform-positive sample location.

As with the current TCR, the proposed RTCR allows reduced monitoring for some small ground water systems. The proposed RTCR is expected to improve public health protection compared to the current TCR by requiring small ground water systems that are on or wish to conduct reduced monitoring to meet certain eligibility criteria. Examples of the criteria include a sanitary survey

showing that the system is free of sanitary defects, a clean TCR compliance history for 12 months, and a recurring annual site visit by the State and/or a voluntary Level 2 assessment for systems on annual monitoring.

For small ground water systems, the proposed RTCR requires increased monitoring for high-risk systems that meet certain criteria such as unacceptable compliance history under the RTCR. The proposed RTCR specifies conditions under which systems will no longer be eligible for reduced monitoring and be required to return to routine monitoring or to monitor at an increased frequency.

The proposed RTCR requires systems on a quarterly or annual monitoring frequency (applicable only to ground water systems serving 1,000 or fewer people) to conduct additional routine monitoring the month following one or more total coliform-positive samples. Under the proposed RTCR, systems must collect at least three routine samples during the next month, unless the State waives the additional routine monitoring. This is a reduction in the required number of additional routine samples from the current TCR, which requires at least five routine samples in the month following a total coliform-positive sample for all systems serving 4,100 or fewer people.

The current TCR requires all systems serving 1,000 or fewer people to collect at least four repeat samples while PWSs serving 1,000 people or greater to collect three repeat samples. The proposed rule requires three repeat samples after a routine total coliform-positive sample, regardless of the system type and size.

Seasonal Systems
The proposed RTCR establishes monitoring requirements for seasonal systems for the first time. Seasonal systems represent a special case in that the shutdown and start-up of these water systems present additional opportunities for contamination to enter or spread through the distribution system. Seasonal systems must demonstrate completion of a State-approved start-up procedure. In addition, they must designate the time period(s) for monitoring based on site-specific considerations (such as during periods of highest demand or highest vulnerability to contamination) in their State-approved sample siting plan. See section III.A.3 of this preamble for a detailed discussion of seasonal systems.

Assessment and Corrective Action
As part of a treatment technique, all PWSs are required to assess their systems when monitoring results show that the system may be vulnerable to contamination. Systems must conduct a simple self-assessment (Level 1) or a more detailed assessment (Level 2) depending on the severity and frequency of contamination. The system is responsible for correcting any sanitary defect(s) found through either a Level 1 or Level 2 assessment.

Violations and Public Notification
The proposed RTCR establishes an E. coli MCL violation, a treatment technique violation, a monitoring violation, and a reporting violation. Public notification is required for each type of violation, with the type of notification dependent on the degree of potential public health concern. This is consistent with EPA's current public notification requirements under 40 CFR part 141 subpart Q. The proposed RTCR also modifies the public notification and Consumer Confidence Report language to reflect the construct of the proposed rule. See sections III.A.6 and III.A.7 of this preamble for detailed discussions of violations and public notification under the proposed RTCR.

Transition to the RTCR
The proposed RTCR allows all systems to transition to the new rule at their current TCR monitoring frequency, including systems on reduced monitoring under the current TCR. States will then evaluate the monitoring frequency during each sanitary survey conducted after the compliance effective date of the RTCR. This process reduces State burden by not requiring the

State to determine appropriate monitoring frequency at the same time as when the State is trying to adopt primacy, develop policies, and train their own staff and the PWSs in the State.

The provisions of the proposed RTCR are contained in the new 40 CFR part 141 subpart Y, superseding 40 CFR 141.21 beginning three years following the publication of the final revised rule.

A. Proposed Rule Provisions and Rationale
1. Terms used in the proposed RTCR
a. Provisions. i. Clean compliance history. For the purposes of the proposed RTCR, EPA is proposing to define "clean compliance history" as a record of no maximum contaminant level (MCL) violations under 40 CFR 141.63; no monitoring violations under 40 CFR 141.21 or subpart Y; and no coliform treatment technique trigger exceedances or coliform treatment technique violations under subpart Y.
ii. Sanitary defect. EPA is proposing to define "sanitary defect" as a "defect that could provide a pathway of entry for microbial contamination into the distribution system or that is indicative of a failure or imminent failure in a barrier that is already in place" (USEPA 2008c).
iii. Seasonal systems. EPA is proposing to define a seasonal system as a non-community water system that is operated in three or fewer calendar quarters per calendar year.
b. EPA's rationale. i. Clean compliance history. EPA is proposing a definition of "clean compliance history" because without a definition, the use of the phrase could result in multiple interpretations. Clean compliance history is one of the criteria a system must meet to be eligible for reduced monitoring. The advisory committee recommended this definition (USEPA 2008c, AIP p. 10).
ii. Sanitary defect. The advisory committee recommended the definition of sanitary defect. The proposed RTCR takes a more preventive approach to protect public health by establishing a framework for the assessment of public water systems to identify sanitary defects and to correct them as appropriate. The first part of the proposed definition of a "sanitary defect" focuses on problems in the distribution system that may provide a pathway for contaminants to enter the distribution system and its implication for potential exposure to both microbial and chemical contaminants. The second part of the definition also recognizes the importance of having barriers in place to prevent the entry of microbial contaminants into the distribution system. Indications of failure or imminent failure of these barriers are defects that require corrective action.

Sanitary Defect
Sanitary defect is a term specific to the proposed RTCR assessment and corrective action provisions. Sanitary defects are not intended to be linked directly to "significant deficiencies" under the Interim Enhanced Surface Water Treatment Rule (IESWTR) (USEPA 1998b, 63 FR 69389, December 16, 1998) and Ground Water Rule (GWR) (USEPA 2006c, 71 FR 65574, November 8, 2006), although some problems could meet either definition. Nothing in this proposed rule is intended to limit the existing authorities of States under other regulations. The following is a list of examples of sanitary defects and defects in the distribution system coliform monitoring practices.

Examples of sanitary defects:
- ✓ Cross connection and backflow issues such as a required backflow prevention device not in place or not operating properly; or an unprotected cross connection found. Operator issues such as failure to follow standard operating procedures (SOPs) that protect distribution system integrity and sanitary conditions.

- ✓ Distribution system issues such as inadequate inspection and maintenance of the distribution system; loss of distribution system integrity such as main breaks; failure to maintain adequate pressure; improper flushing operations; improper construction of new, replaced, or renovated lines; inadequate disinfection during and after repair/replacement activities; or inability to maintain required residual throughout the distribution system.

- ✓ Storage issues such as overflow, vents, hatches, and other penetrations not properly configured, screened, or sealed; inadequate maintenance of storage facilities; or inadequate disinfection during and after repair/replacement activities

- ✓ Disinfection issues such as inability to maintain required residual throughout the distribution system.

iii. Seasonal systems. Seasonal systems fall under the broader category of non-community water systems (NCWS) and therefore are subject to provisions applicable to that category of systems. However, seasonal systems have unique characteristics and timetables that make them particularly susceptible to contamination. Seasonal systems represent a special case in that the shut down and start-up of the water system present opportunities for contamination to enter or spread through the distribution system. For example, loss of pressure after a system's shut down can lead to intrusion of contaminants. Microbial growth prior to start-up can result in biofilm formation, which can lead to the accumulation of contaminants. These systems are also more susceptible to contamination due to changes in the conditions of the source water (such as variable contaminant loading due to increased septic tank or septic field use), the seasonal nature of the demand, and the stress that the system experiences. As a result, the Agency is establishing a definition for seasonal systems and setting forth provisions that mitigate the risk associated with the unique characteristics of this type of system. The advisory committee recommended that such provisions pertain to seasonal systems.

c. Request for comment. EPA requests comment on the proposed definitions and whether they work within the construct of the proposed RTCR. Specifically, EPA requests comment on the proposed definition of seasonal systems. The advisory committee recommended that seasonal systems be identified and be subject to additional regulatory requirements because the shutdown and startup of the system presents opportunities for contaminants to enter or spread through the distribution system. These results are possible in any system that shuts down and does not maintain adequate pressure throughout the distribution system. The AIP describes a seasonal system as “one which operates less than four calendar quarters per year” (USEPA 2008c). EPA has interpreted this to mean that a seasonal system is one which is shut down for at least one full calendar quarter (i.e., it operates in three or fewer calendar quarters). EPA requests comment on whether this proposed definition of “seasonal system” is adequate to address the concern that motivated the advisory committee's recommendation and is consistent with its intent. For example, a system that operated from March to October would operate in all four calendar quarters and would not be considered a seasonal system, but would be subject to the same possibility of distribution system intrusion as a seasonal system that operated April to November (i.e., in only three calendar quarters).

Should EPA modify the definition to address this issue? If so, how should the definition be modified? Should systems that close for some specified period (e.g., 30 days, 60 days, 90 days) be subject to seasonal system requirements? What should that specified period be?

Systems that operate intermittently (e.g., only on weekends or only when a camp is open) may also be subject to distribution system contamination due to lack of adequate pressure. Should this be addressed? If so, how should it be addressed—through regulation, guidance, or some other approach? Is there a specific shutdown time that should be considered for intermittent systems in developing the approach and determining which systems should be included?

In addition to the public health benefits associated with these requirements, EPA is aware of the burden that States will have in determining which systems must comply and in tracking compliance. Therefore, EPA requests comment on ways to reduce State burden and facilitate implementation of seasonal system provisions.

2. MCLG and MCL for E. coli, and Coliform Treatment Technique

a. Provisions. The current TCR established a maximum contaminant level goal (MCLG) of zero for total coliforms (including fecal coliforms and E. coli) and an MCL for total coliforms. EPA is proposing in the RTCR to eliminate the MCLG for total coliforms (including fecal coliforms) and the MCL for total coliforms. Under the proposed RTCR, EPA establishes an MCLG of zero and an MCL for E. coli and a treatment technique for coliform. The proposed MCL for E. coli is based on the monitoring results for total coliforms and E. coli.

A system is in compliance with the E. coli MCL unless any of the following conditions occur:

- A system has an E. coli positive repeat sample following a total coliform-positive routine sample; or
- A routine sample is E. coli-positive and one of its associated repeat samples is total coliform-positive; or
- A system fails to test for E. coli when any repeat sample tests positive for total coliforms; or
- A system fails to take all required repeat samples following a routine sample that is positive for E. coli.

The proposed MCL is similar to the criteria that define the conditions (if exceeded) when a Tier 1 acute MCL violation occurs under the current TCR but with two modifications. First, the proposed MCL excludes fecal coliforms. Second, the proposed MCL also includes an additional condition by which a system violates the MCL, namely failing to collect all repeat samples following an initial E. coli-positive sample. Although not explicitly stated, as a logical consequence of the second condition, a system also violates the MCL when an E. coli- positive routine sample is followed by an E. coli-positive repeat sample because E. coli are a subset of total coliforms. EPA is also proposing a coliform treatment technique, which uses total coliforms and E. coli as indicators of a possible breach in the distribution system that could lead to fecal contamination.

b. EPA's rationale. i. Inclusion of MCLG for E. coli and removal of MCLG for total coliforms (including fecal coliforms). EPA is proposing in the RTCR to include an MCLG of zero for E. coli and to remove the current MCLG of zero for total coliforms (including fecal coliforms). This is because E. coli is a more specific indicator of fecal contamination and potential harmful pathogens in drinking water than are total coliforms (including fecal coliforms). Many of the organisms detected by total coliform and fecal coliform methods are not of fecal origin and do not have any direct public health implication. See also the discussion of fecal coliforms in section III.A.9 of this preamble. New information has become available since promulgation of the current TCR in 1989 that indicates that measurement of fecal coliforms sometimes detects organisms that may not have any connection to fecal contamination (Edberg et al. 2000). An MCLG of zero for E. coli is more appropriate than an MCLG of zero for total coliforms (including fecal coliforms) since E. coli is a more specific indicator of the presence of fecal contamination.

Total coliforms (including fecal coliforms) do not in and of themselves pose a public health risk, but they may indicate the presence of a pathway by which fecal contamination can occur. Therefore, the removal of the MCLG for total coliforms (including fecal coliforms) would prevent possible public confusion as a result of attributing greater public health significance to the presence of total coliforms than is warranted. EPA believes that the removal of the MCLG for total coliforms, along with the other proposed changes discussed in the succeeding paragraphs, leads to a rule that is more protective of public health, and is less confusing to the public. The proposed MCLG of zero for E. coli and the removal of the MCLG for total coliforms (including fecal coliforms) are also consistent with the recommendation made by the advisory committee in the AIP.

ii. Inclusion of MCL for E. coli and removal of MCLs for total coliforms and fecal coliforms. EPA is proposing to include in the RTCR an MCL for E. coli because approved analytical methods continue to be available to measure the presence of E. coli in water samples, i.e., the presence of E. coli is technologically feasible to ascertain. Violation of the proposed MCL for E. coli signifies fecal contamination occurrence and a possible high risk of exposure to pathogens. EPA is proposing to eliminate the MCLs for total coliforms and fecal coliforms because under the proposal there is no longer an MCLG for either total coliforms or fecal coliforms, for the reasons explained earlier. The proposed MCL for E. coli is consistent with the recommendation made by the advisory committee in the AIP.

iii. Coliform treatment technique. The 1996 SDWA amendments authorize EPA to promulgate a treatment technique in lieu of an MCL if EPA determines that "it is not economically or technologically feasible to ascertain the level of the contaminant" (SDWA 1412(b)(7)(A)). While it is technologically feasible to ascertain levels of E. coli (i.e., analytical methods continue to be available to measure the presence of E. coli in water samples), because of the intermittent nature of fecal contamination, it is not economically feasible to ascertain the level of E. coli occurrence below which the water may be deemed safe. This is because it is not economically feasible to monitor E. coli with sufficient frequency to ensure such safety.

Because total coliform bacteria are part of the soil ecosystem, positive samples are indicators of fecal contaminant entry into drinking water via a pathway from the soil. EPA is proposing a coliform treatment technique, supplemental to directly measuring E. coli, to provide additional protection against fecal contamination. Under the proposed coliform treatment technique, as specified in the AIP, total coliform-positive samples, in the absence of E. coli, are still indicators of an E. coli or other fecal contaminant pathway.

A PWS that exceeds a specified frequency of total coliform occurrence must conduct a Level 1 or Level
2 assessment to determine if any sanitary defect(s) exist(s) and, if found, to correct the defect(s). In addition, under the proposed treatment technique requirements, a PWS that incurs an E. coli MCL violation must conduct a Level 2 assessment and take remedial action if any sanitary defects are found.

The treatment technique requirements as proposed enhance public health protection beyond the E. coli MCL for the following reasons:

- ✓ The assessment and corrective action provisions of the treatment technique when the MCL for E. coli is exceeded require PWSs to investigate the potential causes of the fecal contamination and require timely remedial action if any sanitary defects are found. Under the current TCR, there are no requirements for investigation and corrective action after an MCL exceedance. Without such a find-and-fix provision, the pathway for contamination may not be identified and eliminated as sampling alone may not be adequate to identify intermittent sources of fecal contamination. The assessment and corrective action provisions of the proposed rule increase the likelihood of finding and correcting any sanitary defect and reduce the chance of recurrence of fecal contamination in the future.

- ✓ Using total coliforms in addition to E. coli as an indicator to prompt assessment and corrective action increases the sensitivity for identifying potential pathways for contamination. As discussed in section II.D.2 of this preamble, the presence of total coliforms indicates the potential existence of a pathway through which fecal contamination could follow. The absence (versus the presence) of total coliforms in the distribution system indicates a reduced likelihood that fecal contamination and/or waterborne pathogens are occurring in the distribution system. Analyses from EPA's

2005 Six-Year Review 2 data (USEPA 2006b; USEPA 2010e) (see section VI.B of this preamble for details on the Six-Year Review 2 data) and from the proposed RTCR Economic Analysis (EA) occurrence modeling show that total coliform presence in drinking water is approximately 20 to 40 times higher than E. coli occurrence in drinking water (see chapter 4 of the Proposed RTCR EA (USEPA 2010a)). Similarly, under the current TCR, non-acute MCL (also referred to as monthly MCL) violations (informed by total coliform occurrence) occur roughly 10 times more often than acute MCL violations (informed by total coliform and E. coli occurrence, essentially equivalent to the occurrence that triggers an E. coli MCL violation under this proposed rule). Thus, including monitoring of total coliforms, as well as E. coli, as part of a treatment technique to indicate when systems must find and fix any sanitary defects, substantially increases the likelihood of identifying such defects.

- ✓ The proposed treatment technique was supported by the advisory committee and is consistent with the recommendations in the AIP.

c. Request for comment. EPA requests comment on its proposal to eliminate the MCLG and MCL provisions for total coliforms and fecal coliforms and to include an MCLG and MCL for E. coli and coliform treatment technique provisions based on monitoring for total coliforms and E. coli. EPA also requests comment on its proposed definition of the E. coli MCL.

3. Monitoring

a. Provisions. As with the current TCR, the proposed RTCR requires all PWSs to collect and test samples for total coliforms and E. coli according to a sample siting plan and schedule specific to the system. Under the proposed RTCR, all PWSs are still required to take repeat samples within 24 hours of learning of any routine monitoring sample that is total coliform-positive. PWSs must comply with the repeat monitoring requirements and E. coli analytical requirement, discussed in detail in section III.A.4 of this preamble. All samples taken for proposed RTCR compliance (routine and repeat) may occur at a customer's premises, dedicated sampling station, or other designated compliance sampling location.

Under the proposed RTCR, system sample siting plans must include routine and repeat sample sites and any sampling points necessary to meet the Ground Water Rule (GWR) requirements. The sample siting plan is subject to State review and revision. The PWS may propose repeat monitoring locations that are expected to be representative of a pathway for contamination into the distribution system (for example, near a storage tank). Instead of identifying set repeat sampling locations (i.e., within five service connections upstream and downstream of the original sampling location that tested total coliform-positive), systems may elect to specify criteria for selecting their repeat sampling locations on a situational basis in a standard operating procedure (SOP), which is part of the sample siting plan. Upon State review, the PWS must demonstrate to the State's satisfaction that the sample siting plan remains representative of the water quality in the distribution system. The State may modify the SOP as needed. To address access issues, small systems must specify in their sampling plans where the two additional samples will be taken. The State may determine that monitoring at the entry point to the distribution system (especially for undisinfected ground water systems) is effective to differentiate between potential source water and distribution problems.

Under the proposed RTCR, PWSs may take more than the minimum required number of routine samples and include the results in calculating whether the total coliform treatment technique trigger for conducting an assessment has been exceeded only if the samples are taken in accordance with the sample siting plan and are representative of water throughout the distribution system.

EPA is not proposing to make substantive changes to the current TCR requirements for (1) special purpose samples, and (2) invalidation of total coliform samples. EPA is proposing a minor modification to the provision for special purpose samples by changing "total coliform MCL" to "coliform treatment technique trigger."

The following are the proposed monitoring requirements for different categories of systems.
i. Ground water NCWSs serving ? 1,000 people. (a). Routine monitoring. The proposed RTCR requires ground water NCWS serving 1,000 or fewer people to routinely monitor each quarter for total coliforms and E. coli. Seasonal systems under this category must routinely monitor every month (seasonal systems are discussed later in this section).
(b). Transition to the RTCR. The proposed RTCR requires all ground water NCWSs serving 1,000 or fewer people, including seasonal systems, to continue with their TCR monitoring schedules as of the compliance date of the RTCR, unless or until any of the conditions for increased monitoring discussed later on in this section are triggered on or after the compliance date or unless otherwise directed by the State, including through the special monitoring evaluation conducted under a sanitary survey. In addition, systems on annual monitoring, including seasonal systems, must have an initial annual site visit by the State within one year of the compliance date (or an annual voluntary Level 2 assessment by a party approved by the State) and an annual site visit each year thereafter to remain on annual monitoring.

This rule proposes that after the compliance date of the final RTCR, during each sanitary survey the State (which would be either EPA or a State that has received primacy for this rule) must perform a special monitoring evaluation to review the status of the water system, including the distribution system, to determine whether the system is on an appropriate monitoring schedule and modify the monitoring schedule as necessary. States must evaluate system factors such as the pertinent water quality and compliance history, the establishment and maintenance of contamination barriers, and other appropriate protections and validate the appropriateness of the water system's existing monitoring schedule and modify as necessary. For seasonal systems on quarterly or annual monitoring, this evaluation must also include review of the approved sample siting plan which designates the time period(s) for monitoring based on site-specific considerations (such as during periods of highest demand or highest vulnerability to contamination). The system must collect compliance samples during these time periods.
(c). Reduced monitoring. The State has the discretion to reduce the monitoring frequency for well-operated ground water NCWSs from the quarterly routine monitoring to no less than annual monitoring, if the water system can demonstrate that it meets the criteria for reduced monitoring provided in this section.

To be eligible to qualify for and remain on annual monitoring after the compliance date, a ground water NCWS serving 1,000 or fewer people must meet all of the following criteria:

- The most recent sanitary survey shows the system is free of sanitary defects, has a protected water source and meets approved construction standards;

- The system must have a clean compliance history (no MCL violations or monitoring violations under the current TCR and/or proposed RTCR, no Level 1 or Level 2 trigger exceedances or treatment technique violations under the proposed RTCR) for a minimum of 12 months. (For a more detailed discussion on Level 1 and Level 2 triggers, see section III.A.5 of this preamble); and

- An initial site visit by the State within the last 12 months to qualify for reduced annual monitoring, and recurring annually to stay on reduced annual monitoring; and correction of all identified sanitary defects. A voluntary Level 2 assessment by a party approved by the State may be substituted for the State annual site visit in any given year.

(d). Increased monitoring. Ground water NCWS serving 1,000 or fewer people on quarterly or annual monitoring that experience any of the following events must begin monthly monitoring the month following the event:

- ✓ The system triggers a Level 2 assessment or two Level 1 assessments in a rolling 12 month period;

- ✓ The system has an E. coli MCL violation;

- ✓ The system has a coliform treatment technique violation (for example, if the system fails to conduct a Level 1 assessment or correct for sanitary defects if required to do so); or

- ✓ The system on quarterly monitoring has two monitoring violations in a rolling 12-month period or system on annual monitoring has one monitoring violation.

The system must continue monthly monitoring until the requirements in this section for returning to quarterly or annual monitoring are met.

(e). Requirements for returning to quarterly monitoring. To be eligible to return to quarterly monitoring, ground water NCWSs serving 1,000 or fewer people must meet all of the following criteria:

* Within the last 12 months, the system must have a completed sanitary survey or a site visit by the State or a voluntary Level 2 assessment by a party approved by the State. The system is free of sanitary defects, and has a protected water source; and
* The system has a clean compliance history (no E. coli MCL violations, Level 1 or 2 triggers, coliform treatment technique violations or monitoring violations) for a minimum of 12 months.

(f). Requirements for returning to reduced annual monitoring. To be eligible to return to reduced annual monitoring after being placed on increased monitoring, the system must meet the criteria to return to routine quarterly monitoring plus the following criteria:

- ✓ An annual site visit (recurring) by the State and correction of all identified sanitary defects. An annual voluntary Level 2 assessment may be substituted for the State annual site visit in any given year; and

- ✓ The system must have in place or adopt one or more additional enhancements to the water system barriers to contamination as approved by the State. These measures could include but are not limited to the following:

—Cross connection control, as approved by the State;
—An operator certified by an appropriate State certification program, which may include regular visits by a circuit rider;
—Continuous disinfection entering the distribution system and a residual in the distribution system in accordance with criteria specified by the State; and
—Maintenance of at least a 4-log inactivation or removal of viruses each day of the month based on daily monitoring as specified in the GWR (with allowance for a 4-hour exception).
—Other equivalent enhancements to water system barriers as approved by the State.

(g). Seasonal systems. The proposed rule requires all seasonal systems to demonstrate completion of a State-approved start-up procedure on and after the compliance date of the final RTCR. Seasonal systems may continue with their TCR monitoring frequency after the compliance date of the final RTCR unless or until any of the conditions for increased monitoring discussed previously are triggered on or after the compliance date or as directed by the State. Under the proposed RTCR, seasonal systems are required to take routine samples monthly.

To be eligible for reduced monitoring after the compliance date, seasonal systems must meet the following criteria:

- ✓ The system must have an approved sample siting plan that designates the time period for monitoring based on site-specific considerations (e.g., during periods of highest demand

or highest vulnerability to contamination). The system must collect compliance samples during this time period; and

- ✓ To be eligible for reduced quarterly monitoring, the system must also meet all the reduced monitoring criteria discussed in section III.A.3.a.i.(e) of this preamble, Requirements for returning to quarterly monitoring.

- ✓ To be eligible for reduced annual monitoring, the system must also meet all the reduced monitoring criteria discussed in section III.A.3.a.i.(f) of this preamble, Requirements for returning to reduced annual monitoring.

(h). Additional routine monitoring. All systems collecting samples on a quarterly or annual frequency must conduct additional routine monitoring following a single total coliform-positive sample (with or without a Level 1 trigger event). The additional routine monitoring consists of three samples in the month following the total coliform-positive sample at routine monitoring locations identified in the sample siting plan. This is a change from the current TCR additional routine monitoring requirement of taking a total of five samples the month following a total coliform-positive sample for systems that take four or fewer samples per month.

In this proposal, consistent with the current TCR, the State may waive the additional routine monitoring requirement if:

- ➢ The State, or an agent approved by the State, performs a site visit before the end of the next month the system provides water to the public. Although a sanitary survey need not be performed, the site visit must be sufficiently detailed to allow the State to determine whether additional monitoring and/or any corrective action is needed. The State cannot approve an employee of the system to perform this site visit, even if the employee is an agent approved by the State to perform sanitary surveys.

- ➢ The State has determined why the sample was total coliform-positive and establishes that the system has corrected the problem or will correct the problem before the end of the next month the system serves water to the public. In this case, the State must document this decision to waive the following month's additional monitoring requirement in writing, have it approved and signed by the supervisor of the State official who recommends such a decision, and make this document available to the EPA and public. The written documentation must describe the specific cause of the total coliform-positive sample and what action the system has taken and/or will take to correct this problem.

The State may not waive the requirement to collect three additional routine samples the next month in which the system provides water to the public solely on the grounds that all repeat samples are total coliform-negative. If the State determines that the system has corrected the contamination problem before the system takes the set of repeat samples required in § 141.858, and all repeat samples were total coliform-negative, the State may waive the requirement for additional routine monitoring the next month.

All additional routine samples are included in determining compliance with the MCL and coliform treatment technique requirements.

ii. Ground water CWSs serving 1,000 people. (a). Routine monitoring. The proposed RTCR requires ground water CWSs serving 1,000 or fewer people to routinely monitor each month for total coliforms and E. coli.

The State may reduce the monitoring frequency for ground water CWS from the monthly routine monitoring to quarterly reduced monitoring if the water system can demonstrate that it meets the criteria for reduced monitoring provided later in this section
(b). Transition to the RTCR. All ground water CWSs serving 1,000 or fewer people continue with their current TCR monitoring schedules unless or until any of the increased monitoring requirements in this section occur or as directed by the State.

After the compliance date of the final RTCR, the State must determine whether the system is on an appropriate monitoring schedule by performing a special monitoring evaluation during each sanitary survey to review the status of the PWS, including the distribution system. The State must evaluate system factors such as the pertinent water quality and compliance history, the establishment and maintenance of barriers to contamination, and other appropriate protections to validate the water system's existing monitoring schedule or require more frequent monitoring.
(c). Reduced monitoring. The State has the flexibility to reduce the monitoring frequency for well-operated ground water CWS from the monthly routine monitoring to no less than quarterly monitoring if the water system can demonstrate that it meets the criteria for reduced monitoring provided in this section.

To be eligible for quarterly reduced monitoring, ground water CWSs serving 1,000 or fewer people on monthly monitoring after the compliance date must be in compliance with State-certified operator provisions and meet each of the following criteria:

- The most recent sanitary survey shows the system is free of sanitary defects (or has an approved plan and schedule to correct them), has a protected water source, and meets approved construction standards;
- The system must have a clean compliance history (no MCL violations or monitoring violations under the current TCR and/or proposed RTCR, no Level 1 or Level 2 trigger exceedances or treatment technique violations under the proposed RTCR) for a minimum of 12 months; and
- The system must meet at least one of the following criteria:

—An annual site visit by the State or a voluntary Level 2 assessment by a party approved by the State or meeting criteria established by the State and correction of all identified sanitary defects (or an approved plan and schedule to correct them), or
—A cross connection control program, as approved by the State,
—The system must maintain continuous disinfection entering the distribution system and a residual in the distribution system in accordance with criteria specified by the State,—The system must maintain at least a 4-log inactivation or removal of viruses each day of the month based on daily monitoring as specified in the GWR (with allowance for a 4-hour exception) (USEPA 2006c, 71 FR 65574, November 8, 2006); or
—Other equivalent enhancements to water systems as approved by the State.

(d). Return to routine monitoring requirements. When a system on quarterly monitoring experiences any of the following events the system must begin monthly monitoring:

- System triggers a Level 2 assessment or two Level 1 assessments in a rolling 12 month period;
- System has an E. coli MCL violation;
- System has a coliform treatment technique violation (e.g., fails to conduct a Level 1 or Level 2 assessment or to correct for a sanitary defect if required to do so); or
- System has two routine monitoring violations in a rolling 12-month period.

The system must continue monthly monitoring until all the reduced monitoring requirements discussed previously in this section are met. A system that loses its certified operator must also return to monthly monitoring the month following the loss.
(e). Additional routine monitoring. All systems collecting samples on a quarterly frequency must conduct additional routine monitoring following a single total coliform-positive sample (with or without a Level 1 trigger event). The additional routine monitoring consists of three samples in the month following the total coliform-positive sample at routine monitoring locations identified in the sample siting plan. The current TCR additional routine monitoring requirements consist of taking a total of five samples the month following a total coliform-positive sample for systems that take four or fewer samples per month.

In this proposal, consistent with the current TCR, the State may waive the additional routine monitoring requirement if:

- The State, or an agent approved by the State, performs a site visit before the end of the next month the system provides water to the public. Although a sanitary survey need not be performed, the site visit must be sufficiently detailed to allow the State to determine whether additional monitoring and/or any corrective action is needed. The State cannot approve an employee of the system to perform this site visit, even if the employee is an agent approved by the State to perform sanitary surveys.
- The State has determined why the sample was total coliform-positive and establishes that the system has corrected the problem or will correct the problem before the end of the next month the system serves water to the public. In this case, the State must document this decision to waive the following month's additional monitoring requirement in writing, have it approved and signed by the supervisor of the State official who recommends such a decision, and make this document available to the EPA and public. The written documentation must describe the specific cause of the total coliform-positive sample and what action the system has taken and/or will take to correct this problem.

The State may not waive the requirement to collect three additional routine samples the next month in which the system provides water to the public solely on the grounds that all repeat samples are total coliform-negative. If the State determines that the system has corrected the contamination problem before the system takes the set of repeat samples required in § 141.858, and all repeat samples were total coliform-negative, the State may waive the requirement for additional routine monitoring the next month.

All additional routine samples are included in determining compliance with the MCL and the coliform treatment technique requirements.

iii. Subpart H systems of this part serving 1,000 people. The monitoring requirements for subpart H systems of this part (PWSs supplied by surface water source or ground water source under the direct influence of surface water (GWUDI)) serving 1,000 or fewer people remain the same as under the current rule (see§ 141.856). These systems are not eligible for reduced monitoring. In addition, the proposed rule requires all seasonal systems, on and after the compliance date of the final RTCR, to demonstrate completion of a State-approved start-up procedure.
iv. PWSs serving 1,000 people. The monitoring requirements for PWSs serving more than 1,000 people remain the same as under the current TCR (see§ 141.857), with the exception of the applicable revisions to the repeat sampling locations provided in § 141.858 and additional routine monitoring provisions. Systems on monthly monitoring are not required to take additional routine samples the month following a total coliform-positive sample. These systems are not eligible for reduced monitoring. In addition, the proposed rule requires all seasonal systems, on and after the compliance date of the final RTCR, to demonstrate completion of a State-approved start-up procedure.

b. EPA's rationale. i. Sampling sites and monitoring plans. Consistent with current practice, the proposed RTCR requires systems to develop a sample siting plan that is representative of the

water throughout the distribution system. EPA is proposing to maintain the provision from the current TCR that indicates that sample siting plans are subject to State review and revision. The advisory committee recommended that States review and revise sample siting plans consistent with current practice and that the State develops and implements a process to ensure the adequacy of sample siting plans including a periodic review. The advisory committee also recommended that specific elements be included in the sampling plans such as the routine and repeat sample sites and sampling locations necessary to meet the requirements of the GWR. Alternative repeat monitoring locations (e.g., at storage tanks and entry points to the distribution system) are subject to State approval. The system must demonstrate to the State's satisfaction that these alternative monitoring locations are representative of the water quality in the distribution system.

By allowing systems to specify criteria for selecting their repeat sampling locations in their SOP instead of setting fixed repeat sampling locations, systems can provide a more flexible and more protective response. The system can focus the repeat samples at locations that will best verify and determine the extent of potential contamination of the distribution system based on specific situations. In addition, EPA is proposing to require State approval if a ground water system serving 1,000 or fewer people wants to use a single sample to meet both the repeat monitoring requirements of the RTCR and the source water monitoring requirements of the GWR. EPA is proposing to allow the use of dedicated sampling locations for the following reasons:

To reduce potential contamination of the taps. Utilities will have more control to prevent contamination of the tap by preventing its use by unauthorized persons and allowing no routine use of the tap except for sampling;

- To facilitate access to sampling taps. Currently systems may be constrained by where they sample, e.g., only at public buildings or in certain individual customer's houses.
- To improve sampling representation of the distribution system. Allowing dedicated sample taps in areas where systems have not been able to gain access will facilitate better sampling representation of the distribution system.
- ii. Ground water PWSs serving 1,000 people. (a). Routine monitoring. The advisory committee recommended that ground water NCWSs serving 1,000 or fewer people remain under a routine quarterly monitoring as provided in the current TCR. They believed that in conjunction with the assessment and corrective action requirements, public health protection would be maintained or improved without increasing sampling costs over current TCR requirements. The advisory committee also recognized that current sampling costs are not insignificant for small systems, and wanted to recognize the good performance of systems by allowing them to be able to continue to qualify for reduced monitoring, but under the more specific and rigorous criteria described previously. To continue to provide adequate health protection, systems on reduced monitoring must adhere to criteria that ensure that barriers are in place and are effective. Furthermore, systems with problems that may indicate poor system integrity, maintenance, or operations, or systems that fail to monitor, are triggered into monthly monitoring. This approach leverages the limited resources of these small ground water NCWSs and of States, so that systems with minimal problems can minimize their costs and States can focus their resources on systems needing the greatest attention, such as systems with problems or vulnerabilities.

The advisory committee thought it best to continue with existing routine monthly monitoring requirements for ground water CWSs serving 1,000 or fewer people in order to maintain the current levels of effort to identify potential problems. Since sanitary surveys are required under the GWR and these surveys provide substantial diagnostic value and corrective action response for problems identified, specifying higher routine monitoring frequency for these systems was not deemed necessary.

These systems may also qualify for reduced monitoring if they meet certain criteria.

(b). Transition to the RTCR. The advisory committee was concerned about the ability of the States and systems to adopt the new regulations and to make all the determinations that may be necessary to determine the appropriate monitoring frequency within three years of rule promulgation. Requiring significant changes in monitoring frequencies in a short period (i.e., without a transition period) could overwhelm State resources. The advisory committee recommended phasing in the requirements and using the sanitary survey process to facilitate a successful transition and implementation. The advisory committee, therefore, recommended that these systems continue with their current monitoring frequency during a transition period and that the State review the monitoring frequency to determine whether it is appropriate during each sanitary survey (USEPA 2008c, AIP p.9). This gives the systems the opportunity to address operation and maintenance issues to maintain existing monitoring frequency or qualify for reduced monitoring. Systems on reduced TCR monitoring stay on reduced monitoring during the transition period if they continue to meet the reduced monitoring criteria. During the special monitoring evaluation conducted as part of the periodic sanitary survey, the State will determine whether the individual systems are on the proper monitoring schedule.

(c) Reduced monitoring. The reduced monitoring requirements are intended to recognize that well-operated systems may be less vulnerable to contamination. Therefore, certain conditions are specified under which reduced monitoring could be allowed. These include a clean compliance history for a minimum of 12 months, and an annual visit from the State for systems taking one sample per year and correction of all identified sanitary defects. Ground water NCWSs serving 1,000 or fewer people, with a routine quarterly monitoring frequency, could qualify for reduced annual monitoring, while ground water CWSs serving 1,000 or fewer people, with a routine monthly monitoring frequency, could qualify for reduced quarterly monitoring.

For NCWSs on annual monitoring, the advisory committee believed that requiring a system to have an annual site visit or a Level 2 assessment provides at least an equivalent level of diagnosis of problems and vulnerabilities that might exist as compared to quarterly monitoring without an annual site visit. Several States have elected to conduct annual site visits while also doing annual monitoring for some NCWSs.

(d) Increased monitoring requirements for NCWSs. The advisory committee wanted to recognize that if certain vulnerabilities are identified in a system, the system should be required to conduct more frequent monitoring to identify and correct its problems and better protect public health. Other than sanitary surveys or other site visits, monitoring is the primary means to identify pathways for potential contamination. If the system is deemed more vulnerable to such pathways, as indicated by the increased monitoring criteria, it must conduct more monitoring.

(e) Requirements for returning to routine monitoring and reduced monitoring. The advisory committee believed that systems that address or correct vulnerabilities as indicated by a clean compliance history should be allowed to return to routine monitoring, and subsequently to reduced monitoring (for NCWS). This provision allows for reduced monitoring costs.

(f) Seasonal systems. The advisory committee recognized that seasonal systems have unique characteristics that make them more susceptible to contamination. These systems do not maintain pressure while not in operation, which can result in the intrusion of contaminants. During the time when a seasonal system is not in operation, septic tank drain fields or other pollution sources may accumulate that could affect the conditions or quality of the source water (especially for intermittent contaminants) that infrequent monitoring may not be able to capture. If monitoring is done only at the start-up, there may not be enough time for the system to reach equilibrium (i.e., there might not be enough time to recognize if microorganisms from a septic tank moved to the wellhead in seasonally operated systems). Therefore, the proposed rule requires seasonal systems to monitor routinely at a monthly frequency. Seasonal systems can qualify for reduced monitoring if they meet certain criteria. For a seasonal system to be allowed to monitor at a reduced frequency, the proposed rule requires the system to have an approved sample siting plan that designates the time period for monitoring and takes into consideration site-specific

conditions. A system on a reduced monitoring schedule (less than monthly) must collect samples when there is the greatest chance that contamination could be identified and, due to the variability in water demands, when systems could be most challenged.

(g) Additional routine monitoring. EPA is proposing to retain the requirement of taking additional routine samples the month following a total coliform-positive sample for systems on quarterly or annual monitoring. The advisory committee recognized both the benefits and the limitations of additional routine monitoring. Additional routine samples are meant to enhance the diagnostic ability and supplement the infrequent routine monitoring of systems on quarterly or annual monitoring. Without the provision of additional monitoring, systems on annual or quarterly monitoring with a total coliform-positive sample would not take any samples the following month. The advisory committee believed that additional samples collected the following month are appropriate to help recognize the problem if it still persists.
For systems required to take the additional routine samples the following month (i.e., systems on quarterly or annual monitoring), the proposed RTCR changes the requirement from taking a total of five routine samples to a requirement of just three routine samples. The advisory committee recognized that it is appropriate to drop from five to three samples the following month to reduce monitoring costs while still maintaining a substantial likelihood of identifying a problem if a problem persists. EPA recognizes that a reduction in the number of samples taken could also mean a reduction in the number of positive samples found. However, the reduction in the number of additional routine samples in conjunction with the new assessment and corrective action provisions of the proposed RTCR.

For systems taking at least one sample monthly, the advisory committee recommended no additional routine samples for these systems for the following reason. Taking no additional routine samples the following month substantially reduces monitoring costs. The assessment and corrective action provisions will give systems the ability to identify and prevent the occurrence of problems. EA modeling results show that although there is a decrease in the number of E. coli MCL violations found with the decrease in the number of additional routine samples taken (i.e., going from five samples to one during the month following a total coliform-positive), the assessment and corrective action provisions lead to more E. coli MCL violations being prevented compared to the current TCR.

In addition, whenever a total coliform-positive occurs during routine sampling, there is also a requirement to conduct repeat sampling to determine the extent of contamination or if potential pathways to contamination persist. For small systems serving 1,000 or fewer people on monthly monitoring, if a repeat sample is total coliform-positive, at least a Level 1 assessment will be triggered. If a sanitary defect(s) is (are) found, the system is required to correct the sanitary defect(s).

For systems on monthly monitoring, the assessment and corrective action provisions and the repeat sampling provisions mitigate the need for additional routine sampling for the following month.

iii. Subpart H systems of this part serving ? 1,000 people. EPA is not proposing to change the routine monitoring requirements for systems using surface water or GWUDI serving 1,000 or fewer people, which include not allowing reduced monitoring for these systems. Since systems using surface water or ground water under the influence of surface water tend to have much higher levels of contaminants in their source water, and in general have more complex operations than ground water systems, it is appropriate to allow reduced routine monitoring for ground water systems but not for subpart H systems of this part. The advisory committee recommended that no reduced routine monitoring provisions be allowed for subpart H systems of this part serving 1,000 or fewer people.
iv. Public water systems serving 1,000 people. EPA is proposing to eliminate the additional routine samples the month following a total coliform-positive sample for PWSs serving between 1,000 and 4,100 people for the same reasons discussed previously for small ground water

systems monitoring monthly. PWSs serving more than 1,000 people are currently required to routinely monitor monthly (one to four samples per month depending on size) and continue to do so under the proposed RTCR.

c. Request for comment. EPA requests comment on the proposed monitoring requirements for PWSs. Specifically, EPA requests comment on the following questions: Are there other issues that EPA should consider in its approach to help systems transition to the RTCR? Should EPA develop guidance that would help States identify seasonal systems and implement the RTCR requirements (e.g., suggestions for startup procedures and identifying vulnerable time periods)? What start-up procedures or other provisions regarding seasonal systems would be appropriate for inclusion in such guidance? EPA also requests comment on whether seasonal systems should be required to comply with State-directed shut down procedures (in addition to start-up procedures).

EPA requests comment on the following additional questions: Should daily measurement of chlorine residual count toward the maximum residual disinfectant level (MRDL) monitoring and be one of the criteria for reduced monitoring? Should NTNCWSs be required to comply with the CWS requirements (as they are in other rules such as DBP rules) since NTNCWSs serve the same people over time and include populations that may be at greater risk (e.g., schools, hospitals, nursing homes)? Will the reduced, routine, and increased monitoring requirements for NCWSs shift the fixed State resources from CWS oversight to NCWS oversight in those States with large numbers of NCWSs? If so, what might be done to limit the impact? Should EPA develop guidance on how to develop a sample siting plan? Should sample siting plans require State approval.

EPA and the advisory committee did not identify any specific issues regarding consecutive systems in the proposed RTCR. EPA requests comment on whether there are such issues and how they should be addressed in the RTCR.

4. Repeat Samples

a. Provisions. Under the proposed RTCR, all systems must take at least three repeat samples for each routine total coliform-positive sample. This is a change from the current TCR requirements where systems serving 1,000 or fewer people must collect at least four repeat samples while the rest of the systems must collect three repeat samples. EPA is not changing the following provisions: The 24-hour limit within which the system must collect the repeat samples; the authority of the State to extend this limit on a case-by-case basis; and the non-waiver by the State of the requirement for a system to collect repeat samples.

In addition to taking repeat samples, systems must test each routine total coliform-positive sample for E. coli. They must also test any repeat total coliform-positive sample for E. coli. As with the current TCR, if E. coli is present, the system must notify the State the same day it learns of the positive result or by the end of the next business day at the latest. The proposed rule is not changing the provision that a State has the discretion to allow the system to forgo E. coli testing in cases where the system assumes that the total coliform-positive sample is E. coli-positive. If the State allows a system to forgo E. coli testing, the system must still notify the State and comply with the E. coli MCL requirements specified in § 141.858.

As with the current TCR, the system must collect at least one repeat sample from the sampling tap where the original total coliform-positive sample was taken. Unless different locations are specified in its sample siting plan, the system must also collect at least one repeat sample at a tap within five service connections upstream, and at least one repeat sample at a tap within five service connections downstream of the original sampling site. The State may waive the requirement to collect at least one repeat sample upstream or downstream of the original sampling site if the total coliform-positive sample is at the end of the distribution system, or one service connection away from the end of the distribution system.

Under the proposed rule, ground water systems (GWSs) required to conduct triggered source monitoring under the GWR must take ground water source samples in addition to the repeat samples. However, a ground water system serving 1,000 or fewer people may use a repeat sample collected from a ground water source to meet both the repeat monitoring requirements of the proposed RTCR and the source water monitoring requirements of the GWR, but only if the State approves the use of a single sample to meet both rule requirements (i.e., a dual purpose sample) and the use of E. coli as a fecal indicator for source water monitoring. If the sample is E. coli-positive, the system violates the E. coli MCL under the proposed RTCR and must also comply with the GWR requirements following a fecal indictor-positive sample. These provisions are consistent with the GWR.

If a system with a limited number of monitoring locations (such as a system with only one service connection or a campground with only one tap) takes more than one repeat sample at the triggered source water monitoring location, the system may reduce the number of additional source water samples by the number of repeat samples taken at that location that were not E. coli-positive. For example, if a system takes two dual purpose samples and one is E. coli- positive and the other is E. coli-negative, the system has an E. coli MCL violation under the proposed RTCR and is required to take four additional source water samples, rather than five, under the GWR (see 40 CFR 141.402(a)(3)). If the system takes more than one of these repeat samples at the triggered source water monitoring location and has more than one repeat sample that is E. coli-positive, then the system would have both an E. coli MCL violation under the proposed RTCR and a second fecal indicator-positive source sample under the GWR. The system would then need to also comply with the treatment technique requirements under 40 CFR 141.403.

Under the proposed rule, the system must collect all repeat samples on the same day consistent with current TCR requirements. The State may allow systems with a single service connection to collect the required set of repeat samples over a three-day period or to collect a larger volume repeat sample(s) in one or more sample containers of any size, as long as the total volume collected is at least 300 ml.
The proposed RTCR is not changing the requirement that systems collect an additional set of repeat samples for each total coliform-positive repeat sample. As with the original set of repeat samples, the system must collect the additional repeat samples within 24 hours of being notified of the positive result, unless the State extends the time limit. The system must repeat this process until either total coliforms are not detected in one complete set of repeat samples or the system determines that the coliform treatment technique trigger has been exceeded and notifies the State. After a trigger (see section III.A.5 of this preamble) is reached, the system is required to conduct only one round of repeat monitoring after each total coliform-positive or E. coli- positive routine sample. If a trigger is reached as a result of a repeat sample being total coliform- or E. coli-positive, no further repeat monitoring related to that sample is necessary.

The proposed RTCR is also not changing the current TCR provision that a subsequent routine sample, which is within five service connections of the initial routine sample and is collected after an initial routine sample but before the system learns the initial routine sample is total coliform-positive, may count as a repeat sample instead.

Results of all routine and repeat samples not invalidated by the State must be used to determine whether the coliform treatment technique trigger has been exceeded (see section III.A.5 of this preamble for a discussion of the coliform treatment technique triggers).

b. EPA's rationale. i. Why EPA is maintaining a provision for repeat sampling. As with the current TCR, the proposed RTCR requires systems to take repeat samples after a total coliform-positive sample. EPA believes that sampling immediately after an initial positive sample (i.e., conducting repeat sampling) increases the likelihood of identifying the source and/or nature of the possible contamination. Analysis conducted by EPA indicated that once a total coliform-positive is found, there is a much greater likelihood of finding another total coliform-positive within a short period of

time of the initial finding. Repeat sampling (when total coliform-positive) can indicate a current pathway for potential external contamination into the distribution system.

EPA used the Six-Year Review 2 (USEPA 2010e) data to support statistical modeling which produced estimates of average occurrence of routine total coliform-positive samples and repeat total coliform-positive samples and to characterize how occurrence varies from system to system. EPA's occurrence model assumes that, among similar systems, the positive rate for total coliforms in routine samples varies as a beta random variable. EPA used the Six-Year Review 2 data (USEPA 2010e) to estimate the parameters for the distribution of occurrences of routine and repeat total coliform-positive samples.

ii. Frequency of repeat samples. The advisory committee recommended that the current TCR requirement for systems serving more than 1,000 people to take three repeat samples subsequent to a routine total coliform-positive be retained. The advisory committee recommended that systems serving 1,000 or fewer people also be required to take three repeat samples rather than the four required under the current TCR. This view is supported by analysis of repeat sample records from the Six-Year Review 2 data (USEPA 2010e).

Repeat sampling helps utility operators to better understand the extent and duration of potential pathways of contamination into the distribution system. The Six-Year Review 2 data (USEPA 2010e) show that the average percentage of samples that are positive among repeat samples is much higher than that of routine samples, demonstrating that when operators are required to take a second look at their systems following the positive routine sample, they find, on average, a higher rate of coliform presence than during routine sampling. In other words, the high repeat total coliform-positive rate indicates the persistence of total coliforms at such locations in the distribution system.

Further analysis of the data shows that for all PWSs serving 1,000 or fewer people, two or more of the repeat samples are positive in 75 percent of those instances in which there are any positive repeat samples. For those 75 percent of instances, reducing the number of repeat samples from four to three would have no effect on the number of systems that would be triggered to conduct an assessment of the system under the proposed RTCR. In these cases, at least one of the remaining repeat samples would still be total coliform-positive, and only one positive repeat sample is required to trigger an assessment.

The data show that one repeat sample is positive in 25 percent of the instances in which any of the four repeat samples is positive. For these instances, EPA estimates that if only three repeat samples had been taken instead of four, three out of four (or 75 percent) of these positive samples would still have been encountered.

Although dropping the required number of repeat samples from four to three means that some fraction of triggers may be missed, the other provisions of the proposed RTCR compensate for that change and, taken as a whole, the provisions of the proposed RTCR provide for greater protection of public health. One such provision includes enhanced consequences for monitoring violations. For example, systems that do not take all of their repeat samples under the proposed RTCR are triggered to conduct a Level 1 assessment. This permits an increase in public health protection over the current TCR because PWSs are required to assess their systems when monitoring results show that the PWS may be vulnerable to contamination (indicated by exceeding the trigger). Moreover, because of the substantial cost of this potential consequence, systems would be more likely to take all of their required repeat samples in the first place.

It is important to point out that the majority of systems in this category are ground water systems treating to less than 4-log inactivation for viruses (see Exhibit 4.1 of the Proposed RTCR EA (USEPA 2010a)). Because of the triggered source monitoring provision under the GWR, these systems are required to collect a fecal indicator sample from the source water following a total coliform-positive sample in the distribution system in addition to the repeat samples. Under the

existing GWR and TCR, systems taking four repeat samples are permitted to take the fourth repeat sample at the source water if they measure for E. coli as the fecal indicator and if they have State approval. Under the proposed RTCR, systems would continue to take this source water sample to comply with the GWR in addition to the required repeat samples in the distribution system to comply with the TCR.

A positive sample at the source that is not also considered a repeat sample would not trigger an assessment under the proposed RTCR, but it would provide diagnostic value to the system in addition to triggering additional requirements under the GWR (i.e., corrective action or five additional source water fecal indicator samples).

As under the existing GWR and current TCR, with State approval, ground water systems serving 1,000 or fewer people may use the sample taken at the location required for triggered source monitoring to also count toward the repeat monitoring requirements of the proposed RTCR. In this case, the State must also approve the use of E. coli as the fecal indicator under the GWR, and the system would comply with both GWR and the proposed RTCR when a total coliform-positive or E. coli-positive sample occurs. The advisory committee recommended this flexibility to reduce the burden on small ground water systems that in most cases will have a very limited distribution system and only one source, consistent with the GWR.

iii. Location of repeat samples. The advisory committee believed that requiring repeat samples to be taken within five service connections up and downstream of the original total coliform-positive location can be difficult for systems to implement within the required 24 hours for a repeat sample because of issues such as access to the site. Therefore, the advisory committee recommended that systems be allowed to develop standard operating procedures (SOPs) as part of their sample siting plan to identify alternative monitoring sites and facilitate the identification of the source and extent of any problem. EPA is not requiring prior State approval for this provision since there is no reduction in monitoring and the SOP is expected to be used only by larger systems with the technical resources to justify alternative monitoring sites.

The advisory committee also recommended that ground water systems have the flexibility to propose repeat sampling locations that differentiate potential source water and distribution system contamination (e.g., by sampling at entry points to the distribution system). See section III.A.3 of this preamble for additional discussion on this topic. Consistent with its understanding of the intent of the TCRDSAC, EPA has proposed that systems be allowed to exercise this flexibility only with prior State approval. State approval is required because this constitutes a reduction in monitoring (no separate triggered source water samples). EPA believes that this reduction in monitoring is appropriate only if the State determines that the dual purpose sample provides public health protection equivalent to that provided by separate repeat and source water samples. EPA believes that many ground water systems serving 1,000 or fewer people, such as systems with extensive distribution systems, will not be able to show that this reduction in monitoring (i.e., a loss of repeat sample that is near the total coliform-positive routine sample site, but not near the source water sample site) will provide public health protection equivalent to separate samples. EPA believes that systems with limited or no distribution systems are the best candidate for approval.

c. Request for comment. EPA requests comment on the foregoing proposed repeat sampling requirements. Specifically, EPA requests comment on the proposal to allow samples taken at the ground water source to serve both as a triggered source sample under the GWR and as one of the repeat samples under the proposed RTCR. EPA is also requesting comment on whether systems should be allowed to use a dual purpose sample simply by including that in the sample siting plan, without prior State approval. Also, should systems using repeat monitoring sites more than five connections upstream or downstream from the routine total coliform-positive site be required to get prior State approval?

5. Treatment Technique Requirements

a. Provisions. i. Coliform treatment technique triggers. The non-acute MCL violation for total coliforms under the current TCR is effectively replaced by a coliform treatment technique involving monitoring for total coliforms under the proposed RTCR. Under the proposed treatment technique framework, the presence of total coliforms is used as an indicator of a potential pathway of contamination into the distribution system. As discussed in section III.A.2 of this preamble, the proposed RTCR eliminates the associated MCLG and MCL for total coliforms. The proposed revision specifies two levels of treatment technique triggers, Level 1 and Level 2, and their corresponding levels of response. Whether systems are required to conduct either a Level 1 or Level 2 assessment is based on the degree of potential pathway for contamination. The proposed rule further lays out an additional trigger for a Level 1 assessment and defines Level 2 triggers that require a more in-depth examination of the system and its monitoring and operational practices.

The system has exceeded the trigger immediately once any of the following conditions have been met:

Level 1 treatment technique triggers:

- ✓ For systems taking 40 or more samples per month, the PWS exceeds 5.0 percent total coliform-positive samples for the month; or
- ✓ For systems taking fewer than 40 samples per month, the PWS has two or more total coliform-positive samples in the same month; or
- ✓ The PWS fails to take every required repeat sample after any single routine total coliform-positive sample.

Level 2 treatment technique triggers:

- ➢ The PWS has an E. coli MCL violation (see section III.A.6 of this preamble for description of what constitutes an E. coli MCL violation); or
- ➢ The PWS has a second Level 1 treatment technique trigger within a rolling 12-month period, unless the first Level 1 treatment technique trigger was based on exceeding the allowable number of total coliform-positive samples, the State has determined a likely reason for the total coliform-positive samples that caused the initial Level 1 treatment technique trigger, and the State establishes that the system has fully corrected the problem.
- ➢ For PWSs with approved reduced annual monitoring, a Level 1 treatment technique trigger in two consecutive years.

ii. Assessment. EPA is proposing an assessment process in the RTCR to strengthen public health protection. Under the current TCR, a system is not required to perform an assessment following a monthly/non-acute MCL violation or an acute MCL violation. In contrast, the proposed RTCR requires systems to conduct assessments following the triggers specified above.
EPA is proposing two levels of assessment based on the associated treatment technique trigger: Level 1 assessment for a Level 1 treatment technique trigger and Level 2 assessment for a Level 2 treatment technique trigger.

At a minimum, both Level 1 and 2 assessments must include review and identification of the following elements:

- ➢ Inadequacies in sample sites, sampling protocol, and sample processing,
- ➢ Atypical events that may affect distributed water quality or indicate that distributed water quality was impaired,

- Changes in distribution system maintenance and operation that may affect distributed water quality, including water storage,

- Source and treatment considerations that bear on distributed water quality, where appropriate, and

- Existing water quality monitoring data.

EPA expects that States will tailor specific assessment elements to the size and type of the system and that each public water system in turn will tailor its assessment activities based on the characteristics of its distribution system.

Level 1 assessment:
A Level 1 assessment must be conducted when a PWS exceeds one or more of the Level 1 treatment technique triggers specified above. Under the proposed rule, this self-assessment shall consist of a basic examination of the source water, treatment, distribution system and relevant operational practices. The PWS might look at conditions that could have occurred prior to and caused the total coliform-positive sample. Example conditions include treatment process interruptions, loss of pressure, maintenance and operation activities, recent operational changes, etc. In addition, the PWS might check the conditions of the following elements: sample sites, distribution system, storage tanks, source water, etc.

The PWS must complete the Level 1 assessment as soon as practical after notification of its monitoring results or failure to take repeat samples. The PWS must submit the completed assessment form to the State for review within 30 days after determination that the PWS has exceeded the trigger. Failure to submit the completed assessment form within 30 days is a reporting violation. If the State determines that the assessment is insufficient, the State will consult with the PWS. If necessary after consultation, the PWS must submit a revised assessment to the State on an agreed upon schedule not to exceed 30 days from the date of the initial consultation.

The completed assessment form must include assessments conducted, all sanitary defects identified (or a statement that no sanitary defects were identified), corrective actions completed, and a timetable for any corrective actions not already completed. Upon completion and submission of the assessment form by the PWS to the State, the State shall determine if the system has identified the likely cause(s) for the Level 1 treatment technique trigger and establish whether the system has corrected the problem(s).
Level 2 assessment:
A Level 2 assessment must be conducted when a PWS exceeds one or more of the Level 2 treatment technique triggers specified previously.

A Level 2 assessment is a more comprehensive examination of the system, its monitoring and operational practices than the Level 1 assessment. The level of effort and resources committed to undertaking a Level 2 assessment will be commensurate with the more comprehensive investigation and review of available information, and engage additional parties and expertise relative to the Level 1 assessment. Level 2 assessments must be conducted by a party approved by the State: The State itself, a third party, or the PWS where the system has staff or management with the required certification or qualifications specified by the State. If the PWS or a third party conducts the Level 2 assessment, the PWS or third party must follow the State requirements for conducting the Level 2 assessment.

The PWS must complete the Level 2 assessment as soon as practical after notification that the PWS has exceeded a Level 2 treatment technique trigger. The PWS must submit the completed assessment form to the State for review within 30 days after determination that the PWS has exceeded the trigger. Failure to submit the completed assessment form after the PWS properly

conducts the assessment is a reporting violation. The State may direct expedited action or additional actions such as in the case of an E. coli MCL violation. If the State determines that the assessment is insufficient, the State will consult with the PWS. If necessary after consultation, the PWS must submit a revised assessment to the State on an agreed upon schedule not to exceed 30 days from the date of the initial consultation.

The completed assessment form must include assessments conducted, all sanitary defects (or a statement that no sanitary defects were identified), corrective actions completed, and a timetable for any corrective actions not already completed. Upon completion and submission of the assessment form by the PWS to the State, the State shall determine if the system has identified the likely cause(s) for the Level 2 treatment technique trigger and, if so, establish that the system has corrected the problem(s).

iii. Corrective action. The current TCR does not require systems that have MCL violations to perform corrective actions. Under this proposal, EPA is requiring PWSs to correct sanitary defects found through either a Level 1 or Level 2 assessment. Ideally, systems will be able to correct any sanitary defects found in the assessment within 30 days and report that correction on the assessment form. When the correction of sanitary defects is not completed by the time the PWS submits the completed assessment form to the State, the PWS must complete the corrective action(s) on a schedule determined by the State. This schedule may be developed in consultation with the PWS. The schedule must include when the corrective action will be completed and any necessary milestones and temporary public health protection measures. The PWS must notify the State when each scheduled corrective action is completed.

At any time during the assessment or corrective action phase, either the PWS or the State may request a consultation with the other entity to discuss and determine the appropriate actions to be taken. The system may consult with the State on all relevant steps that the system is considering to complete the corrective action, including the method of accomplishment, an appropriate timeframe, and other relevant information.

E. coli detection in the distribution system indicates a public health hazard and can result in an E. coli MCL violation. Under the proposed rule, when an E. coli MCL violation has occurred, the system must complete a Level 2 assessment and corrective action must be implemented as soon as practical. The Agency encourages systems to promptly find the source of E. coli and fix the problem before the completed assessment form is due to the State.

b. EPA's rationale. i. Coliform treatment technique. The advisory committee indicated that the conditions leading to a monthly/non-acute MCL under the current TCR should trigger an assessment under the RTCR for several reasons. First, the advisory committee recognized that presence of total coliform indicates the potential presence of a pathway for contamination from external sources such as source water or through a loss of distribution system integrity. The change to a coliform treatment technique construct that uses total coliforms as an indicator of distribution system integrity places the emphasis on systems to take more preventive actions to address problems. These actions would better protect public health than the additional monitoring with no corrective action that is required under the current TCR. To address the high and constant number of PWSs with violations over the years under the current TCR, the proposed changes would be more protective by requiring systems to correct deficiencies associated with the non-acute MCL (see Exhibit VI.1 in section VI.C.1 of this preamble).

Second, the advisory committee indicated that the public notice associated with non-acute violations is confusing because the presence of total coliforms is not necessarily an indication of a potential public health threat; however, it is an indicator of a potential pathway for fecal contamination to enter into the distribution system. Under the treatment technique requirement, the presence of total coliforms (at the level equivalent to a non-acute violation) indicates a need to assess whether a problem exists. When a system fails to conduct the assessment, the system will then incur a violation and be required to issue public notification. If the system does conduct

the assessment and satisfies the requirements of the treatment technique (including corrective action when a sanitary defect is identified), no public notification is required. Third, the occurrence of total coliforms in the context of the coliform treatment technique requirement continues to inform and further the original objectives of the TCR: to evaluate the effectiveness of treatment, determine the integrity of the distribution system, and signal the possible presence of fecal contamination. Finally, total coliform presence indicates the potential presence of a pathway for contaminants from external sources such as source water or through a loss of distribution system integrity.

ii. Assessment. The proposed rule requires assessments to ensure that specific action is taken to identify whether potential pathways of contamination into the distribution system exist. The advisory committee indicated that assessments are significant actions that protect public health. Under the current rule, when a system has a non-acute MCL violation and if any subsequent sampling did not detect total coliform, the problem may persist due to the intermittent nature of total coliform and remain unaddressed. However, the absence of total coliform-positive samples subsequent to an initial positive finding is not a reliable indicator that a contamination pathway no longer exists. In contrast, the proposed revisions would ensure that systems examine and assess the cause of the total coliform occurrence (that is equivalent to the current non-acute MCL level) and take any corrective action if necessary.

Under the proposed rule, the system will also be required to conduct an assessment if it fails to conduct repeat monitoring following an initial total coliform-positive sample result. As discussed in section III.A.4 of this preamble, repeat monitoring is critical in identifying the extent, source, and characteristics of fecal contamination in a timely manner. Since the revised rule proposes to eliminate additional routine monitoring for systems that monitor at least monthly and decrease the number of additional routine monitoring and repeat monitoring samples for the smallest systems, the need to conduct repeat monitoring is more crucial than ever in providing immediate and useful information needed to protect public health. The cost for collecting and analyzing a repeat sample would be considerably less than the cost for conducting a Level 1 assessment. EPA expects that systems will want to ensure that assessments are conducted only when potential problems may exist rather than for failure to take repeat samples.

The proposed rule specifies two different levels of assessments—Level 1 and Level 2—to recognize that a higher level of effort to diagnose a problem applies to situations of greater potential of public health concern such as repeated Level 1 triggers or an E. coli MCL violation. Level 2 assessments are conducted by a party approved by the State, which may be the PWS where it has staff or management with the certification or qualifications as determined by the State. The Level 2 assessments may also be conducted by the State or a third party approved by the State.

To make more transparent what the Level 1 and Level 2 assessments entail and to facilitate consistent implementation among States, the proposed rule specifies minimum elements for these assessments. The advisory committee recommended that the minimum elements identified previously in this preamble be included in the Level 1 and Level 2 assessments to identify potential flaws in monitoring or specific pathways of contamination. Although the proposed RTCR specifies the same minimum elements for both the Level 1 and Level 2 assessments, the Level 2 assessment involves a more in-depth examination of these elements compared to a Level 1 assessment. Specific examples of how the Level 2 assessments are more in-depth than Level 1 assessments may be found in Appendix X of the AIP (USEPA 2008c).

EPA recognizes that not every assessment will identify a sanitary defect or find a reason or cause for the presence of total coliforms. If no sanitary defect is identified, the system must document that fact in the completed assessment form and provide supporting evidence for this conclusion. EPA expects that only systems that adhere to proper procedures and standards set by the State are eligible to arrive at this determination, and only after providing sufficient supporting evidence.

The advisory committee recommended that the Level 1 and Level 2 assessments be conducted as soon as practical after the PWS receives notice that the system has exceeded the treatment technique trigger. The advisory committee also recommended that systems submit the completed assessment forms to the State within 30 days after determination that the PWS has exceeded the trigger. The rationale for the 30-day interval is to allow sufficient time for problem identification and potential remediation of the problem in conjunction with the follow-up assessment, in most cases.

To help States and PWSs conduct assessments, EPA intends to develop a draft assessment and corrective action guidance manual and to make it available for public comment prior to promulgation of the final rule and to finalize the guidance manual after the rule is finalized.

iii. Corrective action. The advisory committee recognized that not every assessment will identify a sanitary defect. However, the advisory committee recommended that the RTCR require all sanitary defects be corrected by the system in a timely manner. The system, in consultation with the State as needed, identifies and determines the specific corrective action.

Under the proposed rule, the State may allow the PWS additional time to conduct the corrective action if needed. EPA recognizes that some systems may not be able to fix sanitary defects before submitting the completed assessment form within the 30-day interval due to the extent and cost of the corrective action.

In such situations, EPA encourages the State and PWS to work together to determine the appropriate schedule for corrective actions (which may include additional or more detailed assessment or engineering studies) to be completed as soon as possible. The system must comply with the agreed upon schedule and notify the State when each scheduled corrective action is completed.

Either the PWS or the State may request consultation with the other party to determine the appropriate actions to be taken. EPA is not requiring this to be a mandatory consultation to provide ease of implementation for States. In many cases, consultation may not be necessary because the type of corrective action for the sanitary defect will be clear and can be fixed right away (for example, replacement of a missing screen).

c. Request for comment. EPA requests comment on the: (1) Proposed change from the non-acute MCL for total coliforms to a coliform treatment technique requirement that uses total coliforms as an indicator of a pathway of contamination; (2) proposed requirement for systems to conduct an assessment following a trigger condition; (3) proposed levels of assessment required; and (4) proposed requirement for systems to correct all sanitary defects found during an assessment. In addition, EPA requests comment on how to ensure that a Level 2 assessment is more comprehensive than a Level 1 assessment (e.g., should a Level 2 assessment include additional elements such as asset management and capacity development?). Should EPA provide more detail, either in guidance or rule language, on the elements and differences between a Level 1 and Level 2 assessments? If in rule language, how should the rule language distinguish the two levels of assessments? Please provide examples. Additionally, should EPA provide additional guidance on how systems might address the situation where a Level 1 or Level 2 assessment fails to identify any sanitary defects (i.e., the trigger event remains unexplained). If so, what should such guidance say?

6. Violations

a. Provisions. EPA is proposing to modify the definition of the existing MCL violation, establish a treatment technique violation, and revise the monitoring and reporting violations. EPA is proposing that public notice be required for each type of violation (see section III.A.7 of this preamble for detail information on public notification).

i. E. coli MCL violation. A violation of the E. coli MCL occurs when:

- ✓ A routine sample is total coliform-positive and one of its associated repeat samples is E. coli- positive; or
- ✓ A routine sample is E. coli-positive and one of its associated repeat samples is total coliform-positive; or
- ✓ A system fails to take all required repeat samples following a routine sample that is positive for E. coli; or
- ✓ A system fails to test for E. coli when any repeat sample tests positive for total coliforms.

ii. Coliform treatment technique violation. A coliform treatment technique violation occurs when:

- ✓ A system fails to conduct a required assessment within 30 days of notification of the system exceeding the trigger (see section III.A.5 of this preamble for conditions under which monitoring results trigger a required assessment); or
- ✓ A system fails to correct any sanitary defect found through either a Level 1 or 2 assessment within 30 days (see also section III.A.6 of this preamble) or in accordance with State-derived schedule.

There would be no treatment technique violation associated solely with a system exceeding one or more action triggers (Level 1 or Level 2 triggers).

iii. Monitoring violation. Under the current TCR, a monitoring violation occurs when a system fails to comply with the total coliform monitoring requirements, including the sanitary survey requirement. Under the proposed RTCR, a monitoring violation occurs when a system fails to take every required routine or additional routine sample in a compliance period, or when it fails to test for E. coli following a routine sample that is total coliform-positive.

In addition, if a system on quarterly monitoring has a monitoring violation in two or more quarters, or if a system on annual monitoring misses its annual monitoring, it must begin monthly monitoring until it meets criteria for less frequent monitoring. See section III.A.3 of this preamble for a detailed discussion on monitoring frequency.

iv. Reporting violation. A reporting violation occurs when a system that properly conducts monitoring or an assessment fails to submit a monitoring report or a correctly completed assessment form by the required deadline. The PWS is responsible for reporting this information to the State regardless of any arrangement with a laboratory. A reporting violation also occurs when a system fails to notify the State following an E. coli-positive sample.

b. EPA's rationale. To define violations, the advisory committee built upon the principles underlying the current TCR violations and current TCR public notification and suggested changes to improve public health protection where they saw a specific need. This proposal specifies responses to different degrees of potential public health concern. As described in the next section on providing information and notification to the public, Tier 1, Tier 2, and Tier 3 public notices are required following violations corresponding to the severity of each violation type.

i. E. coli MCL violation. An E. coli MCL violation (which includes failure to take all required repeat samples following an E. coli-positive sample) creates concern of an immediate potential public health threat. For this reason, an E. coli MCL violation is considered an acute violation requiring immediate response by the system. Including an E. coli MCL violation condition for systems failing to collect all repeat samples following an initial E. coli- positive sample enhances public health protection by preventing a system from incurring only a monitoring violation when there is an indication of fecal contamination. As discussed previously in section II.D of this preamble, the presence of E. coli indicates a pathway of fecal contamination and should be taken seriously. Systems need to follow up with repeat samples to characterize the extent and source of such contamination. Failure to take the required repeat samples following an initial E. coli-positive sample is not protective of public health and is a serious violation.

ii. Coliform treatment technique violation. A coliform treatment technique violation occurs when a potential pathway of contamination in the distribution system is unexplored and/or uncorrected. Performing the Level 1 and 2 assessments and taking corrective action are essential aspects of compliance with the treatment technique. A system which neglects to perform the prescribed assessment or corrective action is in violation of the proposed RTCR's treatment technique requirements.

iii. Monitoring violation and reporting violation. Monitoring and reporting violations occur when a system fails to comply with the routine monitoring requirements or when a system fails to submit monitoring reports or completed assessment forms. EPA believes that monitoring violations and reporting violations need to be addressed so that a system is held accountable to take actions to reduce public health risk, including regular monitoring of water quality.
c. Request for comment. EPA requests comment on the proposed violation determinations.

7. Providing Notification and Information to the Public
a. Provisions. To correspond to the changes in the proposed revised rule, EPA is proposing some modifications to the public notice (PN) requirements contained in 40 CFR part 141 subpart Q. Tier 1 PN is required for an E. coli MCL violation. Tier 2 PN is required for a treatment technique violation for failure to conduct assessments or corrective actions. Tier 3 PN is required for a monitoring violation or a reporting violation.

In the current TCR, if a system has an acute MCL violation which is based on the presence of fecal coliforms or E. coli, or which is based on the system's failure to test for fecal coliforms or E. coli following a total coliform-positive repeat sample, the system is required to publish Tier 1 PN. Under the proposed RTCR, a system is required to publish Tier 1 PN when it has an E. coli MCL violation (see section III.A.6 of this preamble for what constitutes an E. coli MCL violation). In addition, the system will continue to be required to notify the State after learning of an E. coli-positive sample, as currently is required under the TCR. As mentioned earlier in section III.A.2 of this preamble, EPA is proposing to eliminate the MCL for fecal coliforms. Under the proposed rule, the standard health effects language, which is required to be included in all public notification actions, is modified to delete the reference to the fecal coliform MCL and fecal coliforms. The language for a non-acute violation under the current TCR is modified to apply to a violation of the assessments and corrective action requirements of the coliform treatment technique.

In the current TCR, a system is required to publish a Tier 2 PN when the system has a non-acute MCL violation, which is based on total coliform presence. Under the proposed rule, a system is required to publish a Tier 2 PN if the system violates the coliform treatment technique requirements. Also, EPA is proposing to modify the standard health effects language for coliform to emphasize the assessment and corrective action requirements of the proposed rule.

In the current TCR, a system is required to publish a Tier 3 PN when the system has a monitoring or reporting violation. In the proposed rule, the Tier 3 PN requirements are changed to

incorporate the recommendation in the AIP that monitoring violations be considered distinct from reporting violations under the proposed RTCR. Both types of violations require Tier 3 PN.

Consumer confidence report (CCR) requirements are also modified. Health effects language for the CCR, which is identical to the health effects language required for PN, is updated in the same way as described for PN. In addition, the proposed RTCR removes the CCR requirements that require the inclusion of total numbers of positive samples, or highest monthly percentage of positive samples for total coliforms as well as total number of positive samples for fecal coliforms. These provisions are replaced by requirements to include the number of Level 1 and Level 2 assessments required and completed, the corrective actions required and completed, and the total number of positive samples for E. coli. Unchanged and consistent with existing provisions under the current TCR, a CWS may provide Tier 3 PN using the annual CCR.

b. EPA's rationale. The proposed public notification requirements are consistent with the AIP language as well as with the tier system described in 40 CFR part 141 subpart Q. These changes are appropriate because some of the types of violations in the proposed RTCR are different from the current TCR. The standard health effects language for the public notification is also revised as appropriate given the changes to what constitutes a violation under the proposed RTCR.

The proposed Tier 1 PN requirement for an E. coli MCL violation is consistent with the current TCR. Tier 1 PN is required for NPDWR violations and situations with significant potential to have serious adverse effects on human health as a result of short term exposure. The existing Tier 1 PN requires public notice as soon as possible but no later than 24 hours after the system learns of the violation. Exposure to E. coli in drinking water can possibly result in serious, acute health effects, such as diarrhea, cramps, nausea, headaches, or other symptoms and possible greater health risks for infants, young children, some of the elderly, and people with severely compromised immune systems.

Tier 2 PN is required for all NPDWR violations and situations with potential to have serious adverse effects on human health not requiring Tier 1 PN.

The system must provide public notice as soon as practical, but no later than 30 days after the system learns of the violation. A treatment technique violation under the proposed RTCR meets these criteria because it is an indication that the public water system failed to conduct an assessment or complete corrective action following identification of sanitary defects. Identification of a sanitary defect indicates that a problem may exist in the distribution system that has potential to cause public health concern.

Tier 3 PN is required for all other NPDWR violations and situations not included in Tier 1 or Tier 2. The existing Tier 3 PN requires a system to provide public notice no later than one year after the system learns of the violation or situation or begins operating under a variance or exemption. Monitoring violations and reporting violations meet these criteria because, while they do represent a violation of the proposed RTCR, the risk to public health is not as clearly linked as those that are Tier 1 or 2. Therefore, EPA believes that a public notice given at least annually fulfills the public's right-to-know about these violations.

Consumer confidence report requirements are updated to reflect the advisory committee's recommendations that total coliforms be used as an indicator to start an evaluation process that, where necessary, will require the PWS to correct sanitary defects. EPA believes it is most appropriate to inform the public about actions taken, in the form of assessments and corrective actions, since failure to conduct these activities lead to treatment technique violations under the proposed RTCR. Because the proposed RTCR no longer includes the total coliform MCL but now includes a trigger, EPA believes that systems no longer need to report the number of total coliform-positive samples via the CCR, since that could cause confusion or inappropriate changes in behavior among consumers. In addition, the CCR requirements will also reflect the removal of fecal coliform provisions under the proposed RTCR.

c. Request for comment. EPA requests comment on whether the PN and CCR language revisions are consistent with the provisions of the proposed RTCR that reflect the use of total coliforms as an indicator within a coliform treatment technique. Since EPA is not aware of health effects resulting solely from exposure to total coliforms, the proposed RTCR eliminates the public notification requirement for detection of total coliforms, but provides for public notification upon detection of E. coli, and for violation of the coliform treatment technique.
The Agency does request comment, however, on the loss of information to consumers resulting from elimination of public notification requirements following positive sample results for total coliforms.

EPA also requests comment on whether the proposed RTCR should require special notice to the public of sanitary defects, in addition to the PN requirements, similar to the GWR special notice requirements. This would be consistent with current requirements for other regulations that limit pathogens in ground water systems. Under 40 CFR 141.403(a)(7)(i), a CWS must inform the public of the significant deficiency and/or fecal indicator-positive sample. The CWS must continue to inform the public annually until the significant deficiency is corrected or the fecal contamination in the ground water source has been determined by the State to be corrected. Under 40 CFR 141.403(a)(7)(ii), an NCWS that receives notice from the State of a significant deficiency must inform the public of any significant deficiency that has not been corrected within 12 months of being notified by the State, or earlier if directed by the State. The NCWS must continue to inform the public annually until the significant deficiency is corrected.

8. Reporting and Recordkeeping Requirements for Systems
a. Provisions. i. Reporting. In addition to the existing general reporting requirements provided in 40 CFR 141.31, the proposed RTCR requires a PWS to:

- ✓ Notify the State no later than the end of the next business day after it learns of an E. coli-positive sample.

- ✓ Report to the State an E. coli MCL violation no later than the end of the next business day after learning of the violation. The PWS is also required to notify the public according to the provisions laid out in 40 CFR part 141 subpart Q.

- ✓ Report to the State a treatment technique violation no later than the end of the next business day after it learns of the violation. The PWS must also notify the public in accordance with 40 CFR part 141 subpart Q.

- ✓ Report to the State monitoring violations within ten days after the system discovers the violation, and notify the public in accordance with 40 CFR part 141 subpart Q.

- ✓ Notify the State when each scheduled corrective action is completed for corrections not completed by the time of the submission of the assessment form.

In addition, systems triggered into conducting an assessment are required to submit the completed assessment form within 30 days after determination that the coliform treatment technique trigger has been exceeded (see section III.A.3 of this preamble for additional discussion).

ii. Recordkeeping. EPA is proposing to maintain the current TCR requirements regarding retention of sample results and records of decisions related to monitoring schedules found in 40 CFR 141.33, including provisions that address the new requirements of the proposed RTCR pertaining to reduced and increased monitoring, treatment technique, etc. In addition, systems are required to maintain on file for State review the assessment form or other available summary documentation of the sanitary defects and corrective actions taken. Systems are required to

maintain these documents for a period not less than five years after completion of the assessment or corrective action.

b. EPA's rationale. In the case of an E. coli-positive sample, the proposed RTCR maintains the current TCR requirement that systems must notify the State by the end of the day when they are notified of the E. coli-positive result or by the end of the next business day if the State office is already closed. The advisory committee believed that this requirement is important to maintain because of the potential for immediate public health risk associated with E. coli presence and the desire for States to consider quickly whether additional actions might be appropriate. The same rationale applies to E. coli MCL violations.

Since there are new requirements for conducting assessments and corrective actions, and new conditions for obtaining increased or reduced monitoring provisions, the proposed rule includes reporting and recordkeeping requirements to facilitate tracking of Level 1 and Level 2 triggers and compliance with treatment technique requirements. Systems are required to maintain these files no less than five years. Since systems have to maintain these files no shorter than the maximum period allowed between sanitary surveys (i.e., five years; see 40 CFR 142.16(b)(3) and 40 CFR 142.16(o)(2)), States have the opportunity to look at and review these files during sanitary surveys and/or annual visits. The five year period is also consistent with the recordkeeping requirements for microbiological analyses under 40 CFR 141.33(a).

The timeframe by which reporting and recordkeeping are required under the proposed rule is consistent with EPA's practice regarding reporting and recordkeeping requirements in other regulations under SDWA.

c. Request for comment. EPA requests comment on whether the timeframe required for reporting and recordkeeping requirements are appropriate.

9. Analytical Methods

a. AIP-related method issues. i. Evaluation of currently-approved methods. The AIP contains several recommendations by the advisory committee regarding the analytical methods approved under the proposed RTCR. The advisory committee noted that the methods currently approved under the current TCR have varying sensitivities and specificities, and recommended that " * * * the Agency evaluate all currently approved coliform analytical methods to determine whether these methods continue to be appropriate for use in drinking water compliance monitoring" (USEPA 2008c, AIP p. 7).

In the twenty years since the current TCR was promulgated, many methods have been developed and approved for use. Most of the approved methods that are used to support the current TCR were evaluated under EPA's Alternate Test Procedure (ATP) process. Under this process, a proposed method is evaluated in comparison to a reference method. A favorable comparison serves as the basis for subsequent approval of the method for use in regulatory compliance monitoring.

The ATP evaluations are designed based on the ATP Microbiology Protocol (USEPA 2004), an EPA guidance document that outlines how the evaluation study should be conducted. In the years the ATP program has been in place, the ATP guidance document has been revised several times. As a result of different protocols being used over time, the current set of approved methods have not all been evaluated under identical conditions.

In addition to the concerns expressed by the advisory committee that the approved methods may not be equivalent to each other, EPA notes that there have been additional concerns with some of the methods currently approved. This includes allegations that some of the approved methods may have been modified since approval without EPA's knowledge. EPA is also aware of reports of varying performance of some enzyme-based methods (Oldstadt et al. 2007; Fricker et al. 2003). Lastly, EPA is aware of at least one circumstance where the manufacturer of an approved

method placed a “product hold” and recall on the medium after the product was reported to be experiencing reduced recovery of E. coli.

For these reasons, EPA believes that additional information may be needed regarding the performance of the currently approved methods in order to justify their continued approval. Among the options, EPA is considering a complete, side-by-side method evaluation study, whereby all the methods are compared to each other under identical conditions, according to the same protocol.

EPA is considering an approach under which vendors of all currently approved methods would have the option of voluntarily participating in an independent, third-party laboratory evaluation through EPA's Environmental Technology Verification (ETV) Program. The goal of the ETV Program is to provide independent, objective, and credible performance data for commercial-ready environmental technologies. More information on this program is available on EPA's Web site at www.epa.gov/etv/index.

Under the ETV approach, EPA anticipates that participating vendors would generally fund the majority of the cost of their method evaluation. Based on the results of the ETV study, as documented in the verification report, EPA would judge the appropriateness of each analytical method and would determine which should continue to be approved for future monitoring. EPA would then make any changes to the analytical methods approved under the RTCR through later rulemaking.

If a vendor chooses not to participate in the ETV study, EPA would allow the vendor to propose, for EPA's consideration, an equivalent alternative approach for method evaluation. EPA will determine whether the proposed approach will provide an independent, effective, and credible evaluation. EPA emphasizes that any alternative approach would need to be equivalent in scope and rigor to the ETV program. As with the ETV study, EPA would use the results from an alternative study to judge the appropriateness of each analytical method and would determine which methods warrant approval for future monitoring under this regulation.

iii. Approval of “24-hour” methods. The AIP also recommends that EPA “consider approving methods that allow the timely (e.g. on the order of 24 hours) analytical results for E. coli and TC and that provide relatively concurrent analyses, without significantly sacrificing accuracy, precision and specificity” (USEPA 2008c, AIP p. 7). EPA notes that many of the approved methods that may be used in compliance with the proposed rule can be completed in approximately 24 hours. However, the methods that detect lactose fermentation include a confirmation step that involves transfer of a presumptively positive culture into a more inhibitory confirmation medium which serves to ensure the initial positive was correct. As a result of this confirmatory step, lactose fermentation methods can take up to 96 hours to obtain a result. The enzyme based methods do not require this confirmation step, and their results can be obtained in a 24 to 48 hour time period.

EPA is aware of some concerns that methods with a 24 hour incubation time may not be able to detect as many coliform bacteria as methods with a 48 hour incubation period. Since many of the coliform bacteria found in a distribution system are injured or stressed due to disinfection practices, and since injured/stressed organisms may take longer to detect than 24 hours, this concern is of interest to EPA.

As part of, or in addition to, the method evaluation previously described, EPA may therefore further investigate the impact of incubation time on the recovery of stressed/injured organisms in drinking water using approved media. At this time, EPA believes that it is premature to conclude that either enzyme-based or lactose-based methods are inherently preferable.

As discussed during the advisory committee meetings, the analysis time of the analytical methods is just one aspect in the overall amount of time it takes before a PWS obtains sample results from

the laboratory and subsequently collects repeat samples. Factors that can impact how quickly the PWS receives notification of a positive result include whether the PWS uses an in-house laboratory or must ship the sample to a distant contract laboratory, and whether the sample results are reported via an electronic means or via traditional mail. In addition, the turnaround time for repeat sampling can be affected by such factors as the laboratory daily hours of operation. The current TCR specifies that repeat samples be collected within 24 hours, but States currently have the flexibility to extend this timeline. The current TCR does not contain provisions for how quickly the laboratory must notify the PWS when a positive result is obtained. This proposal does not change these provisions.

iv. Elimination of fecal coliforms under the proposed RTCR. The AIP also contains a recommendation that EPA remove all provisions related to fecal coliforms under the proposed RTCR. Consistent with this recommendation, and for the following reasons, EPA is proposing to eliminate all fecal coliform provisions in the RTCR.

First, the fecal coliform group can contain bacteria not associated with fecal contamination. E. coli is the most prominent member of the fecal coliform group. However, other coliform bacteria, such as thermotolerant strains of Klebsiella spp., have been shown to occur in the fecal coliform group (Warren et al. 1978). These non-E. coli bacteria are often found in environmental sources (for example, soil, vegetation, water) and, therefore, are not exclusively associated with feces. Due to the presence of these non-fecal bacteria, the fecal coliform group may not always provide the public water system with meaningful data regarding the vulnerability of their distribution system to fecal contamination.

Secondly, when the current TCR was developed, there were few E. coli methods available. Many public water systems were familiar with and preferred to use the fecal coliform methods. However, since the current TCR was promulgated, many E. coli methods have been developed and approved for use. EPA believes that most systems nationwide currently test for E. coli, while few test for fecal coliform bacteria. Since the methods used to test for E. coli have approximately the same cost as those used to test for fecal coliform bacteria, this proposed change is not expected to create an additional burden on PWSs.
EPA is proposing to eliminate all analytical method provisions for fecal coliforms that are included in the current TCR. EPA proposes instead to allow testing only for E. coli following a total coliform-positive sample. This change will provide the water system with more meaningful information regarding potential fecal contamination of the distribution system.

The current TCR specifies a number of analytical methods that can be used for compliance sample analysis (in 40 CFR 141.21(f)). Since fecal coliform bacteria are not regulated contaminants under this proposed rule, the analytical methods for fecal coliforms are no longer applicable and are removed from the list of analytical methods. All other methods used for compliance with the current TCR are maintained for compliance sample analysis under the proposed RTCR.

v. Request for comment on AIP-related method issues. EPA is requesting comment on the following RTCR analytical method issues related to recommendations from the TCRDSAC in its AIP:

- The use of an ETV approach for a reevaluation of analytical methods.
- Whether the RTCR should include provisions to ensure a more expedited results notification process. The RTCR could, for example, include language requiring that PWSs arrange to be notified of a positive result by their laboratory within 24 hours.
- Whether the RTCR should require repeat samples be taken within 24 hours of a total coliform-positive with no (or limited) exceptions.

b. Other method issues. In addition to addressing the recommendations of the advisory committee, EPA is proposing some minor technical changes related to analytical methods.

Many of these changes document practices that are already followed by PWSs and laboratories, and are consistent with the Manual for the Certification of Laboratories Analyzing Drinking Water (referred to as the "Laboratory Certification Manual") (USEPA 2005), an EPA document that outlines method requirements and good laboratory practices for certified laboratories conducting drinking water compliance sample analyses.

Some of these changes were brought to the attention of EPA by EPA Regions and States involved in the implementation of the drinking water certification program. Other minor changes have been proposed to make the analytical methods section of this regulation easier to understand and implement. Each proposed change is described as follows with a discussion of the rationale for the change.

i. Holding time. The proposed RTCR continues to provide a 30-hour holding time limit for the samples collected in compliance with this regulation (40 CFR 141.21(f)(3)). However, EPA is proposing to change the definition for holding time from "the time from sample collection to initiation of analysis may not exceed 30 hours" to "the time from sample collection to initiation of test medium incubation may not exceed 30 hours."

ii. Dechlorinating agent for sample preservation of chlorinated water supplies. The proposed RTCR establishes the following provision: "If chlorinated water is to be analyzed, sufficient sodium thiosulfate (Na 2 S 2 O 3) must be added to the sample bottle before sterilization to neutralize any residual chlorine in the water sample." Dechlorination procedures are addressed in section 9060A.2 of Standard Methods for the Examination of Water and Wastewater (20th and 21st editions) (Clesceri et al. 1998; Eaton et al. 2005).

iii. Filtration funnels. EPA is proposing to add the following footnote to the analytical methods table (§ 141.852) under the revised rule:

All filtration series must begin with membrane filtration equipment that has been sterilized by autoclaving. Exposure of membrane filtration equipment to UV light is not adequate to ensure sterilization. Subsequent to the initial autoclaving, exposure of the filtration equipment to UV light may be used to sanitize the funnels between filtrations within a filtration series.
iv. Analytical methods table changes. EPA is proposing the following changes to the analytical methods table:

- ✓ The table is organized by methodology (e.g., lactose-fermentation methods vs. enzyme-substrate methods).
- ✓ E. coli methods are included in the table.
- ✓ 18th and 19th editions of Standard Methods for the Examination of Water and Wastewater are no longer approved and have been removed.
- ✓ The references to Standard Methods 9221A and 9222A are removed.
- ✓ The reference to Standard Methods 9221B is changed to 9221B.1, B.2.
- ✓ The reference to Standard Methods 9221D is changed to 9221D.1, D.2.
- ✓ The table proposes to allow Standard Methods 9221D in the multiple tube format as described in Standard Methods 9221B.
- ✓ The citation for MI agar is changed to EPA Method 1604 for clarity and consistency.

- ✓ The table clarifies that Standard Methods 9221 F.1 and 9222 G.1 a (1), (2) may be used for E. coli analysis.

- ✓ The table clarifies the correct formulation for EC-MUG broth, when used in conjunction with Standard Methods 9222G.1 a (2), through the addition of the following footnote:

The following changes must be made to the EC broth with MUG (EC-MUG) formulation: Potassium dihydrogen phosphate, KH_2PO_4 must be 1.5g and 4-methylumbelliferyl-Beta-D-glucuronide must be 0.05 g.

- ➢ The table reflects the approval of a modified Colitag method for the simultaneous detection of E. coli and other total coliforms.

v. EPA's rationale for proposed changes related to other method issues. (a). Holding time. The current rule states "The time from sample collection to initiation of analysis may not exceed 30 hours" (40 CFR 141.21 (f)(3)). Since promulgation of the current TCR, some States and EPA Regions have commented that "initiation of analysis" may be interpreted several different ways, which can lead to the sample being held longer than the 30 hours intended by the rule. The proposed language more clearly defines the amount of time that the sample may be held and is consistent with section 6.4.1 of the Manual for the Certification of Laboratories Analyzing Drinking Water which states: "For the analysis of total coliform in drinking water, the time between sample collection and the placement of sample in the incubator must not exceed 30 hours."

EPA believes that changing the definition of holding time from "the time from sample collection to initiation of analysis" to "the time from sample collection to initiation of test medium incubation" may slightly decrease the amount of time a PWS has to get the sample to a laboratory. EPA does not believe that this change will significantly reduce the amount of time a water system has to get a sample to the laboratory, as most of the methods approved under this rule require 30 minutes or less to process and prepare the sample for the incubation step. Thus, the initial analytical steps should not constitute a large portion of the holding time as a whole. EPA recommends that PWSs that have difficulty meeting the holding time notify the laboratory that the samples are in transit and need to be given priority. The laboratory could begin analysis immediately upon sample arrival so that the samples could be placed in the incubator in time to meet the 30 hour holding time. EPA notes that a laboratory may have to make specific accommodations in their processes in order to properly analyze a sample received close to the end of the holding time. EPA believes that this is feasible with proper planning.

(b). Dechlorinating agent for sample preservation of chlorinated water supplies. Under this proposal, EPA would require that chlorinated water samples be collected in bottles that contain the dechlorinating agent sodium thiosulfate. This is consistent with section 3.15.4 of the Laboratory Certification Manual, which states "If chlorinated water is to be analyzed, sufficient sodium thiosulfate (Na 2 S 2 O 3) must be added to the sample bottle before sterilization to neutralize any residual chlorine in the water sample." Neutralization ceases the bactericidal action of the chlorine during sample transit, thus allowing a more accurate assessment of what the true microbial content of the water sample was at the time of sample collection. Implementation of this new requirement should be straightforward since PWSs need only ask the laboratory for pre-treated sample containers. EPA does not believe this provision will cause an increase in cost to PWSs, as the cost of the bottles with the sodium thiosulfate is essentially the same as the cost of the bottles without the sodium thiosulfate.

(c). Filtration funnels. Under this proposal, EPA is requiring that membrane filtration equipment be autoclaved before beginning a filtration series. This requirement is consistent with section 4.1.3 of the Laboratory Certification Manual, which states: "Membrane filter equipment must be autoclaved before the beginning of a filtration series."

Under the current TCR, not all of the approved membrane filtration methods require that a filtration series begin with membrane filtration units that have been sterilized by autoclave. Some of the approved methods allow the laboratory to use ultraviolet (UV) radiation exposure in lieu of autoclaving to sterilize filtration units between filtration series. EPA does not believe that ultraviolet radiation is sufficient to properly sterilize the membrane filtration equipment. Additionally, EPA believes that when ultraviolet radiation is used, not all areas of the membrane filtration equipment are exposed, and therefore microorganisms may persist and contaminate other water samples and the laboratory. For these reasons, EPA is proposing to include a footnote to the analytical methods table in order to ensure proper sterilization.

EPA does, however, believe that ultraviolet light can be used to sanitize the filtration equipment between filtrations within a filtration series, as stated in section 4.1.4 of the Laboratory Certification Manual: "Ultraviolet light (254 nm) may be used to sanitize equipment (after initial autoclaving for sterilization), if all supplies are pre-sterilized. Ultraviolet light may be used to reduce bacterial carry-over between samples during a filtration series."

(d). Analytical methods table. In this proposal, EPA is identifying a number of changes to the analytical methods table for clarity and accuracy.

In the current TCR, the methods are listed by date approved and the E. coli methods are listed in a text format. In this proposal, the analytical methods table is organized by methodology (e.g., lactose-fermentation methods vs. enzyme-substrate methods), and the E. coli methods are included in the table.
Standard Methods for the Examination of Water and Wastewater is a reference document designed to represent "the best current practice of American water analysts." Periodically, new editions are published in order to incorporate improvements in the methods contained within this manual. Thus, new editions of this publication contain more current and improved versions of the methods. Under the current TCR, four editions of this publication are approved, resulting in different, oftentimes outdated, versions of the same method being approved.

Having multiple editions of this manual approved under this regulation also creates a burden for the laboratory certification officers who must understand the differences between the versions of the method for which the laboratory may be seeking certification. For these reasons, EPA is proposing to remove the 18th and 19th editions of Standard Methods for the Examination of Water and Wastewater for use in compliance sample analysis under the RTCR. EPA expects that the burden associated with this change will be minimal as most laboratories have already procured the newer editions or have arranged for access to the online publication.

In this proposed regulation, the reference to Standard Methods 9221A and 9222A are removed. These sections of the methods contain only introductory information, not any actual methodology. They do not represent methods approved for use under this regulation.

The references to Standard Methods 9221B and 9221D are modified in this proposed regulation. In the current TCR, the methods are referenced as 9221B and 9221D with footnote 5 denoting that the "completed phase" called for in the methods is not required. By more specifically citing Standard Methods 9221 B.1, B.2, and 9221 D.1, D.2 (which contain the applicable, required steps of these methods) EPA is able to eliminate the original footnote and improve clarity.

EPA is proposing to allow Standard Methods 9221D (Presence-Absence broth) to be used in a multiple tube format. This method has traditionally been used in a single bottle, allowing only the qualitative detection of total coliforms. However, there are published reports showing this method can be used in a multiple tube format for the quantitative detection of total coliforms (Rice et al. 1987; Rice et al. 1993). This medium would be used in the same manner that Lauryl Tryptose Broth (LTB) is described as being used in Standard Methods 9221B. Allowing the use of this method in a multiple tube format would allow PWSs that use this method to quantitate any total coliforms that may occur in the water sample.

EPA is proposing to change the citation for MI Agar. Under the current TCR, this method is cited as Standard Methods 9222, with a footnote citing the Applied and Environmental Microbiology article where the method was initially described. In this proposal, the method is now cited as EPA Method 1604, consistent with section 5.4.2.1.3 of the Laboratory Certification Manual. EPA Method 1604 is identical to the citation in the TCR and does not require the use of the original footnote. This change is also consistent with the citation of this method as listed in the Ground Water Rule (see 40 CFR 141.402).

The current TCR describes the use of "EC medium supplemented with 50 µg/mL of 4-methylumbelliferyl-Beta-D-glucuronide (MUG)" (see 40 CFR 141.21(f)(6)(i)). This proposal clarifies that this medium, included in both Standard Methods 9221F and Standard Methods 9222G.1 a (2), is approved for use under this regulation. This is consistent with the Laboratory Certification Manual, particularly section 5.1.8, which describes both of these methods as approved for use in the detection of E. coli under this regulation.

Lastly, EPA is clarifying the formulation for EC broth with MUG (EC-MUG) given in Standard Methods 9222G.1 a (2) to correct an error in the publication. The Standard Methods 9222G.1 a (2) formulation calls for 0.1 g of 4-methylumbelliferyl-Beta-D-glucuronide, and 1.4g KH 2 PO 4. This formulation differs from that given in Standard Methods 9221F.1, which calls for 0.05 g and 1.5 g, respectively. EPA believes that the correct formulation is given in Standard Methods 9221F and has confirmed this with Standard Methods committee members (Rice 2009). Accordingly, EPA has added a footnote to the 9222G.1 a (2) stating the proper formulation.

EPA anticipates that these changes to the analytical methods table will not cause any additional burden to the PWSs.

vi. Request for comment regarding holding temperature. The current TCR states the following regarding sample shipment: "Systems are encouraged but not required to hold samples below 10 deg. C during transit." Other national primary drinking water regulations requiring microbial sampling require that the samples be shipped in cold conditions, and require the sample be maintained at a temperature of 10 degrees Celsius (C) or less. Maintaining the sample temperature below 10 degrees C serves to preserve the bacterial population by minimizing both bacterial cell death and cell multiplication, thus allowing for a more accurate representation of the microbial population in the sample at the time of sample collection. Also, Standard Methods for Examination of Water and Wastewater, 21st edition (Eaton et al. 2005) recommends that samples be shipped at less than 8 degrees C but not frozen.

In the years since the promulgation of the current TCR, EPA has heard concern that at times, samples collected under the TCR may reach high temperatures during transit to the laboratory due to the lack of a requirement to ship samples on ice. High temperatures that may be reached during transit could have a deleterious or prolific effect on the bacterial cells present in the samples such that the samples may no longer represent the microbial content of the water at the time of sample collection.

EPA recognizes that requiring the samples under the proposed RTCR to be held at 10 degrees C or less, but above freezing, would result in an increased cost to the water systems (for shipping, supplies, etc.), but believes the extra burden may be warranted. EPA is seeking public comment on whether this passage should remain as is in the current TCR or whether the RTCR should require that the samples collected for compliance with this regulation be shipped in cold conditions, i.e., requiring a temperature of 10 degrees C or less, but above freezing to be maintained for better sample preservation. EPA also welcomes comments and supporting data on what the acceptable temperature range should be when samples are in transit.

B. Proposed Compliance Date
Consistent with SDWA section 1412(b)(10), EPA proposes that the compliance date of the final RTCR be three years from the date on which the regulation is promulgated (i.e., the publication date of the final rule in the Federal Register). PWSs must comply with the requirements of the rule by the compliance date.

EPA believes that capital improvements generally are not necessary to ensure compliance with the proposed RTCR. However, a State may allow individual systems up to two additional years to comply with the RTCR if the State determines that additional time is necessary for capital improvements, in accordance with SDWA section 1412(b)(10).

EPA requests comment on the proposed compliance date of the proposed RTCR.

C. Links to Other Drinking Water Rule Requirements
The proposed RTCR recognizes that existing NPDWRs contain linkages among monitoring requirements in different rules. The current residual disinfectant monitoring must be conducted at the same time and location at which TCR samples are taken, as provided for in the Surface Water Treatment Rule (SWTR) (USEPA 1989b, 54 FR 27486, June 29, 1989) and the Stage 1 Disinfectants and Disinfection Byproducts Rule (Stage 1 DBPR) (USEPA 1998a, 63 FR 69389, December 16, 1998). Under the GWR, TCR distribution system monitoring results determine whether a system is required to conduct source water monitoring. Under the SWTR, high measurements of turbidity in an unfiltered subpart H system of this part trigger additional total coliform samples. Sanitary survey provisions exist in surface water and ground water drinking water regulations. The proposed RTCR does not change the existing sanitary survey requirements except to add the special monitoring evaluation that States must conduct at systems serving 4,100 or fewer people. These evaluations do not increase the burden to conduct sanitary surveys because of the relatively simple nature of these systems and their monitoring requirements.

1. SWTR, Stage 1 and Stage 2 DBPRs, ADWR
After considering the possible linkages among the proposed RTCR and the SWTR, Stage 1 DBPR, Stage 2 DBPR (USEPA 2006e, 71 FR 388, January 4, 2006), and Airline Drinking Water Rule (ADWR) (USEPA 2009), EPA has concluded that the only necessary revision is to update the reference to the current TCR at 40 CFR 141.21, which is superseded by 40 CFR part 141 subpart Y beginning three years following publication of the final rule. EPA is also proposing several revisions to other NPDWRs, discussed below, that are not necessary but would facilitate implementation of all applicable NPDWRs.

2. GWR
As with the other drinking water rules mentioned above, EPA is proposing to update the references in the GWR to the current TCR at 40 CFR 141.21, which will be superseded by 40 CFR part 141 subpart Y.

3. Sanitary Surveys
Sanitary survey requirements are not included in the proposed RTCR. Under the current TCR, community water systems and non-community water systems that serve 4,100 or fewer people are required to conduct periodic sanitary surveys. Since the promulgation of the TCR in 1989, new sanitary survey requirements for surface water systems and ground water systems have been established for all system sizes and types under the Interim Enhanced Surface Water Treatment Rule (IESWTR) (USEPA 1998b, 63 FR 69477, December 16, 1998) (40 CFR 142.16(b)(3)), and the Ground Water Rule (GWR) (40 CFR 142.16(o)(2)(i)). Public water systems began implementing the IESWTR sanitary survey requirements in 2001. Therefore, for surface water systems, the current TCR sanitary survey requirements have phased out since that time. Implementation of the GWR sanitary survey requirements began in December 2009 for ground water systems. Therefore, for ground water systems, the GWR sanitary survey requirements will be in effect by the time the RTCR is finalized.

D. Best Available Technology (BAT)

1. Provisions

The proposed RTCR would maintain the provisions set forth in 40 CFR 141.63(d) (proposed to be in § 141.63(e)), regarding the best technology, treatment techniques, or other means available for achieving compliance with the MCL of either total coliforms or E. coli. EPA is proposing the following modifications:

- ✓ 40 CFR 141.63(d)(1) (proposed § 141.63(e)(1)) would be modified by replacing "coliforms" with "fecal contaminants."

- ✓ 40 CFR 141.63(d)(3) (proposed § 141.63(e)(3)) would be modified by including "cross connection control" in the list of proper maintenance practices for the distribution system.

- ✓ 40 CFR 141.63(d)(4) (proposed § 141.63(e)(4)) would be modified by including the subparts P, T, and W that describe filtration and/or disinfection of surface water, and subpart S for disinfection of ground water.

2. EPA's Rationale

a. Change "coliform" to "fecal contaminants." This change reflects the approach of the proposed RTCR that the presence of total coliforms does not necessarily have a direct public health implication. Instead, total coliform is used as an indicator of a potential pathway of contamination within a treatment technique requirement. For additional discussion on this topic, see section III.A.2 of this preamble.

b. Inclusion of cross connection control. EPA believes that adding cross connection control to the list of proper maintenance practices for distribution systems is appropriate because of the significant contribution of cross connections and backflow to waterborne disease outbreaks. From 1981 to 1998, the CDC documented 9, 734 detected and reported illnesses from 57 waterborne outbreaks related to cross connections (NRC 2006). From 1970 to 2001, approximately 12,000 illnesses resulted from 459 incidents of waterborne outbreaks from backflow events (NRC 2006).

c. Addition of other relevant subparts of 141. This change adds references to subparts that contain provisions for the other drinking water rules promulgated since 1989 when the TCR was promulgated (in particular, subpart P for the IESWTR, subpart S for the GWR, subpart T for the Long Term Enhanced Surface Water Treatment Rule (USEPA 2002, 67 FR 1812, January 14, 2002, and subpart W for the Long Term 2 Enhanced Surface Water Treatment Rule (USEPA 2006d, 71 FR 654, January 5, 2006)). These drinking water rules contain updated filtration and disinfection standards that were not part of the current TCR when it was promulgated in 1989.

3. Request for Comment

EPA requests comment on the modifications to the existing BATs and whether there is a need to add or otherwise update the list of BATs.

E. Variances and Exemptions

1. Provisions

EPA is proposing to not allow variances or exemptions to the E. coli MCL. EPA is also proposing to eliminate the variance provisions in 40 CFR 141.4(b) that allow systems to demonstrate to the State that the violation of the monthly/non-acute total coliform MCL is due to biofilm and not fecal or pathogenic contamination. This change will also result in a parallel change in 40 CFR 142.63(b).

2. EPA's Rationale

Under the proposed RTCR, E. coli is used as an indicator of fecal contamination that may contain waterborne pathogens. To the extent a variance or exemption would permit the continued presence of E. coli, the potential for pathogens to be present also would remain. EPA believes

that water which exceeds the MCL for E. coli poses an unreasonable risk to public health. Therefore, EPA is not allowing any variances or exemptions to the E. coli MCL. This provision is consistent with the existing requirement, since the provision that allows variances applies only to the monthly/non-acute total coliform MCL violation and not to the acute violation associated with the presence of E. coli.

Under the current TCR, EPA allows variances to the MCL for total coliforms when a system has demonstrated to the State that the violation of the total coliform MCL is due to a persistent growth of total coliforms in the distribution system (i.e., biofilm) rather than fecal or pathogenic contamination, a treatment lapse or deficiency, or a problem in the operation or maintenance of the distribution system.

EPA is proposing to eliminate the variance in 40 CFR 141.4(b) because under the proposed RTCR, there would no longer be an MCL for total coliforms (see section III.A.2 of this preamble). The current TCR MCL for total coliforms was based on the presence or absence of total coliforms in a sample (see 40 CFR 141.63 for details). In the proposed RTCR, the presence of total coliforms at a certain level requires the system to comply with the coliform treatment technique requirements (see section III.A.5 of this preamble). The assessment and corrective action requirements under this proposed rule include the possibility of recognizing that the total coliform presence is associated with biofilm. EPA plans to include this information in a new assessment and corrective action guidance manual related to the RTCR.

3. Request for Comment

EPA requests comment on its proposal to allow no variance or exemption to the E. coli MCL and to eliminate the variance provisions associated with the monthly/non-acute total coliform MCL.

F. Request for Comment on Other Issues Related to the Proposed RTCR

1. Consistency Between the Proposed RTCR and the GWR

EPA requests comment on the need for general consistency between the proposed RTCR and the GWR. Please provide specific examples. For example, under the current TCR, States are required to keep records of their decision to either waive or extend the 24-hour limit for collecting samples (that is, for repeat samples following a total coliform-positive sample, or for follow-up samples after high levels of turbidity) (see 40 CFR 142.14(a)(5)(i)(A) and 142.14(a)(5)(ii)(D)). The proposed RTCR also requires States to keep records of decisions to either waive or extend the 24-hour limit for repeat samples following a total coliform-positive sample, for samples following invalidation, or for follow-up samples after high levels of turbidity (see§§ 142.14(a)(10)(i)(A) and 142.14(a)(10)(ii)(D) of the proposed RTCR). Under the GWR, there are no recordkeeping requirements for the decision to waive or extend the 24-hour limit. Instead, the GWR includes special primacy requirements to describe criteria the State will use to extend the 24-hour limit (see 40 CFR 142.16(o)(3)(i)). EPA requests comment on whether it is appropriate to have States describe their criteria for waiving or extending the 24-hour limit as a primacy condition, or instead have States keep records of decisions to waive and/or extend the 24-hour limit.

2. Storage Tank Inspection and Cleaning

EPA requests comment on the value and cost of periodic storage tank inspection and cleaning. There are instances of storage tanks being the source of waterborne disease outbreaks at PWSs. In December 1993, a Salmonella typhimurium outbreak in Gideon, Missouri resulted in over 600 people affected by diarrhea, 31 cases of laboratory-confirmed salmonellosis and seven deaths of nursing home residents who had exhibited diarrheal illness (four deaths were confirmed by culture). The larger of the two storage tanks had a breach in the roof hatch that allowed pigeon droppings to be carried into the tank and likely accumulated in the several inches of sediment. This contaminated sediment, more than likely, was pulled into the distribution system by a flushing program that drained the tank (Clark et al. 1996). Salmonella typhimurium was isolated from the sediment of one of the towers, and tap water tested positive for fecal coliforms (CDC 1996).

In March 2008, Alamosa, Colorado (with a population of about 9,000 people) experienced a waterborne disease outbreak associated with Salmonella. The report released by the Colorado Department of Public Health and Environment (Falco and Williams 2009) indicated that the outbreak resulted in 442 reported cases of illnesses, 122 of which were laboratory confirmed, and one fatality. The State epidemiologist estimated that a total of 1,300 people may have been ill. Two storage tanks in Alamosa had several inches of sediment and breaches; one tank had breaches large enough for birds and animals to enter. Some of the key factors that contributed to these two outbreaks include significant levels of sediment (several inches to feet) and the presence of breaches of the integrity of the storage tank.

Sediment accumulation occurs within storage facilities due to quiescent conditions which promote particle setting. Over time sediment continues to accumulate in a tank, even if the finished water is consistently treated to below 0.1 nephelometric turbidity unit (NTU). For surface water systems, it is not uncommon to have1/4to1/2inch or more of sediment accumulate after two to three years (Kirmeyer et al. 1999). While there are no turbidity regulations for ground water systems (except for ground water under the direct influence of surface water (GWUDI)), the levels of turbidity can be significant in the water pumped from an aquifer. Sand particles, if allowed to accumulate, provide pore spaces that house diverse populations of biota (which may include pathogenic microorganisms) (Kirmeyer et al. 1999; van der Kooij 2003). Periodic high flows in the storage tank may scour, stir up, and suspend the sediment (along with entrapped bacteria and pathogens) and carry it into the distribution system, with greater accumulation of sediment being a more significant concern. Other water quality problems associated with sediment accumulation include increased disinfectant demand and disinfection byproduct formation.

The storage tank's vulnerability to contamination increases when breaches of the storage tank allow insects, animals, and birds and their associated diseases to enter. Contamination from bird and other animal excrement can potentially transmit disease-causing organisms to the finished water. Waterfowl, for example, are known carriers of many different waterborne pathogens including Vibrio cholerae (Ogg et al. 1989).

Based on the potential public health implications associated with poorly maintained storage tanks (e.g., as indicated by significant sediment accumulation and breaches), EPA is interested in receiving comments and supporting information regarding the state and condition of tanks that have been cleaned and inspected, costs of storage tank inspection and cleaning, and how public health can be better protected. EPA requests information on whether there are States that recommend or require periodic inspection and cleaning of storage tanks. If so, what are the requirements, the frequency of inspection and cleaning, and how successful are they? Are inspections and cleaning done by individual PWSs or by contractors?

3. States Under EPA Direct Implementation

EPA does not have the authorities provided to other primacy agencies under 40 CFR part 142 to use in implementing rules in direct implementation entities (e.g., Tribal systems and Wyoming). To provide EPA the flexibility of other primacy agencies to modify monitoring requirements as necessary to protect public health (e.g., to require more stringent monitoring or to develop criteria such as those that primacy States develop under the special primacy conditions requirement in 40 CFR 142.16) and facilitate implementation of this rule. EPA is requesting comment on whether the Agency should have the same authorities specified in subpart Y, as States have in 40 CFR 142.16, for PWSs for which the Agency has direct implementation responsibilities. EPA is requesting comment on whether this authority should be added to subpart Y specifically.

G. Limitations to the Public Comment on the Proposed RTCR

The proposed revisions to other drinking water regulations (SWTR, Stage 1 DBPR, Stage 2 DBPR, and ADWR) are made solely to update the reference to the current TCR at 40 CFR 141.21, which will be superseded by 40 CFR part 141 subpart Y beginning three years following publication of the final rule. This proposed rule would not change any substantive requirements of those rules and EPA is not soliciting public comments on those rules other than their proposed

revised references to the current TCR or any other references to the current TCR that EPA may need to revise.
IV.

The proposed RTCR provides States with flexibility to implement the requirements of the rule in a manner that maximizes the efficiency of the rule for the States and water systems while increasing the effectiveness of the rule to protect public health. While the proposed rule provides some reduction in monitoring relative to the current TCR, overall, the proposed rule is more stringent and better protects public health. As a result, States must adopt these revisions, when final, or adopt or maintain more stringent requirements, in order to maintain primacy. This section describes the regulations and other procedures and policies States must adopt in order to obtain primacy to implement the RTCR, if finalized as proposed today.

SDWA section 1413 establishes requirements that States or eligible Indian Tribes must meet to assume and maintain primary enforcement responsibility (primacy) for its PWSs. These requirements include:

- ✓ Adopting drinking water regulations that are no less stringent than Federal drinking water regulations;
- ✓ Adopting and implementing adequate procedures for enforcement;
- ✓ Keeping records and making reports available on activities that EPA requires by regulation;
- ✓ Issuing variances and exemptions (if allowed by the State), under conditions no less stringent than allowed under SDWA; and
- ✓ Adopting and being capable of implementing an adequate plan for the provisions of safe drinking water under emergency situations.

States may adopt more stringent requirements (e.g., requiring all systems to conduct routine monthly monitoring). Many States have used this authority in the past to improve public health protection and/or simplify implementation.

Section 1413(a)(1) of SDWA provides two years (plus up to two more years if the Administrator approves) after promulgation of the final RTCR for the State to adopt corresponding drinking water regulations in order to obtain primacy for the final RTCR. To implement the final RTCR, States would be required to adopt or maintain requirements that are at least as stringent as the following revisions to 41 CFR part 141:

- ➢ Section 141.4—Variances and exemptions (if allowed by the State).
- ➢ Section 141.21—Coliform sampling.
- ➢ Section 141.52—Maximum contaminant level goals for microbiological contaminants.
- ➢ Section 141.63—Maximum contaminant levels (MCLs) for microbiological contaminants.
- ➢ Section 141.74—Analytical and monitoring requirements.
- ➢ Section 141.132—Monitoring requirements.
- ➢ Subpart 141.153—Content of the reports.
- ➢ Subpart 141.202—Tier 1 Public Notice—Form, manner, and frequency of notice.

- Subpart 141.203—Tier 2 Public Notice—Form, manner, and frequency of notice.
- Subpart 141.204—Tier 3 Public Notice—Form, manner, and frequency of notice.
- Subpart O—Consumer Confidence Reports, Appendix A, Regulated Contaminants.
- Subpart Q—Public Notification of Drinking Water Violations, Appendix A, NPDWR Violations and Other Situations.
- Subpart Q—Public Notification of Drinking Water Violations, Appendix B, NPDWR Violations and Other Situations.
- Subpart Y—Revised Total Coliform Rule.

EPA's regulation at 40 CFR part 142 sets out the specific program implementation requirements for States to obtain primacy for the public water supply supervision program as authorized under SDWA section 1413. In addition to adopting basic primacy requirements specified in 40 CFR part 142, States may be required to adopt special primacy provisions pertaining to specific regulations where implementation of the rule involves activities beyond general primacy provisions. States must include these regulation-specific provisions in their application for approval of their program revision. States must continue to meet all other conditions of primacy for all other rules in 40 CFR part 142. Primacy requirements for the proposed RTCR are described below.

The advisory committee recognized that this rule will require more tracking to ensure effective implementation. Therefore, EPA plans to release an upgrade to SDWIS/State and SDWIS/FED (the State and Federal versions of the Safe Drinking Water Information System, respectively) within 18 months of final rule promulgation to accommodate monitoring data, tracking, compliance determinations and reporting of all rule related requirements, as appropriate.

A. State Special Primacy Requirements

To ensure that a State program includes all the elements necessary for an effective and enforceable program under the proposed RTCR, a State primacy application must include a description of how the State will perform the following:

- ✓ Sample Siting Plans—States must describe the frequency and process used to review and revise sample siting plans in accordance with 40 CFR 141, subpart Y to determine adequacy.
- ✓ Reduced Monitoring Criteria—The primacy application must indicate whether the State will adopt the reduced monitoring provisions of subpart Y. If the State adopts the reduced monitoring provisions, it must describe the specific types or categories of water systems that will be covered by reduced monitoring and whether the State will use all or a reduced set of the optional criteria. For each of the reduced monitoring criteria, both mandatory and optional, the State must describe how the criteria will be evaluated to determine when systems qualify.
- ✓ Assessments and Corrective Actions—States must describe their process to implement the new assessment and corrective action phase of the rule. The description must include examples of sanitary defects, examples of assessment forms or formats, and methods that systems may use to consult with the State on appropriate corrective actions.
- ✓ Invalidation of routine and repeat samples collected under subpart Y—States must describe their criteria and process to invalidate total coliform-positive and E. coli- positive samples under subpart Y. This includes criteria to determine if a sample was improperly processed by the laboratory, reflects a domestic or other non-distribution system plumbing problem or reflects circumstances or condition that does not reflect water quality in the distribution system.

- ✓ Approval of individuals allowed to conduct subpart Y Level 2 assessments—States must describe their criteria and process for approval of individuals allowed to conduct subpart Y Level 2 assessments.
- ✓ Special monitoring evaluation—States must describe how they will perform special monitoring evaluations during sanitary surveys for ground water systems serving 1,000 or fewer people to determine whether systems are on an appropriate monitoring schedule.
- ✓ Seasonal systems—States must describe how they will identify seasonal systems, how they will determine when systems on less than monthly monitoring must monitor, and what will be the seasonal system start-up provisions.
- ✓ Additional criteria for reduced monitoring—States must describe how they will require systems on reduced monitoring to demonstrate:
  —Continuous disinfection entering the distribution system and a residual in the distribution system;
  —Cross connection control;
  —Other enhancements to water system barriers; and
  —Procedures for seasonal systems to start up operations at the beginning of each season.

B. State Recordkeeping Requirements

The current regulations in 40 CFR 142.14 require States with primacy to keep records, including: Analytical results to determine compliance with MCLs, MRDLs, and treatment technique requirements; PWS inventories; State approvals; enforcement actions; and the issuance of variances and exemptions. The proposed RTCR requires States to keep additional records, including all supporting information and an explanation of the technical basis for each decision as follows. Records of the following decisions or activities must be retained for five years, consistent with recordkeeping requirements for existing regulations:

- ✓ Any decision to waive the 24-hour time limit for collecting repeat samples after a total coliform-positive routine sample, or to extend the 24-hour limit for collection of samples following invalidation, or for an unfiltered subpart H system of this part to collect a total coliform sample following a turbidity measurement exceeding 1 NTU.
- ✓ Any decision to allow a system to waive the requirement for three routine samples the month following a total coliform-positive sample. The record of the waiver decision must contain all the items listed in §§ 141.854(j) and 141.855(f) of the proposed RTCR.
- ✓ Any decision to invalidate a total coliform-positive sample. If the State decides to invalidate a total coliform-positive sample as provided in § 141.853(c)(1) of the proposed RTCR, the record of the decision must contain all the items listed in that paragraph.
- ✓ Completed and approved 40 CFR part 141 subpart Y assessments, including reports from the system that corrective action has been completed.

States must retain records of each of the following decisions in such a manner so that each system's current status may be determined at any time:

- ➢ Any decision to reduce the total coliform monitoring frequency for a community water system serving 1,000 or fewer people to less than once per month, as provided in § 141.855(d) of the proposed RTCR; and what the reduced monitoring frequency is. A copy of the reduced monitoring frequency must be provided to the system.
- ➢ Any decision to reduce the total coliform monitoring frequency for a non-community water system using only ground water and serving 1,000 or fewer people to less than once per quarter, as provided in § 141.854(e) of the proposed RTCR, and what the reduced monitoring frequency is. A copy of the reduced monitoring frequency must be provided to the system.
- ➢ Any decision to reduce the total coliform monitoring frequency for a non-community water system using only ground water and serving more than 1,000 persons during any month the system serves 1,000 or fewer people, as provided in § 141.857(d) of the proposed RTCR. A copy of the reduced monitoring frequency must be provided to the system.
- ➢ Any decision to waive the 24-hour limit for taking a total coliform sample for a public water system that uses surface water, or ground water under the direct influence of

surface water, and that does not practice filtration in accordance with part 141, subparts H, P, T, and W, and that measures a source water turbidity level exceeding 1 NTU near the first service connection.

- Any decision to allow a public water system to forgo E. coli testing on a total coliform-positive sample if that system assumes that the total coliform-positive sample is E. coli-positive.

C. State Reporting Requirements

EPA currently requires at 40 CFR 142.15 that States report to EPA information such as violations, variance and exemption status, and enforcement actions. The proposed RTCR requires States to develop and maintain a list of public water systems that the State is allowing to monitor less frequently than once per month for community water systems or less frequently than once per quarter for non-community water systems, including the compliance date (the date that reduced monitoring was approved) of the reduced monitoring requirement for each system.

D. Interim Primacy

On April 28, 1998, EPA amended its State primacy regulations at 40 CFR 142.12 to incorporate the new process identified in the 1996 SDWA Amendments for granting primary enforcement authority to States while their applications to modify their primacy programs are under review (USEPA 1998c, 63 FR 23361, April 28, 1998). The new process grants interim primary enforcement authority for a new or revised regulation during the period in which EPA is making a determination with regard to primacy for that new or revised regulation. This interim enforcement authority begins on the date of the primacy application submission or the effective date of the new or revised State regulation, whichever is later, and ends when EPA makes a final determination. However, this interim primacy authority is only available to a State that has primacy (including interim primacy) for every existing NPDWR in effect when the new regulation is promulgated.

As a result, States that have primacy (including interim primacy) for every existing NPDWR already in effect may obtain interim primacy for the RTCR, beginning on the date that the State submits the application for this rule to EPA, or the effective date of its revised regulations, whichever is later. A State that wishes to obtain interim primacy for future NPDWRs must obtain primacy for this rule.

E. Request for Comment

EPA requests comment on the adequacy of the proposed RTCR requirements for State implementation, including but not limited to State special primacy requirements and State reporting and recordkeeping requirements. Specifically, EPA requests comment on whether there are any requirements that should be added to assure proper State oversight, or any that can be removed without detriment to implementation of the rule.

V. Distribution System Research and Information Collection Activities

A. Research and Information Collection Partnership

The advisory committee recommended that a Research and Information Collection Partnership (RICP) be formed to inform and support the drinking water community in developing future national risk management decisions pertaining to drinking water distribution systems. The advisory committee recommended seven priority areas for research and information collection. These seven priority areas are: (1) Cross-connection and backflow of contaminated water; (2) contamination due to storage facility design, operation, or maintenance; (3) contamination due to main installation, repair, or rehabilitation practices; (4) contaminant intrusion due to pressure conditions and physical gaps in distribution system infrastructure; (5) significance and control of biofilm and microbial growth; (6) nitrification issues that lead to public health effects; and (7) accumulation and release of contaminants from distribution system scales and sediments (USEPA 2008c, AIP p. 30).

In January 2009, EPA and the Water Research Foundation (WRF or the Foundation) signed a memorandum of understanding (MOU) to form the RICP in response to recommendations from

the advisory committee contained in the AIP (USEPA and WRF 2009). The MOU conveys the partners' agreement to collaborate and identify, define, prioritize, coordinate, and communicate critical decision-relevant distribution system research and information collection needs of the drinking water community. The RICP is directed by a steering committee comprised of nine members: Three members from EPA, three members from water utilities, and three additional members representing the public health, environmental advocate, and State regulator perspectives.

B. Distribution System Optimization Activities

As part of the AIP, the advisory committee encouraged "the development of national and regional distribution system optimization partnerships that focus on protecting the integrity of drinking water quality once it is delivered to the distribution system. The purpose of the partnerships should be to inform and inspire proactive systems to implement best management practices that emphasize protection of public health. These partnerships, comprised, for example, of representatives from utilities, communities, academia, and regulatory organizations could develop continuous improvement programs that encompass water distribution optimization principles and practices for system design, operations, and maintenance. These partnerships should foster continuous review of distribution system issues and should define excellence in distribution system operation in terms of processes, systems, procedures, as well as measures. The optimization partnerships should encourage voluntary program participation of all drinking water utilities regardless of system size" (USEPA 2008c, AIP p. 25).

EPA is aware of two distribution system optimization programs that are currently being developed. EPA and the Foundation are concurrently developing distribution system optimization programs that focus on protecting public health in the distribution system. Developmental activities to support these efforts are occurring through the EPA's National Area Wide Optimization Program (AWOP) and the Foundation's project #4109. While these programs are being developed independently with differing measures of performance, both are founded on the optimization principles of improving water systems, and go beyond the regulatory requirements, while using existing staff and facilities. These principles and practices are currently being used through the in-plant treatment optimization programs operated through AWOP and the American Water Works Association's (AWWA) Partnership for Safe Water (the Partnership).

The goal of EPA's optimization program is to protect public health by addressing both the technical and management issues that limit the water system's ability to meet water quality performance goals. EPA has started developing a distribution system optimization program, which is currently focused on improving water treatment plant finished water quality while maintaining disinfectant residual and minimizing disinfection byproduct formation in the distribution system. Future work may focus on other water quality parameters or issues of concern. An outcome of this effort will be the identification of the key technical and management skills, practices, and tools that a water system should implement to achieve long-term distribution system optimization. Ultimately, participating AWOP States will be introduced to distribution system optimization methods developed by EPA. At this time, additional development activities are needed before a distribution system optimization program will be available for State implementation.

In 2007, the Foundation initiated project #4109 to identify a limited number of straightforward criteria that can be used by water utilities to measure distribution system optimization performance and to develop a self-assessment approach using standards of excellence. The results from this project will also be used to expand the Partnership for Safe Water Program treatment plant optimization program into distribution system optimization. The Foundation anticipates project #4109 to be completed by early 2010. With the results of project #4109, the Partnership anticipates finalizing a preliminary set of optimization goals and a model assessment process in calendar year 2010. Prior to finalizing the goals and assessment process, the Partnership will conduct trials at several volunteer utilities. The optimization goals and

assessment process will be evaluated and refined based on those trials prior to consideration by the Partnership for adoption and implementation.

C. Request for Comment
EPA requests comment about these distribution system optimization projects and information about or suggestions for other possible approaches to distribution system optimization.

VI. Economic Analysis (Health Risk Reduction and Cost Analysis)
This section summarizes the Health Risk Reduction and Cost Analysis (HRRCA) in support of the proposed RTCR as required by section 1412(b)(3)(C) of the SDWA. EPA has prepared the RTCR Economic Analysis (EA) (USEPA 2010a) to comply with this requirement. The EA document for the proposed RTCR is available in the docket and is also published on the government's Web site at http://www.regulations.gov.

The HRRCA consists of seven elements:
(1) quantifiable and nonquantifiable health risk reduction benefits;
(2) quantifiable and nonquantifiable health risk reduction benefits from reductions in co-occurring contaminants;
(3) quantifiable and nonquantifiable costs that are likely to occur solely as a result of compliance;
(4) incremental costs and benefits of rule options;
(5) effects of the contaminant on the general population and sensitive subpopulations including infants, children, pregnant women, elderly, and individuals with a history of serious illness;
(6) any increased health risks that may occur as a result of compliance, including risks associated with co-occurring contaminants; and
(7) other relevant factors such as uncertainties in the analysis and factors with respect to the degree and nature of risk. See SDWA section 1412(b)(3)(C). A summary of these elements is provided in this section of the preamble, and a complete discussion can be found in the Proposed RTCR EA (USEPA 2010a).

The benefits described in this section are discussed qualitatively, and reductions in detection of total coliforms and E. coli and in Level 2 assessments are used to describe the benefits, as described later in this section. The costs discussed in this section are presented as annualized present values in 2007 dollars. Both benefit and cost measures are adjusted using social discounting. In social discounting, future values of a rule's or policy's effects are multiplied by discount factors. The discount factors reflect both the amount of time between the present and the point at which these events occur and the degree to which current consumption is more highly valued than future consumption (USEPA 2000c). This process allows comparison of cost and benefit streams that are variable over a given time period. EPA uses social discount rates of both three percent and seven percent to calculate present values from the stream of benefits and costs and also to annualize the present value estimates. Historically, the use of three percent is based on rates of return on relatively risk-free financial instruments, while seven percent is generally an estimate of before-tax rate of return to incremental private investment. For further information, see USEPA 2000c and OMB 1996.

In the Proposed RTCR EA (USEPA 2010a), EPA also presents the undiscounted stream of benefits and costs over the 25-year time frame (i.e., the year-to-year realization of benefits and costs presented in constant terms).

The time frame used for both benefit and cost comparisons in this rule is 25 years. This time interval accounts for rule implementation activities occurring soon after promulgation (e.g., States adopting the criteria of the regulation) and the time for different types of compliance actions (e.g., assessments and corrective actions) to be realized up through the 25th year following rule promulgation.

EPA was unable to quantify health benefits for the proposed RTCR because there are insufficient data reporting the co-occurrence in a single sample of fecal indicator E. coli and pathogenic

organisms. In addition, the available fecal indicator E. coli data from the Six-Year Review 2 dataset (USEPA 2010e) described in this preamble were limited to presence-absence data because the current TCR requires only the reporting of presence or absence of fecal indicator E. coli using EPA-approved standard methods.

However, as discussed in chapter 6 of the Proposed RTCR EA (USEPA 2010a), even though health benefits could not be directly quantified, the potential benefits from the proposed RTCR include avoidance of a full range of health effects from the consumption of fecally contaminated drinking water, including the following: acute and chronic illness, endemic and epidemic disease, waterborne disease outbreaks, and death. Also, since fecal contamination may contain waterborne pathogens including bacteria, viruses, and parasitic protozoa, in general, a reduction in fecal contamination should reduce the risk from these other contaminants.

The net costs of the rule stem mostly from the new assessment and corrective action requirements as well as the revised monitoring provisions described earlier in this preamble.

This section of the preamble includes elements as follows: (A) Regulatory Options Considered, (B) Major Sources of Data and Information used in Supporting Analyses, (C) Occurrence and Predictive Modeling, (D) Baseline Profiles, (E) Anticipated Benefits of the Proposed RTCR, (F) Anticipated Costs of the Proposed RTCR, (G) Potential Impact of the Proposed RTCR on Households, (H) Incremental Costs and Benefits, (I) Benefits from Simultaneous Reduction of Co-occurring Contaminants, (J) Change in Risk from Other Contaminants, (K) Effects of Fecal Contamination and/or Waterborne Pathogens on the General Population and Sensitive Subpopulations, (L) Uncertainties in the Benefit and Cost Estimates for the Proposed RTCR, (M) Benefit Cost Determination for the Proposed RTCR, and (N) Request for Comment.

A. Regulatory Options Considered

EPA evaluated the following three regulatory options as part of this revised rule proposal: (1) The current TCR option, (2) the AIP option, and (3) an Alternative option. EPA discusses the three regulatory options briefly in this preamble and in greater detail in the Proposed RTCR EA (USEPA 2010a).

First, the current TCR option reflects EPA's understanding of how the current TCR (USEPA 1989a, 54 FR 27544, June 29, 1989) is currently being implemented. That is, the current TCR option is assumed to include “status quo” PWS and State implementation practices. Next, the AIP option is a revised TCR based on the recommendations of the advisory committee. The provisions of this proposed rule are based on the AIP option and are described in detail in section III of this preamble. Third, the Alternative option parallels the AIP in most ways but includes variations of some of the provisions that were discussed by the advisory committee before consensus was reached on the AIP.

The Alternative option differs from the AIP option in two ways. First, under the Alternative option, at the compliance date all PWSs are required to sample monthly for an initial period until they meet the eligibility criteria for reduced monitoring. EPA assumes that eligibility for reduced monitoring is determined during the next sanitary survey following the RTCR compliance date.
This more stringent approach differs from the AIP option that allows PWSs to continue to monitor at their current frequencies (with an additional annual site visit or voluntary Level 2 assessment requirement for PWSs wishing to remain on annual monitoring) until they are triggered into an increased sampling frequency. Second, under the Alternative option, no PWSs are allowed to reduce monitoring to an annual basis. EPA defined the Alternative option this way and included it in the Proposed RTCR EA (USEPA 2010a) to assess the relative impacts of a more stringent rule and to better understand the balance between costs and public health protection.

To understand the relative impacts of the options, EPA gathered available data and information to develop and provide input into an occurrence and predictive model. EPA estimated both baseline conditions and changes to these conditions anticipated to occur over time as a result of these

revised rule options. The analysis is described in more detail in the Proposed RTCR EA (USEPA 2010a).

B. Major Sources of Data and Information Used in Supporting Analyses
This section of the preamble briefly discusses the data sources that EPA used in its supporting analyses for the proposed RTCR. For a more detailed discussion, see chapter 4 of the Proposed RTCR EA (USEPA 2010a).

1. Safe Drinking Water Information System Federal Version Data
Safe Drinking Water Information System Federal Version (SDWIS/FED) is EPA's national regulatory compliance database for the drinking water program and is the main source of PWS inventory and violation data for the proposed RTCR baseline. SDWIS/FED contains information on each of the approximately 155,000 active PWSs as reported by primacy agencies, EPA Regions, and EPA headquarters personnel. SDWIS/FED includes records of MCL violations and monitoring and reporting (MR) violations (both routine and repeat and minor and major). It does not include sample results. It also contains information to characterize the US inventory of PWSs including system name and location, retail population served, source water type (ground water (GW), surface water (SW), or ground water under the direct influence of surface water (GWUDI)), disinfection status, and PWS type (community water system (CWS), transient non-community water system (TNCWS), and non-transient non-community water system (NTNCWS)).

To create the PWS and population baseline, EPA used the fourth quarter of SDWIS/FED 2007 (USEPA 2007b), which was the most current PWS inventory data available when EPA began developing the Proposed RTCR EA. These data represent all current, active PWSs and the population served by these systems.

EPA also used the MCL violation data from SDWIS/FED to validate model predictions for systems serving 4,100 or fewer people and to predict E. coli (acute)_-MCL violations (current TCR, AIP, and Alternative option), total coliform (non-acute or monthly) MCL violations (current TCR), and Level 1 and Level 2 assessment triggers (AIP and Alternative option) for systems serving more than 4,100 people.

2. Six-Year Review 2 Data
Through an Information Collection Request (USEPA 2006b), States voluntarily submitted electronically available TCR monitoring data (sample results) that were collected between January 1998 and December 2005. EPA requested the TCR monitoring results with the intent of conducting analyses and developing models to assess the potential impacts of changes to the current TCR. EPA received data from 46 States, Tribes, and territories. A Data Quality Report (USEPA 2010c) describes how TCR monitoring data were obtained, evaluated, and modified where necessary to make the database internally consistent and usable for analysis. Exhibit 2.1 in the Data Quality Report provides a complete list of States or territories that submitted data and a description of the use of these data.

In this EA, EPA included data from 37 primacy agencies (35 States and 2 Tribes). Records included data for:

- PWS information (system type, population served, source water type)
- Sample type (routine, repeat, special purpose)
- Analytical result
- Sampling location—entry point, distribution system and, for repeat samples, original location, downstream, upstream, and other
- Analytical method

- Disinfectant residual data collected at TCR monitoring sites

As discussed in greater detail in section 4.2.2.1 of the Proposed RTCR EA (USEPA 2010a), EPA used 2005 data exclusively in the analyses supporting the proposed RTCR because the 2005 data set was the most complete year of data among the Six-Year Review 2 data (USEPA 2010e). The 2005 data was also the most recent data available suggesting that it may be the most representative of present conditions.

The Six-Year Review 2 data (USEPA 2010e) also informed EPA's assumptions regarding the proportions of GWSs serving 1,000 or fewer people that sample monthly, quarterly, or annually.

Disinfection Byproduct Regulations

Drinking water chlorination has contributed to a dramatic decline in waterborne disease rates and increased life expectancy in the United States. Largely because of this success, many Americans take it for granted that their tap water will be free of disease-causing organisms. In recent years, regulators and the general public have focused greater attention on potential health risks from chemical contaminants in drinking water. One such concern relates to disinfection byproducts (DBPs), chemical compounds formed unintentionally when chlorine and other disinfectants react with certain organic matter in water.

In the early 1970s, EPA scientists first determined that drinking water chlorination could form a group of byproducts known as trihalomethanes (THMs), including chloroform. Concerned that these chemicals may be carcinogenic to humans, EPA set the first regulatory limits for THMs in 1979. Since that time, a wealth of research has improved our understanding of how DBPs are formed, their potential health risks, and how they can be controlled. It is now recognized that all chemical disinfectants form some potentially harmful byproducts. The byproducts of chlorine disinfection are by far the most thoroughly studied.

While the available evidence does not prove that DBPs in drinking water cause adverse health effects in humans, high levels of these chemicals are certainly undesirable. Cost-effective methods to reduce DBP formation are available and should be adopted where possible. However, the International Programme on Chemical Safety (IPCS), a joint venture of the United Nations Environment Programme, the International Labor Organization, and the World Health Organization (IPCS 2000, p. 13) strongly cautions:

The health risks from these byproducts at the levels at which they occur in drinking water are extremely small in comparison with the risks associated with inadequate disinfection. Thus, it is important that disinfection not be compromised in attempting to control such byproducts.

Recent EPA regulations have further limited THMs and other DBPs in drinking water. Most water systems are meeting these new standards by controlling the amount of natural organic matter prior to disinfection, while ensuring that microbial protection remains the top priority. DBPs and Human Cancer Risk Toxicology studies have reported that high doses of some DBPs, including THMs and haloacetic acids (HAAs), can cause cancer in laboratory animals. Based largely on these animal data, EPA considers individual THMs and HAAs to be either possible or probable human carcinogens, although any risk from the low levels found in drinking water would be slight. After reviewing the full body of toxicology studies, the IPCS concluded, “None of the chlorination byproducts studied to date is a potent carcinogen at concentrations normally found in drinking water” (IPCS 2000, p. 376).

Some epidemiology studies have reported an association between human exposure to DBPs and elevated cancer risks, while other studies have found no association. EPA evaluated the existing cancer epidemiology studies and found that only for bladder cancer were associations with chlorinated water somewhat consistent. Even in these studies, cancer risks were not strongly correlated to measured THM levels, indicating that other factors cannot be ruled out (Craun et al., 2001). EPA has concluded, “The present epidemiologic data do not support a causal relationship between exposure to chlorinated drinking water and development of cancer at this time” (EPA 1998). The IPCS reached a similar conclusion in 2000, noting that a causal relationship between DBPs and increased cancer remains an open question (IPCS 2000).

Balancing DBP and Microbial Risks

Continuing evidence of waterborne disease occurrence suggests that microbial risks should receive a much higher level of attention than disinfection byproducts. For this reason, The American Academy of Microbiology (Ford and Colwell, 1996) has recommended, the health risks posed by microbial pathogens should be placed as the highest priority in water treatment to

protect public health. A report published by the International Society of Regulatory Toxicology and Pharmacology (Coulston and Kolbye, 1994) stated "The reduction in mortality due to waterborne infectious diseases, attributed largely to chlorination of potable water supplies, appears to outweigh any theoretical cancer risks (which may be as low as zero) posed by the minute quantities of chlorinated organic chemicals reported in drinking waters disinfected with chlorine."

The IPCS (IPCS 2000, p. 375) reached similar conclusions:
Disinfection is unquestionably the most important step in the treatment of water for drinking water supplies. The microbial quality of drinking water should not be compromised because of concern over the potential long-term effects of disinfectants and DBPs. The risk of illness and death resulting from exposure to pathogens in drinking water is very much greater than the risks from disinfectants and DBPs.

Controlling Disinfection Byproducts
Treatment techniques are available that provide water suppliers the opportunity to maximize potable water safety and quality while minimizing the risk of DBP risks. Generally, the best approach to reduce DBP formation is to remove natural organic matter precursors prior to disinfection. EPA has published a guidance document for water system operators entitled, Controlling Disinfection byproducts and Microbial Contaminants in Drinking Water (EPA, 2001).

The EPA guidance discusses three processes to effectively remove natural organic matter prior to disinfection:

1. Coagulation and Clarification
Most treatment plants optimize their coagulation process for turbidity (particle) removal. However, coagulation processes can also be optimized for natural organic matter removal with higher doses of inorganic coagulants (such as alum or iron salts), and optimization of pH.

2. Absorption
Activated carbon can be used to absorb soluble organics that react with disinfectants to form byproducts.

3. Membrane Technology
Membranes, used historically to desalinate brackish waters, have also demonstrated excellent removal of natural organic matter. Membrane processes use hydraulic pressure to force water through a semi-permeable membrane that rejects most contaminants. Variations of this technology include reverse osmosis (RO), nanofilitration (low pressure RO), and microfiltration (comparable to conventional sand filtration).

Other conventional methods of reducing DBP formation include changing the point of chlorination and using chloramines for residual disinfection. EPA predicts that most water systems will be able to achieve compliance with new DBP regulations through the use of one or more of these relatively low cost methods (EPA, 1998).

Water system managers may also consider switching from chlorine to alternative disinfectants to reduce formation of THMs and HAAs. However, all chemical disinfectants form some DBPs. Much less is known about the byproducts of these alternatives than is known about chlorination byproducts. Furthermore, each disinfection method has other distinct advantages and disadvantages.

Common Water Quality and Sampling Questions and Review

These statements will be more explained in the previous chapters.

1. What are the correct procedures to follow in collecting bacteriological samples?
Use a sterile plastic or glass bottle. Sodium thiosulfate should be added to neutralize the chorine residual. Refrigerate the sample to 4^{o} C. The regulations call for a minimum of five samples for the month from any system that has positive sample results. Small systems that take only one sample per month have to take four (4) repeats when they get a total coliform positive test result. If any system has to take repeat samples, it must also take a minimum of five (5) routine samples the following month. Small systems that normally take less than 5 samples/month will have to increase the number to 5 samples. They can return to normal sampling schedules the following month if no repeats are required.

2. What are the proper sampling techniques for microbiological sampling?
Proper sampling techniques are extremely important in obtaining accurate water quality information. An improperly taken coliform sample may indicate bacteriological contamination of your water when the water is actually safe. You can avoid the cost of additional testing by using good sampling procedures. Carefully follow these steps in taking a sample for bacteriological testing:
1. Select the sampling point. The sampling point must be a faucet from which water is commonly taken for public use.

The sampling point should be a non-swivel faucet.

Remove any aerator or screen and flush.

It should not be a faucet that leaks, permitting water to run over the outside of the faucet. Leaking faucets can promote bacterial growth.

If an outside faucet must be used, disconnect any hoses or other attachments and be sure to flush the line thoroughly.

Do not use fire hydrants as sampling points. Do not dip the bottle in reservoirs, spring boxes or storage tanks in order to collect the sample.

3. What do the following abbreviations stand for and what do they mean: gpm, MGD, TTHM, psi, HAA, NTU, and mg/L.
Gallons per minute- Million Gallons a Day - Total Trihalomethanes – Pounds Per Square Inch – Haloacetic acids - Nephelometric turbidity unit -Milligrams Per Liter

4. What is the relationship between mg/L and ppm; ug/L and ppb?
Milligram per liter: Milligram per liter of substance and part per million are equals amounts in water. While you can easily convert between micrograms/liter and milligrams/liter, and between PPM and PPB, it's not so easy to convert between the different types of units such as milligrams/liter to PPM.

To convert micrograms per liter to milligrams per liter, divide by 1000.

To convert to PPM, you would first need to know the density of the substance, and the density of what the substance is in.

5. Ug/L: Represents the concentration of something in water or soil. One ppb represents one microgram of something per liter of water (ug/l), or one microgram of something per kilogram of soil (ug/kg).

Parts per million (ppm) or Milligrams per liter (mg/l) - one part per million corresponds to one minute in two years or a single penny in $10,000.

Parts per billion (ppb) or Micrograms per liter - one part per billion corresponds to one minute in 2,000 years, or a single penny in $10,000,000.

Parts per trillion (ppt) or Nanograms per liter (nanograms/l) - one part per trillion corresponds to one minute in 2,000,000 years, or a single penny in $10,000,000,000.

6. What do the following terms represent in reference to water quality.
Total coliform: The coliform family has been divided into two groups. Results may come back as either total coliform positive (TC positive) or fecal coliform positive, or (FC positive or *E. coli* positive.) Total coliform positive means that no human coliform are present.

7. Fecal Coliform: Fecal coliform positive indicates the presence of *E. coli*, which means there is a greater chance of pathogens being present. The laboratory tests for coliform include the MPN method, the Membrane Filter test, the Colilert test, and the presence-absence test.

8. Presence-absence Test: Presence-Absence Broth is used for the detection of coliform bacteria in water treatment plants or distribution systems using the presence-absence coliform test.

9. Physical Characteristics of Water: A characteristic of water defined by the temperature, turbidity, color, taste, and odor of the water.

10. Point-of-entry sample (POE): A type of water sample taken after treatment and before reaching the first consumer.

11. Acute Health Effect: An immediate (i.e. within hours or days) effect that may result from exposure to certain drinking water contaminants (e.g., pathogens).

12. Non-acute violation: If the MCL is exceeded and none of the positive results indicated a presence of Fecal Coliform, a Tier 2 violation has occurred. This level of violation used to be called a non-acute violation.

13. Routine Sample: Samples collected on a routine basis to monitor for contamination. Collection should be in accordance with an approved sampling plan.

14. Repeat Sample: Short answer… Samples collected following a '***coliform present'*** routine sample. The number of repeat samples to be collected is based on the number of routine samples you normally collect. Long Answer. Anytime a microbiological sample result comes back positive, indicating the presence of total or fecal coliform/ E.coli, repeat samples must be taken. Three repeats are usually required. One must be taken at the site of the positive sample. The two samples must be taken upstream and downstream of the original site (within five service connections). These repeat samples must be taken within 24 hours of notification of positive results. They must be identified as a Repeat Sample on the sample form. Repeat samples may be required to be sealed with a red evidentiary seal tape. The tape must cover the cap and extend down the sides of the bottle. The sample forms must also include the reference number for the positive sample.

There is an important exception to the three repeat samples rule. The regulations also state that when repeats are taken the minimum number of samples is raised to five for the month. A system

that collects just one sample a month must collect four repeat samples, when the sample is positive, in order to have five samples as required.

Whenever a system has to take repeat samples, a minimum of five routine samples must also be submitted the following month. This is only an issue for systems that normally turn in four or fewer samples each month. If the five samples are negative the system can return to its normal sampling schedule the next month.

Small systems that have fewer than four sampling sites have a problem complying with the "upstream and downstream" aspects of the repeat sampling requirements. In this case, samples should be taken at as many separate sites as possible and then wait a minimum of 2 hours before resampling enough sites to get the required number of samples. Repeat sample with red seal tape.

15. Treatment technique: An enforceable procedure or level of technical performance which public water systems must follow to ensure control of a contaminant.

16. Action level: The level of lead or copper which, if exceeded, triggers treatment or other requirements that a water system must follow.

17. What does the membrane filter test analyze with regards to bacteriological sampling?
Membrane Filter Technique: A standard test used for measuring coliform numbers (quantity) in water is the membrane filter technique. This technique involves filtering a known volume of water through a special sterile filter. These filters are made of nitrocellulose acetate and polycarbonate, are 150 µm thick, and have 0.45 µm diameter pores. A grid pattern is printed on these filter disks in order to facilitate colony counting. When the water sample is filtered, bacteria (larger than 0.45 µm) in the sample are trapped on the surface of the filter. The filter is then carefully removed, placed in a sterile petri plate on a pad saturated with a liquid medium, and incubated for 20-24 hours at 37°C.

One assumes that each bacterium trapped on the filter will then grow into a separate colony. By counting the colonies one can directly determine the number of bacteria in the water sample that was filtered. The broth medium usually employed in detecting total coliforms is M-Endo Broth MF. Total coliform colonies will be pink to dark red in color and will appear to have a golden green color.

18. What do the following terms mean in relation to drinking water quality: disinfection, pathogenic, toxic, pH, aesthetic, culinary and potable.
Disinfection: The chemical process of killing or inactivating most microorganisms in water. See also Sterilization.

19. Pathogenic: Organisms or bugs that cause disease. These include bacteria, viruses, cysts and anything capable of causing disease in humans, like cryptosporidiosis, typhoid, cholera and so on. There are other organisms that do not create disease, these are called non-pathogenic organisms.

20. Toxic: Stuff that will kill you. A substance which is poisonous to living organisms.

21. pH: A measure of the acidity of water. The pH scale runs from 0 to 14 with 7 being the mid-point or neutral. A pH of less than 7 is on the acid side of the scale with 0 as the point of greatest acid activity. A pH of more than 7 is on the basic (alkaline) side of the scale with 14 as the point of greatest basic activity. For example, the acidity of a sample with a pH of **5** is ten times greater than that of a sample with a pH of **6**. A difference of 2 units, from **6** to **4**, would mean that the acidity is one hundred times greater, and so on. Normal rain has a pH of **5.6** – slightly acidic because of the carbon dioxide picked up in the earth's atmosphere by the rain.

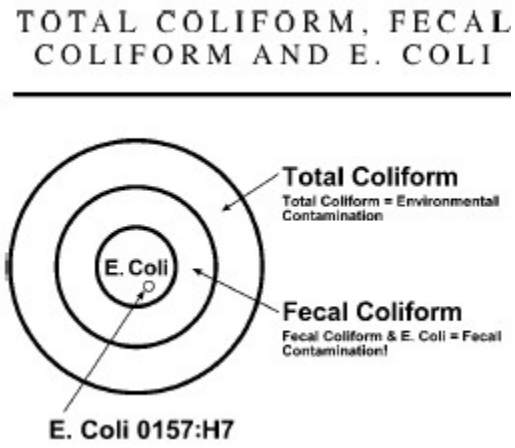
22. Aesthetic: Attractive or appealing water or things in water that will not make you sick but may appear to change the water's color or taste.

23. Culinary: Having to do with cooking food. Potable water is often called culinary water.

24. Potable: Water that is free of objectionable pollution, contamination, or infective agents. Generally speaking, we serve only potable water and not palatable water. Palatable is pleasant tasting water.

25. What is hardness in water and what chemicals cause it?
Hardness: Water that contains high amounts of dissolved minerals, specifically calcium and magnesium. **Ion Exchange:** A method of water softening where hardness causing ions are exchanged with sodium ions; also effective in removing many inorganic contaminants such as nitrates, copper, and lead; and treating aesthetic water problems.

26. What is Escherichia Coliform and what does it indicate in relation to drinking water?
E. coli is a sub-group of the fecal coliform group. Most *E. coli* bacteria are harmless and are found in great quantities in the intestines of people and warm-blooded animals. Some strains, however, can cause illness. The presence of *E. coli* in a drinking water sample almost always indicates recent fecal contamination meaning there is a greater risk that pathogens are present.



Total coliform, fecal coliform, and *E. coli* are all indicators of drinking water quality. The total coliform group is a large collection of different kinds of bacteria. Fecal coliforms are types of total coliform that mostly exist in feces. *E. coli* is a sub-group of fecal coliform. When a water sample is sent to a lab, it is tested for total coliform. If total coliform is present, the sample will also be tested for either fecal coliform or *E. coli*, depending on the lab testing method.

27. What problems are associated with Hydrogen Sulfide in the water?
Hydrogen sulfide is a gas which, when dissolved in water, gives it a "rotten egg" odor. Chlorination will remove this gas from the water but the effectiveness of the chlorine for disinfection is lessened.

28. When Hydrogen sulfide reacts with chlorine, it produces Sulfuric acid and elemental Sulfur: It is therefore recommended that aeration be applied prior to the addition of chlorine for the most effective disinfection.

29. Why is it important to know what the turbidity of the water is when using chlorine?
To be careful not to overdose with chlorine or properly dose with chlorine.

30. What is the log removal for Cryptosporidium?
The LT1ESWTR extends further this necessary protection from Cryptosporidium to communities of fewer than 10,000 persons. Today's rule for the first time establishes Cryptosporidium control requirements for systems serving less than 10,000 persons by requiring a minimum 2-log removal for Cryptosporidium. The rule also strengthens filter performance requirements to ensure 2-log Cryptosporidium removal, establishes individual filter monitoring to minimize poor performance in individual units, includes Cryptosporidium in the definition of GWUDI, and explicitly considers unfiltered system watershed control provisions. The rule also reflects a commitment to the importance of maintaining existing levels of microbial protection in public water systems as plants take steps to comply with newly applicable DBP standards.

31. What is the log removal?
This log-reduction terminology was developed by engineers as a way to express levels of decreased biological contamination in water by factors of 10 that could be easily converted to percent reduction. The most commonly used logarithmic base is 10 because it is compatible with our base-10 decimal system. The log of 10 in the base 10 logarithmic system is 1 and the log of 100 is 2, with the log of 1000 being 3, etc. A 1-log reduction is nine out of 10 and would be equivalent to a 90 percent reduction. A 2-log reduction would be 99 out of 100 or 99 percent reduction and a 3-log reduction would be 999 out of 1000 or 99.9 percent reduction. A 99.99 percent reduction would be called a 4-log reduction.

32. What are the turbidity requirements for Direct and Conventional filtration plants?
For conventional and direct filtration systems (including those systems utilizing in-line filtration), the turbidity level of representative samples of a system's filtered water (measured every four hours) must be less than or equal to ***0.3 NTU*** in at least 95 percent of the measurements taken each month. The turbidity level of representative samples of a system's filtered water must not exceed ***1 NTU*** at any time. Conventional filtration is defined as a series of processes including coagulation, flocculation, sedimentation, and filtration resulting in substantial particulate removal. Direct filtration is defined as a series of processes including coagulation and filtration but excluding sedimentation resulting in substantial particle removal.

33. What are chloramines, how are they formed, and do they have any beneficial use?
Chloramines: Ammonia and Chlorine are combined. Cl_2NH_3 Yes, limited use and this chemical will create less THMS than chlorine alone. Chloramine is a disinfectant used to treat drinking water. It is formed by mixing chlorine with ammonia. Although it is a weaker disinfectant than chlorine, it is more stable and extends disinfectant benefits throughout a water utility's distribution system (a system of pipes water is delivered to homes through). Some water systems use chloramine as a secondary disinfectant to maintain a disinfectant residual throughout the distribution system so that drinking water remains safe as it travels from the treatment facility to the customer. Chloramine has been used by water systems for almost 90 years, and its use is closely regulated. Since chloramine is not as reactive as chlorine, it forms fewer disinfection byproducts. Some disinfection byproducts, such as the trihalomethanes (THMs) and haloacetic acids (HAAs), may have adverse health effects and are closely regulated. Because a chloramine residual is more stable and longer lasting than free chlorine, it provides better protection against bacterial regrowth in systems with large storage tanks and dead-end water mains. Chloramine, like chlorine, is effective in controlling biofilm, which is a coating in the pipe caused by bacteria. Controlling biofilm also tends to reduce coliform bacteria concentrations and biofilm-induced corrosion of pipes.

Symbols and Characters

These symbols and characters are specific to water sampling methods but have been conformed to common usage as much as possible.

Units of weight and measure and their abbreviations
Symbols

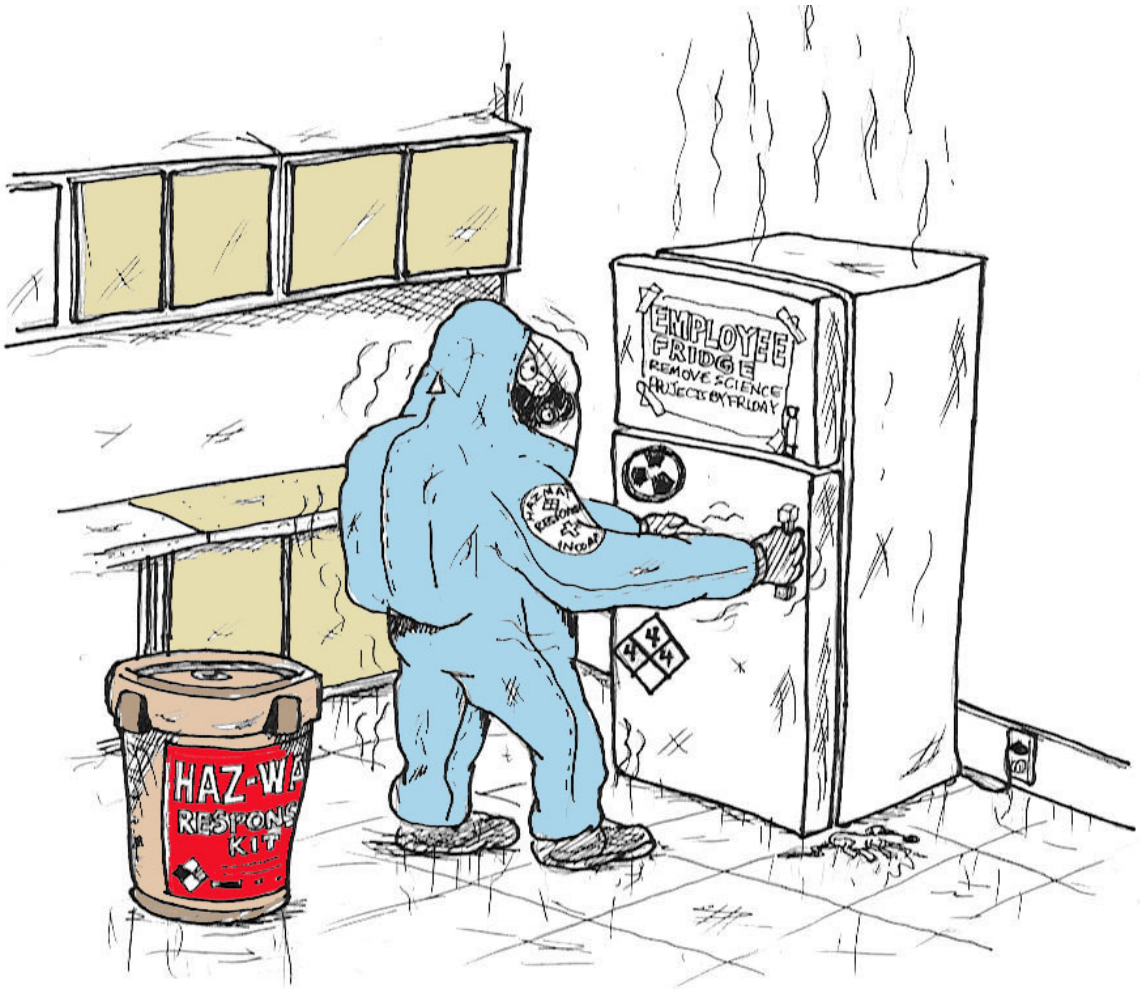
°C degrees Celsius
µL microliter
< less than
> greater than
% percent

Alphabetical characters

ACUK Acid chrome violet K
AECL Alternate enhanced coagulant level
AOC Assimilable organic carbon
ASDWA Association of State Drinking Water Administrators
AWWA American Water Works Association
AWWARF AWWA Research Foundation
BAC Biologically active carbon
BAF Biologically active filtration
BAT Best Available Technology
BCAA Bromochloroacetic acid
BDOC Biodegradable organic carbon
BMP Best management practice
BOM Biodegradable Organic Matter (=BDOC + AOC)
Br- Bromide ion
BrO^2- Bromite ion
BrO^3- Bromate ion
CI Confidence interval
Cl^2 Chlorine
ClO^2 Chlorine Dioxide
cm centimeter
CT Concentration-Time
CWS Community Water System
D/DBP Disinfectants/disinfection byproducts
DBP Disinfection byproduct
DBPFP Disinfection byproduct formation potential
DBPP Disinfection byproduct precursors
DBPR Disinfectants/disinfection byproducts rule
DBPRAM DBP Regulatory Assessment Model
DBPs Disinfection byproducts
DOC Dissolved organic carbon
DPD N,N-diethyl-p-phenylenediamine
DWEL Drinking Water Equivalent Level
EBCT Empty bed contact time
EMSL EPA Environmental Monitoring and Support Laboratory (Cincinnati)
EPA United States Environmental Protection Agency
ESWTR Enhanced Surface Water Treatment Rule
FBR Filter Backwash Rule

FY Fiscal year
G acceleration due to gravity
g gram
GAC Granular activated carbon
GWR Ground Water Rule
GWSS Ground Water Supply Survey
H2O2 Hydrogen Peroxide
HAA5 Haloacetic acids (five)
HOBr Hypobromous acid
HOCl Hypochlorous acid
hr hour
IC Ion chromotography
ICR Information Collection Rule
ID inside diameter
IESWTR Interim Enhanced Surface Water Treatment Rule
in. inch
IOA International Ozone Association
IOC Inorganic chemical
KMnO4 Potassium permanganate
L liter
LOAEL Lowest observed adverse effect level
LOQ Limit of quantitation
LT1ESWTR Long Term Stage 1 Enhanced Surface Water Treatment Rule
m meter
MCL Maximum Contaminant Level
MCLG Maximum Contaminant Level Goal
M-DBP Microbial and disinfection byproducts
MDL Method Detection Limit
mg milligram
mg/L Milligrams per liter
mgd Million gallons per day
MIB Methylisoborneol
mL milliliter
mm millimeter
mM millimolar
MRDL Maximum Residual Disinfectant Level (as mg/l)
MRDLG Maximum Residual Disinfectant Level Goal
MRL Minimum Reporting Level
MX 3-chloro-4-(dichloromethyl)-5-hydroxyl-2(5H)-furanone
N normal; gram molecular weight of solute divided by hydrogen equivalent of solute, per liter of solution
NaCl Sodium chloride
NCI National Cancer Institute
ND Not detected
NH^2Cl Monochloramine
NIOSH National Institute for Occupational Safety and Health
NIPDWR National Interim Primary Drinking Water Regulation
NOAEL No Observed Adverse Effect Level
NOM Natural Organic Matter
NOMS National Organic Monitoring Survey
NORS National Organics Reconnaissance Survey for Halogenated Organics

NPDWR National Primary Drinking Water Regulation
NTNCWS Nontransient noncommunity water system
NTP Normal Temperature and Pressure
O^2 Oxygen
O^3 Ozone
OBr- Hypobromite ion
OCl- Hypochlorite ion
PCE Perchloroethylene
PE Performance evaluation
POE Point-of-Entry Technologies
POU Point-of-Use Technologies
ppb Parts per billion
ppm Parts per million
PQL Practical Quantitation Level
PTA Packed Tower Aeration
PWS Public water system
RIA Regulatory Impact Analysis
RMCL Recommended Maximum Contaminant Level
RNDB Regulations Negotiation Data Base
RSC Relative Source Contribution
RSD relative standard deviation
SDWA Safe Drinking Water Act, or the "Act," as amended in 1996
SM Standard Method
SMCL Secondary Maximum Contaminant Level
SMR Standardized mortality ratios
SOC Synthetic Organic Chemical
s_r standard deviation of recovery
SWTR Surface Water Treatment Rule
TCE Trichloroethylene
THM Trihalomethane
THMFP Trihalomethane formation potential
TMV Tobacco mosaic virus
TOC Total organic carbon
TTHM Total trihalomethanes
TWG Technologies Working Group
UV Ultraviolet
VOC Volatile Organic Chemical
WIDB Water Industry Data Base
WS Water supply
X average percent recovery
XDBPs Halogenated DBPs



Never keep food or drinks in your sample refrigerator. I know all of you have done this in the past and I know you've seen someone work without gloves, but you need to be strong and remind personnel that you had enough of tasting all the nastiness. If you are new to this industry, don't fret, you will get a free taste very soon, one way or another. My advice, ask for the hepatitis injections and prepare for a case of the runs that will last for about 1-2 days, after this, you should be good to go. All of us have suffered through this ordeal.

What are the Symptoms of Viral Gastroenteritis?
The main symptoms of viral gastroenteritis are watery diarrhea and vomiting. The affected person may also have headache, fever, and abdominal cramps ("stomach ache"). In general, the symptoms begin 1 to 2 days following infection with a virus that causes gastroenteritis and may last for 1 to 10 days, depending on which virus causes the illness.

Lots of fun…

General Water Treatment Glossary – Sampling Glossary in rear

ABANDONED WELL: Wells that have been or need to be sealed by an approved method.

ABSENCE OF OXYGEN: The complete absence of oxygen in water described as Anaerobic.

ACCURACY: How closely an instrument measures the true or actual value.

ACID AND BASE ARE MIXED: When an acid and a base are mixed, an explosive reaction occurs and decomposition products are created under certain conditions.

ACID: Slowly add the acid to water while stirring. An operator should not mix acid and water or acid to a strong base.

ACID RAIN: A result of airborne pollutants.

ACTIVATED CHARCOAL (GAC or PAC): Granular Activated Charcoal or Powered Activated Charcoal. Used for taste and odor removal. A treatment technique that is not included in the grading of a water facility.

ACTIVATED CARBON FILTRATION: Can remove organic chemicals that produce off-taste and odor. These compounds are not dangerous to health but can make the water unpleasant to drink. Carbon filtration comes in several forms, from small filters that attach to sink faucets to large tanks that contain removable cartridges. Activated carbon filters require regular maintenance or they can become a health hazard.

ADSORPTION: *Not to be confused with absorption.* Adsorption is a process that occurs when a gas or liquid solute accumulates on the surface of a solid or a liquid (adsorbent), forming a film of molecules or atoms (the adsorbate). It is different from absorption, in which a substance diffuses into a liquid or solid to form a solution. The term sorption encompasses both processes, while desorption is the reverse process. Adsorption is present in many natural physical, biological, and chemical systems, and is widely used in industrial applications such as activated charcoal, synthetic resins, and water purification.

ADSORPTION CLARIFIERS: The concept of the adsorption clarifier package plant was developed in the early 1980s. This technology uses an up-flow clarifier with low-density plastic bead media, usually held in place by a screen. This adsorption media is designed to enhance the sedimentation/clarification process by combining flocculation and sedimentation into one step. In this step, turbidity is reduced by adsorption of the coagulated and flocculated solids onto the adsorption media and onto the solids already adsorbed onto the media. Air scouring cleans adsorption clarifiers followed by water flushing. Cleaning of this type of clarifier is initiated more often than filter backwashing because the clarifier removes more solids. As with the tube-settler type of package plant, the sedimentation/ clarification process is followed by mixed-media filtration and disinfection to complete the water treatment.

AIR GAP SEPARATION: A physical separation space that is present between the discharge vessel and the receiving vessel; for an example, a kitchen faucet.

AIR HAMMER: A pneumatic cylindrical hammering device containing a piston used on air rotary rigs. The air hammer's heavy piston moves up and down by the introduction of compressed air creating a hammering action on the bit.

AIR HOOD: The most suitable protection when working with a chemical that produces dangerous fumes.

AIR ENTRAINMENT: The dissolution or inclusion of air bubbles into water.

AIRLIFT: The lifting of water and/or cuttings to the surface by the injection of high pressure bursts of air. Airlift occurs continuously when drilling with air rotary and can be used for well development with a surging technique.

AIR PUMPING: Continuous airlifting to remove water from the well.

AIR ROTARY: A method of rotary well drilling that utilizes compressed air as the primary drilling fluid.

AGGLOMERATION: A jumbled cluster or mass of varied parts. The act or process of agglomerating.

ALKALINITY: Alkalinity or AT is a measure of the ability of a solution to neutralize acids to the equivalence point of carbonate or bicarbonate. Alkalinity is closely related to the acid neutralizing capacity (ANC) of a solution and ANC is often incorrectly used to refer to alkalinity. However, the acid neutralizing capacity refers to the combination of the solution and solids present (e.g., suspended matter, or aquifer solids), and the contribution of solids can dominate the ANC (see carbonate minerals below).

ALTERNATIVE DISINFECTANTS: Disinfectants - other than chlorination (halogens) - used to treat water, e.g. ozone, ultraviolet radiation, chlorine dioxide, and chloramine. There is limited experience and scientific knowledge about the by-products and risks associated with the use of alternatives.

ALGAE: Microscopic plants that are free-living and usually live in water. They occur as single cells floating in water, or as multicellular plants like seaweed or strands of algae that attach to rocks.

ALPHA AND BETA RADIOACTIVITY: Represent two common forms of radioactive decay. Radioactive elements have atomic nuclei so heavy that the nucleus will break apart, or disintegrate spontaneously. When decay occurs, high-energy particles are released. These high-energy particles are called radioactivity. Although radioactivity from refined radioactive elements can be dangerous, it is rare to find dangerous levels of radioactivity in natural waters. An alpha particle is a doubly-charged helium nucleus comprised of two protons, two neutrons, and no electrons. A beta particle is a high-speed electron. Alpha particles do not penetrate matter easily, and are stopped by a piece of paper. Beta particles are much more penetrating and can pass through a millimeter of lead.

ALUMINUM SULFATE: The chemical name for Alum. The molecular formula of Alum is $Al_2(SO_4)3{\sim}14H_2O$. It is a cationic polymer.

AMOEBA: Amoeba (sometimes amœba or ameba, plural amoebae) is a genus of protozoa that moves by means of pseudopods, and is well-known as a representative unicellular organism. The word amoeba or ameba is variously used to refer to it and its close relatives, now grouped as the Amoebozoa, or to all protozoa that move using pseudopods, otherwise termed amoeboids.

AMMONIA: NH_3 A chemical made with Nitrogen and Hydrogen and used with chlorine to disinfect water. Most ammonia in water is present as the ammonium ion rather than as ammonia.

AMMONIATOR: A control device which meters gaseous ammonia directly into water under positive pressure.

ANAEROBIC: An abnormal condition in which color and odor problems are most likely to occur.

ANAEROBIC CONDITIONS: When anaerobic conditions exist in either the metalimnion or hypolimnion of a stratified lake or reservoir, water quality problems may make the water unappealing for domestic use without costly water treatment procedures. Most of these problems are associated with Reduction in the stratified waters.

ANEROID: Using no fluid, as in aneroid barometer.

ASEPTIC: Free from the living germs of disease, fermentation, or putrefaction.

ANNULAR SPACE: The space between the borehole wall and either drill piping or casing within a well.

ANNULUS: See Annular Space.

AMMONIA: A chemical made with Nitrogen and Hydrogen and used with chlorine to disinfect water.

AQUICLUDE: A layer or layers of soils or formations which water cannot pass through (ex - solid bedrock or very stiff clay). The opposite of aquifer.

AQUIFER: A saturated layer or layers of soils or formations which water can pass through and be provided in usable quantities to supply wells or springs (ex – saturated semi consolidated sediment or saturated fractured bedrock.) An underground geologic formation capable of storing significant amounts of water.

AQUIFER PARAMETERS: Referring to such attributes as specific capacity, aquifer storage, transmissivity, hydraulic conductivity, gradient, and water levels. Refers to all of the components of Darcy's Law and related parameters.

ARTESIAN AQUIFER: A confined aquifer in which the pressure head results in a water elevation higher than the land surface.

ARTESIAN WELL: A well constructed within an artesian aquifer. When an artesian well is opened it will flow naturally.

As: The chemical symbol of Arsenic.

AS NITROGEN: An expression that tells how the concentration of a chemical is expressed mathematically. The chemical formula for the nitrate ion is NO_3 , with a mass of 62. The concentration of nitrate can be expressed either in terms of the nitrate ion or in terms of the principal element, nitrogen. The mass of the nitrogen atom is 14. The ratio of the nitrate ion mass to the nitrogen atom mass is 4.43. Thus a concentration of 10 mg/L nitrate expressed as nitrogen would be equivalent to a concentration of 44.3 mg/L nitrate expressed as nitrate ion. When dealing with nitrate numbers it is very important to know how numeric values are expressed.

ASYNCHRONOUS: Not occurring at the same time.

AUGER RIG: A drilling rig, which drives a rotating spiral flange to drill into the earth.

ATOM: The general definition of an ion is an atom with a positive or negative charge. Electron is the name of a negatively charged atomic particle.

BACKFLOW PREVENTION: To stop or prevent the occurrence of, the unnatural act of reversing the normal direction of the flow of liquid, gases, or solid substances back in to the public potable (drinking) water supply. See Cross-connection control.

BACKFLOW: To reverse the natural and normal directional flow of a liquid, gases, or solid substances back in to the public potable (drinking) water supply. This is normally an undesirable effect.

BACKSIPHONAGE: A liquid substance that is carried over a higher point. It is the method by which the liquid substance may be forced by excess pressure over or into a higher point.

BACTERIA: Small, one-celled animals too small to be seen by the naked eye. Bacteria are found everywhere, including on and in the human body. Humans would be unable to live without the bacteria that inhabit the intestines and assist in digesting food. Only a small percentage of bacteria cause disease in normal, healthy humans. Other bacteria can cause infections if they get into a cut or wound. Bacteria are the principal concern in evaluating the microbiological quality of drinking water, because some of the bacteria-caused diseases that can be transmitted by drinking water are potentially life-threatening.

BACTERIOPHAGE: Any of a group of viruses that infect specific bacteria, usually causing their disintegration or dissolution. A bacteriophage (from 'bacteria' and Greek phagein, 'to eat') is any one of a number of viruses that infect bacteria. The term is commonly used in its shortened form, phage. Typically, bacteriophages consist of an outer protein hull enclosing genetic material. The genetic material can be ssRNA (single stranded RNA), dsRNA, ssDNA, or dsDNA between 5 and 500 kilo base pairs long with either circular or linear arrangement. Bacteriophages are much smaller than the bacteria they destroy - usually between 20 and 200 nm in size.

BAILER: A device used to withdrawal water or sediment from a well utilizing a check valve type mechanism.

BARITE: Processed barium sulfate, often used to increase drilling fluid densities in mud rotary.

BATTERY: A source of direct current (**DC**) may be used for standby lighting in a water treatment facility. The electrical current used in a DC system may come from a battery.

BENTONITE: High quality clay composed primarily of montmorillonite. Used to thicken drilling mud in mud rotary drilling and used to form seals in well construction or abandonment.

BEST AVAILABLE TECHNOLOGY ECONOMICALLY ACHIEVABLE (BAT): A level of technology based on the best existing control and treatment measures that are economically achievable within the given industrial category or subcategory.

BEST MANAGEMENT PRACTICES (BMPs): Schedules of activities, prohibitions of practices, maintenance procedures, and other management practices to prevent or reduce the pollution of waters of the U.S. BMPs also include treatment requirements, operating procedures and practices to control plant site runoff, spillage or leaks, sludge or waste disposal, or drainage from raw material storage.

BEST PRACTICABLE CONTROL TECHNOLOGY CURRENTLY AVAILABLE (BPT): A level of technology represented by the average of the best existing wastewater treatment performance levels within an industrial category or subcategory.

BEST PROFESSIONAL JUDGMENT (BPJ): The method used by a permit writer to develop technology-based limitations on a case-by-case basis using all reasonably available and relevant data.

BIT: The primary cutting edge of a drill string.

BLANK CASING: A section of well casing that is solid.

BLOWDOWN: The discharge of water with high concentrations of accumulated solids from boilers to prevent plugging of the boiler tubes and/or steam lines. In cooling towers, blowdown is discharged to reduce the concentration of dissolved salts in the recirculating cooling water.

BOREHOLE DEVIATION: A boreholes' orientation deviates from vertical while drilling.

BOREHOLE GEOPHYSICS: A surveying technique of utilizing specialized tools to measure various physical parameters of the aquifer, formation, and well.

BOREHOLE: The hole that is formed when drilling into the earth.

BOULDER: An individual rock or solid mass of rock larger than 10 inches in diameter.

BREAK POINT CHLORINATION: The process of chlorinating the water with significant quantities of chlorine to oxidize all contaminants and organic wastes and leave all remaining chlorine as free chlorine.

BRIDGING: The tendency of sediment, filter, or seal media to create an obstruction if installed in too small an annulus or to rapidly. Also can occur within filter packs requiring development.

BROMINE: Chemical disinfectant (HALOGEN) that kills bacteria and algae. This chemical disinfectant has been used only on a very limited scale for water treatment because of its handling difficulties. This chemical causes skin burns on contact, and a residual is difficult to obtain.

BUCKET AUGER: A single cylindrical type of auger flight consisting of offset cutting blades at the bottom. A bucket auger rig rotates the bucket and its blades cut into the earth and fill the bucket with cuttings, which are dumped on the surface as needed.

BUFFER: Chemical that resists pH change, e.g. sodium bicarbonate

BUTTON BIT: A bit that is constructed with raised (typically carbide) buttons that strengthen the bit and aid in crushing and grinding efficiency. A button bit may be of a roller, hammer, or percussion type.

CABLE TOOL: (Also called Percussion Drilling) A method of drilling which utilizes the consecutive lifting and dropping of a heavy drill string via a system of cables.

CALCIUM HARDNESS: A measure of the calcium salts dissolved in water.

Ca: The chemical symbol for calcium.

CADMIUM: A contaminant that is usually not found naturally in water or in very small amounts.

CALCIUM HARDNESS: A measure of the calcium salts dissolved in water.

CALCIUM ION: Is divalent because it has a valence of +2.

CALCIUM, MAGNESIUM AND IRON: The three elements that cause hardness in water.

$CaOCl_2.4H_2O$: The molecular formula of Calcium hypochlorite.

CAPILLARY ACTION: The occurrence of an upward movement of fluid into previously unsaturated soil due to adhesion and surface tension which develops between the fluid and soil particles.

CAPILLARY FRINGE: The uppermost portion of an aquifer where the vadose zone ends. The capillary action of soils permits moisture to extend upwards into the vadose zone within the capillary fringe.

CARBON DIOXIDE GAS: The pH will decrease and alkalinity will change as measured by the Langelier index after pumping carbon dioxide gas into water.

CARBONATE HARDNESS: Carbonate hardness is the measure of Calcium and Magnesium and other hard ions associated with carbonate (CO_32^-) and bicarbonate (HCO_3^-) ions contained in a solution, usually water. It is usually expressed either as parts per million (ppm or mg/L), or in degrees (KH - from the German "Karbonathärte"). One German degree of carbonate hardness is equivalent to about 17.8575 mg/L. Both measurements (mg/L or KH) are usually expressed "as $CaCO_3$" – meaning the amount of hardness expressed as if calcium carbonate was the sole source of hardness. Every bicarbonate ion only counts for half as much carbonate hardness as a carbonate ion does. If a solution contained 1 liter of water and 50 mg $NaHCO_3$ (baking soda), it would have a carbonate hardness of about 18 mg/L as $CaCO_3$. If you had a liter of water containing 50 mg of Na_2CO_3, it would have a carbonate hardness of about 29 mg/L as $CaCO_3$.

CARBONATE, BICARBONATE AND HYDROXIDE: Chemicals that are responsible for the alkalinity of water.

CARBONATE ROCK: Rock that is composed primarily of calcium carbonate.

CASING DRIVER: A percussion or hammering device used to force casing into the subsurface.

CASING: A column of specially designed pipe of metal or plastic material installed in wells in order to keep a borehole open to permit serviceability of and/or construction and completion of a well within it.

CATHEAD: A specially designed auxiliary reel that normally utilizes heavy rope rather than steel cable. Often used on cable tool or percussion drilling rigs for the operation of drive blocks.

CATHODIC PROTECTION: An operator should protect against corrosion of the anode and/or the cathode by painting the copper cathode. Cathodic protection interrupts corrosion by supplying an electrical current to overcome the corrosion-producing mechanism. Guards against stray current corrosion.

CAUSTIC: NaOH (also called Sodium Hydroxide) is a strong chemical used in the treatment process to neutralize acidity, increase alkalinity or raise the pH value.

CAUSTIC SODA: Also known as sodium hydroxide and is used to raise pH.

CAVERN: Large open spaces (>5ft.) encountered while drilling. More often associated with limestone formations in a karst environment.

CEILING AREA: The specific gravity of ammonia gas is 0.60. If released, this gas will accumulate first at the ceiling area. Cl_2 gas will settle on the floor.

CEMENT GROUT: Cement of fine consistency, capable of being pumped. Used to seal in and around wells.

CENTRALIZER: Stand offs attached to well casing and screen to maintain annular space. In drilling, it has the same meaning as stabilizer or drill collar.

CENTRIFUGAL FORCE: That force when a ball is whirled on a string that pulls the ball outward. On a centrifugal pump, it is that force which throws water from a spinning impeller.

CENTRIFUGAL PUMP: A pump consisting of an impeller fixed on a rotating shaft and enclosed in a casing, having an inlet and a discharge connection. The rotating impeller creates pressure in the liquid by the velocity derived from centrifugal force.

CESIUM (also Caesium)**:** Symbol Cs- A soft, silvery-white ductile metal, liquid at room temperature, the most electropositive and alkaline of the elements, used in photoelectric cells and to catalyze hydrogenation of some organic compounds.

CHAIN OF CUSTODY (COC): A record of each person involved in the possession of a sample from the person who collects the sample to the person who analyzes the sample in the laboratory.

CHAIN OF CUSTODY (COC): A record of each person involved in the possession of a sample from the person who collects the sample to the person who analyzes the sample in the laboratory.

CHECK VALVE: Allows water to flow in only one direction.

CHELATION: A chemical process used to control scale formation in which a chelating agent "captures" scale-causing ions and holds them in solution.

CHEMICAL FEED RATE: Chemicals are added to the water in order to improve the subsequent treatment processes. These may include pH adjusters and coagulants. Coagulants are chemicals, such as alum, that neutralize positive or negative charges on small particles, allowing them to stick together and form larger particles that are more easily removed by sedimentation (settling) or filtration. A variety of devices, such as baffles, static mixers, impellers and in-line sprays, can be used to mix the water and distribute the chemicals evenly.

CHEMICAL OXIDIZER: KMnO4 or Potassium Permanganate is used for taste and odor control because it is a strong oxidizer which eliminates many organic compounds.

CHEMICAL REATION RATE: In general, when the temperature decreases, the chemical reaction rate also decreases. The opposite is true for when the temperature increases.

CHEMISORPTION: (or chemical adsorption) Is adsorption in which the forces involved are valence forces of the same kind as those operating in the formation of chemical compounds.

CHLORAMINATION: Treating drinking water by applying chlorine before or after ammonia. This creates a persistent disinfectant residual called chloramines.

CHLORAMINES: A group of chlorine ammonia compounds formed when chlorine combines with organic wastes in the water. Chloramines are not effective as disinfectants and are responsible for eye and skin irritation as well as strong chlorine odors.

CHLORINATION: The process in water treatment of adding chlorine (gas or solid hypochlorite) for purposes of disinfection.

CHLORINE: A chemical used to disinfect water. Chlorine is extremely reactive, and when it comes in contact with microorganisms in water it kills them. Chlorine is added to swimming pools to keep the water safe for swimming. Chlorine is available as solid tablets for swimming pools. Some public water system's drinking water treatment plants use chlorine in a gas form because of the large volumes required. Chlorine is very effective against algae, bacteria and viruses. Protozoa are resistant to chlorine because they have thick coats; protozoa are removed from drinking water by filtration.

CHLORINE DEMAND: Amount of chlorine required to react on various water impurities before a residual is obtained. Also, means the amount of chlorine required to produce a free chlorine residual of 0.1 mg/l after a contact time of fifteen minutes as measured by iodmetic method of a sample at a temperature of twenty degrees in conformance with Standard methods.

CHLORINE FEED: Chlorine may be delivered by vacuum-controlled solution feed chlorinators. The chlorine gas is controlled, metered, introduced into a stream of injector water and then conducted as a solution to the point of application.

CHLORINE, FREE: Chlorine available to kill bacteria or algae. The amount of chlorine available for sanitization after the chlorine demand has been met. Also known as chlorine residual.

CIRCULATION: The continual flow of drilling fluid from injection to recovery and recirculation at the surface.

CLEAR WELL: A large underground storage facility sometimes made of concrete. A clear well or a plant storage reservoir is usually filled when demand is low. The final step in the conventional filtration process, the clearwell provides temporary storage for the treated water. The two main purposes for this storage are to have filtered water available for backwashing the filter and to provide detention time (or contact time) for the chlorine (or other disinfectant) to kill any microorganisms that may remain in the water.

ClO_2: The molecular formula of Chlorine dioxide.

COAGULATION: The best pH range for coagulation is between 5 and 7. Mixing is an important part of the coagulation process you want to complete the coagulation process as quickly as possible.

COBBLES: A rock smaller than a boulder but larger than a pebble. A cobble is greater than 2.5 inches in diameter and smaller than 10 inches in diameter.

COLIFORM: Bacteria normally found in the intestines of warm-blooded animals. Coliform bacteria are present in high numbers in animal feces. They are an indicator of potential contamination of water. Adequate and appropriate disinfection effectively destroys coliform bacteria. Public water systems are required to deliver safe and reliable drinking water to their customers 24 hours a day, 365 days a year. If the water supply becomes contaminated, consumers can become seriously ill. Fortunately, public water systems take many steps to ensure that the public has safe, reliable drinking water. One of the most important steps is to regularly test the water for coliform bacteria. Coliform bacteria are organisms that are present in the environment and in the feces of all warm-blooded animals and humans. Coliform bacteria will not likely cause illness. However, their presence in drinking water indicates that disease-causing organisms (pathogens) could be in the water system. Most pathogens that can contaminate water supplies come from the feces of humans or animals. Testing drinking water for all possible pathogens is complex, time-consuming, and expensive. It is relatively easy and inexpensive to test for coliform bacteria. If coliform bacteria are found in a water sample, water system operators work to find the source of contamination and restore safe drinking water. There are three different groups of coliform bacteria; each has a different level of risk.

COLIFORM TESTING: The effectiveness of disinfection is usually determined by Coliform bacteria testing. A positive sample is a bad thing and indicates that you have bacteria contamination.

COLLOIDAL SUSPENSIONS: Because both iron and manganese react with dissolved oxygen to form insoluble compounds, they are not found in high concentrations in waters containing dissolved oxygen except as colloidal suspensions of the oxide.

COLORIMETRIC MEASUREMENT: A means of measuring an unknown chemical concentration in water by measuring a sample's color intensity.

COMMUTATOR: A device for reversing the direction of a current. (in a DC motor or generator) a cylindrical ring or disk assembly of conducting members, individually insulated in a supporting structure with an exposed surface for contact with current-collecting brushes and mounted on the armature shaft, for changing the frequency or direction of the current in the armature windings.

CHRONIC: A stimulus that lingers or continues for a relatively long period of time, often one-tenth of the life span or more. Chronic should be considered a relative term depending on the life span of an organism. The measurement of chronic effect can be reduced growth, reduced reproduction, etc., in addition to lethality.

COMBINED CHLORINE: The reaction product of chlorine with ammonia or other pollutants, also known as chloramines.

COMMUNITY WATER SYSTEM: A water system which supplies drinking water to 25 or more of the same people year-round in their residences.

COMPLIANCE CYCLE: A 9-calendar year time-frame during which a public water system is required to monitor. Each compliance cycle consists of 3 compliance periods.

COMPLAINCE PERIOD: A 3-calendar year time-frame within a compliance cycle.

COMPLETION (WELL COMPLETION): Refers to the final construction of the well including the installation of pumping equipment.

COMPOSITE SAMPLE: A water sample that is a combination of a group of samples collected at various intervals during the day.

CONDENSATION: The process that changes water vapor to tiny droplets or ice crystals.

CONE OF DEPRESSION: That portion of the water table or potentiometric surface that experiences drawdown from a pumped well.

CONFINED AQUIFER: An aquifer that is isolated by confining layers on both its top and bottom. Pressures within a confined aquifer are normally greater than atmospheric pressure resulting in a potentiometric head.

CONFINING LAYER: An extensive layer of soil or formation that resists the movement of water from an aquifer below or above it. Confining layers isolate aquifers thereby confining them. May or may not be an aquiclude. (ex – Clay or silt rich layer)

CONSOLIDATED: Soil, sediment, or formation that is solidified or cemented together as a unit.

CONTACT TIME, pH and LOW TURBIDITY: Factors which are important in providing good disinfection using chlorine.

CONTACT TIME: If the water temperature decreases from 70°F (21°C) to 40°F (4°C). The operator needs to increase the detention time to maintain good disinfection of the water.

CONTAINS THE ELEMENT CARBON: A simple definition of an organic compound.

CONTAMINANT: Any natural or man-made physical, chemical, biological, or radiological substance or matter in water, which is at a level that may have an adverse effect on public health, and which is known or anticipated to occur in public water systems.

CONTAMINATE: tr.v. con·tam·i·nated, con·tam·i·nat·ing, con·tam·i·nates
1. To make impure or unclean by contact or mixture.
2. To expose to or permeate with radioactivity.

CONTAMINATION: A degradation in the quality of groundwater in result of the it's becoming polluted with unnatural or previously non-existent constituents.

CONTINUOUS SLOT SCREEN: A wire wrapped or plastic slotted screen in which the slot openings completely encircle the inner ribs of the screen.

CONTROL TASTE AND ODOR PROBLEMS: $KMnO_4$ Potassium permanganate is a strong oxidizer commonly used to control taste and odor problems.

CONVENTIONAL: A standard or common procedure to a group of more complex methods. (ex – Direct Rotary *conventional* vs. Reverse *non-conventional*)

COPPER: The chemical name for the symbol Cu.

CORROSION: The removal of metal from copper, other metal surfaces and concrete surfaces in a destructive manner. Corrosion is caused by improperly balanced water or excessive water velocity through piping or heat exchangers.

CORROSIVITY: The Langelier Index measures corrosivity.

COUPON: A coupon placed to measure corrosion damage in the water mains.

CROSS-CONNECTION: A physical connection between a public water system and any source of water or other substance that may lead to contamination of the water provided by the public water system through backflow. Might be the source of an organic substance causing taste and odor problems in a water distribution system.

CROSS-CONTAMINATION: The mixing of two unlike qualities of water. For example, the mixing of good water with a polluting substance like a chemical.

CUTTING HEAD (CUTTER HEAD): The bit portion of auger flighting that serves as the primary cutting edge of the auger.

CUTTING SHOE: A hardened steel sleeve with a wedged or armored cutting edge that is installed on well casing that is to be driven into the earth.

CUTTINGS: Crushed rock, soil, or formation material generated by the drilling action of a bit.

CRYPTOSPORIDIUM: A disease-causing parasite, resistant to chlorine disinfection. It may be found in fecal matter or contaminated drinking water. Cryptosporidium is a protozoan pathogen of the Phylum Apicomplexa and causes a diarrheal illness called cryptosporidiosis. Other apicomplexan pathogens include the malaria parasite Plasmodium, and Toxoplasma, the causative agent of toxoplasmosis. Unlike Plasmodium, which transmits via a mosquito vector, Cryptosporidium does not utilize an insect vector and is capable of completing its life cycle within a single host, resulting in cyst stages which are excreted in feces and are capable of transmission to a new host.

CYANURIC ACID: White, crystalline, water-soluble solid, $C_3H_3O_3N_3 \cdot 2H_2O$, used chiefly in organic synthesis. Chemical used to prevent the decomposition of chlorine by ultraviolet (UV) light.

CYANOBACTERIA: Cyanobacteria, also known as blue-green algae, blue-green bacteria or Cyanophyta, is a phylum of bacteria that obtain their energy through photosynthesis. The name "cyanobacteria" comes from the color of the bacteria (Greek: kyanós = blue). They are a significant component of the marine nitrogen cycle and an important primary producer in many areas of the ocean, but are also found on land.

DAILY MAXIMUM LIMITATIONS: The maximum allowable discharge of pollutants during a 24 hour period. Where daily maximum limitations are expressed in units of mass, the daily discharge is the total mass discharged over the course of the day. Where daily maximum limitations are expressed in terms of a concentration, the daily discharge is the arithmetic average measurement of the pollutant concentration derived from all measurements taken that day.

DANGEROUS CHEMICALS: The most suitable protection when working with a chemical that produces dangerous fumes is to work under an air hood or fume hood.

DARCY'S LAW: (Q=KIA) A fundamental equation used in the groundwater sciences to determine aquifer characteristics, where Q=Flux, K=Hydraulic Conductivity (Permeability), I = Hydraulic Gradient (change in head), and A = Cross Sectional Area of flow.

DECIBELS: The unit of measurement for sound.

DECOMPOSE: To decay or rot.

DECOMPOSTION OF ORGANIC MATERIAL: The decomposition of organic material in water produces taste and odors.

DEMINERALIZATION PROCESS: Mineral concentration of the feed water is the most important consideration in the selection of a demineralization process. Acid feed is the most common method of scale control in a membrane demineralization treatment system.

DENTAL CARIES PREVENTION IN CHILDREN: The main reason that fluoride is added to a water supply.

DEPOLARIZATION: The removal of hydrogen from a cathode.

DESICCANT: When shutting down equipment which may be damaged by moisture, the unit may be protected by sealing it in a tight container. This container should contain a desiccant.

DESORPTION: Desorption is a phenomenon whereby a substance is released from or through a surface. The process is the opposite of sorption (that is, adsorption and absorption). This occurs in a system being in the state of sorption equilibrium between bulk phase (fluid, i.e. gas or liquid solution) and an adsorbing surface (solid or boundary separating two fluids). When the concentration (or pressure) of substance in the bulk phase is lowered, some of the sorbed substance changes to the bulk state. In chemistry, especially chromatography, desorption is the ability for a chemical to move with the mobile phase. The more a chemical desorbs, the less likely it will adsorb, thus instead of sticking to the stationary phase, the chemical moves up with the solvent front. In chemical separation processes, stripping is also referred to as desorption as one component of a liquid stream moves by mass transfer into a vapor phase through the liquid-vapor interface.

DEVELOPMENT: The cleaning of the well and bore once construction is complete.

DETENTION LAG: Is the period of time between the moment of change in a chlorinator control system and the moment when the change is sensed by the chlorine residual indicator.

DETENTION LAG TIME: The minimum detention time range recommended for flocculation is 5 – 20 minutes for direct filtration and up to 30 minutes for conventional filtration.

DIATOMACEOUS EARTH: A fine silica material containing the skeletal remains of algae.

DIRECT CURRENT: A source of direct current (**DC**) may be used for standby lighting in a water treatment facility. The electrical current used in a DC system may come from a battery.

DIRECT ROTARY: The conventional method of rotary drilling involving the rotation of a drill string and standard use of drilling fluid to penetrate the earth.

DISCHARGE HEAD: See Total Dynamic Head.

DISINFECT: The application of a chemical to kill most, but not all, microorganisms that may be present. Chlorine is added to public water drinking systems drinking water for disinfection. Depending on your state rule, drinking water must contain a minimum of 0.2 mg/L free chlorine. Disinfection makes drinking water safe to consume from the standpoint of killing pathogenic microorganisms including bacteria and viruses. Disinfection does not remove all bacteria from drinking water, but the bacteria that can survive disinfection with chlorine are not pathogenic bacteria that can cause disease in normal healthy humans.

DISINFECTION: The treatment of water to inactivate, destroy, and/or remove pathogenic bacteria, viruses, protozoa, and other parasites.

DISINFECTION BY-PRODUCTS (DBPs): The products created due to the reaction of chlorine with organic materials (e.g. leaves, soil) present in raw water during the water treatment process. The EPA has determined that these DBPs can cause cancer. Chlorine is added to drinking water to kill or inactivate harmful organisms that cause various diseases. This process is called disinfection. However, chlorine is a very active substance and it reacts with naturally occurring substances to form compounds known as disinfection byproducts (DBPs). The most common DBPs formed when chlorine is used are trihalomethanes (THMs), and haloacetic acids (HAAs).

DISSOLVED OXYGEN: Can be added to zones within a lake or reservoir that would normally become anaerobic during periods of thermal stratification.

DISSOLUTION : The chemical and physical process of dissolving rock. Typically, limestone or carbonate rocks can be dissolved via the percolation or movement of groundwater that, in its infancy, is slightly acidic. As time goes on, the rock may also be physically worn away by the rapid movement of groundwater through the interconnected open spaces created by the initial chemical dissolving process.

DISTILLATION, REVERSE OSMOSIS AND FREEZING: Processes that can be used to remove minerals from the water.

DRAG BIT: A style of drill bit used in rotary drilling when soil or formation conditions are loosely consolidated and are comprised of fine-grained sediments.

DRAWDOWN: The change in water level from static to pumping level.

DRILL COLLAR: A section of the drill string that provides sufficient mass and diameter to maintain vertical borehole alignment and consistent borehole diameter.

DRILL FOAM: Surfactant used in air rotary drilling and well development.

DRILL PIPE: Sections of the drill string that are connected one to another in order to achieve a desired length while also providing a pathway for the circulation of drilling fluid.

DRILL STEM: The complete drill string or, in cable drilling, the equivalent of a drill collar.

DRILL STRING: The complete drilling assembly in rotary drilling including drill pipe, subs, collars, and bit.

DRILLER: A specially trained individual that operates the drilling rig.

DRILLING FLUID: Fluid circulated through the borehole in rotary drilling methods used to lift cuttings to the surface, provide borehole stability, and cool the bit. Drilling Fluid may consist of mud, water, air, foam, or other additives.

DRILLING PERMIT: A certificate of approval to drill and construct a well often required by the state or local regulating authority.

DRILLING PRESSURE: The pressure exerted within the borehole during drilling. The pressure required to circulate drilling fluid to the surface.

DRIVE BLOCK: A heavy collar that attaches over the drill pipe and is dropped successively to advance casing into the earth. Used primarily in cable tool or percussion drilling methods.

DRIVE CLAMP: A fitting that is attached to the top of a drill string or stem serving as a striking surface for driving casing into the earth.

DRIVE UNIT: The portion of a rotary rig that provides the rotation to the drill string. (ex – top drive or table drive unit). Also may be called the drive head.

DRIVING: The installation of a well or casing via forcing of it into the earth by repeated striking.

DRY ACID: A granular chemical used to lower pH and or total alkalinity.

E. COLI, Escherichia coli: A bacterium commonly found in the human intestine. For water quality analyses purposes, it is considered an indicator organism. These are considered evidence of water contamination. Indicator organisms may be accompanied by pathogens, but do not necessarily cause disease themselves.

EFFECTIVENESS OF CHLORINE: The factors which influence the effectiveness of chlorination the most are pH, turbidity and temperature. Effectiveness of Chlorine decreases occurs during disinfection in source water with excessive turbidity.

ELECTRON: The name of a negatively charged atomic particle.

ELEMENTARY BUSINESS PLAN: Technical Capacity, Managerial Capacity, and Financial Capacity make up the elementary business plan. To become a new public water system, an owner shall file an elementary business plan for review and approval by state environmental agency.

EMERGENCY RESPONSE TEAM: A local team that is thoroughly trained and equipped to deal with emergencies, e.g. chlorine gas leak. In case of a chlorine gas leak, get out of the area and notify your local emergency response team in case of a large uncontrolled chlorine leak.

ENHANCED COAGULATION: The process of joining together particles in water to help remove organic matter.

ENTAMOEBA HISTOLYTICA: Entamoeba histolytica, another water-borne pathogen, can cause diarrhea or a more serious invasive liver abscess. When in contact with human cells, these amoebae are cytotoxic. There is a rapid influx of calcium into the contacted cell, it quickly stops all membrane movement save for some surface blebbing. Internal organization is disrupted, organelles lyse, and the cell dies. The ameba may eat the dead cell or just absorb nutrients released from the cell.

ENTEROVIRUS: A virus whose presence may indicate contaminated water; a virus that may infect the gastrointestinal tract of humans.

EUGLENA: Euglena are common protists, of the class Euglenoidea of the phylum Euglenophyta. Currently, over 1000 species of Euglena have been described. Marin et al. (2003) revised the genus so and including several species without chloroplasts, formerly classified as Astasia and Khawkinea. Euglena sometimes can be considered to have both plant and animal features. Euglena gracilis has a long hair-like thing that stretches from its body. You need a very powerful microscope to see it. This is called a flagellum, and the euglena uses it to swim. It also has a red eyespot. Euglena gracilis uses its eyespot to locate light. Without light, it cannot use its chloroplasts to make itself food.

EVOLUTION: Any process of formation or growth; development: the evolution of a language; the evolution of the airplane. A product of such development; something evolved: The exploration of space is the evolution of decades of research.

Biology. Change in the gene pool of a population from generation to generation by such processes as mutation, natural selection, and genetic drift. A process of gradual, peaceful, progressive change or development, as in social or economic structure or institutions, a motion incomplete in itself, but combining with coordinated motions to produce a single action, as in a machine. A pattern formed by or as if by a series of movements: the evolutions of a figure skater.

An evolving or giving off of gas, heat, etc. **evolutional**, adjective ev·o·lu·tion·al·ly, adverb Synonyms 1. unfolding, change, progression, metamorphosis. Antonyms 1. stasis, inactivity, changelessness.

F: The chemical symbol of Fluorine.

FAUCET WITH AN AERATOR: When collecting a water sample from a distribution system, a faucet with an aerator should not be used as a sample location.

FAULT: A break in the earth's crust where movement has occurred.

FAULTING: A geological process involving the breaking and displacement of rock or formation through movements within the earth's crust along a fault.

FECAL COLIFORM: A group of bacteria that may indicate the presence of human or animal fecal matter in water. Total coliform, fecal coliform, and E. coli are all indicators of drinking water quality. The total coliform group is a large collection of different kinds of bacteria. Fecal coliforms are types of total coliform that mostly exist in feces. E. coli is a sub-group of fecal coliform. When a water sample is sent to a lab, it is tested for total coliform. If total coliform is present, the sample will also be tested for either fecal coliform or E. coli, depending on the lab testing method.

FILTRATION: The process of passing water through materials with very small holes to strain out particles. Most conventional water treatment plants used filters composed of gravel, sand, and anthracite. These materials settle into a compact mass that forms very small holes. Particles are filtered out as treated water passes through these holes. These holes are small enough to remove microorganisms including algae, bacteria, and protozoans, but not viruses. Viruses are eliminated from drinking water through the process of disinfection using chlorine. A series of processes that physically removes particles from water. A water treatment step used to remove turbidity, dissolved organics, odor, taste and color.

FILTER CLOGGING: An inability to meet demand may occur when filters are clogging.

FILTRATION METHODS: The conventional type of water treatment filtration method includes coagulation, flocculation, sedimentation, and filtration. Direct filtration method is similar to conventional except that the sedimentation step is omitted. Slow sand filtration process does not require pretreatment, has a flow of 0.1 gallons per minute per square foot of filter surface area, and is simple to operate and maintain. The Diatomaceous earth method uses a thin layer of fine siliceous material on a porous plate. This type of filtration medium is only used for water with low turbidity. Sedimentation, adsorption, and biological action treatment methods are filtration processes that involve a number of interrelated removal mechanisms. Demineralization is primarily used to remove total dissolved solids from industrial wastewater, municipal water, and seawater.

FINISHED WATER: Treated drinking water that meets minimum state and federal drinking water regulations.

FLIGHTING: The spiral flanged drill pipe used in auger drilling.

FLOATING SUB: A collapsible section of drill pipe shorter than primary drill pipe. Used to provide a cushion between the drive unit and the drill string.

FLOCCULATION: The process of bringing together destabilized or coagulated particles to form larger masses that can be settled and/or filtered out of the water being treated. Conventional coagulation–flocculation-sedimentation practices are essential pretreatments for many water purification systems—especially filtration treatments. These processes agglomerate suspended solids together into larger bodies so that physical filtration processes can more easily remove them. Particulate removal by these methods makes later filtering processes far more effective. The process is often followed by gravity separation (sedimentation or flotation) and is always followed by filtration. A chemical coagulant, such as iron salts, aluminum salts, or polymers, is added to source water to facilitate bonding among particulates. Coagulants work by creating a chemical reaction and eliminating the negative charges that cause particles to repel each other. The coagulant-source water mixture is then slowly stirred in a process known as flocculation. This water churning induces particles to collide and clump together into larger and more easily removable clots, or “flocs.” The process requires chemical knowledge of source water characteristics to ensure that an effective coagulant mix is employed. Improper coagulants make these treatment methods ineffective. The ultimate effectiveness of coagulation/flocculation is also determined by the efficiency of the filtering process with which it is paired.

FLOCCULANTS: Flocculants, or flocculating agents, are chemicals that promote flocculation by causing colloids and other suspended particles in liquids to aggregate, forming a floc. Flocculants are used in water treatment processes to improve the sedimentation or filterability of small particles. For example, a flocculant may be used in swimming pool or drinking water filtration to aid removal of microscopic particles which would otherwise cause the water to be cloudy and which would be difficult or impossible to remove by filtration alone. Many flocculants are multivalent cations such as aluminum, iron, calcium or magnesium. These positively charged molecules interact with negatively charged particles and molecules to reduce the barriers to aggregation. In addition, many of these chemicals, under appropriate pH and other conditions such as temperature and salinity, react with water to form

insoluble hydroxides which, upon precipitating, link together to form long chains or meshes, physically trapping small particles into the larger floc.

Long-chain polymer flocculants, such as modified polyacrylamides, are manufactured and sold by the flocculant producing business. These can be supplied in dry or liquid form for use in the flocculation process. The most common liquid polyacrylamide is supplied as an emulsion with 10-40 % actives and the rest is a carrier fluid, surfactants and latex. Emulsion polymers require activation to invert the emulsion and allow the electrolyte groups to be exposed.

FLOC SHEARING: Likely to happen to large floc particles when they reach the flocculation process.

FLOCCULATION BASIN: A compartmentalized basin with a reduction of speed in each compartment. This set-up or basin will give the best overall results.

FLOOD RIM: The point of an object where the water would run over the edge of something and begin to cause a flood.

FLOW MUST BE MEASURED: A recorder that measures flow is most likely to be located in a central location.

FLUORIDE: High levels of fluoride may stain the teeth of humans. This is called Mottling. This chemical must not be overfed due to a possible exposure to a high concentration of the chemical. The most important safety considerations to know about fluoride chemicals are that all fluoride chemicals are extremely corrosive**.** These are the substances most commonly used to furnish fluoride ions to water: Sodium fluoride, Sodium silicofluoride and Hydrofluosilicic acid.

FLUORIDE FEEDING: Always review fluoride feeding system designs and specifications to determine whether locations for monitoring readouts and dosage controls are convenient to the operation center and easy to read and correct.

FLUX: The term flux describes the rate of water flow through a semipermeable membrane. When the water flux decreases through a semipermeable membrane, it means that the mineral concentration of the water is increasing.

FORMATION: A series of layers, deposits, or bodies of rock, which are geologically similar and related in depositional environment or origin. A formation can be clearly distinguished relative to bounding deposits or formations due to its particular characteristics and composition.

FORMATION OF TUBERCLES: This condition is of the most concern regarding corrosive water effects on a water system. It is the creation of mounds of rust inside the water lines.

FRACTURE: A discrete break in a rock or formation.

FRACTURED AQUIFER: An aquifer within and otherwise massive block that has been made permeable due to the concentrated presence of fractures typically resultant of faulting or concentrated joints.

FREE CHLORINE: In disinfection, chlorine is used in the form of free chlorine or as hypochlorite ion.

FREE CHLORINE RESIDUAL: Regardless of whether pre-chloration is practiced or not, a free chlorine residual of at least 10 mg/L should be maintained in the clear well or distribution reservoir immediately downstream from the point of post-chlorination. The reason for chlorinating past the breakpoint is to provide protection in case of backflow.

GATE VALVE: The most common type of valve used in isolating a small or medium sized section of a distribution system and is the only linear valve used in water distribution. All the other valves are in the rotary classification.

GIARDIA LAMLIA: Giardia lamblia (synonymous with Lamblia intestinalis and Giardia duodenalis) is a flagellated protozoan parasite that colonizes and reproduces in the small intestine, causing giardiasis. The giardia parasite attaches to the epithelium by a ventral adhesive disc, and reproduces via binary fission. Giardiasis does not spread via the bloodstream, nor does it spread to other parts of the gastro-intestinal tract, but remains confined to the lumen of the small intestine. Giardia trophozoites absorb their nutrients from the lumen of the small intestine, and are anaerobes.

GIARDIASAS, HEPATITIS OR TYHOID: Diseases that may be transmitted through the contamination of a water supply but not AIDS.

GIS – GRAPHIC INFORMATION SYSTEM: Detailed information about the physical locations of structures such as pipes, valves, and manholes within geographic areas with the use of satellites.

GEOTECHNICAL: Characteristics of soil, rock, or formation such as grain size, shear strength, porosity, and compressibility, etc. Of particular concern to a geologist or engineer relative to soil or aquifer characteristics.

GLOBE VAVLVE: The main difference between a globe valve and a gate valve is that a globe valve is designed as a controlling device.

GOOD CONTACT TIME, pH and LOW TURBIDITY: These are factors that are important in providing good disinfection when using chlorine.

GPM: Gallons per minute.

GRAB SAMPLE: A sample which is taken from a water or wastestream on a one-time basis with no regard to the flow of the water or wastestream and without consideration of time. A single grab sample should be taken over a period of time not to exceed 15 minutes.

GRAINSIZE: The dimension of particle classifications such as gravel, sand, silt, and clay. Often based on the unified soil classification system.

GROUNDWATER: Water that percolates through and exists within saturated portions of the earth's crust and is replenished by the hydrologic cycle.

GROUT: A type of cement that is normally fine grained and used to effectively construct well seals and used in well abandonment. Grout may also be used to stabilize otherwise unstable boreholes, permitting continued drilling.

GT: Represents (Detention time) x (mixing intensity) in flocculation.

H_2SO_4: The molecular formula of Sulfuric acid.

HALIDES: A halide is a binary compound, of which one part is a halogen atom and the other part is an element or radical that is less electronegative than the halogen, to make a fluoride, chloride, bromide, iodide, or astatide compound. Many salts are halides. All Group 1 metals form halides with the halogens and they are white solids. A halide ion is a halogen atom bearing a negative charge. The halide anions are fluoride (F), chloride (Cl), bromide (Br), iodide (I) and astatide (At). Such ions are present in all ionic halide salts.

HALL EFFECT: Refers to the potential difference (Hall voltage) on the opposite sides of an electrical conductor through which an electric current is flowing, created by a magnetic field applied perpendicular to the current. Edwin Hall discovered this effect in 1879.

HALOACETIC ACIDS: Haloacetic acids are carboxylic acids in which a halogen atom takes the place of a hydrogen atom in acetic acid. Thus, in a monohaloacetic acid, a single halogen would replace a hydrogen atom. For example, chloroacetic acid would have the structural formula CH_2ClCO_2H. In the same manner, in dichloroacetic acid two chlorine atoms would take the place of two hydrogen atoms ($CHCl_2CO_2H$).

HAMMER BIT: The bit driven by the hammer to cut into rock or formation.

HAMMER: See Air Hammer

HARD ROCK: Consolidated formation or solid rock.

HARD WATER: Hard water causes a buildup of scale in household hot water heaters. Hard water is a type of water that has high mineral content (in contrast with soft water). Hard water primarily consists of calcium (Ca2+), and magnesium (Mg2+) metal cations, and sometimes other dissolved compounds such as bicarbonates and sulfates. Calcium usually enters the water as either calcium carbonate ($CaCO_3$), in the form of limestone and chalk, or calcium sulfate ($CaSO_4$), in the form of other mineral deposits. The predominant source of magnesium is dolomite ($CaMg(CO_3)2$). Hard water is generally not harmful. The simplest way to determine the hardness of water is the lather/froth test: soap or toothpaste, when agitated, lathers easily in soft water but not in hard water. More exact measurements of hardness can be obtained through a wet titration. The total water 'hardness' (including both Ca2+ and Mg2+ ions) is read as parts per million or weight/volume (mg/L) of calcium carbonate (CaCO3) in the water. Although water hardness usually only measures the total concentrations of calcium and magnesium (the two most prevalent, divalent metal ions), iron, aluminum, and manganese may also be present at elevated levels in some geographical locations.

HARDNESS: A measure of the amount of calcium and magnesium salts in water. More calcium and magnesium lead to greater hardness. The term "hardness" comes from the fact that it is hard to get soap suds from soap or detergents in hard water. This happens because calcium and magnesium react strongly with negatively-charged chemicals like soap to form insoluble compounds.

HARTSHORN: The antler of a hart, formerly used as a source of ammonia. Ammonium carbonate.

HAZARDS OF POLYMERS: Slippery and difficult to clean-up are the most common hazards associated with the use of polymers in a water treatment plant.

HEAD: The measure of the pressure of water expressed in feet of height of water. 1 PSI = 2.31 feet of water or 1 foot of head equals about a half a pound of pressure or .433 PSI. There are various types of heads of water depending upon what is being measured. Static (water at rest) and Residual (water at flow conditions).

HEADWORKS: The facility at the "**head**" of the water source where water is first treated and routed into the distribution system.

HEALTH ADVISORY: An EPA document that provides guidance and information on contaminants that can affect human health and that may occur in drinking water, but which the EPA does not currently regulate in drinking water.

HERTZ: The term used to describe the frequency of cycles in an alternating current (AC) circuit.

HETEROTROPHIC PLATE COUNT: A test performed on drinking water to determine the total number of all types of bacteria in the water.

HF: The molecular formula of Hydrofluoric acid.

HIGH TURBIDITY CAUSING INCREASED CHLORINE DEMAND: May occur or be caused by the inadequate disinfection of water.

HOLLOW STEM (AUGER): An auger form of drilling in which the flighting is hollow.

HOLLOW STEM FLIGHT: The hollow spiral flanged drill pipe used on hollow stem auger rigs.

HOMOPOLAR: Of uniform polarity; not separated or changed into ions; not polar in activity. Electricity. unipolar.

HYDRAULIC CONDUCTIVITY: A primary factor in Darcy's Law, the measure of a soil or formations ability to transmit water, measured in gallons per day (gpd) See also Permeability and Darcy's Law.

HYDRIDES: Hydride is the name given to the negative ion of hydrogen, H. Although this ion does not exist except in extraordinary conditions, the term hydride is widely applied to describe compounds of hydrogen with other elements, particularly those of groups 1–16. The variety of compounds formed by hydrogen is vast, arguably greater than that of any other element. Various metal hydrides are currently being studied for use as a means of hydrogen storage in fuel cell-powered electric cars and batteries. They also have important uses in organic chemistry as powerful reducing agents, and many promising uses in hydrogen economy.

HYDROCHLORIC AND HYPOCHLOROUS ACIDS: HCL and HOCL The compounds that are formed in water when chlorine gas is introduced.

HYDROFLUOSILIC ACID: (H_2SiF_6) a clear, fuming corrosive liquid with a pH ranging from 1 to 1.5. Used in water treatment to fluoridate drinking water.

HYDROGEN SULFIDE OR CHLORINE GAS: These chemicals can cause olfactory fatigue.

HYDROLOGIC CYCLE: (Water Cycle) The continual process of precipitation (rain and snowfall), evaporation (primarily from the oceans), peculation (recharge to groundwater), runoff (surface water), and transpiration (plants) constituting the renew ability and recycling of each component.

HYDROPHOBIC: Does not mix readily with water.

HYGROSCOPIC: Absorbing or attracting moisture from the air.

HYPOCHLORITE (OCL-) AND ORGANIC MATERIALS: Heat and possibly fire may occur when hypochlorite is brought into contact with an organic material.

HYPOLIMNION: The layer of water in a thermally stratified lake that lies below the thermocline, is noncirculating, and remains perpetually cold.

IMPELLERS: The semi-open or closed props or blades of a turbine pump that when rotated generate the pumping force.

IMPERVIOUS: Not allowing, or allowing only with great difficulty, the movement of water.

IN SERIES: Several components being connected one to the other without a bypass, requiring each component to work dependent on the one before it.

INFILTRATION: The percolation of fluid into soil or formation. See also percolation.

INFECTIOUS PATHOGENS/MICROBES/GERMS: Are considered disease-producing bacteria, viruses and other microorganisms.

INFLATABLE PACKER: A rubber or fiber bladder device that is inflated to seal against either casing or borehole walls.

INFORMATION COLLECTION RULE: ICR EPA collected data required by the Information Collection Rule (May 14, 1996) to support future regulation of microbial contaminants, disinfectants, and disinfection byproducts. The rule was intended to provide EPA with information on chemical byproducts that form when disinfectants used for microbial control react with chemicals already present in source water (disinfection byproducts (DBPs)); disease-causing microorganisms (pathogens), including Cryptosporidium; and engineering data to control these contaminants.

INITIAL MONITORING YEAR: An initial monitoring year is the calendar year designated by the Department within a compliance period in which a public water system conducts initial monitoring at a point of entry.

INORGANIC CONTAMINANTS: Mineral-based compounds such as metals, nitrates, and asbestos. These contaminants are naturally-occurring in some water, but can also get into water through farming, chemical manufacturing, and other human activities. EPA has set legal limits on 15 inorganic contaminants.

INORGANIC IONS: Present in all waters. Inorganic ions are essential for human health in small quantities, but in larger quantities they can cause unpleasant taste and odor or even illness. Most community water systems will commonly test for the concentrations of seven inorganic ions: nitrate, nitrite, fluoride, phosphate, sulfate, chloride, and bromide. Nitrate and nitrite can cause an illness in infants called methemoglobinemia. Fluoride is actually added to the drinking water in some public water systems to promote dental health. Phosphate, sulfate, chloride, and bromide have little direct effect on health, but high concentrations of inorganic ions can give water a salty or briny taste.

INSOLUBLE COMPOUNDS: Are types of compounds cannot be dissolved. When iron or manganese reacts with dissolved oxygen (DO) insoluble compound are formed.

INTAKE FACILITIES: One of the more important considerations in the construction of intake facilities is the ease of operation and maintenance over the expected lifetime of the facility. Every intake structure must be constructed with consideration for operator safety and for cathodic protection.

ION EXCHANGE: An effective treatment process used to remove iron and manganese in a water supply. The hardness of the source water affects the amount of water an ion exchange softener may treat before the bed requires regeneration.

IRON: Fe The elements iron and manganese are undesirable in water because they cause stains and promote the growth of iron bacteria.

IRON AND MANGANESE: Fe and Mn In water they can usually be detected by observing the color of the inside walls of filters and the filter media. If the raw water is pre-chlorinated, there will be black stains on the walls below the water level and a black coating over the top portion of the sand filter bed. When significant levels of dissolved oxygen are present, iron and manganese exist in an oxidized state and normally precipitate into the reservoir bottom sediments. The presence of iron and manganese in water promote the growth of Iron bacteria. Only when a water sample has been acidified then you can perform the analysis beyond the 48 hour holding time. Iron and Manganese in water may be detected by observing the color of the of the filter media. Maintaining a free chlorine residual and regular flushing of water mains may control the growth of iron bacteria in a water distribution system.

IRON BACTERIA: Perhaps the most troublesome consequence of iron and manganese in the water is they promote the growth of a group of microorganism known as Iron Bacteria.

IRON FOULING: You should look for an orange color on the resin and backwash water when checking an ion exchange unit for iron fouling

JARS (DRILLING JARS): Metal sections of a drill string that when released provide a jarring force or action to aid in removing drill string. Used primarily in cable tool or percussion drilling methods.

JETTING: The process of injecting high velocity streams of water and/or air through a system of nozzles or jets into the well screen and filter pack for well development.

KARST TOPOGRAPHY: The visual presence of karst on the surface.

KARST: The presence of caverns, voids, sink holes as characteristic features of a weathered limestone or other carbonate formation on or beneath the surface.

KELLY: A multi-faceted section of drill pipe driven by a kelly drive (table or top drive).

KILL = C X T: Where other factors are constant, the disinfecting action may be represented by: Kill=C x T. C= Chlorine T= Contact time.

KINETIC ENERGY: The ability of an object to do work by virtue of its motion. The energy terms that are used to describe the operation of a pump are pressure and head.

LACRIMATION: The secretion of tears, esp. in abnormal abundance Also, lachrymation, lachrimation.

LANGELIER INDEX: A measurement of Corrosivity. The water is becoming corrosive in the distribution system causing rusty water if the Langelier index indicates that the pH has decreased from the equilibrium point. Mathematically derived factor obtained from the values of calcium hardness, total alkalinity, and pH at a given temperature. A Langelier index of zero indicates perfect water balance (i.e., neither corroding nor scaling). The Langelier Saturation Index (sometimes Langelier Stability Index) is a calculated number used to predict the calcium carbonate stability of water. It indicates whether the water will precipitate, dissolve, or be in equilibrium with calcium carbonate. Langelier developed a method for predicting the pH at which water is saturated in calcium carbonate (called pHs). The LSI is expressed as the difference between the actual system pH and the saturation pH.
LSI = pH - pHs

LEACHING: A chemical reaction between water and metals that allows for removal of soluble materials.

LEAD AND COPPER: Initial tap water monitoring for lead and copper must be conducted during 2 consecutive 6-month periods.

LIME: Is a chemical that may be added to water to reduce the corrosivity. When an operator adds lime to water, Calcium and magnesium become less soluble.

LIME SODA SOFTENING: In a lime soda softening process, to the pH of the water is raised to 11.0. In a lime softening process, excess lime is frequently added to remove Calcium and Magnesium Bicarbonate. The minimum hardness which can be achieved by the lime-soda ash process is 30 to 40 mg/L as calcium carbonate. The hardness due to noncarbonate hardness is most likely to determine the choice between lime softening and ion exchange to remove hardness.

LIME SOFTENING: Lime softening is primarily used to “soften” water—that is to remove calcium and magnesium mineral salts. But it also removes harmful toxins like radon and arsenic. Though there is no consensus, some studies have even suggested that lime softening is effective at removal of Giardia. Hard water is a common condition responsible for numerous problems. Users often recognize hard water because it prevents their soap from lathering properly. However, it can also cause buildup (“scale”) in hot water heaters, boilers, and hot water pipes. Because of these inconveniences, many treatment facilities use lime softening to soften hard water for consumer use. Before lime softening can be used, managers must determine the softening chemistry required. This is a relatively easy task for groundwater sources, which remain more constant in their composition. Surface waters, however, fluctuate widely in quality and may require frequent changes to the softening chemical mix. In lime softening, lime and sometimes sodium carbonate are added to the water as it enters a combination solids contact clarifier. This raises the pH (i.e., increases alkalinity) and leads to the precipitation of calcium carbonate. Later, the pH of the effluent from the clarifier is reduced again, and the water is then filtered through a granular media filter. The water chemistry requirements of these systems require knowledgeable operators, which may make lime softening an economic challenge for some very small systems.

LINE SHAFT TURBINE: See vertical turbine.

LOGGED (LOGGING): The assessment and documentation of geological and water production data obtained while drilling progresses or following drilling through the use of borehole geophysical logging tools.

L.O.T.O.: Lock Out, Tag Out. If a piece of equipment is locked out, the key to the lock-out device the key should be held by the person who is working on the equipment. The tag is an identification device and the lock is a physical restraint.

M-ENDO BROTH: The coliform group is used as indicators of fecal pollution in water, for assessing the effectiveness of water treatment and disinfection, and for monitoring water quality. m-Endo Broth is used for selectively isolating coliform bacteria from water and other specimens using the membrane filtration technique. m-Endo Broth is prepared according to the formula of Fifield and Schaufus.1 It is recommended by the American Public Health Association in standard total coliform membrane filtration procedure for testing water, wastewater, and foods.2,3 The US EPA specifies using m-Endo Broth in the total coliform methods for testing water using single-step, two-step, and delayed incubation membrane filtration methods.

MAGNESIUM HARDNESS: Measure of the magnesium salts dissolved in water – it is not a factor in water balance.

MAGNETIC STARTER: Is a type of motor starter should be used in an integrated circuit to control flow automatically.

MARBLE AND LANGELIER TESTS: Are used to measure or determine the corrosiveness of a water source.

MAXIMUM CONTAMINANT LEVEL (MCLs): The maximum allowable level of a contaminant that federal or state regulations allow in a public water system. If the MCL is exceeded, the water system must treat the water so that it meets the MCL.

MAXIMUM CONTAMINANT LEVEL GOAL (MCLG): The level of a contaminant at which there would be no risk to human health. This goal is not always economically or technologically feasible, and the goal is not legally enforceable.

MCL for TURBIDITY: Turbidity is undesirable because it causes health hazards. An MCL for turbidity was established by the EPA because turbidity does not allow for proper disinfection.

MEASURE CORROSION DAMAGE: A coupon such as a strip of metal and is placed to measure corrosion damage in the distribution system in a water main.

MECHANICAL SEAL: A mechanical device used to control leakage from the stuffing box of a pump. Usually made of two flat surfaces, one of which rotates on the shaft. The two flat surfaces are of such tolerances as to prevent the passage of water between them. Held in place with spring pressure.

MEDIUM WATER SYSTEM: More than 3,300 persons and 50,000 or fewer persons.

MEGGER: Is a portable instrument used to measure insulation resistance. The megger consists of a hand-driven DC generator and a direct reading ohm meter. Used to test the insulation resistance on a motor.

M-ENDO BROTH: The media shall be brought to the boiling point when preparing M-Endo broth to be used in the membrane filter test for total coliform.

METALIMNION: Thermocline, middle layer of a thermally stratified lake which is characterized by a rapid decrease in temperature in proportion to depth.

METALLOID: Metalloid is a term used in chemistry when classifying the chemical elements. On the basis of their general physical and chemical properties, nearly every element in the periodic table can be termed either a metal or a nonmetal. A few elements with intermediate properties are, however, referred to as metalloids. (In Greek metallon = metal and eidos = sort)

METHANE: Methane is a chemical compound with the molecular formula CH_4. It is the simplest alkane, and the principal component of natural gas. Methane's bond angles are 109.5 degrees. Burning methane in the presence of oxygen produces carbon dioxide and water. The relative abundance of methane and its clean burning process makes it a very attractive fuel. However, because it is a gas at normal temperature and pressure, methane is difficult to transport from its source. In its natural gas form, it is generally transported in bulk by pipeline or LNG carriers; few countries still transport it by truck.

MILLILITER: One one-thousandth of a liter; A liter is a little more than a quart. A milliliter is about two drops from an eye dropper.

Mg/L: Stands for "**milligrams per liter**." A common unit of chemical concentration. It expresses the mass of a chemical that is present in a given volume of water. A milligram (one one-thousandth of a gram) is equivalent to about 18 grains of table salt. A liter is equivalent to about one quart.

MICROBIOLOGICAL: Is a type of analysis in which a composite sample unacceptable.

MICROBE OR MICROBIAL: Any minute, simple, single-celled form of life, especially one that causes disease.

MICROBIAL CONTAMINANTS: Microscopic organisms present in untreated water that can cause waterborne diseases.

MICROORGANISMS: Very small animals and plants that are too small to be seen by the naked eye and must be observed using a microscope. Microorganisms in water include algae, bacteria, viruses, and protozoa. Algae growing in surface waters can cause off-taste and odor by producing the chemicals MIB and geosmin. Certain types of bacteria, viruses, and protozoa can cause disease in humans. Bacteria are the most common microorganisms found in treated drinking water. The great majority of bacteria are not harmful. In fact, humans would not be able to live without the bacteria that inhabit the intestines. However, certain types of bacteria called coliform bacteria can signal the presence of possible drinking water contamination.

MILLILITER: One one-thousandth of a liter. A liter is a little more than a quart. A milliliter is about two drops from an eye dropper.

MOISTURE: If a material is hygroscopic, it must it be protected from water.

MOISTURE AND POTASSIUM PERMANGANATE: The combination of moisture and potassium permanganate produces heat.

MOLECULAR WEIGHT: The molecular mass (abbreviated Mr) of a substance, formerly also called molecular weight and abbreviated as MW, is the mass of one molecule of that substance, relative to the unified atomic mass unit u (equal to 1/12 the mass of one atom of carbon-12). This is distinct from the relative molecular mass of a molecule, which is the ratio of the mass of that molecule to 1/12 of the mass of carbon 12 and is a dimensionless number. Relative molecular mass is abbreviated to Mr.

MOTTLING: High levels of fluoride may stain the teeth of humans.

M.S.D.S.: Material Safety Data Sheet. A safety document must an employer provide to an operator upon request.

MUD BALLS IN FILTER MEDIA: Is a possible result of an ineffective or inadequate filter backwash.

MUD CAKE: A film of mud drilling fluid that builds up on borehole walls adding to borehole stability and limits the groundwater's ability to enter the borehole while drilling.

MUD CAKING: The process of building up the mud cake.

MUD ENGINEER: A specially trained individual who's responsible for maintaining proper drilling fluid densities and viscosity.

MUD PIT: Single or multiple subsurface or surface containment system used for settling cuttings out of drilling fluid and for recirculation of drilling fluid.

MUD PUMP: A specially designed pump that can pass particles of mud and cuttings (drilling fluid) at variable pressures, serving as the primary component in a mud rotary drilling system (similar to a grout or cement pump).

MUD ROTARY: The method of rotary drilling with mud circulation as the drilling fluid.

MURIATIC ACID: An acid used to reduce pH and alkalinity. Also used to remove stain and scale.

MYCOTOXIN: A toxin produced by a fungus.

NaOCl: Is the molecular formula of Sodium hypochlorite.

NaOH: Is the molecular formula of Sodium hydroxide.

NASCENT: Coming into existence; emerging.

NATURAL GRAVEL PACK (NATURALLY PACKED): Refers to a well that has no gravel pack installed but is simply allowed to develop a filter pack composed of the aquifer particles itself. Usually coarse grained and hard rock aquifers are naturally packed.

NH_3: The molecular formula of Ammonia.

NH_4+: The molecular formula of the Ammonium ion.

NITRATES: A dissolved form of nitrogen found in fertilizers and sewage by-products that may leach into groundwater and other water sources. Nitrates may also occur naturally in some waters. Over time, nitrates can accumulate in aquifers and contaminate groundwater.

NITROGEN: Nitrogen is a nonmetal, with an electronegativity of 3.0. It has five electrons in its outer shell and is therefore trivalent in most compounds. The triple bond in molecular nitrogen (N_2) is one of the strongest in nature. The resulting difficulty of converting (N_2) into other compounds, and the ease (and associated high energy release) of converting nitrogen compounds into elemental N_2, have dominated the role of nitrogen in both nature and human economic activities.

NITROGEN AND PHOSPHORUS: Pairs of elements and major plant nutrients that cause algae to grow.

NO_3^-: The molecular formula of the Nitrate ion.

NON-CARBONATE HARDNESS: The portion of the total hardness in excess of the alkalinity.

NON-CARBONATE IONS: Water contains non-carbonate ions if it cannot be softened to a desired level through the use of lime only.

NON-POINT SOURCE POLLUTION: Air pollution may leave contaminants on highway surfaces. This non-point source pollution adversely impacts reservoir water and groundwater quality.

NON-TRANSIENT, NON-COMMUNITY WATER SYSTEM: A water system which supplies water to 25 or more of the same people at least six months per year in places other than their residences. Some examples are schools, factories, office buildings, and hospitals which have their own water systems.

NORMALITY: It is the number of equivalent weights of solute per liter of solution. Normality highlights the chemical nature of salts: in solution, salts dissociate into distinct reactive species (ions such as H^+, Fe_3^+, or Cl^-). Normality accounts for any discrepancy between the concentrations of the various ionic species in a solution. For example, in a salt such as $MgCl_2$, there are two moles of Cl^- for every mole of Mg_2^+, so the concentration of Cl- as well as of Mg_2^+ is said to be 2 N (read: "two normal"). Further examples are given below. A normal is one gram equivalent of a solute per liter of solution. The definition of a gram equivalent varies depending on the type of chemical reaction that is discussed - it can refer to acids, bases, redox species, and ions that will precipitate. It is critical to note that normality measures a single ion which takes part in an overall solute. For example, one could determine the normality of hydroxide or sodium in an aqueous solution of sodium hydroxide, but the normality of sodium hydroxide itself has no meaning. Nevertheless it is often used to describe solutions of acids or bases, in those cases it is implied that the normality refers to the H+ or OH- ion. For example, 2 Normal sulfuric acid (H_2SO_4), means that the normality of H+ ions is 2, or that the molarity of the sulfuric acid is 1. Similarly for 1 Molar H_3PO_4 the normality is 3 as it contains three H+ ions.

NTNCWS: Non-transient non-community water system.

NTU (Nephelometric turbidity unit)**:** A measure of the clarity or cloudiness of water.

O_3: The molecular formula of ozone.

OIL TUBE: A tubular enclosure that houses the line shaft and bearings of a vertical turbine pump. Oil is allowed to pass through the oil tube in order to lubricate the pumps drive shaft and bearings.

OLIGOTROPHIC: A reservoir that is nutrient-poor and contains little plant or animal life. An oligotrophic ecosystem or environment is one that offers little to sustain life. The term is commonly utilized to describe bodies of water or soils with very low nutrient levels. It derives etymologically from the Greek oligo (small, little, few) and trophe (nutrients, food). Oligotrophic environments are of special interest for the alternative energy sources and survival strategies upon which life could rely.

ORGANIC PRESURSORS: Natural or man-made compounds with chemical structures based upon carbon that, upon combination with chlorine, leading to trihalomethane formation.

OSMOSIS: Osmosis is the process by which water moves across a semi permeable membrane from a low concentration solute to a high concentration solute to satisfy the pressure differences caused by the solute.

OVERBURDEN: Normally a thin loosely consolidated or unconsolidated sediment overlying competent formation.

OVER-RANGE PROTECTION DEVICES: Mechanical dampers, snubbers and an air cushion chamber are examples of surging and overrange protection devices.

OXIDE: An oxide is a chemical compound containing at least one oxygen atom as well as at least one other element. Most of the Earth's crust consists of oxides. Oxides result when elements are oxidized by oxygen in air. Combustion of hydrocarbons affords the two principal oxides of carbon, carbon monoxide and carbon dioxide. Even materials that are considered to be pure elements often contain a coating of oxides. For example, aluminum foil has a thin skin of Al_2O_3 that protects the foil from further corrosion.

OXIDIZED:
1. to convert (an element) into an oxide; combine with oxygen.
2. to cover with a coating of oxide or rust.
3. to take away hydrogen, as by the action of oxygen; add oxygen or any nonmetal.
4. to remove electrons from (an atom or molecule), thereby increasing the valence. Compare REDUCE (def. 12).

–verb (used without object)

5. to become oxidized.

OXIDIZING: The process of breaking down organic wastes into simpler elemental forms or by products. Also used to separate combined chlorine and convert it into free chlorine.

OXYGEN DEFICIENT ENVIRONMENT: One of the most dangerous threats to an operator upon entering a manhole.

OZONE: Ozone or trioxygen (O_3) is a triatomic molecule, consisting of three oxygen atoms. It is an allotrope of oxygen that is much less stable than the diatomic O_2. Ground-level ozone is an air pollutant with harmful effects on the respiratory systems of animals. Ozone in the upper atmosphere filters potentially damaging ultraviolet light from reaching the Earth's surface. It is present in low concentrations throughout the Earth's atmosphere. It has many industrial and consumer applications. Ozone, the first allotrope of a chemical element to be recognized by science, was proposed as a distinct chemical compound by Christian Friedrich Schönbein in 1840, who named it after the Greek word for smell (ozein), from the peculiar odor in lightning storms. The formula for ozone, O_3, was not determined until 1865 by Jacques-Louis Soret and confirmed by Schönbein in 1867. Ozone is a powerful oxidizing agent, far better than dioxygen. It is also unstable at high concentrations, decaying to ordinary diatomic oxygen (in about half an hour in atmospheric conditions):

$2\ O_3 = 3\ O_2$

This reaction proceeds more rapidly with increasing temperature and decreasing pressure. Deflagration of ozone can be triggered by a spark, and can occur in ozone concentrations of 10 wt% or higher.

OZONE DOES NOT PROVIDE A RESIDUAL: One of the major drawbacks to using ozone as a disinfectant.

OZONE, CHLORINE DIOXIDE, UV, CHLORAMINES: These chemicals may be used as alternative disinfectants.

PAC: A disadvantage of using PAC is it is very abrasive and requires careful maintenance of equipment. One precaution that should be taken in storing PAC is that bags of carbon should not be stored near bags of HTH. Removes tastes and odors by adsorption only. Powered activated carbon frequently used for taste and odor control because PAC is non-specific and removes a broad range of compounds. Jar tests and threshold odor number testing determines the application rate for powdered activated carbon. Powdered activated carbon, or PAC, commonly used for in a water treatment plant for taste and odor control. Powdered activated carbon may be used with some success in removing the precursors of THMs

PACKING: Material, usually of woven fiber, placed in rings around the shaft of a pump and used to control the leakage from the stuffing box.

PARAMECIUM: Paramecia are a group of unicellular ciliate protozoa formerly known as slipper animalcules from their slipper shape. They are commonly studied as a representative of the ciliate group. Simple cilia cover the body which allows the cell to move with a synchronous motion (like a caterpilla). There is also a deep oral groove containing inconspicuous compound oral cilia (as found in other peniculids) that is used to draw food inside. They generally feed upon bacteria and other small cells. Osmoregulation is carried out by a pair of contractile vacuoles, which actively expel water absorbed by osmosis from their surroundings. Paramecia are widespread in freshwater environments, and are especially common in scums. Paramecia are attracted by acidic conditions. Certain single-celled eukaryotes, such as Paramecium, are examples for exceptions to the universality of the genetic code (translation systems where a few codons differ from the standard ones).

PATHOGENS: Disease-causing pathogens; waterborne pathogens A pathogen may contaminate water and cause waterborne disease.

Pb: The chemical symbol of Lead.

PCE: Perchloroethylene. Known also as perc or tetrachloroethylene, perchloroethylene is a clear, colorless liquid with a distinctive, somewhat ether-like odor. It is non-flammable, having no measurable flashpoint or flammable limits in air. Effective over a wide range of applications, perchloroethylene is supported by closed loop transfer systems, stabilizers and employee exposure monitoring.

PEAK DEMAND: The maximum momentary load placed on a water treatment plant, pumping station or distribution system.

PERCOLATION: The process of fluid penetrating or slowly flowing through soil, rock, or formation. See also infiltration.

PERCUSSION RIG: See Cable Tool.

PERFORATED SCREEN: Well screen that has openings mechanically cut into it.

PERFORMANCE CURVE: A graphical representation of a pumps efficiency relative to gpm and feet of head.

PEPTIDOGLYCAN: A polymer found in the cell walls of prokaryotes that consists of polysaccharide and peptide chains in a strong molecular network. Also called *mucopeptide*, *murein*.

PERMEATE: The term for water which has passed through the membrane of a reverse osmosis unit.

PERMEABILITY: A measure of a soil or formation's capacity to transmit water, typically in volume per time units. Equivalent to Darcy's hydraulic conductivity.

PERMEABLE: Soil or formation of which water can pass through.

pH: A unit of measure which describes the degree of acidity or alkalinity of a solution. The pH scale runs from 0 to 14 with 7 being the mid-point or neutral. A pH of less than 7 is on the acid side of the scale with 0 as the point of greatest acid activity. A pH of more than 7 is on the basic (alkaline) side of the scale with 14 as the point of greatest basic activity. The term pH is derived from "p", the mathematical symbol of the negative logarithm, and "H", the chemical symbol of Hydrogen. The definition of pH is the negative logarithm of the Hydrogen ion activity. $pH=-\log[H^+]$.

pH OF SATURATION: The ideal pH for perfect water balance in relation to a particular total alkalinity level and a particular calcium hardness level, at a particular temperature. The pH where the Langelier Index equals zero.

PHENOLPHTHALEIN/TOTAL ALKALINITY: The relationship between the alkalinity constituent's bicarbonate, carbonate, and hydroxide can be based on the P and T alkalinity measurement.

PHENOL RED: Chemical reagent used for testing pH in the range of 6.8 - 8.4.

PHOSPHATE, NITRATE AND ORGANIC NITROGEN: Nutrients in a domestic water supply reservoir may cause water quality problems if they occur in moderate or large quantities.

PHYSISORPTION: (Or physical adsorption) Is adsorption in which the forces involved are intermolecular forces (van der Waals forces) of the same kind as those responsible for the imperfection of real gases and the condensation of vapors, and which do not involve a significant change in the electronic orbital patterns of the species involved. The term van der Waals adsorption is synonymous with physical adsorption, but its use is not recommended.

PICOCURIE: A unit of radioactivity. "Pico" is a metric prefix that means one one-millionth of one one-millionth. A picocurie is one one-millionth of one one-millionth of a Curie. A Curie is that quantity of any radioactive substance that undergoes 37 billion nuclear disintegrations per second. Thus a picocurie is that quantity of any radioactive substance that undergoes 0.037 nuclear disintegrations per second.

pCi/L: Picocuries per liter A curie is the amount of radiation released by a set amount of a certain compound. A picocurie is one quadrillionth of a curie.

PICOCURIE: A unit of radioactivity. "Pico" is a metric prefix that means one one-millionth of one one-millionth. A picocurie is one one-millionth of one one-millionth of a Curie. A Curie is that quantity of any radioactive substance that undergoes 37 billion nuclear disintegrations per second. Thus a picocurie is that quantity of any radioactive substance that undergoes 0.037 nuclear disintegrations per second.

PIEZOMETRIC SURFACE: See potentiometric surface.

PILOT BIT: A bit used on auger rigs to cut a pilot hole ahead of the cutter head when drilling into more resistant formations.

PIPELINE APPURTENANCE: Pressure reducers, bends, valves, regulators (which are a type of valve), etc.

PITLESS ADAPTER: A fitting installed on a section of column pipe and well casing permitting piping from the well to be installed below grade. (Often requires a special permit for construction)

PLANKTON: The aggregate of passively floating, drifting, or somewhat motile organisms occurring in a body of water, primarily comprising microscopic algae and protozoa.

PLATFORM: The portion of the drilling rig where a driller and crew operate the drill rig.

PLUG: A removable cap installed behind the pilot and cutter bits on hollow stem auger flighting.

PLUNGER: See Surge-block.

POINT OF ENTRY: POE.

POLLUTION: To make something unclean or impure. See Contaminated.

POLYPHOSPHATES: Chemicals that may be added to remove low levels of iron and manganese.

POLYMER: A type of chemical when combined with other types of coagulants aid in binding small suspended particles to larger particles to help in the settling and filtering processes.

PORE SPACE: The interstitial space between sediments and fractures that is capable of storing and transmitting water.

POROSITY: A factor representing a rock, soil, or formations percentage of open space available for the percolation and storage of groundwater.

POST-CHLORINE: Where the water is chlorinated to make sure it holds a residual in the distribution system.

POTABLE: Good water which is safe for drinking or cooking purposes. Non-Potable: A liquid or water that is not approved for drinking.

POTENTIAL ENERGY: The energy that a body has by virtue of its position or state enabling it to do work.

POTENTIOMETRIC SURFACE: An imaginary surface representing the height a column of water will reach at any location within a confined aquifer. The measured surface of a confined aquifer related to the aquifer's pressure head.

PPM: Abbreviation for parts per million.

PRE-CHLORINE: Where the raw water is dosed with a large concentration of chlorine.

PRE-CHLORINATION: The addition of chlorine before the filtration process will help:
> Control algae and slime growth
> Control mud ball formation
> Improve coagulation
> Precipate iron
The addition of chlorine to the water prior to any other plant treatment processes.

PERKINESIS: The aggregation resulting from random thermal motion of fluid molecules.

PRESSURE: Pressure is defined as force per unit area. It is usually more convenient to use pressure rather than force to describe the influences upon fluid behavior. The standard unit for pressure is the Pascal, which is a Newton per square meter. For an object sitting on a surface, the force pressing on the surface is the weight of the object, but in different orientations it might have a different area in contact with the surface and therefore exert a different pressure.

PRESSURE HEAD: The height of a column of water capable of being maintained by pressure. See also Total Head, Total Dynamic Head.

PRESSURE MEASUREMENT: Bourdon tube, Bellows gauge and Diaphragm are commonly used to measure pressure in waterworks systems. A Bellows-type sensor reacts to a change in pressure.

PREVENTION: To take action; stop something before it happens.

PROTON, NEUTRON AND ELECTRON: Are the 3 fundamental particles of an atom.

PRODUCING ZONE: A specific productive interval.

PRODUCTIVE INTERVAL: The portion or portions of an aquifer in which significant water production is obtained within the well.

PROTIST: Any of a group of eukaryotic organisms belonging to the kingdom Protista according to some widely used modern taxonomic systems. The protists include a variety of unicellular, coenocytic, colonial, and multicellular organisms, such as the protozoans, slime molds, brown algae, and red algae. A unicellular protoctist in taxonomic systems in which the protoctists are considered to form a kingdom.

PROTOCTIST: Any of various unicellular eukaryotic organisms and their multicellular, coenocytic, or colonial descendants that belong to the kingdom Protoctista according to some taxonomic systems. The protoctists include the protozoans, slime molds, various algae, and other groups. In many new classification systems, all protoctists are considered to be protists.

PROTOZOA: Microscopic animals that occur as single cells. Some protozoa can cause disease in humans. Protozoa form cysts, which are specialized cells like eggs that are very resistant to chlorine. Cysts can survive the disinfection process, then "hatch" into normal cells that can cause disease. Protozoa must be removed from drinking water by filtration, because they cannot be effectively killed by chlorine.

PUBLIC NOTIFICATION: An advisory that EPA requires a water system to distribute to affected consumers when the system has violated MCLs or other regulations. The notice advises consumers what precautions, if any, they should take to protect their health.

PUBLIC WATER SYSTEM (PWS): Any water system which provides water to at least 25 people for at least 60 days annually. There are more than 170,000 PWSs providing water from wells, rivers and other sources to about 250 million Americans. The others drink water from private wells. There are differing standards for PWSs of different sizes and types.

PUMP SURGING: A process of well development whereby water is pumped nearly to the surface and then is allowed to fall back into the well. The process creates a backwashing action that cleans the well and nearby formation.

PUMPING LIFT: The height to which water must be pumped or lifted to, feet of head.

PWS: 3 types of public water systems. Community water system, non-transient non-community water system, transient non community water system.

RADIOCHEMICALS: (Or radioactive chemicals) Occur in natural waters. Naturally radioactive ores are particularly common in the Southwestern United States, and some streams and wells can have dangerously high levels of radioactivity. Total alpha and beta radioactivity and isotopes of radium and strontium are the major tests performed for radiochemicals. The federal drinking water standard for gross alpha radioactivity is set at 5 picocuries per liter.

RADIUS OF INFLUENCE: The distance away from a pumping well that water levels are affected by a wells cone of depression.

RAWHIDING: See Pump Surging.

RAW TURBIDITY: The turbidity of the water coming to the treatment plant from the raw water source.

RAW WATER: Water that has not been treated in any way; it is generally considered to be unsafe to drink.

REAGENT: A substance used in a chemical reaction to measure, detect, examine, or produce other substances.

REAM: The process of enlarging a borehole.

REAMER BIT: A special bit designed to ream existing boreholes.

RECHARGE: The infiltration component of the hydrologic cycle. Often used in the context of referring to: The infiltration of water back into an aquifer, resulting in the restoration of lost storage and water levels which had been decreased due to pumping and/or natural discharges from the aquifer.

RECIRCULATING SYSTEM: A system of constructed or surface mud pits that settle out cuttings from drilling fluid to be circulated back down the borehole.

RECORDER, FLOW: A flow recorder that measures flow is most likely to be located anywhere in the plant where a flow must be measured and in a central location.

RED WATER AND SLIME: Iron bacteria are undesirable in a water distribution system because of red water and slime complaints.

REDOX POTENTIAL: Reduction potential (also known as redox potential, oxidation / reduction potential or ORP) is the tendency of a chemical species to acquire electrons and thereby be reduced. Each species has its own intrinsic reduction potential; the more positive the potential, the greater the species' affinity for electrons and tendency to be reduced. In aqueous solutions, the reduction potential is the tendency of the solution to either gain or lose electrons when it is subject to change by introduction of a new species. A solution with a higher (more positive) reduction potential than the new species will have a tendency to gain electrons from the new species (i.e. to be reduced by oxidizing the new species) and a solution with a lower (more negative) reduction potential will have a tendency to lose electrons to the new species (i.e. to be oxidized by reducing the new species).

RELAY LOGIC: The name of a popular method of automatically controlling a pump, valve, chemical feeder, and other devices.

RESERVOIR: An impoundment used to store water.

RESIDUAL DISINFECTION PROTECTION: A required level of disinfectant that remains in treated water to ensure disinfection protection and prevent recontamination throughout the distribution system (i.e., pipes).

REVERSE MUD ROTARY: A non-conventional drilling method in which drilling fluid is injected through the borehole annulus downward through the bit and circulated back to the surface through the drill string.

REVERSE OSMOSIS: Forces water through membranes that contain holes so small that even salts cannot pass through. Reverse osmosis removes microorganisms, organic chemicals, and inorganic chemicals, producing very pure water. For some people, drinking highly purified water exclusively can upset the natural balance of salts in the body. Reverse osmosis units require regular maintenance or they can become a health hazard.

RIBBED STABILIZER: A stabilizer or drill collar that has cutting ribs attached to its side. Ribs are normally installed in vertical or spiral arrangements.

ROLLER BIT: A rotary drill bit having rotating cutting heads.

ROTAMETER: The name of transparent tube with a tapered bore containing a ball is often used to measure the rate of flow of a gas or liquid.

ROTARY RIG: A conventional rotary drill rig. Can be either an air or mud rotary rig.

ROTIFER: Rotifers get their name (derived from Greek and meaning "wheel-bearer"; they have also been called wheel animalcules) from the corona, which is composed of several ciliated tufts around the mouth that in motion resemble a wheel. These create a current that sweeps food into the mouth, where it is chewed up by a characteristic pharynx (called the mastax) containing a tiny, calcified, jaw-like structure called the trophi. The cilia also pull the animal, when unattached, through the water. Most free-living forms have pairs of posterior toes to anchor themselves while feeding. Rotifers have bilateral symmetry and a variety of different shapes. There is a well-developed cuticle which may be thick and rigid, giving the animal a box-like shape, or flexible, giving the animal a worm-like shape; such rotifers are respectively called loricate and illoricate.

RUNOFF: Surface water sources such as a river or lake are primarily the result of natural processes of runoff.

SAFE YIELD: A possible consequence when the “safe yield” of a well is exceeded and water continues to be pumped from a well, is land subsidence around the well will occur. Safe yield refers to a long-term balance between the water that is naturally and artificially recharged to an aquifer and the groundwater that is pumped out. When more water is removed than is recharged, the aquifer is described as being out of safe yield. When the water level in the aquifer then drops, we are said to be mining groundwater.

SALTS ARE ABSENT: Is a strange characteristic that is unique to water vapor in the atmosphere.

SAMPLE: The water that is analyzed for the presence of EPA-regulated drinking water contaminants. Depending on the regulation, EPA requires water systems and states to take samples from source water, from water leaving the treatment facility, or from the taps of selected consumers.

SAMPLING LOCATION: A location where soil or cuttings samples may be readily and accurately collected.

SAND, ANTHRACITE AND GARNET: Mixed media filters are composed of these three materials.

SANITARY SURVEY: Persons trained in public health engineering and the epidemiology of waterborne diseases should conduct the sanitary survey. The importance of a detailed sanitary survey of a new water source cannot be overemphasized. An on-site review of the water sources, facilities, equipment, operation, and maintenance of a public water systems for the purpose of evaluating the adequacy of the facilities for producing and distributing safe drinking water. The purpose of a non-regulatory sanitary survey is to identify possible biological and chemical pollutants which might affect a water supply.

SANITIZER: A disinfectant or chemical which disinfects (kills bacteria), kills algae and oxidizes organic matter.

SATURATION INDEX: See Langelier's Index.

SATURATOR: A device which produces a fluoride solution for the fluoride process. Crystal-grade types of sodium fluoride should be fed with a saturator. Overfeeding must be prevented to protect public health when using a fluoridation system.

SATURATED ZONE: Where an unconfined aquifer becomes saturated beneath the capillary fringe.

SCADA: A remote method of monitoring pumps and equipment. 130 degrees F is the maximum temperature that transmitting equipment is able to with stand. If the level controller may be set with too close a tolerance 45 could be the cause of a control system that is frequently turning a pump on and off.

SCALE: Crust of calcium carbonate, the result of unbalanced water. Hard insoluble minerals deposited (usually calcium bicarbonate) which forms on pool and spa surfaces and clog filters, heaters and pumps. Scale is caused by high calcium hardness and/or high pH. The regular use of stain prevention chemicals can prevent scale.

SCHMUTZDECKE: German, "grime or filth cover", sometimes spelt schmutzedecke) is a complex biological layer formed on the surface of a slow sand filter. The schmutzdecke is the layer that provides the effective purification in potable water treatment, the underlying sand providing the support medium for this biological treatment layer. The composition of any particular schmutzdecke varies, but will typically consist of a gelatinous biofilm matrix of bacteria, fungi, protozoa, rotifera and a range of aquatic insect larvae. As a schmutzdecke ages, more algae tend to develop, and larger aquatic organisms may be present including some bryozoa, snails and annelid worms.

SCROLL AND BASKET: The two basic types of centrifuges used in water treatment.

SEAL: For wells: to abandon a well by filling up the well with approved seal material including cementing with grout from a required depth to the land surface.

SECONDARY DRINKING WATER STANDARDS: Non-enforceable federal guidelines regarding cosmetic effects (such as tooth or skin discoloration) or aesthetic effects (such as taste, odor, or color) of drinking water.

SECTIONAL MAP: The name of a map that provides detailed drawings of the distribution system's zones. Sometimes we call these quarter-sections.

SEDIMENTATION BASIN: Where the thickest and greatest concentration of sludge will be found. Twice a year sedimentation tanks should be drained and cleaned if the sludge buildup interferes with the treatment process.

SEDIMENTATION: The process of suspended solid particles settling out (going to the bottom of the vessel) in water.

SEDIMENT: Grains of soil, sand, gravel, or rock deposited by and generated by water movement.

SENSOR: A float and cable system are commonly found instruments that may be used as a sensor to control the level of liquid in a tank or basin.

SESSILE: *Botany*. attached by the base, or without any distinct projecting support, as a leaf issuing directly from the stem. *Zoology*. permanently attached; not freely moving.

SETTLED SOLIDS: Solids that have been removed from the raw water by the coagulation and settling processes.

SHAKER: A device used in mud containment systems that vibrates various sized screens as drilling fluid passes through it, thereby separating cuttings from drilling fluid and providing a good sampling location.

SHOCK: Also known as superchlorination or break point chlorination. Ridding a water of organic waste through oxidization by the addition of significant quantities of a halogen.

SHORT-CIRCUITING: Short Circuiting is a condition that occurs in tanks or basins when some of the water travels faster than the rest of the flowing water. This is usually undesirable since it may result in shorter contact, reaction or settling times in comparison with the presumed detention times.

SHROUD: A baffle or piece of pipe installed over a pump to force water to pass the pumps motor.

SIEVE ANALYSIS: The process of sifting soil or formation samples through a series of screens to determine percentages of particle sizes.

SINGLE PHASE POWER: The type of power used for lighting systems, small motors, appliances, portable power tools and in homes.

SINUSOID: A curve described by the equation y = a sin x, the ordinate being proportional to the sine of the abscissa.

SINUSOIDAL: Mathematics. Of or pertaining to a sinusoid. Having a magnitude that varies as the sine of an independent variable: a sinusoidal current.

SLUDGE BASINS: After cleaning sludge basins and before returning the tanks into service the tanks should be inspected, repaired if necessary, and disinfected.

SLUDGE REDUCTION: Organic polymers are used to reduce the quantity of sludge. If a plant produces a large volume of sludge, the sludge could be dewatered, thickened, or conditioned to decrease the volume of sludge. Turbidity of source water, dosage, and type of coagulant used are the most important factors which determine the amount of sludge produced in a treatment of water.

SLURRY: A mixture of crushed rock and water.

SMALL WATER SYSTEM: 3,300 or fewer persons.

SOC: Synthetic organic chemical. A common way for a synthetic organic chemical such as dioxin to be introduced to a surface water supply is from an industrial discharge, agricultural drainage, or a spill.

SODA ASH: Chemical used to raise pH and total alkalinity (sodium carbonate)

SODIUM BICARBONATE: Commonly used to increase alkalinity of water and stabilize pH.

SODIUM BISULFATE: Chemical used to lower pH and total alkalinity (dry acid).

SODIUM HYDROXIDE: Also known as caustic soda, a by-product chlorine generation and often used to raise pH.

SOIL MOISTURE: A relative consideration of the degree to which a soil is saturated.

SOFTENING WATER: When the water has a low alkalinity it is advantageous to use soda ash instead of caustic soda for softening water.

SOFTENING: The process that removes the ions which cause hardness in water.

SOLAR DRYING BEDS OR LAGOONS: Are shallow, small-volume storage pond where sludge is concentrated and stored for an extended periods.

SOLAR DRYING BEDS, CENTRIFUGES AND FILTER PRESSES: Are procedures used in the dewatering of sludge.

SOLID, LIQUID AND VAPOR: 3 forms of matter.

SOLDER: A fusible alloy used to join metallic parts.

SOLID STEM (AUGER): An auger that is constructed of solid stem drill flights.

SPADNS: The lab reagent called SPADNS solution is used in performing the Fluoride test.

SPECIFIC CAPACITY (Sc): A measure of a well's pumping performance in gallons per minute per foot of drawdown.

SPIDER: A bearing or flange used in vertical turbine pumps to stabilize the drive shaft or shaft tube and seal column joints.

SPIRAL FLANGE: A continuous blade that wraps spirally around auger flighting.

SPIRIT OF HARTSHORN: A colorless, pungent, suffocating, aqueous solution of about 28.5 percent ammonia gas: used chiefly as a detergent, for removing stains and extracting certain vegetable coloring agents, and in the manufacture of ammonium salts.

SPLIT SPOON: A sampling device that is driven into the earth and operated by a wire line for the retrieval of soil or formation samples.

SPLIT FLOW CONTROL SYSTEM: This type of control system is to control the flow to each filter influent which is divided by a weir.

SPRAY BOTTLE OF AMMONIA: An operator should use ammonia to test for a chlorine leak around a valve or pipe. You will see white smoke if there is a leak.

SPRING PRESSURE: Is what maintains contact between the two surfaces of a mechanical seal.

STABILE: Reference to formation, soil, or sediments of sufficient strength to remain in place under its own weight and existing pressures.

STABILIZE: Actions taken to enhance borehole stability or vertical rotational when drilling.

STABILIZER: The portion of a drill string used to stabilize rotation.

STANDPIPE: A water tank that is taller than it is wide. Should not be found in low point.

STERILIZED GLASSWARE: The only type of glassware that should be used in testing for coliform bacteria.

STORAGE TANKS: Three types of water usage that determine the volume of a storage tank are fire suppression storage, equalization storage, and emergency storage. Equalization storage is the volume of water needed to supply the system for periods when demand exceeds supply. Generally, a water storage tank's interior coating (paint) protects the interior about 3-5 years.

S.T.P.: Standard temperature and pressure standard temperature and pressure the temperature of 0°C and pressure of 1 atmosphere, usually taken as the conditions when stating properties of gases.

STRATIFIED: Layered.

STUFFING BOX: That portion of the pump that houses the packing or mechanical seal.

SUB: A small section of drill pipe used to connect larger sections.

SUBMERSIBLE PUMP: A turbine pump that has the motor attached directly to it and therefore is operated while submerged.

SULFATE: Will readily dissolve in water to form an anion. Sulfate is a substance that occurs naturally in drinking water. Health concerns regarding sulfate in drinking water have been raised because of reports that diarrhea may be associated with the ingestion of water containing high levels of sulfate. Of particular concern are groups within the general population that may be at greater risk from the laxative effects of sulfate when they experience an abrupt change from drinking water with low sulfate concentrations to drinking water with high sulfate concentrations.

Sulfate in drinking water currently has a secondary maximum contaminant level (SMCL) of 250 milligrams per liter (mg/L), based on aesthetic effects (i.e., taste and odor). This regulation is not a federally enforceable standard, but is provided as a guideline for States and public water systems. EPA estimates that about 3% of the public drinking water systems in the country may have sulfate levels of 250 mg/L or greater. The Safe Drinking Water Act (SDWA), as amended in 1996, directs the U.S. Environmental Protection Agency (EPA) and the Centers for Disease Control and Prevention (CDC) to jointly conduct a study to establish a reliable dose-response relationship for the adverse human health effects from exposure to sulfate in drinking water, including the health effects that may be experienced by sensitive subpopulations (infants and travelers). SDWA specifies that the study be based on the best available peer-reviewed science and supporting studies, conducted in consultation with interested States, and completed in February 1999.

SULFIDE: The term sulfide refers to several types of chemical compounds containing sulfur in its lowest oxidation number of -2. Formally, "sulfide" is the dianion, S_2^-, which exists in strongly alkaline aqueous solutions formed from H_2S or alkali metal salts such as Li_2S, Na_2S, and K_2S. Sulfide is exceptionally basic and, with a pKa > 14, it does not exist in appreciable concentrations even in highly alkaline water, being undetectable at pH < ~15 (8 M NaOH). Instead, sulfide combines with electrons in hydrogen to form HS, which is variously called hydrogen sulfide ion, hydrosulfide ion, sulfhydryl ion, or bisulfide ion. At still lower pH's (<7), HS- converts to H_2S, hydrogen sulfide. Thus, the exact sulfur species obtained upon dissolving sulfide salts depends on the pH of the final solution. Aqueous solutions of transition metals cations react with sulfide sources (H_2S, NaSH, Na_2S) to precipitate solid sulfides. Such inorganic sulfides typically have very low solubility in water and many are related to minerals. One famous example is the bright yellow species CdS or "cadmium yellow". The black tarnish formed on sterling silver is Ag_2S. Such species are sometimes referred to as salts. In fact, the bonding in transition metal sulfides is highly covalent, which gives rise to their semiconductor properties, which in turn is related to the practical applications of many sulfide materials.

SUPERNATANT: The liquid layer which forms above the sludge in a settling basin.

SURFACE SEAL: The upper portion of a wells construction where surface contaminants are adequately prevented from entering the well, normally consisting of surface casing and neat cement grout.

SURFACE WATER SOURCES: Surface water sources such as a river or lake are primarily the result of Runoff.

SURFACE WATER: Water that is open to the atmosphere and subject to surface runoff; generally, lakes, streams, rivers.

SURFACTANT: Surfactants reduce the surface tension of water by adsorbing at the liquid-gas interface. They also reduce the interfacial tension between oil and water by adsorbing at the liquid-liquid interface. Many surfactants can also assemble in the bulk solution into aggregates. Examples of such aggregates are vesicles and micelles. The concentration at which surfactants begin to form micelles is known as the critical micelle concentration or CMC. When micelles form in water, their tails form a core that can encapsulate an oil droplet, and their (ionic/polar) heads form an outer shell that maintains favorable contact with water. When surfactants assemble in oil, the aggregate is referred to as a reverse micelle. In a reverse micelle, the heads are in the core and the tails maintain favorable contact with oil. Surfactants are also often classified into four primary groups; anionic, cationic, non-ionic, and zwitterionic (dual charge).

SUSCEPTIBILITY WAIVER: A waiver that is granted based upon the results of a vulnerability assessment.

SURGE-BLOCK: A disc shaped device that fits tightly into a well and is moved up and down to agitate the water column in order to develop a well.

SURGING: The process of purging a well rapidly for well development.

SWAB: See Surge-block.

SWING ARM: A large moveable arm on a bucket auger rig that pulls the bucket auger out away from the drilling rig for dumping.

SYNCHRONY: Simultaneous occurrence; synchronism.

TABLE DRIVE: A drilling rig that uses a rotating table within the platform to turn a kelly driven drill string.

TABLE: The back portion of a drill rig where the drill pipe is inserted (or driven if a table drive), adjacent to or within the driller's platform.

TAPPING VALVE: The name of the valve that is specifically designed for connecting a new water main to an existing main that is under pressure.

TARGET DEPTH: The proposed construction depth of a well prior to drilling.

TASTE AND ODORS: The primary purpose to use potassium permanganate in water treatment is to control taste and odors. Anaerobic water undesirable for drinking water purposes because of color and odor problems are more likely to occur under these conditions. Taste and odor problems in the water may happen if sludge and other debris are allowed to accumulate in a water treatment plant.

TCE, *trichloroethylene*: A solvent and degreaser used for many purposes; for example dry cleaning, it is a common groundwater contaminant. Trichloroethylene is a colorless liquid which is used as a solvent for cleaning metal parts. Drinking or breathing high levels of trichloroethylene may cause nervous system effects, liver and lung damage, abnormal heartbeat, coma, and possibly death. Trichloroethylene has been found in at least 852 of the 1,430 National Priorities List sites identified by the Environmental Protection Agency (EPA).

TDS-TOTAL DISSOLVED SOLIDS: An expression for the combined content of all inorganic and organic substances contained in a liquid which are present in a molecular, ionized or micro-granular (colloidal sol) suspended form. Generally, the operational definition is that the solids (often abbreviated TDS) must be small enough to survive filtration through a sieve size of two micrometers. Total dissolved solids are normally only discussed for freshwater systems, since salinity comprises some of the ions constituting the definition of TDS. The principal application of TDS is in the study of water quality for streams, rivers and lakes, although TDS is generally considered not as a primary pollutant (e.g. it is not deemed to be associated with health effects), but it is rather used as an indication of aesthetic characteristics of drinking water and as an aggregate indicator of presence of a broad array of chemical contaminants. Ion exchange is an effective treatment process used to remove iron and manganese in a water supply. This process is ideal as long as the water does not contain a large amount of TDS. When determining the total dissolved solids, a sample should be filtered before being poured into an

evaporating dish and dried. Demineralization may be necessary in a treatment process if the water has a very high value Total Dissolved Solids.

TELEMETERING: The use of a transmission line with remote signaling to monitor a pumping station or motors. Can be used to accomplish accurate and reliable remote monitoring and control over a long distribution system.

TEMPERATURE SAMPLE: This test should be performed immediately in the field, this is a grab sample.

TELESCOPING KELLY: A kelly with successively smaller sized pipe within itself that drops out as a borehole is drilled. This permits that drilling may proceed without adding drill pipe. Normally found on bucket auger rigs.

TELESCOPING: The successive decrease in borehole size with depth.

THE RATE DECREASES: In general, when the temperature decreases, the chemical reaction rate decreases also.

THICKENING, CONDITIONING AND DEWATERING: Common processes that are utilized to reduce the volume of sludge.

TIME FOR TURBIDITY BREAKTHROUGH AND MAXIMUM HEADLOSS: Are the two factors which determine whether or not a change in filter media size should be made.

TITRATION: A method of testing by adding a reagent of known strength to a water sample until a specific color change indicates the completion of the reaction.

TITRIMETRIC: Chemistry. Using or obtained by titration. Titrimetrically, adverb.

TOP DRIVE: A rotary type drill head that moves freely up and down the rigs mast while driving the drill string.

TOROID: A surface generated by the revolution of any closed plane curve or contour about an axis lying in its plane. The solid enclosed by such a surface.

TOTAL ALKALINITY: A measure of the acid-neutralizing capacity of water which indicates its buffering ability, i.e. measure of its resistance to a change in pH. Generally, the higher the total alkalinity, the greater the resistance to pH change.

TOTAL COLIFORM: Total coliform, fecal coliform, and E. coli are all indicators of drinking water quality. The total coliform group is a large collection of different kinds of bacteria. Fecal coliforms are types of total coliform that mostly exist in feces. E. coli is a sub-group of fecal coliform. When a water sample is sent to a lab, it is tested for total coliform. If total coliform is present, the sample will also be tested for either fecal coliform or E. coli, depending on the lab testing method.

TOTAL DISSOLVED SOLIDS (TDS): The accumulated total of all solids that might be dissolved in water.

TOTAL DYNAMIC HEAD: The pressure (psi) or equivalent feet of water, required for a pump to lift water to its point of storage overcoming elevation head, friction loss, line pressure, drawdown and pumping lift.

TRANSIENT, NON-COMMUNITY WATER SYSTEM: TNCWS A water system which provides water in a place such as a gas station or campground where people do not remain for long periods of time. These systems do not have to test or treat their water for contaminants which pose long-term health risks because fewer than 25 people drink the water over a long period. They still must test their water for microbes and several chemicals. A Transient Non-community Water System: Is not required to sample for VOC's.

TREATED WATER: Disinfected and/or filtered water served to water system customers. It must meet or surpass all drinking water standards to be considered safe to drink.

TRIHALOMETHANES (THM): Four separate compounds including chloroform, dichlorobromomethane, dibromochloromethane, and bromoform. The most common class of disinfection by-products created when chemical disinfectants react with organic matter in water during the disinfection process. See Disinfectant Byproducts.

TRICONE BIT: A roller bit with three independent rolling bits with teeth or buttons that intermesh for efficient rock crushing and cutting.

TUBE SETTLERS: This modification of the conventional process contains many metal tubes that are placed in the sedimentation basin, or clarifier. These tubes are approximately 1 inch deep and 36 inches long, split-hexagonal shape and installed at an angle of 60 degrees or less. These tubes provide for a very large surface area upon which particles may settle as the water flows upward. The slope of the tubes facilitates gravity settling of the solids to the bottom of the basin, where they can be collected and removed. The large surface settling area also means that adequate clarification can be obtained with detention times of 15 minutes or less. As with conventional treatment, this sedimentation step is followed by filtration through mixed media.

TUBERCLES: The creation of this condition is of the most concern regarding corrosive water effects on a water system. Tubercles are formed due to joining dissimilar metals, causing electro-chemical reactions. Like iron to copper pipe. We have all seen these little rust mounds inside cast iron pipe.

TURBIDIMETER: Monitoring the filter effluent turbidity on a continuous basis with an in-line instrument is a recommended practice. Turbidimeter is best suited to perform this measurement.

TURBIDITY: A measure of the cloudiness of water caused by suspended particles.

TURBINE PUMP: A pump that utilizes rotating impellers on a shaft that generate centrifugal force for pumping water.

UNCONFINED AQUIFER: An aquifer that exists under atmospheric pressure and is not confined.

UNCONSOLIDATED: Sediment that is not cemented or is loosely arranged.

UNDER-REAM: The process of reaming, from within the borehole, a section of an existing smaller borehole area.

UNSATURATED ZONE: That portion of the subsurface, including the capillary fringe that is not saturated but may contain water in both vapor and liquid form. See also Vadose Zone.

UNSTABLE: Sediment or other material that cannot exit without rapidly decomposing or collapsing in on itself. (ex. unconsolidated sediment)

U.S. ENVIRONMENTAL PROTECTION AGENCY: In the United States, this agency responsible for setting drinking water standards and for ensuring their enforcement. This agency sets federal regulations which all state and local agencies must enforce.

UNDER PRESSURE IN STEEL CONTAINERS: After chlorine gas is manufactured, it is primarily transported in steel containers.

UNIT FILTER RUN VOLUME (UFRV): One of the most popular ways to compare filter runs. This technique is the best way to compare water treatment filter runs.

VADOSE ZONE: A portion of the subsurface above the water table that is not saturated but contains water in both vapor and liquid form. The portion of the subsurface where water percolates through to the saturated zone. See also Unsaturated Zone.

VANE: That portion of an impeller that throws the water toward the volute.

VARIABLE DISPLACEMENT PUMP: A pump that will produce different volumes of water dependent on the pressure head against it.

VELOCITY HEAD: The vertical distance a liquid must fall to acquire the velocity with which it flows through the piping system. For a given quantity of flow, the velocity head will vary indirectly as the pipe diameter varies.

VENTURI: If water flows through a pipeline at a high velocity, the pressure in the pipeline is reduced. Velocities can be increased to a point that a partial vacuum is created.

VERTICAL TURBINE: A type of variable displacement pump in which the motor or drive head is mounted on the wellhead and rotates a drive shaft connected to the pump impellers.

VIRION: A complete viral particle, consisting of RNA or DNA surrounded by a protein shell and constituting the infective form of a virus.

VIRUSES: Very small disease-causing microorganisms that are too small to be seen even with microscopes. Viruses cannot multiply or produce disease outside of a living cell.

VITRIFICATION: Vitrification is a process of converting a material into a glass-like amorphous solid that is free from any crystalline structure, either by the quick removal or addition of heat, or by mixing with an additive. Solidification of a vitreous solid occurs at the glass transition temperature (which is lower than melting temperature, Tm, due to supercooling). When the starting material is solid, vitrification usually involves heating the substances to very high temperatures. Many ceramics are produced in such a manner. Vitrification may also occur naturally when lightning strikes sand, where the extreme and immediate heat can create hollow, branching rootlike structures of glass, called fulgurite. When applied to whiteware ceramics, vitreous means the material has an extremely low permeability to liquids, often but not always water, when determined by a specified test regime. The microstructure of whiteware ceramics frequently contain both amorphous and crystalline phases.

VOC WAIVER: The longest term VOC waiver that a public water system using groundwater could receive is 9 years.

VOLATILE ORGANIC COMPOUNDS: (**VOCs**) Solvents used as degreasers or cleaning agents. Improper disposal of VOCs can lead to contamination of natural waters. VOCs tend to evaporate very easily. This characteristic gives VOCs very distinct chemical odors like gasoline, kerosene, lighter fluid, or dry cleaning fluid. Some VOCs are suspected cancer-causing agents. Volatile organic compounds (VOCs) are organic chemical compounds that have high enough vapor pressures under normal conditions to significantly vaporize and enter the atmosphere. A wide range of carbon-based molecules, such as aldehydes, ketones, and other light hydrocarbons are VOCs. The term often is used in a legal or regulatory context and in such cases the precise definition is a matter of law. These definitions can be contradictory and may contain "loopholes"; e.g. exceptions, exemptions, and exclusions. The United States Environmental Protection Agency defines a VOC as any organic compound that participates in a photoreaction; others believe this definition is very broad and vague as organics that are not volatile in the sense that they vaporize under normal conditions can be considered volatile by this EPA definition. The term may refer both to well characterized organic compounds and to mixtures of variable composition.

VOID: An opening, gap, or space within rock or sedimentary formations formed at the time of origin or deposition.

VOLTAGE: Voltage (sometimes also called electric or electrical tension) is the difference of electrical potential between two points of an electrical or electronic circuit, expressed in volts. It measures the potential energy of an electric field to cause an electric current in an electrical conductor. Depending on the difference of electrical potential it is called extra low voltage, low voltage, high voltage or extra high voltage. Specifically Voltage is equal to energy per unit charge.

VOLUTE: The spiral-shaped casing surrounding a pump impeller that collects the liquid discharge by the impeller.

VORTEX: The helical swirling of water moving towards a pump.

VIRUSES: Are very small disease-causing microorganisms that are too small to be seen even with microscopes. Viruses cannot multiply or produce disease outside of a living cell.

VOLATILE ORGANIC COMPOUNDS: (**VOCs**) Solvents used as degreasers or cleaning agents. Improper disposal of VOCs can lead to contamination of natural waters. VOCs tend to evaporate very easily. This characteristic gives VOCs very distinct chemical odors like gasoline, kerosene, lighter fluid, or dry cleaning fluid. Some VOCs are suspected cancer-causing agents.

VULNERABILITY ASSESSMENT: An evaluation of drinking water source quality and its vulnerability to contamination by pathogens and toxic chemicals.

WAIVERS: Monitoring waivers for nitrate and nitrite are prohibited.

WASHOUT: The rapid erosion of aquifer material from the borehole walls while a well is being drilled, which often results in a loss of circulation.

WATER COURSE: An opening within a cable tool drill string that allows fluid to flow in and out of the drill string thereby minimizing friction loss to the slurry.

WATER HAMMER: A surge in a pipeline resulting from the rapid increase or decrease in water flow. Water hammer exerts tremendous force on a system and can be highly destructive.

WATER PURVEYOR: The individuals or organization responsible to help provide, supply, and furnish quality water to a community.

WATER QUALITY: The 4 broad categories of water quality are: Physical, chemical, biological, radiological. Pathogens are disease causing organisms such as bacteria and viruses. A positive bacteriological sample indicates the presence of bacteriological contamination. Source water monitoring for lead and copper be performed when a public water system exceeds an action level for lead of copper.

WATER QUALITY CRITERIA: Comprised of both numeric and narrative criteria. Numeric criteria are scientifically derived ambient concentrations developed by EPA or States for various pollutants of concern to protect human health and aquatic life. Narrative criteria are statements that describe the desired water quality goal.

WATER QUALITY STANDARD: A statute or regulation that consists of the beneficial designated use or uses of a waterbody, the numeric and narrative water quality criteria that are necessary to protect the use or uses of that particular waterbody, and an antidegradation statement.

WATER TABLE: The measured water level surface of an unconfined aquifer.

WATER VAPOR: A characteristic that is unique to water vapor in the atmosphere is that water does not contain any salts.

WATERBORNE DISEASE: A disease, caused by a virus, bacterium, protozoan, or other microorganism, capable of being transmitted by water (e.g., typhoid fever, cholera, amoebic dysentery, gastroenteritis).

WATERSHED: An area that drains all of its water to a particular water course or body of water. The land area from which water drains into a stream, river, or reservoir.

WEATHERED: The existence of rock or formation in a chemically or physically broken down or decomposed state. Weathered material is in an unstable state.

WELL ABANDONMENT: The process of sealing a well by approved means. The filling of a well to the surface with cement grout.

WELL HEAD: The upper portion of the well that is constructed on the land surface, including the well manifold. Also a term used to refer to the area near the well that is subject to wellhead protection.

WELL HEAD PROTECTION: Programs designed to maintain the quality of groundwater used as public drinking water sources, by managing the land uses around the well field. A government program that encourages the limitation and elimination of activities, near and within a wells recharge area, which present a potential risk to the wells water supply.

WELL MANIFOLD: The piping, valves, and metering equipment used to connect the well to the distribution system, installed on the wellhead.

WELL SCREEN: A section of well casing that contains openings which permit water to enter the well but limit or prevent sediment from entering the well while pumping.

WELL SEAL: The watertight cap or seal installed within and between the well casing and pumping equipment. The metal or plastic plug or seal, which the pumping column rests on the top of casing.

WHOLE EFFLUENT TOXICITY: The total toxic effect of an effluent measured directly with a toxicity test.

YIELD: The volume of water measured in flow rates that are produced from the well.

ZONE OF AERATION: See Saturated Zone or Vadose Zone.

ZONE OF SATURATION: See Saturated Zone.



A cross completely blown out. A major job and a great location for bacteria to enter the system.

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Common Water Quality and Sampling Statements

These statements will be more explained in the previous chapters.

1. What are the correct procedures to follow in collecting bacteriological samples?

Use a sterile plastic or glass bottle. Sodium thiosulfate should be added to neutralize the chorine residual. Refrigerate the sample to 4^{o} C. The regulations call for a minimum of five samples for the month from any system that has positive sample results. Small systems that take only one sample per month have to take four (4) repeats when they get a total coliform positive test result. If any system has to take repeat samples, it must also take a minimum of five (5) routine samples the following month. Small systems that normally take less than 5 samples/month will have to increase the number to 5 samples. They can return to normal sampling schedules the following month if no repeats are required.

2. What are the proper sampling techniques for microbiological sampling?

Proper sampling techniques are extremely important in obtaining accurate water quality information. An improperly taken coliform sample may indicate bacteriological contamination of your water when the water is actually safe. You can avoid the cost of additional testing by using good sampling procedures. Carefully follow these steps in taking a sample for bacteriological testing:

1. Select the sampling point. The sampling point must be a faucet from which water is commonly taken for public use.

The sampling point should be a non-swivel faucet.

Remove any aerator or screen and flush.

It should not be a faucet that leaks, permitting water to run over the outside of the faucet. Leaking faucets can promote bacterial growth.

If an outside faucet must be used, disconnect any hoses or other attachments and be sure to flush the line thoroughly.

Do not use fire hydrants as sampling points. Do not dip the bottle in reservoirs, spring boxes or storage tanks in order to collect the sample.

3. What do the following abbreviations stand for and what do they mean: gpm, MGD, TTHM, psi, HAA, NTU, and mg/L.

Gallons per minute- Million Gallons a Day - Total Trihalomethanes – Pounds Per Square Inch –Haloacetic acids - Nephelometric turbidity unit -Milligrams Per Liter

4. What is the relationship between mg/L and ppm; ug/L and ppb?

Milligram per liter: Milligram per liter of substance and part per million are equals amounts in water. While you can easily convert between micrograms/liter and milligrams/liter, and between PPM and PPB, its not so easy to convert between the different types of units such as milligrams/liter to PPM.

To convert micrograms per liter to milligrams per liter, divide by 1000.

To convert to PPM, you would first need to know the density of the substance, and the density of what the substance is in.

5. Ug/L: Represents the concentration of something in water or soil. One ppb represents one microgram of something per liter of water (ug/l), or one microgram of something per kilogram of soil (ug/kg).

Parts per million (ppm) or Milligrams per liter (mg/l) - one part per million corresponds to one minute in two years or a single penny in $10,000.

Parts per billion (ppb) or Micrograms per liter - one part per billion corresponds to one minute in 2,000 years, or a single penny in $10,000,000.

Parts per trillion (ppt) or Nanograms per liter (nanograms/l) - one part per trillion corresponds to one minute in 2,000,000 years, or a single penny in $10,000,000,000.

6. What do the following terms represent in reference to water quality.
Total coliform: The coliform family has been divided into two groups. Results may come back as either total coliform positive (TC positive) or fecal coliform positive, or (FC positive or *E. coli* positive.) Total coliform positive means that no human coliform are present.

7. Fecal Coliform: Fecal coliform positive indicates the presence of *E. coli*, which means there is a greater chance of pathogens being present. The laboratory tests for coliform include the MPN method, the Membrane Filter test, the Colilert test, and the presence-absence test.

8. Presence-absence Test: Presence-Absence Broth is used for the detection of coliform bacteria in water treatment plants or distribution systems using the presence-absence coliform test.

9. Physical Characteristics of Water: A characteristic of water defined by the temperature, turbidity, color, taste, and odor of the water.

10. Point-of-entry sample (POE): A type of water sample taken after treatment and before reaching the first consumer.

11. Acute Health Effect: An immediate (i.e. within hours or days) effect that may result from exposure to certain drinking water contaminants (e.g., pathogens).

12. Non-acute violation: If the MCL is exceeded and none of the positive results indicated a presence of Fecal Coliform, a Tier 2 violation has occurred. This level of violation used to be called a non-acute violation.

13. Routine Sample: Samples collected on a routine basis to monitor for contamination. Collection should be in accordance with an approved sampling plan.

14. Repeat Sample: Short answer… Samples collected following a '***coliform present'*** routine sample. The number of repeat samples to be collected is based on the number of routine samples you normally collect. Long Answer. Anytime a microbiological sample result comes back positive, indicating the presence of total or fecal coliform/ E.coli, repeat samples must be taken. Three repeats are usually required. One must be taken at the site of the positive sample. The two samples must be taken upstream and downstream of the original site (within five service connections). These repeat samples must be taken within 24 hours of notification of positive results. They must be identified as a Repeat Sample on the sample form. Repeat samples may be required to be sealed with a red evidentiary seal tape. The tape must cover the cap and extend down the sides of the bottle. The sample forms must also include the reference number for the positive sample.

There is an important exception to the three repeat samples rule. The regulations also state that when repeats are taken the minimum number of samples is raised to five for the month. A system that collects just one sample a month must collect four repeat samples, when the sample is positive, in order to have five samples as required.

Whenever a system has to take repeat samples, a minimum of five routine samples must also be submitted the following month. This is only an issue for systems that normally turn in four or fewer samples each month. If the five samples are negative the system can return to its normal sampling schedule the next month.

Small systems that have fewer than four sampling sites have a problem complying with the "upstream and downstream" aspects of the repeat sampling requirements. In this case, samples should be taken at as many separate sites as possible and then wait a minimum of 2 hours before resampling enough sites to get the required number of samples. Repeat sample with red seal tape.

15. Treatment technique: An enforceable procedure or level of technical performance which public water systems must follow to ensure control of a contaminant.

16. Action level: The level of lead or copper which, if exceeded, triggers treatment or other requirements that a water system must follow.

17. What does the membrane filter test analyze with regards to bacteriological sampling?

Membrane Filter Technique: A standard test used for measuring coliform numbers (quantity) in water is the membrane filter technique. This technique involves filtering a known volume of water through a special sterile filter. These filters are made of nitrocellulose acetate and polycarbonate, are 150 µm thick, and have 0.45 µm diameter pores. A grid pattern is printed on these filter disks in order to facilitate colony counting. When the water sample is filtered, bacteria (larger than 0.45 µm) in the sample are trapped on the surface of the filter. The filter is then carefully removed, placed in a sterile petri plate on a pad saturated with a liquid medium, and incubated for 20-24 hours at 37°C.

One assumes that each bacterium trapped on the filter will then grow into a separate colony. By counting the colonies one can directly determine the number of bacteria in the water sample that was filtered. The broth medium usually employed in detecting total

coliforms is M-Endo Broth MF. Total coliform colonies will be pink to dark red in color and will appear to have a golden green color.

18. What do the following terms mean in relation to drinking water quality: disinfection, pathogenic, toxic, pH, aesthetic, culinary and potable.
Disinfection: The chemical process of killing or inactivating most microorganisms in water. See also Sterilization.

19. Pathogenic: Organisms or bugs that cause disease. These include bacteria, viruses, cysts and anything capable of causing disease in humans, like cryptosporidiosis, typhoid, cholera and so on. There are other organisms that do not create disease, these are called non-pathogenic organisms.

20. Toxic: Stuff that will kill you. A substance which is poisonous to living organisms.

21. pH: A measure of the acidity of water. The pH scale runs from 0 to 14 with 7 being the mid point or neutral. A pH of less than 7 is on the acid side of the scale with 0 as the point of greatest acid activity. A pH of more than 7 is on the basic (alkaline) side of the scale with 14 as the point of greatest basic activity. For example, the acidity of a sample with a pH of **5** is ten times greater than that of a sample with a pH of **6**. A difference of 2 units, from **6** to **4**, would mean that the acidity is one hundred times greater, and so on. Normal rain has a pH of **5.6** – slightly acidic because of the carbon dioxide picked up in the earth's atmosphere by the rain.

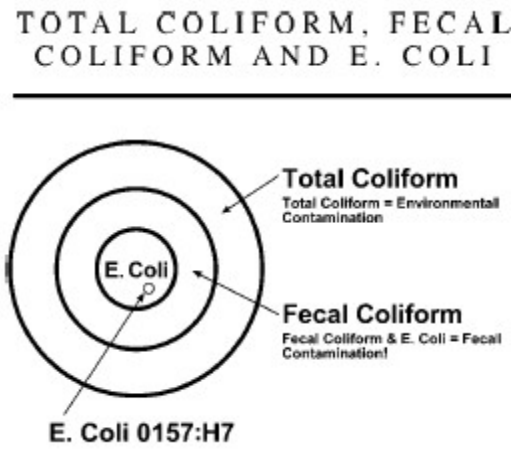
22. Aesthetic: Attractive or appealing water or things in water that will not make you sick but may appear to change the water's color or taste.

23. Culinary: Having to do with cooking food. Potable water is often called culinary water.

24. Potable: Water that is free of objectionable pollution, contamination, or infective agents. Generally speaking, we serve only potable water and not palatable water. Palatable is pleasant tasting water.

25. What is hardness in water and what chemicals cause it?
Hardness: Water that contains high amounts of dissolved minerals, specifically calcium and magnesium. **Ion Exchange:** A method of water softening where hardness causing ions are exchanged with sodium ions; also effective in removing many inorganic contaminants such as nitrates, copper, and lead; and treating aesthetic water problems.

26. What is Escherichia Coliform and what does it indicate in relation to drinking water?
E. coli is a sub-group of the fecal coliform group. Most *E. coli* bacteria are harmless and are found in great quantities in the intestines of people and warm-blooded animals. Some strains, however, can cause illness. The presence of *E. coli* in a drinking water sample almost always indicates recent fecal contamination meaning there is a greater risk that pathogens are present.



Total coliform, fecal coliform, and *E. coli* are all indicators of drinking water quality. The total coliform group is a large collection of different kinds of bacteria. Fecal coliforms are types of total coliform that mostly exist in feces. *E. coli* is a sub-group of fecal coliform. When a water sample is sent to a lab, it is tested for total coliform. If total coliform is present, the sample will also be tested for either fecal coliform or *E. coli*, depending on the lab testing method.

27. What problems are associated with Hydrogen Sulfide in the water?
Hydrogen sulfide is a gas which, when dissolved in water, gives it a “rotten egg” odor. Chlorination will remove this gas from the water but the effectiveness of the chlorine for disinfection is lessened.

28. When Hydrogen sulfide reacts with chlorine, it produces Sulfuric acid and elemental Sulfur: It is therefore recommended that aeration be applied prior to the addition of chlorine for the most effective disinfection.

29. Why is it important to know what the turbidity of the water is when using chlorine?
To be careful not to overdose with chlorine or properly dose with chlorine.

30. What is the log removal for Cryptosporidium?
The LT1ESWTR extends further this necessary protection from Cryptosporidium to communities of fewer than 10,000 persons. Today's rule for the first time establishes Cryptosporidium control requirements for systems serving less than 10,000 persons by requiring a minimum 2-log removal for Cryptosporidium. The rule also strengthens filter performance requirements to ensure 2-log Cryptosporidium removal, establishes individual filter monitoring to minimize poor performance in individual units, includes Cryptosporidium in the definition of GWUDI, and explicitly considers unfiltered system watershed control provisions. The rule also reflects a commitment to the importance of maintaining existing levels of microbial protection in public water systems as plants take steps to comply with newly applicable DBP standards.

31. What is the log removal?

This log-reduction terminology was developed by engineers as a way to express levels of decreased biological contamination in water by factors of 10 that could be easily converted to percent reduction. The most commonly used logarithmic base is 10 because it is compatible with our base-10 decimal system. The log of 10 in the base 10 logarithmic system is 1 and the log of 100 is 2, with the log of 1000 being 3, etc. A 1-log reduction is nine out of 10 and would be equivalent to a 90 percent reduction. A 2-log reduction would be 99 out of 100 or 99 percent reduction and a 3-log reduction would be 999 out of 1000 or 99.9 percent reduction. A 99.99 percent reduction would be called a 4-log reduction.

32. What are the turbidity requirements for Direct and Conventional filtration plants?

For conventional and direct filtration systems (including those systems utilizing in-line filtration), the turbidity level of representative samples of a system's filtered water (measured every four hours) must be less than or equal to ***0.3 NTU*** in at least 95 percent of the measurements taken each month. The turbidity level of representative samples of a system's filtered water must not exceed ***1 NTU*** at any time. Conventional filtration is defined as a series of processes including coagulation, flocculation, sedimentation, and filtration resulting in substantial particulate removal. Direct filtration is defined as a series of processes including coagulation and filtration but excluding sedimentation resulting in substantial particle removal.

33. What are chloramines, how are they formed, and do they have any beneficial use?

Chloramines: Ammonia and Chlorine are combined. Cl_2NH_3 Yes, limited use and this chemical will create less THMS than chlorine alone. Chloramine is a disinfectant used to treat drinking water. It is formed by mixing chlorine with ammonia. Although it is a weaker disinfectant than chlorine, it is more stable and extends disinfectant benefits throughout a water utility's distribution system (a system of pipes water is delivered to homes through). Some water systems use chloramine as a secondary disinfectant to maintain a disinfectant residual throughout the distribution system so that drinking water remains safe as it travels from the treatment facility to the customer. Chloramine has been used by water systems for almost 90 years, and its use is closely regulated. Since chloramine is not as reactive as chlorine, it forms fewer disinfection byproducts. Some disinfection byproducts, such as the trihalomethanes (THMs) and haloacetic acids (HAAs), may have adverse health effects and are closely regulated. Because a chloramine residual is more stable and longer lasting than free chlorine, it provides better protection against bacterial regrowth in systems with large storage tanks and dead-end water mains. Chloramine, like chlorine, is effective in controlling biofilm, which is a coating in the pipe caused by bacteria. Controlling biofilm also tends to reduce coliform bacteria concentrations and biofilm-induced corrosion of pipes.

Simple Math Conversion Factors

1 PSI = 2.31 Feet of Water
1 Foot of Water = .433 PSI
1.13 Feet of Water = 1 Inch of Mercury
454 Grams = 1 Pound
1 Gallon of Water = 8.34 Pounds
1 mg/L = 1 PPM
17.1 mg/L = 1 Grain/Gallon
1% = 10,000 mg/L
694 Gallons per Minute = MGD
1.55 Cubic Feet per Second = 1 MGD
60 Seconds = 1 Minute
1440 Minutes = 1 Day
.746 kW = 1 Horsepower

LENGTH
12 Inches = 1 Foot
3 Feet = 1 Yard
5,280 Feet = 1 Mile

AREA
144 Square Inches = 1 Square Foot
43,560 Square Feet = 1 Acre

VOLUME
1000 Milliliters = 1 Liter
3.785 Liters = 1 Gallon
231 Cubic Inches = 1 Gallon
7.48 Gallons = 1 Cubic Foot
62.38 Pounds = 1 Cubic Foot of Water

Dimensions

SQUARE: Area (sq.ft) = Length X Width
Volume (cu.ft) = Length (ft) X Width (ft) X Height (ft)

CIRCLE: Area (sq.ft) = 3.14 X Radius (ft) X Radius (ft)

CYLINDER: Volume (Cu. ft) = 3.14 X Radius (ft) X Radius (ft) X Depth (ft)

PIPE VOLUME: .785 X Diameter ^{2} X Length = Area X 7.48 for Gallons

SPHERE: $\frac{(3.14)\ (\text{Diameter})^3}{(6)}$

Circumference = 3.14 X Diameter

Flow Rate

Multiply	—>	to get
to get	**<—**	**Divide**
cc/min	1	mL/min
cfm (ft^3/min)	28.31	L/min
cfm (ft^3/min)	1.699	m^3/hr
cfh (ft^3/hr)	472	mL/min
cfh (ft^3/hr)	0.125	GPM
GPH	63.1	mL/min
GPH	0.134	cfh
GPM	0.227	m^3/hr
GPM	3.785	L/min
oz/min	29.57	mL/min

General Conversions

POUNDS PER DAY= Concentration (mg/L) X Flow (MG) X 8.34

PERCENT EFFICIENCY = $\frac{\text{In} - \text{Out}}{\text{In}}$ X 100

TEMPERATURE: $^0F = (^0C \times 9/5) + 32$ 9/5 =1.8
$^0C = (^0F - 32) \times 5/9$ 5/9 = .555

CONCENTRATION: Conc. (A) X Volume (A) = Conc. (B) X Volume (B)

FLOW RATE (Q): Q = A X V (Quantity = Area X Velocity)

FLOW RATE (gpm): Flow Rate (gpm) = $\frac{2.83 (\text{Diameter, in})^2 (\text{Distance, in})}{\text{Height, in}}$

% SLOPE = $\frac{\text{Rise (feet)}}{\text{Run (feet)}} \times 100$

ACTUAL LEAKAGE = $\frac{\text{Leak Rate (GPD)}}{\text{Length (mi.) X Diameter (in)}}$

VELOCITY = $\frac{\text{Distance (ft)}}{\text{Time (Sec)}}$

N = Manning's Coefficient of Roughness
R = Hydraulic Radius (ft.)
S = Slope of Sewer (ft/ft.)

HYDRAULIC RADIUS (ft) = $\frac{\text{Cross Sectional Area of Flow (ft)}}{\text{Wetted pipe Perimeter (ft)}}$

WATER HORSEPOWER = $\frac{\text{Flow (gpm) X Head (ft)}}{3960}$

BRAKE HORSEPOWER = $\frac{\text{Flow (gpm) X Head (ft)}}{3960 \text{ X Pump Efficiency}}$

MOTOR HORSEPOWER = $\frac{\text{Flow (gpm) X Head (ft)}}{3960 \text{ X Pump Eff. X Motor Eff.}}$

MEAN OR AVERAGE = $\frac{\text{Sum of the Values}}{\text{Number of Values}}$

TOTAL HEAD (ft) = Suction Lift (ft) X Discharge Head (ft)

SURFACE LOADING RATE = $\frac{\text{Flow Rate (gpm)}}{\text{Surface Area (sq. ft)}}$
(gal/min/sq.ft)

MIXTURE STRENGTH (%) = $\frac{(\text{Volume 1, gal})(\text{Strength 1, \%}) + (\text{Volume 2, gal})(\text{Strength 2,\%})}{(\text{Volume 1, gal}) + (\text{Volume 2, gal})}$

INJURY FREQUENCY RATE = $\frac{(\text{Number of Injuries}) \ 1{,}000{,}000}{\text{Number of hours worked per year}}$

DETENTION TIME (hrs) = $\frac{\text{Volume of Basin (gals) X 24 hrs}}{\text{Flow (GPD)}}$

FLUORIDE ION PURITY = $\frac{(\text{Molecular weight of Fluoride})(100\%)}{\text{Molecular weight of Chemical}}$
(%)

INJURY FREQUENCY RATE = $\frac{(\text{Number of Injuries}) \ 1{,}000{,}000}{\text{Number of hours worked per year}}$

DETENTION TIME (hrs) = Volume of Basin (gals) X 24 hrs
Flow (GPD)

BY-PASS WATER (gpd) = Total Flow (GPD) X Plant Effluent Hardness (gpg)
Filtered Hardness (gpg)

HARDNESS
HARDNESS (mg/L as $CaCO_3$) = A (mls of titrant) X 1000
Mls of Sample

Ca HARDNESS as mg/L $CaCo_3$ = 2.5 X (Ca, mg/L)

Mg HARDNESS as mg/L $CaCo_3$ = 4.12 (Mg, mg/L)

ALKALINITY TOTAL = Mls of Titrant X Normality X 50,000
(mg/L) Mls of Sample

EXCHANGE CAPACITY (grains) = Resin Volume (cu. ft) X Removal Capacity

HARDNESS TO GRAIN/GALLON = Hardness (mg/L) X gr./gal
17.1 mg/L

LANGELIER INDEX = pH - pH_s

CHEMICAL ADDITION

CHEMICAL FEED RATE = Chemical Feed (ml/min)
(gpm) 3785 ml/gal

CHLORINE DOSE (mg/L) = Chlorine Demand (mg/L) + Chlorine Residual (mg/L)

POLYMER % = Dry Polymer (lbs.)
Dry Polymer (lbs.) + Water (lbs.)

DESIRED PAC = Volume (MG) X Dose (mg/L) X 8.34
(lbs./MG) 1 MG

PAC (lbs./gal) = PAC (mg/L) X 3.785 (1/gallon)
1000 (mg/g) X 454 (g/lb.)

FILTRATION
FILTRATION RATE = Flow Rate (gpm)
(gpm/sq. ft) Surface Area (sq. ft)

BACKWASH PUMPING RATE = Filter Area (sq. ft) X Backwash Rate (gpm/sq. ft)
(gpm)

FILTRATION RATE = Flow Rate (gpm)
(gpm/sq. ft) Filter Area (sq. ft)



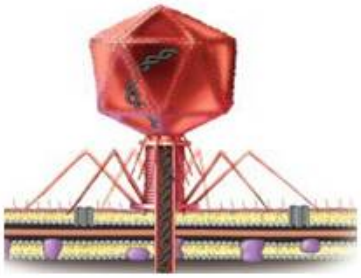
COCCI
(SPHERICAL)

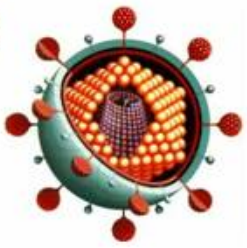


SPIRILLI
(SPIRAL)

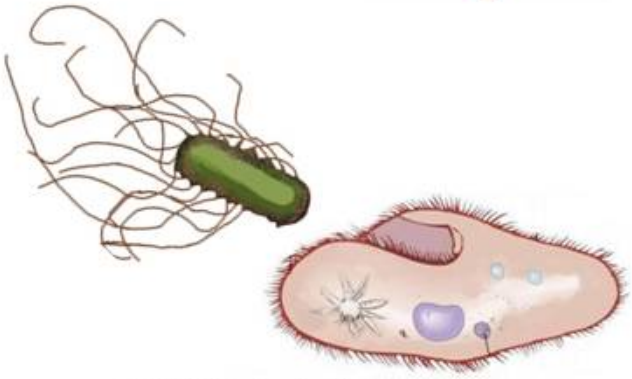


BACILLI
(ROD-SHAPED)

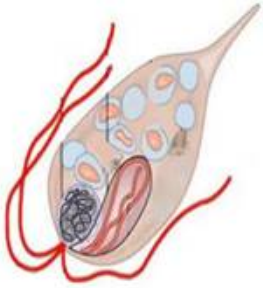




VIRUSES



BACTERIA

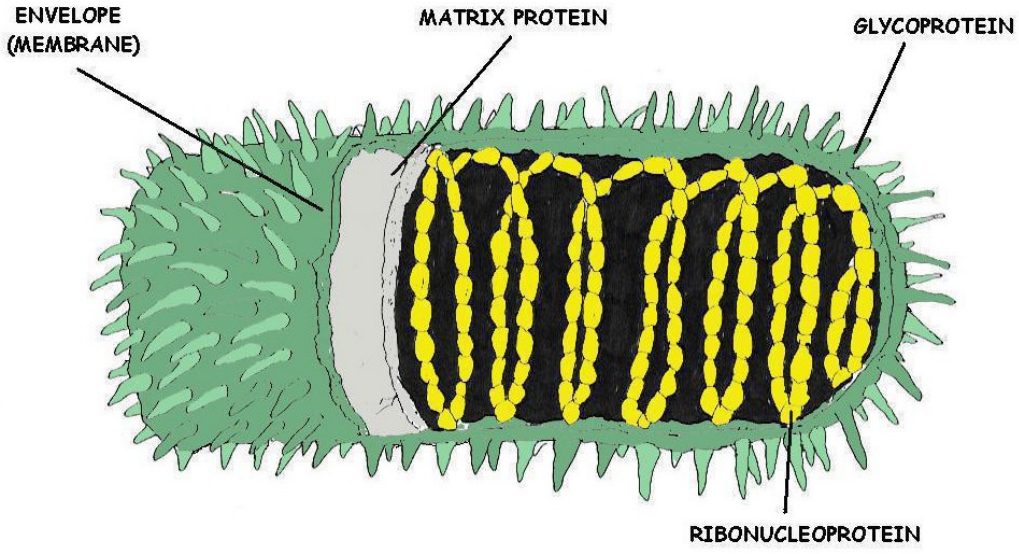


PROTOZOA

BACTERIA TYPES

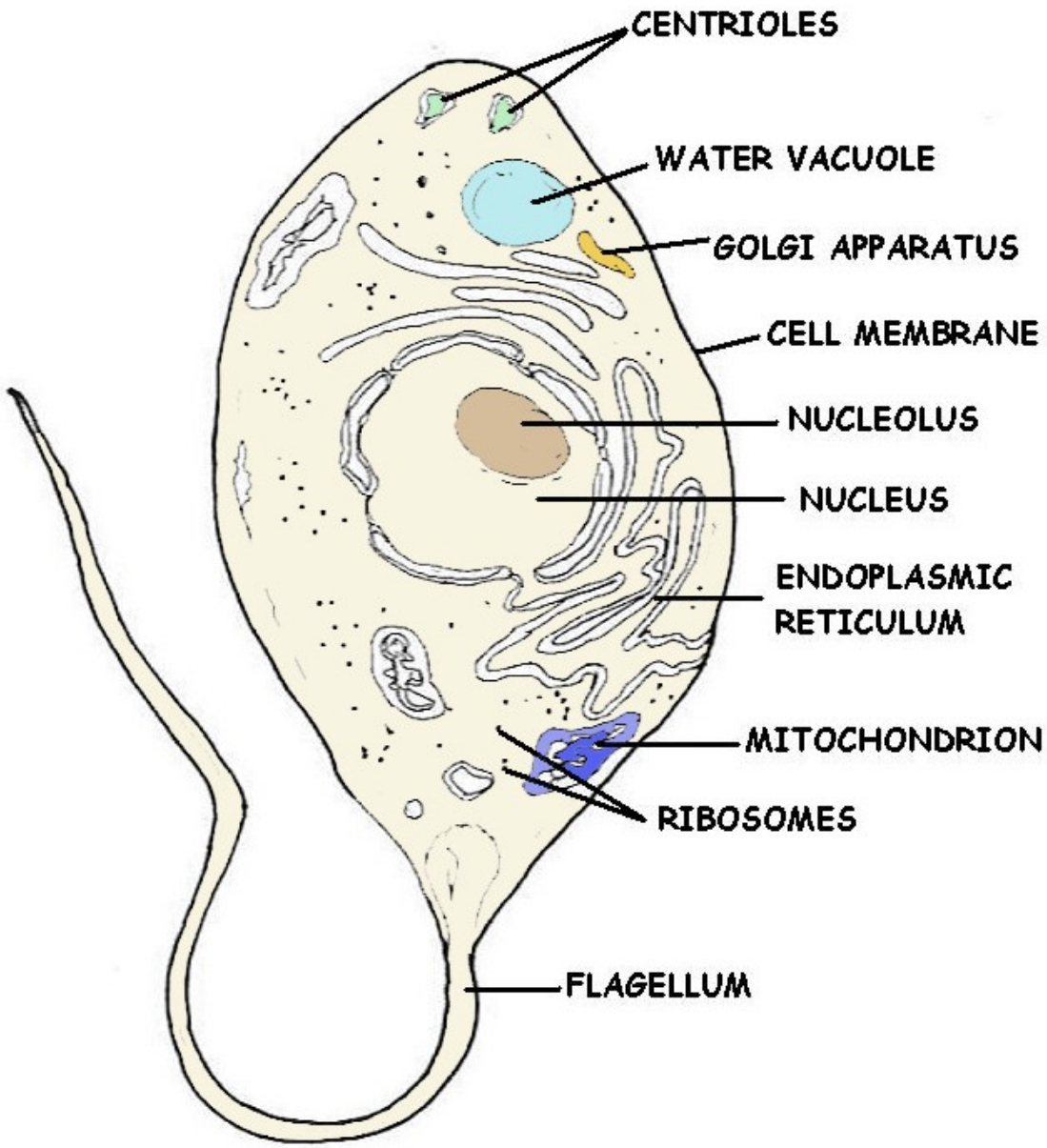


This section will give a close-up and short explanation of the major microorganisms found in water and in wastewater.



VIRUS

Protozoa



PROTOZOAN CELL

Protozoa are around 10–50 micrometer, but can grow up to 1 mm and can easily be seen under a microscope. Protozoa exist throughout aqueous environments and soil. Protozoa occupy a range of trophic levels. As predators, they prey upon unicellular or filamentous algae, bacteria, and microfungi.

Protozoa play a role both as herbivores and as consumers in the decomposer link of the food chain. Protozoa also play a vital role in controlling bacteria populations and biomass. As components of the micro- and meiofauna, protozoa are an important food source for microinvertebrates. Thus, the ecological role of protozoa in the transfer of bacterial and algal production to successive trophic levels is important. Protozoa such as the malaria parasites (Plasmodium spp.), trypanosomes and leishmania are also important as parasites and symbionts of multicellular animals.

Most protozoa exist in 5 stages of life which are in the form of trophozoites and cysts. As cysts, protozoa can survive harsh conditions, such as exposure to extreme temperatures and harmful chemicals, or long periods without access to nutrients, water, or oxygen for a period of time. Being a cyst enables parasitic species to survive outside of the host, and allows their transmission from one host to another. When protozoa are in the form of trophozoites (Greek, tropho=to nourish), they actively feed and grow. The process by which the protozoa takes its cyst form is called encystation, while the process of transforming back into trophozoite is called excystation.

Protozoa can reproduce by binary fission or multiple fission. Some protozoa reproduce sexually, some asexually, and some both (e.g. Coccidia). An individual protozoan is hermaphroditic.

Classification
Protozoa were commonly grouped in the kingdom of Protista together with the plant-like algae and fungus-like water molds and slime molds. In the 21st-century systematics, protozoans, along with ciliates, mastigophorans, and apicomplexans, are arranged as animal-like protists. However, protozoans are neither Animalia nor Metazoa (with the possible exception of the enigmatic, moldy Myxozoa).

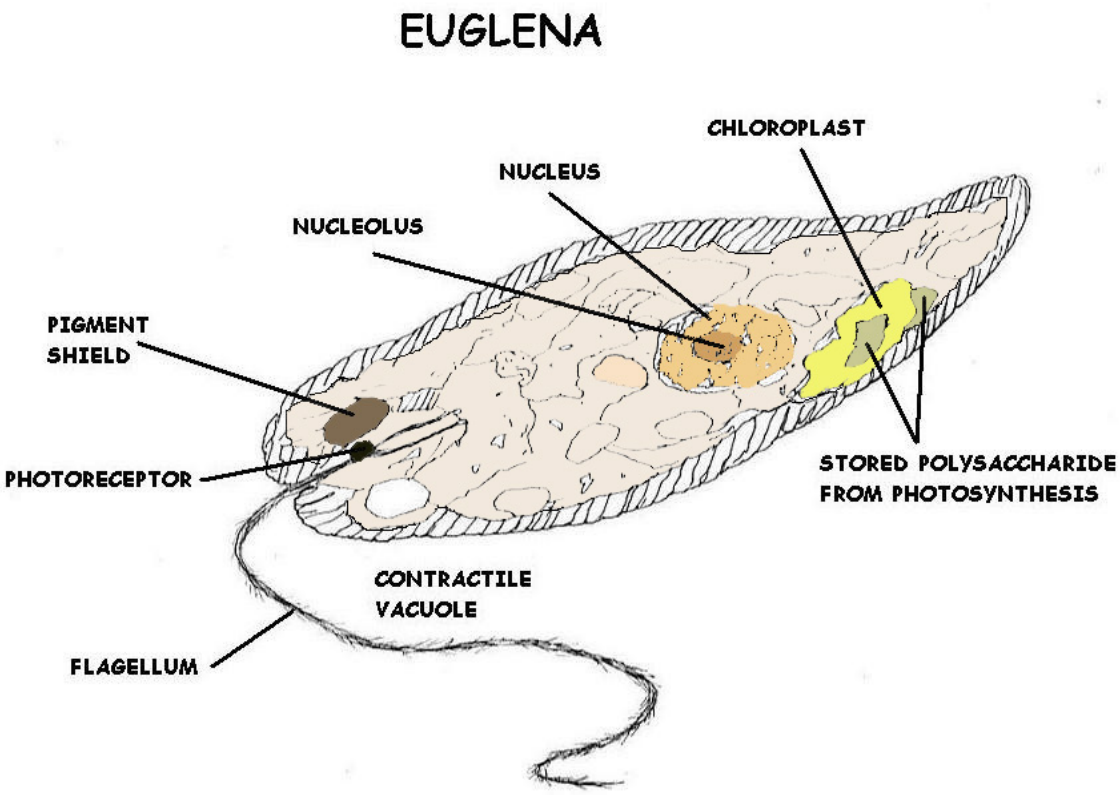
Sub-groups
Protozoa have traditionally been divided on the basis of their means of locomotion, although this is no longer believed to represent genuine relationships:
* Flagellates (e.g. Giardia lambia)
* Amoeboids (e.g. Entamoeba histolytica)
* Sporozoans (e.g. Plasmodium knowlesi)
* Apicomplexa
* Myxozoa
* Microsporidia
* Ciliates (e.g. Balantidium coli)

There are many ways that infectious diseases can spread. Pathogens usually have specific routes by which they are transmitted, and these routes may depend on the type of cells and tissue that a particular agent targets. For example, because cold viruses infect the respiratory tract, they are dispersed into the air via coughing and sneezing.

Once in the air, the viruses can infect another person who is unlucky enough to inhale air containing the virus particles.

Agents vary greatly in their stability in the environment. Some viruses may survive for only a few minutes outside of a host, while some spore-forming bacteria are extremely durable and may survive in a dormant state for a decade or more.

Protozoa Section



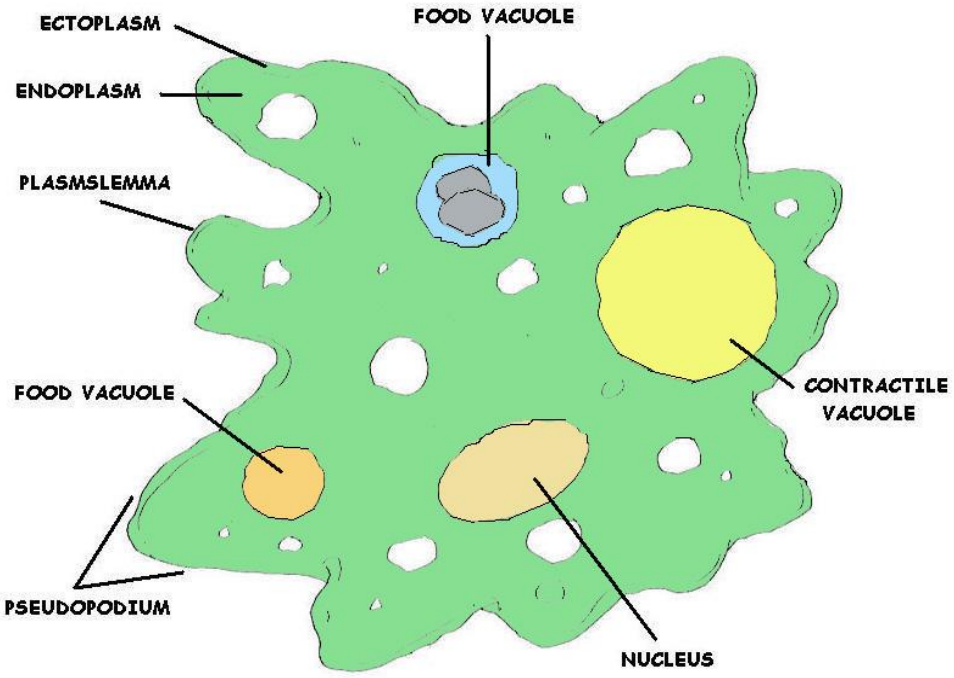
The diverse assemblage of organisms that carry out all of their life functions within the confines of a single, complex eukaryotic cell are called protozoa.

Paramecium, Euglena, and Amoeba are well-known examples of these major groups of organisms. Some protozoa are more closely related to animals, others to plants, and still others are relatively unique. Although it is not appropriate to group them together into a single taxonomic category, the research tools used to study any unicellular organism are usually the same, and the field of protozoology has been created to carry out this research. The unicellular photosynthetic protozoa are sometimes also called algae and are addressed elsewhere. This report considers the status of our knowledge of heterotrophic protozoa (protozoa that cannot produce their own food).

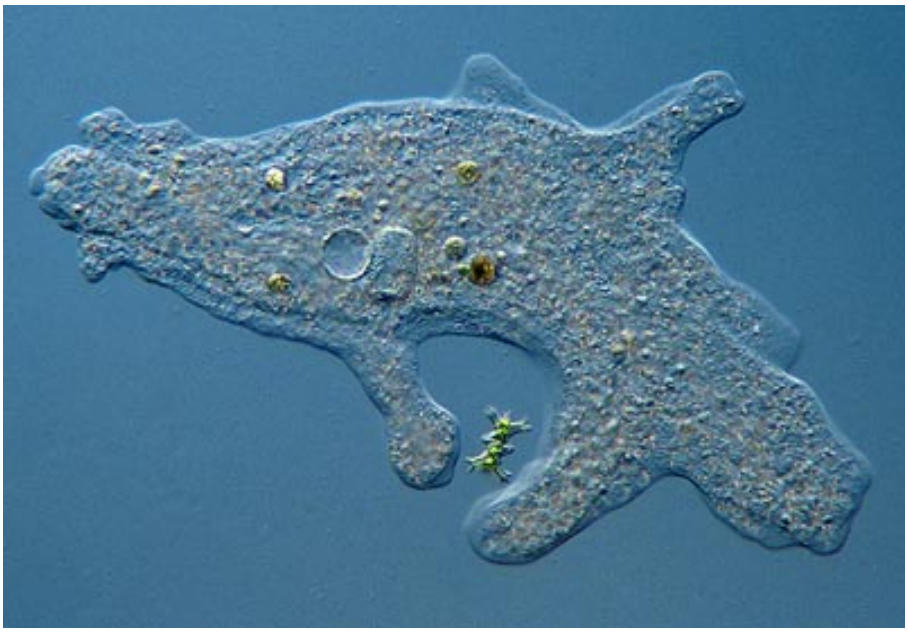
Free-living Protozoa

Protozoans are found in all moist habitats within the United States, but we know little about their specific geographic distribution. Because of their small size, production of resistant cysts, and ease of distribution from one place to another, many species appear to be cosmopolitan and may be collected in similar microhabitats worldwide (Cairns and Ruthven 1972). Other species may have relatively narrow limits to their distribution.

Marine ciliates inhabit interstices of sediment and beach sands, surfaces, deep sea and cold Antarctic environments, planktonic habitats, and the algal mats and detritus of estuaries and wetlands.



AMOEBA



Amoeba proteus*, pseudopods slowly engulf the small desmid *Staurastrum.

Amoebas

Amoebas (Phylum Rhizopoda) are unicellular protists that are able to change their shape constantly. Each species has its own distinct repertoire of shapes.

How does an amoeba locomote?
Amoebas locomote by way of cytoplasmic movement. (cytoplasm is the cell content around the nucleus of the cell) The amoeba forms pseudopods (false feet) with which they 'flow' over a surface. The cytoplasma not only flows, it also changes from a fluid into a solid state.

These pseudopods are also used to capture prey, they simply engulf the food. They can detect the kind of prey and use different 'engulfing tactics'.

The image from the last page shows several cell organelles. Left from the center we can see aspherical water expelling vesicle and just right of it, the single nucleus of this species can be seen. Other species may have many nuclei. The cell is full of brown food vacuoles and also contains small crystals.

Protozoa Information
Our actual knowledge of salinity, temperature, and oxygen requirements of marine protozoa is poor (although some groups, such as the foraminifera, are better studied than others), and even the broadest outlines of their biogeographic ranges are usually a mystery. In general, freshwater protozoan communities are similar to marine communities except the specialized interstitial fauna of the sand is largely missing. In freshwater habitats, the foraminifera and radiolaria common in marine environments are absent or low in numbers while testate amoebae exist in greater numbers. Relative abundance of species in the marine versus freshwater habitat is unknown.

Soil-dwelling protozoa have been documented from almost every type of soil and in every kind of environment, from the peat-rich soil of bogs to the dry sands of deserts. In general, protozoa are found in greatest abundance near the soil surface, especially in the upper 15 cm (6 in), but occasional isolates can be obtained at depths of a meter (yard) or more.

Protozoa do not constitute a major part of soil biomass, but in some highly productive regions such as forest litter, the protozoa are a significant food source for the microinvertebrates, with a biomass that may reach 20 g/m2 of soil surface area there.

Environmental Quality Indicators
Polluted waters often have a rich and characteristic protozoan fauna. The relative abundance and diversity of protozoa are used as indicators of organic and toxic pollution (Cairns et al. 1972; Foissner 1987; Niederlehner et al. 1990; Curds 1992). Bick (1972), for example, provided a guide to ciliates that are useful as indicators of environmental quality of European freshwater systems, along with their ecological distribution with respect to parameters such as amount of organic material and oxygen levels.

Foissner (1988) clarified the taxonomy of European ciliates as part of a system for classifying the state of aquatic habitats according to their faunas.

Symbiotic Protozoa
Parasites
Protozoa are infamous for their role in causing disease, and parasitic species are among the best-known protozoa. Nevertheless, our knowledge has large gaps, especially of normally free-living protozoa that may become pathogenic in immunocompromised individuals. For example, microsporidia comprise a unique group of obligate, intracellular parasitic protozoa. Microsporidia are amazingly diverse organisms with more than 700 species and 80 genera that are capable of infecting a variety of plant, animal, and even other protist hosts.

They are found worldwide and have the ability to thrive in many ecological conditions. Until the past few years, their ubiquity did not cause a threat to human health, and few systematists worked to describe and classify the species. Since 1985, however, physicians have documented an unusual rise in worldwide infections in AIDS patients caused by four different genera of microsporidia (Encephalitozoon, Nosema, Pleistophora, and Enterocytozoon). According to the Centers for Disease Control in the United States, difficulties in identifying microsporidian species are impeding diagnosis and effective treatment of AIDS patients.

Protozoan Reservoirs of Disease
The presence of bacteria in the cytoplasm of protozoa is well known, whereas that of viruses is less frequently reported. Most of these reports simply record the presence of bacteria or viruses and assume some sort of symbiotic relationship between them and the protozoa. Recently, however, certain human pathogens were shown to not only survive but also to multiply in the cytoplasm of free-living, nonpathogenic protozoa. Indeed, it is now believed that protozoa are the natural habitat for certain pathogenic bacteria. To date, the main focus of attention has been on the bacterium Legionella pneumophila, the causative organism of Legionnaires' disease; these bacteria live and reproduce in the cytoplasm of some free-living amoebae (Curds 1992). More on this subject in the following pages.

Symbionts
Some protozoa are harmless or even beneficial symbionts. A bewildering array of ciliates, for example, inhabit the rumen and reticulum of ruminates and the cecum and colon of equids. Little is known about the relationship of the ciliates to their host, but a few may aid the animal in digesting cellulose.

Data on Protozoa
While our knowledge of recent and fossil foraminifera in the U.S. coastal waterways is systematically growing, other free-living protozoa are poorly known. There are some regional guides and, while some are excellent, many are limited in scope, vague on specifics, or difficult to use. Largely because of these problems, most ecologists who include protozoa in their studies of aquatic habitats do not identify them, even if they do count and measure them for biomass estimates (Taylor and Sanders 1991).

Parasitic protozoa of humans, domestic animals, and wildlife are better known although no attempt has been made to compile this information into a single source. Large gaps in our knowledge exist, especially for haemogregarines, microsporidians, and myxosporidians (see Kreier and Baker 1987).

Museum Specimens
For many plant and animal taxa, museums represent a massive information resource. This is not true for protozoa. In the United States, only the National Natural History Museum (Smithsonian Institution) has a reference collection preserved on microscope slides, but it does not have a protozoologist curator and cannot provide species' identification or verification services. The American Type Culture Collection has some protozoa in culture, but its collection includes relatively few kinds of protozoa.

Ecological Role of Protozoa
Although protozoa are frequently overlooked, they play an important role in many communities where they occupy a range of trophic levels. As predators upon unicellular or filamentous algae, bacteria, and microfungi, protozoa play a role both as herbivores and as consumers in the decomposer link of the food chain. As components of the micro- and meiofauna, protozoa are an important food source for microinvertebrates. Thus, the ecological role of protozoa in the transfer of bacterial and algal production to successive trophic levels is important.

Factors Affecting Growth and Distribution
Most free-living protozoa reproduce by cell division (exchange of genetic material is a separate process and is not involved in reproduction in protozoa). The relative importance for population growth of biotic versus chemical-physical components of the environment is difficult to ascertain from the existing survey data. Protozoa are found living actively in nutrient-poor to organically rich waters and in fresh water varying between 0°C (32°F) and 50°C (122°F). Nonetheless, it appears that rates of population growth increase when food is not constrained and temperature is increased (Lee and Fenchel 1972; Fenchel 1974; Montagnes et al. 1988).

Comparisons of oxygen consumption in various taxonomic groups show wide variation (Laybourn and Finlay 1976), with some aerobic forms able to function at extremely low oxygen tensions and to thereby avoid competition and predation.

Many parasitic and a few free-living species are obligatory anaerobes (grow without atmospheric oxygen). Of the free-living forms, the best known are the plagiopylid ciliates that live in the anaerobic sulfide-rich sediments of marine wetlands (Fenchel et al. 1977). The importance of plagiopylids in recycling nutrients to aerobic zones of wetlands is potentially great.

Because of the small size of protozoa, their short generation time, and (for some species) ease of maintaining them in the laboratory, ecologists have used protozoan populations and communities to investigate competition and predation.

The result has been an extensive literature on a few species studied primarily under laboratory conditions. Few studies have been extended to natural habitats with the result that we know relatively little about most protozoa and their roles in natural communities. Intraspecific competition for common resources often results in cannibalism, sometimes with dramatic changes in morphology of the cannibals (Giese 1973). Field studies of interspecific competition are few and most evidence for such species interactions is indirect (Cairns and Yongue 1977).

Contractile Vacuoles

Many protozoa have contractile vacuoles, which collect and expel excess water, and extrusomes, which expel material used to deflect predators or capture prey. In multicellular organisms, hormones are often produced in vesicles. In higher plants, most of a cell's volume is taken up by a central vacuole or tonoplast, which maintains its osmotic pressure. Many eukaryotes have slender motile projections, usually called flagella when long and cilia when short. These are variously involved in movement, feeding, and sensation. These are entirely distinct from prokaryotic flagella. They are supported by a bundle of microtubules arising from a basal body, also called a kinetosome or centriole, characteristically arranged as nine doublets surrounding two singlets. Flagella also may have hairs or mastigonemes, scales, connecting membranes, and internal rods. Their interior is continuous with the cell's cytoplasm.

Centrioles

Centrioles are often present even in cells and groups that do not have flagella. They generally occur in groups of one or two, called kinetids that give rise to various microtubular roots. These form a primary component of the cytoskeletal structure, and are often assembled over the course of several cell divisions, with one flagellum retained from the parent and the other derived from it. Centrioles may also be associated in the formation of a spindle during nuclear division. Some protists have various other microtubule-supported organelles. These include the radiolaria and heliozoa, which produce axopodia used in flotation or to capture prey, and the haptophytes, which have a peculiar flagellum-like organelle called the haptonema.

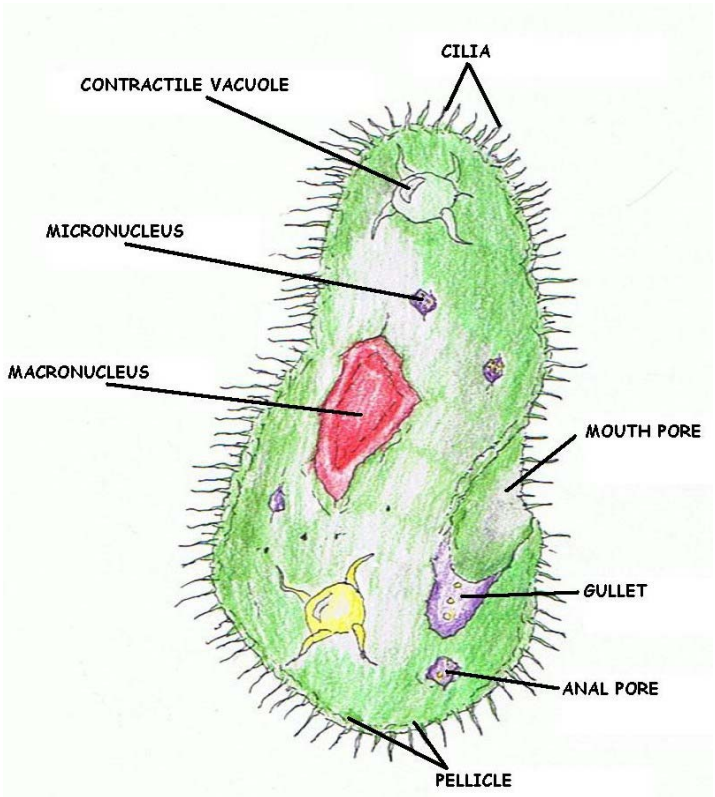
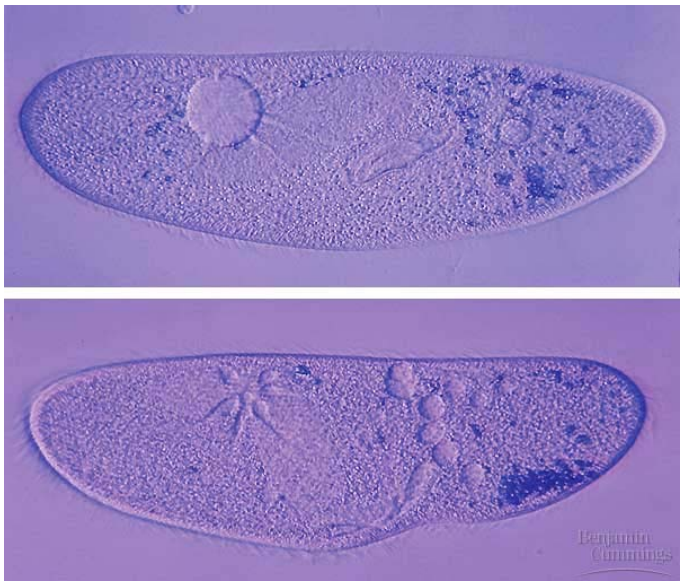


Figure 1. A diagram of *Paramecium* sp. with major organelles indicated.



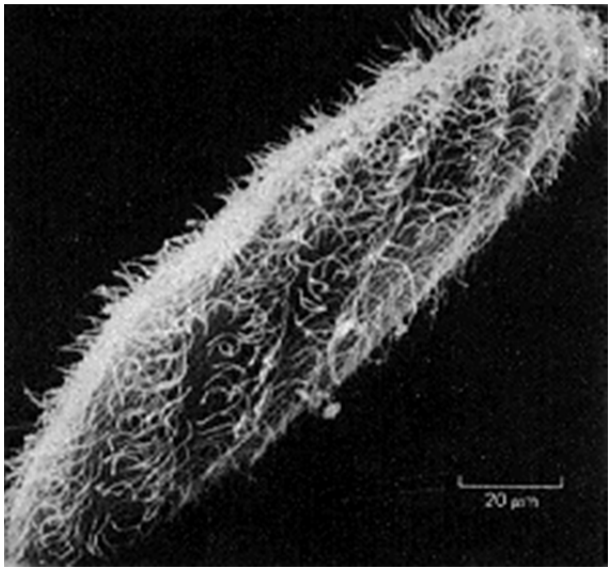
Contractile Vacuoles

Figure 2. The contractile vacuole when full (top) and after contraction (bottom).

Paramecium

Members of the genus *Paramecium* are single-celled, freshwater organisms in the kingdom Protista. They exist in an environment in which the osmotic concentration in their external environment is much lower than that in their cytoplasm. More specifically, the habitat in which they live is **hypotonic** to their cytoplasm. As a result of this, *Paramecium* is subjected to a continuous influx of water, as water diffuses inward to a region of higher osmotic concentration.

If *Paramecium* is to maintain homeostasis, water must be continually pumped out of the cell (against the osmotic gradient) at the same rate at which it moves in. This process, known as **osmoregulation**, is carried out by two organelles in *Paramecium* known as **contractile vacuoles** (Figures 1 and 2).





Here is a wastewater Inspector is utilizing the auto sampler's manual to help adjust the time and adjust for the correct flow for a composite sample. You can see that she is pouring off the pickle jar. This inspector is also the course author.

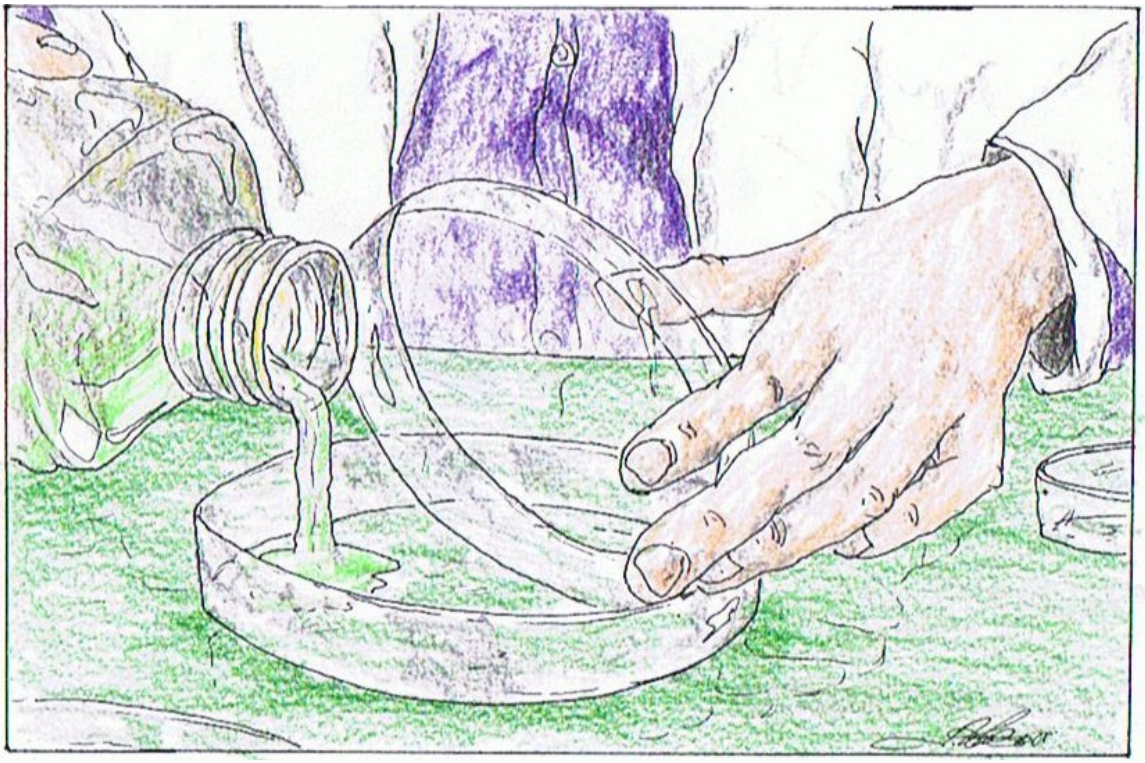
Protozoan Diseases

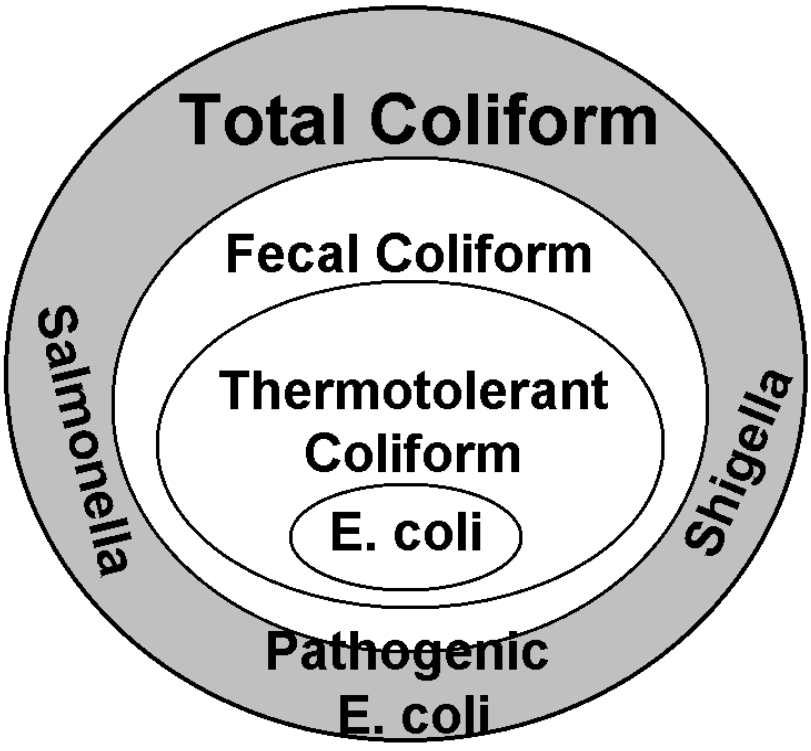
Protozoan pathogens are larger than bacteria and viruses, but still microscopic. They invade and inhabit the gastrointestinal tract. Some parasites enter the environment in a dormant form, with a protective cell wall called a "***cyst***." The cyst can survive in the environment for long periods of time and be extremely resistant to conventional disinfectants such as chlorine. Effective filtration treatment is therefore critical to removing these organisms from water sources.

Giardiasis
Giardiasis is a commonly reported protozoan-caused disease. It has also been referred to as "***backpacker's disease***" and "***beaver fever***" because of the many cases reported among hikers and others who consume untreated surface water. Symptoms include chronic diarrhea, abdominal cramps, bloating, frequent loose and pale greasy stools, fatigue and weight loss. The incubation period is 5-25 days or longer, with an average of 7-10 days. Many infections are asymptomatic (no symptoms). Giardiasis occurs worldwide. Waterborne outbreaks in the United States occur most often in communities receiving their drinking water from streams or rivers without adequate disinfection or a filtration system. The organism, ***Giardia lamblia,*** has been responsible for more community-wide outbreaks of disease in the U.S. than any other pathogen. Drugs are available for treatment but are not 100% effective.

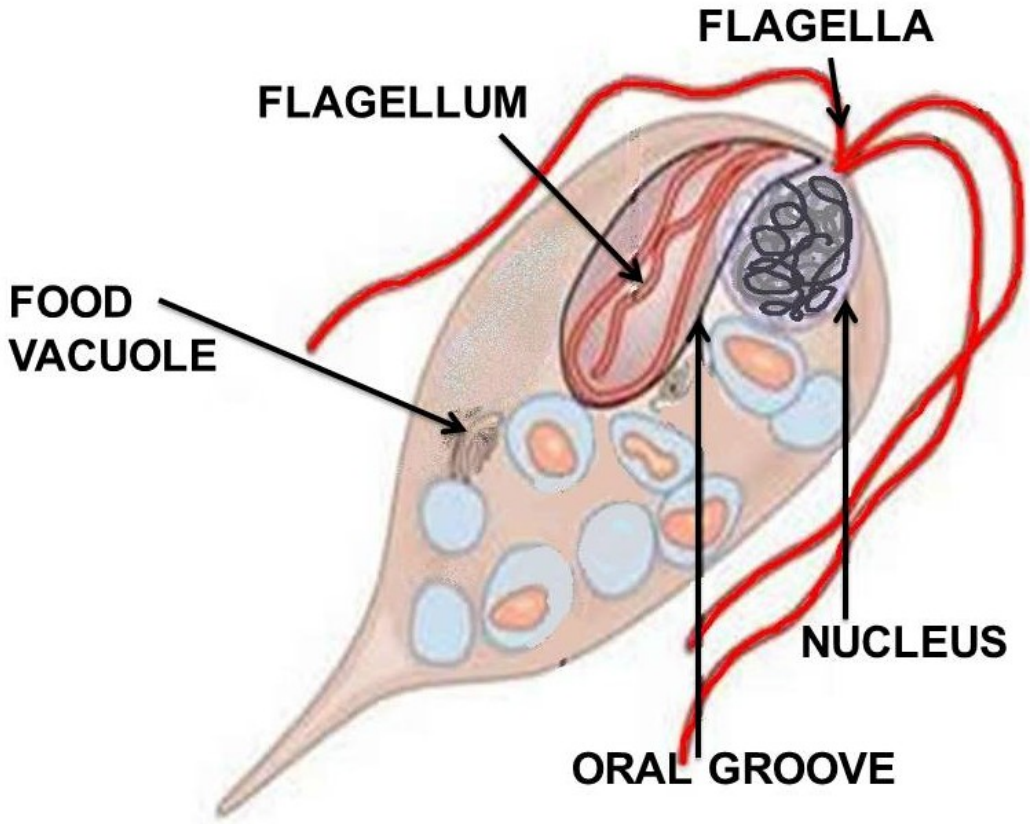
Cryptosporidiosis
Cryptosporidiosis is an example of a protozoan disease that is common worldwide, but was only recently recognized as causing human disease. The major symptom in humans is diarrhea, which may be profuse and watery. The diarrhea is associated with cramping abdominal pain. General malaise, fever, anorexia, nausea, and vomiting occur less often. Symptoms usually come and go, and end in fewer than 30 days in most cases. The incubation period is 1-12 days, with an average of about seven days. ***Cryptosporidium*** organisms have been identified in human fecal specimens from more than 50 countries on six continents. The mode of transmission is fecal-oral, either by person-to-person or animal-to-person. There is no specific treatment for ***Cryptosporidium*** infections.

All of these diseases, with the exception of hepatitis A, have one symptom in common: diarrhea. They also have the same mode of transmission, fecal-oral, whether through person-to-person or animal-to-person contact, and the same routes of transmission, being either foodborne or waterborne. Although most pathogens cause mild, self-limiting disease, on occasion, they can cause serious, even life threatening illness. Particularly vulnerable are persons with weak immune systems such as those with HIV infections or cancer. By understanding the nature of waterborne diseases, the importance of properly constructed, operated and maintained public water systems becomes obvious. While water treatment cannot achieve sterile water (no microorganisms), the goal of treatment must clearly be to produce drinking water that is as pathogen-free as possible at all times. For those who operate water systems with inadequate source protection or treatment facilities, the potential risk of a waterborne disease outbreak is real. For those operating systems that currently provide adequate source protection and treatment, operating and maintaining the system at a high level on a continuing basis is critical to prevent disease.




Total Coliform
Fecal Coliform
Thermotolerant Coliform
E. coli
Salmonella
Shigella
Pathogenic E. coli

Giardia Lamblia



GIARDIA LAMBLIA

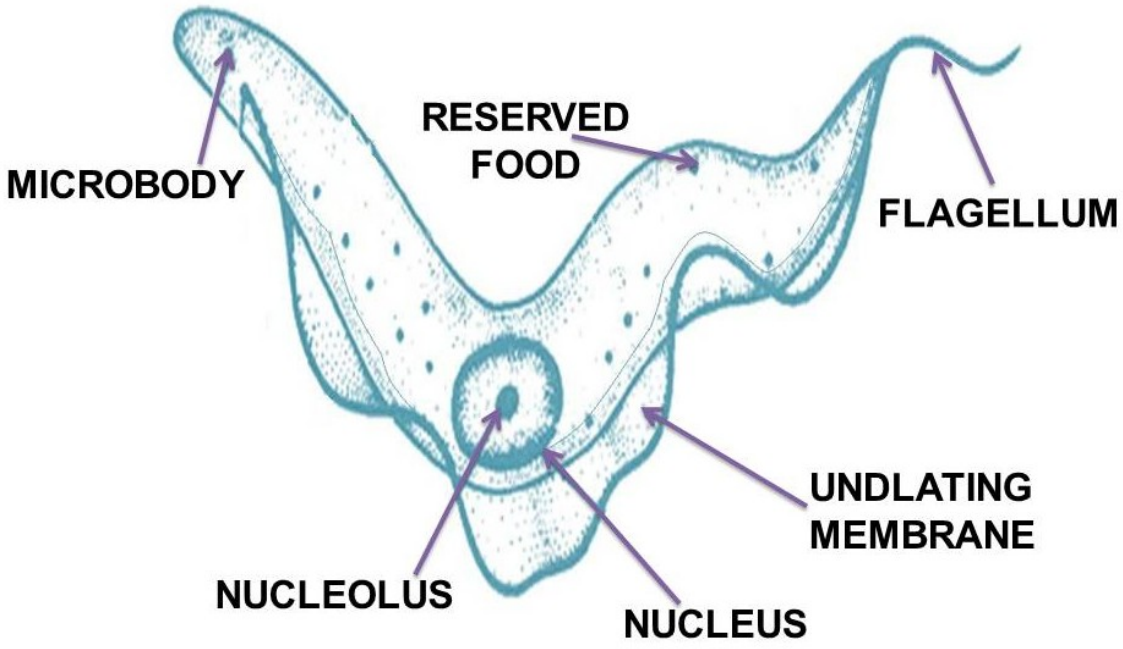
Giardia lamblia (synonymous with Lamblia intestinalis and Giardia duodenalis) is a flagellated protozoan parasite that colonizes and reproduces in the small intestine, causing giardiasis. The giardia parasite attaches to the epithelium by a ventral adhesive disc, and reproduces via binary fission. Giardiasis does not spread via the bloodstream, nor does it spread to other parts of the gastro-intestinal tract, but remains confined to the lumen of the small intestine. Giardia trophozoites absorb their nutrients from the lumen of the small intestine, and are anaerobes.

Giardia infection can occur through ingestion of dormant cysts in contaminated water, or by the fecal-oral route (through poor hygiene practices). The Giardia cyst can survive for weeks to months in cold water and therefore can be present in contaminated wells and water systems, and even clean-looking mountain streams, as well as city reservoirs, as the Giardia cysts are resistant to conventional water treatment methods, such as chlorination and ozonolysis. Zoonotic transmission is also possible, and therefore Giardia infection is a concern for people camping in the wilderness or swimming in contaminated streams or lakes, especially the artificial lakes formed by beaver dams (hence the popular name for giardiasis, "Beaver Fever"). As well as water-borne sources, fecal-oral transmission can also occur, for example in day care centers, where children may have poorer hygiene practices.

Those who work with children are also at risk of being infected, as are family members of infected individuals. Not all Giardia infections are symptomatic, so some people can unknowingly serve as carriers of the parasite.

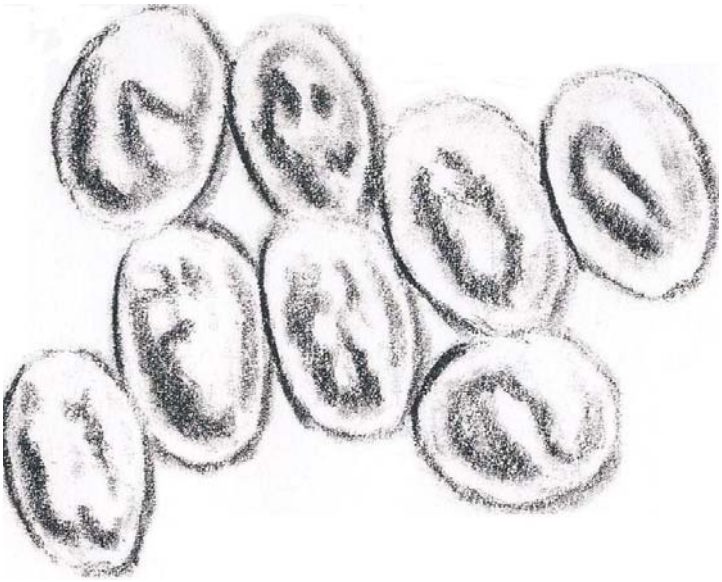
The life cycle begins with a non-infective cyst being excreted with feces of an infected individual. Once out in the environment, the cyst becomes infective. A distinguishing characteristic of the cyst is 4 nuclei and a retracted cytoplasm. Once ingested by a host, the trophozoite emerges to an active state of feeding and motility. After the feeding stage, the trophozoite undergoes asexual replication through longitudinal binary fission. The resulting trophozoites and cysts then pass through the digestive system in the feces. While the trophozoites may be found in the feces, only the cysts are capable of surviving outside of the host.

Distinguishing features of the trophozoites are large karyosomes and lack of peripheral chromatin, giving the two nuclei a halo appearance. Cysts are distinguished by a retracted cytoplasm. This protozoa lacks mitochondria, although the discovery of the presence of mitochodrial remnant organelles in one recent study "indicate that Giardia is not primitively amitochondrial and that it has retained a functional organelle derived from the original mitochondrial endosymbiont"



PROTOZOAN PARASITE

Cryptosporidium

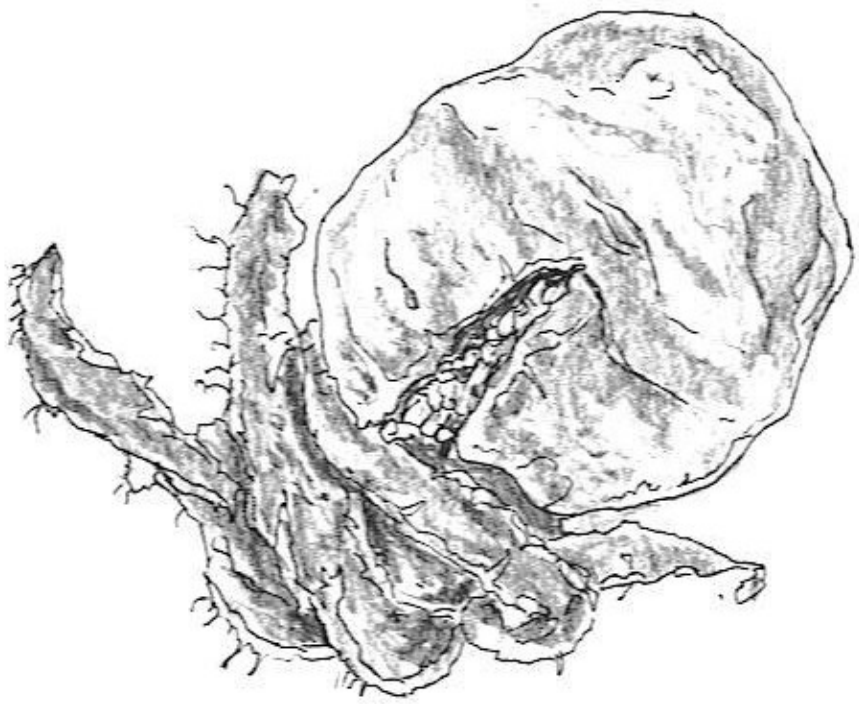


Cryptosporidium is a protozoan pathogen of the Phylum Apicomplexa and causes a diarrheal illness called cryptosporidiosis. Other apicomplexan pathogens include the malaria parasite Plasmodium, and Toxoplasma, the causative agent of toxoplasmosis. Unlike Plasmodium, which transmits via a mosquito vector, Cryptosporidium does not utilize an insect vector and is capable of completing its life cycle within a single host, resulting in cyst stages which are excreted in feces and are capable of transmission to a new host.

A number of species of Cryptosporidium infect mammals. In humans, the main causes of disease are C. parvum and C. hominis (previously C. parvum genotype 1). C. canis, C. felis, C. meleagridis, and C. muris can also cause disease in humans. In recent years, cryptosporidiosis has plagued many commercial Leopard gecko breeders. Several species of the Cryptosporidium family (C. serpentes and others) are involved, and outside of geckos it has been found in monitor lizards, iguanas, tortoises as well as several snake species.

Cryptosporidiosis is typically an acute short-term infection but can become severe and non-resolving in children and immunocompromised individuals. The parasite is transmitted by environmentally hardy cysts (oocysts) that, once ingested, excyst in the small intestine and result in an infection of intestinal epithelial tissue.

The genome of Cryptosporidium parvum was sequenced in 2004 and was found to be unusual amongst Eukaryotes in that the mitochondria seem not to contain DNA. A closely-related species, C. hominis, also has its genome sequence available. CryptoDB.org is a NIH-funded database that provides access to the Cryptosporidium genomics data sets.



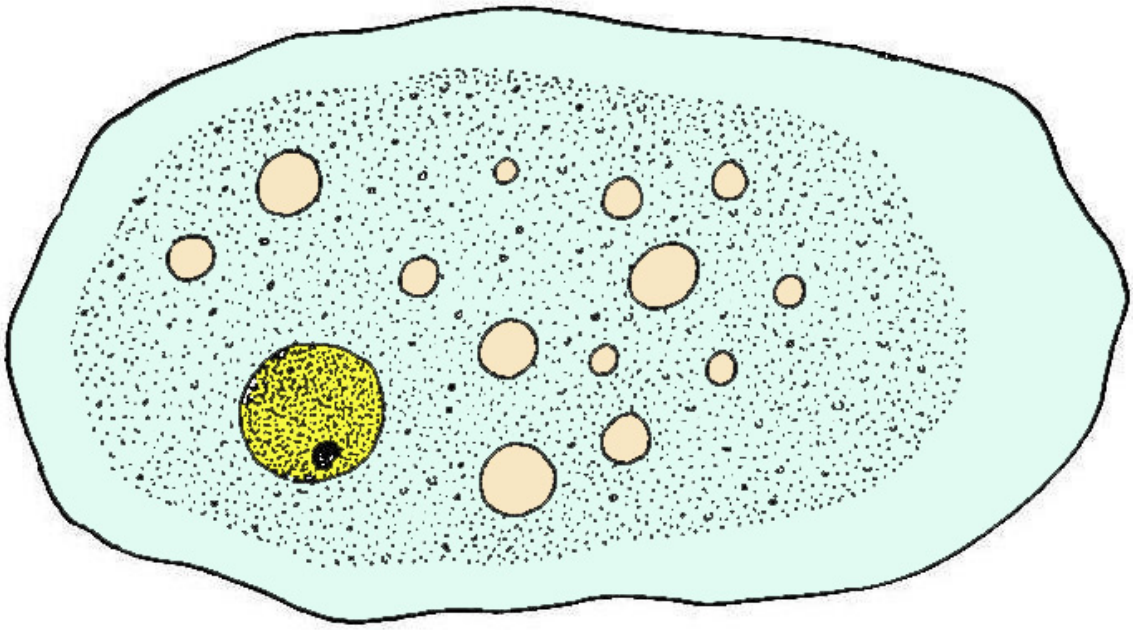
CRYPTO-PARVUM

When *C. parvum* was first identified as a human pathogen, diagnosis was made by a biopsy of intestinal tissue (Keusch, *et al*., 1995). However, this method of testing can give false negatives due the "patchy" nature of the intestinal parasitic infection (Flanigan and Soave, 1993). Staining methods were then developed to detect and identify the oocysts directly from stool samples. The modified acid-fast stain is traditionally used to most reliably and specifically detect the presence of cryptosporidial oocysts.

There have been six major outbreaks of cryptosporidiosis in the United States as a result of contamination of drinking water (Juranek, 1995). One major outbreak in Milwaukee in 1993 affected over 400,000 persons. Outbreaks such as these usually result from drinking water taken from surface water sources such as lakes and rivers (Juranek, 1995). Swimming pools and water park wave pools have also been associated with outbreaks of cryptosporidiosis. Also, untreated groundwater or well water public drinking water supplies can be sources of contamination.

The highly environmentally resistant cyst of *C. parvum* allows the pathogen to survive various drinking water filtrations and chemical treatments such as chlorination. Although municipal drinking water utilities may meet federal standards for safety and quality of drinking water, complete protection from cryptosporidial infection is not guaranteed. In fact, *all* waterborne outbreaks of cryptosporidiosis have occurred in communities where the local utilities met all state and federal drinking water standards (Juranek, 1995).

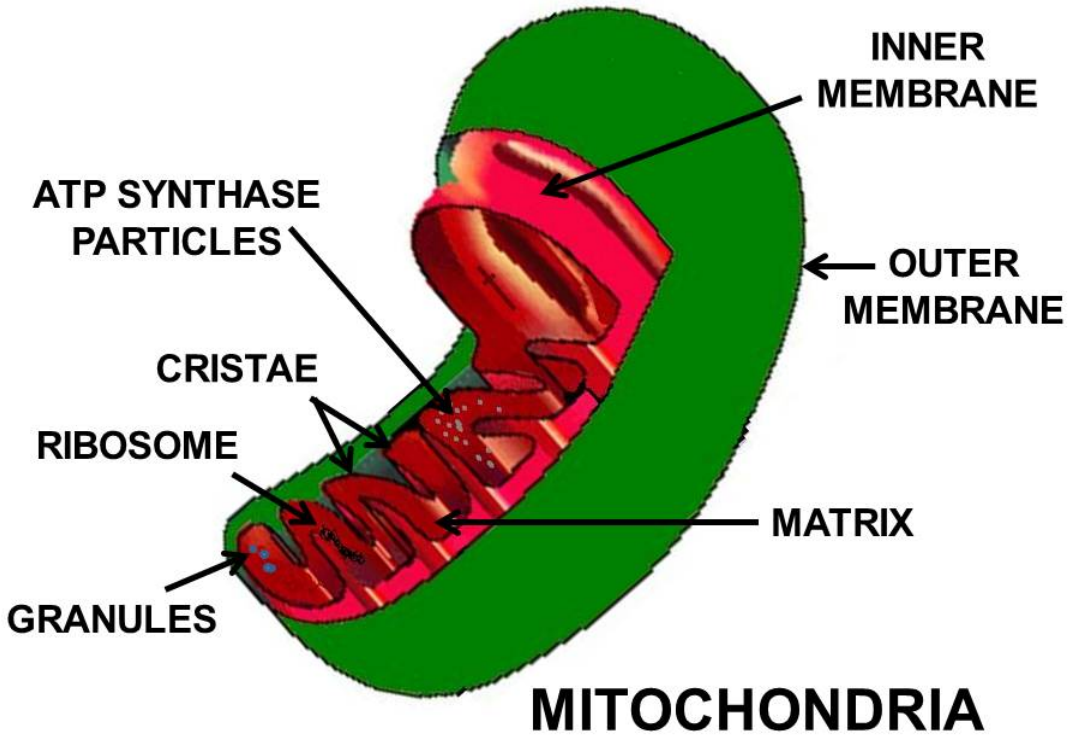
Entamoeba histolytica



Entamoeba histolytica, another water-borne pathogen, can cause diarrhea or a more serious invasive liver abscess. When in contact with human cells, these amoebae are cytotoxic. There is a rapid influx of calcium into the contacted cell, it quickly stops all membrane movement save for some surface blebbing. Internal organization is disrupted, organelles lyse, and the cell dies. The ameba may eat the dead cell or just absorb nutrients released from the cell.

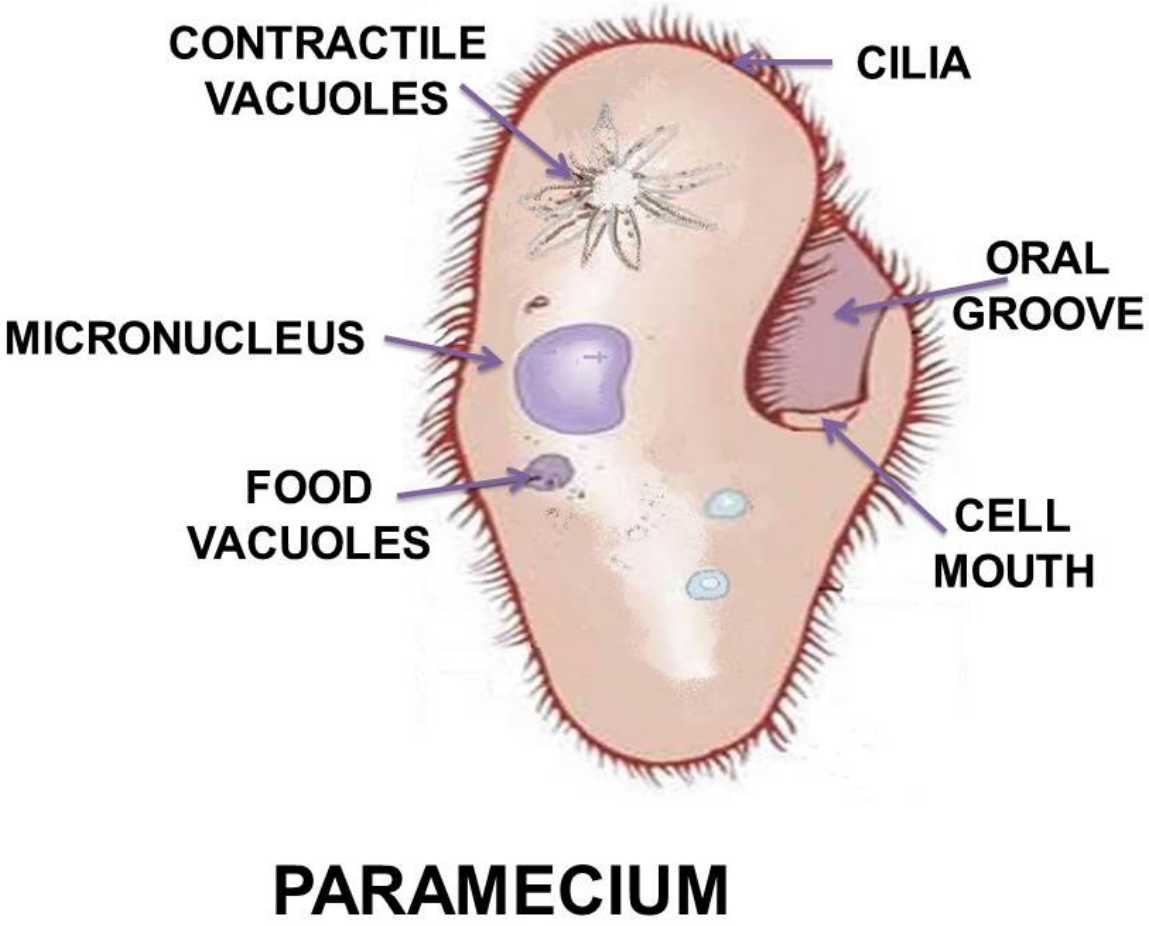
On average, about one in 10 people who are infected with *E. histolytica* becomes sick from the infection. The symptoms often are quite mild and can include loose stools, stomach pain, and stomach cramping. Amebic dysentery is a severe form of amebiasis associated with stomach pain, bloody stools, and fever. Rarely, *E. histolytica* invades the liver and forms an abscess. Even less commonly, it spreads to other parts of the body, such as the lungs or brain.

Scientific classification
Domain: Eukaryota
Phylum: Amoebozoa
Class: Archamoebae
Genus: Entamoeba
Species: E. histolytica



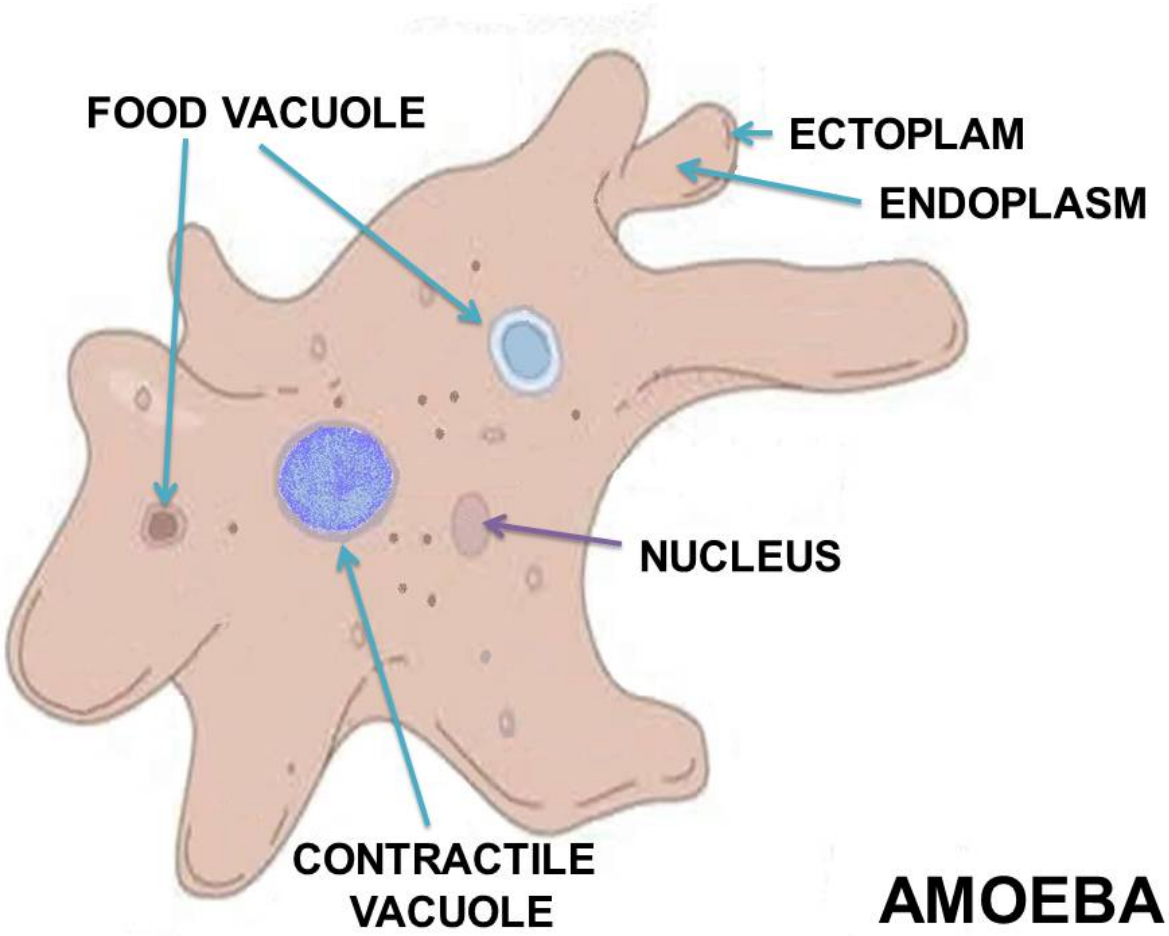
Mitochondria

The bacterial cell is surrounded by a lipid membrane, or cell membrane, which encloses the contents of the cell and acts as a barrier to hold nutrients, proteins and other essential components of the cytoplasm within the cell. As they are prokaryotes, bacteria do not tend to have membrane-bound organelles in their cytoplasm and thus contain few large intracellular structures. They consequently lack a nucleus, mitochondria, chloroplasts and the other organelles present in eukaryotic cells, such as the Golgi apparatus and endoplasmic reticulum.



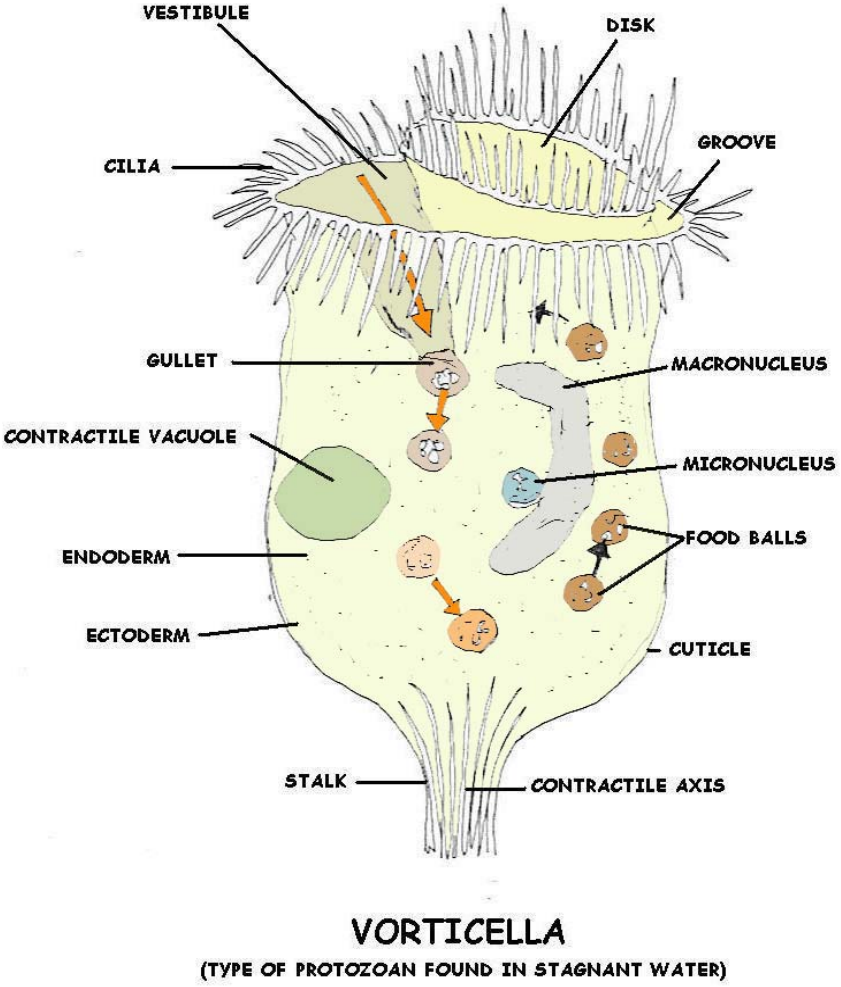
Paramecia

Paramecia are a group of unicellular ciliate protozoa formerly known as slipper animalcules from their slipper shape. They are commonly studied as a representative of the ciliate group. Simple cilia cover the body which allows the cell to move with a synchronous motion (like a caterpilla). There is also a deep oral groove containing inconspicuous compound oral cilia (as found in other peniculids) that is used to draw food inside. They generally feed upon bacteria and other small cells. Osmoregulation is carried out by a pair of contractile vacuoles, which actively expel water absorbed by osmosis from their surroundings. Paramecia are widespread in freshwater environments, and are especially common in scums. Paramecia are attracted by acidic conditions. Certain single-celled eukaryotes, such as Paramecium, are examples for exceptions to the universality of the genetic code (translation systems where a few codons differ from the standard ones).



Amoeba

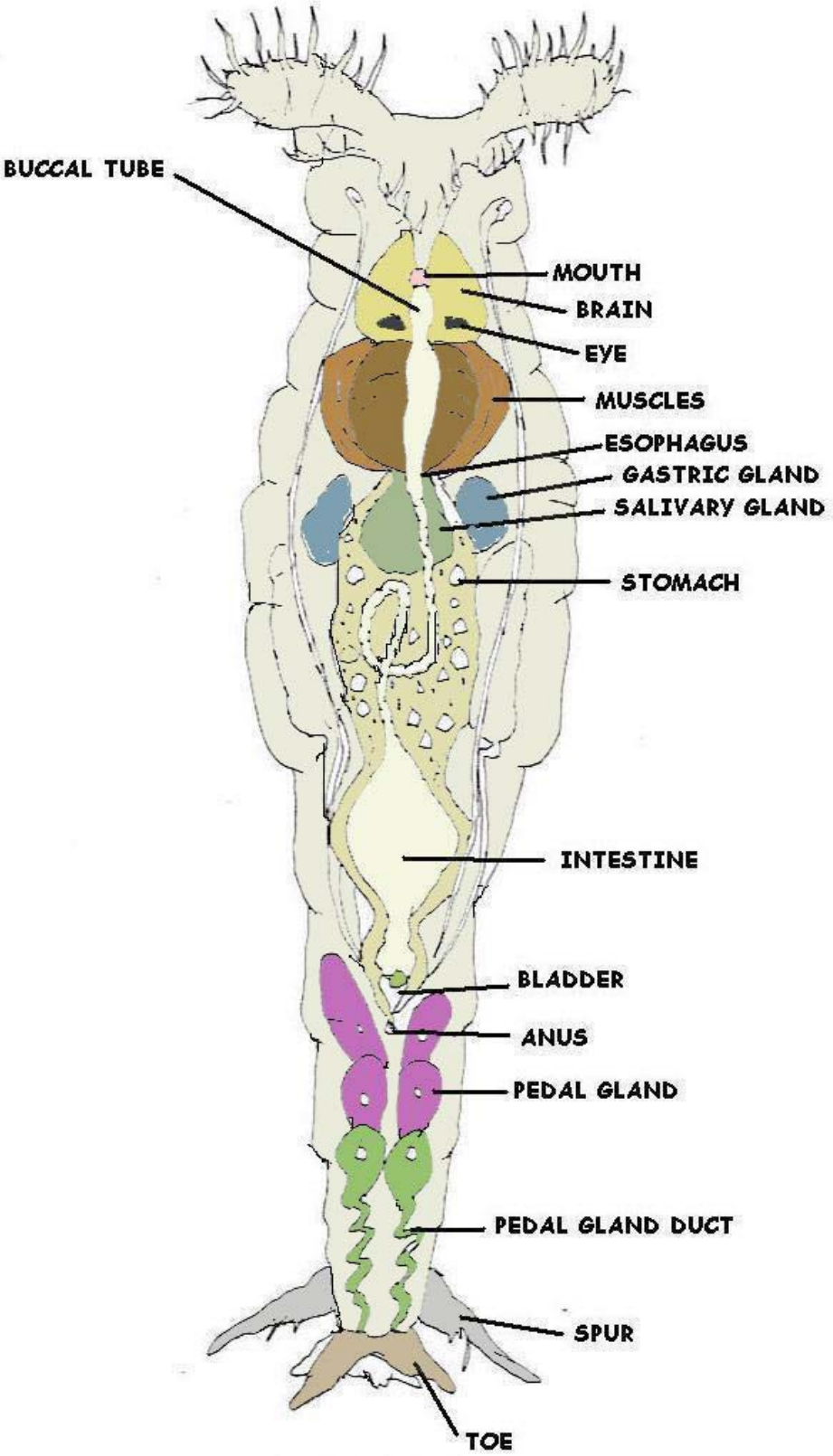
Amoeba (sometimes amœba or ameba, plural amoebae) is a genus of protozoa that moves by means of pseudopods, and is well-known as a representative unicellular organism. The word amoeba or ameba is variously used to refer to it and its close relatives, now grouped as the Amoebozoa, or to all protozoa that move using pseudopods, otherwise termed amoeboids.



Vorticella

Vorticella is a genus of protozoa, with over 100 known species. They are stalked inverted bell-shaped ciliates, placed among the peritrichs. Each cell has a separate stalk anchored onto the substrate, which contains a contracile fibril called a myoneme. When stimulated this shortens, causing the stalk to coil like a spring. Reproduction is by budding, where the cell undergoes longitudinal fission and only one daughter keeps the stalk. Vorticella mainly lives in freshwater ponds and streams - generally anywhere protists are plentiful. Other genera such as Carchesium resemble Vorticella but are branched or colonial.

Domain: Eukaryota
Phylum: Ciliophora
Class: Oligohymenophorea
Subclass: Peritrichia
Order: Sessilida
Family: Vorticellidae
Genus: Vorticella


BUCCAL TUBE
MOUTH
BRAIN
EYE
MUSCLES
ESOPHAGUS
GASTRIC GLAND
SALIVARY GLAND
STOMACH
INTESTINE
BLADDER
ANUS
PEDAL GLAND
PEDAL GLAND DUCT
SPUR
TOE

ROTIFER

Rotifer

The rotifers make up a phylum of microscopic and near-microscopic pseudocoelomate animals. They were first described by John Harris in 1696 (Hudson and Gosse, 1886). Leeuwenhoek is mistakenly given credit for being the first to describe rotifers but Harris had produced sketches in 1703. Most rotifers are around 0.1-0.5 mm long, and are common in freshwater throughout the world with a few saltwater species. Rotifers may be free swimming and truly planktonic, others move by inch worming along the substrate, whilst some are sessile, living inside tubes or gelatinous holdfasts. About 25 species are colonial (e.g. Sinantherina semibullata), either sessile or planktonic.

Rotifers get their name (derived from Greek and meaning "wheel-bearer"; they have also been called wheel animalcules) from the corona, which is composed of several ciliated tufts around the mouth that in motion resemble a wheel. These create a current that sweeps food into the mouth, where it is chewed up by a characteristic pharynx (called the mastax) containing a tiny, calcified, jaw-like structure called the trophi. The cilia also pull the animal, when unattached, through the water. Most free-living forms have pairs of posterior toes to anchor themselves while feeding. Rotifers have bilateral symmetry and a variety of different shapes. There is a well-developed cuticle which may be thick and rigid, giving the animal a box-like shape, or flexible, giving the animal a worm-like shape; such rotifers are respectively called loricate and illoricate.

Like many other microscopic animals, adult rotifers frequently exhibit eutely - they have a fixed number of cells within a species, usually on the order of one thousand. Males in the class Monogononta may be either present or absent depending on the species and environmental conditions. In the absence of males, reproduction is by parthenogenesis and results in clonal offspring that are genetically identical to the parent. Individuals of some species form two distinct types of parthenogenetic eggs; one type develops into a normal parthenogenetic female, while the other occurs in response to a changed environment and develops into a degenerate male that lacks a digestive system, but does have a complete male reproductive system that is used to inseminate females thereby producing fertilized 'resting eggs'. Resting eggs develop into zygotes that are able to survive extreme environmental conditions such as may occur during winter or when the pond dries up. These eggs resume development and produce a new female generation when conditions improve again. The life span of monogonont females varies from a couple of days to about three weeks.

Bdelloid rotifers are unable to produce resting eggs, but many can survive prolonged periods of adverse conditions after desiccation. This facility is termed anhydrobiosis, and organisms with these capabilities are termed anhydrobionts. Under drought conditions, bdelloid rotifers contract into an inert form and lose almost all body water; when rehydrated, however, they resume activity within a few hours. Bdelloids can survive the dry state for prolonged periods, with the longest well-documented dormancy being nine years. While in other anhydrobionts, such as the brine shrimp, this desiccation tolerance is thought to be linked to the production of trehalose, a non-reducing disaccharide (sugar), bdelloids apparently lack the ability to synthesize trehalose. Bdelloid rotifer genomes contain two or more divergent copies of each gene. Four copies of hsp82 are, for example, found. Each is different and found on a different chromosome, excluding the possibility of homozygous sexual reproduction.

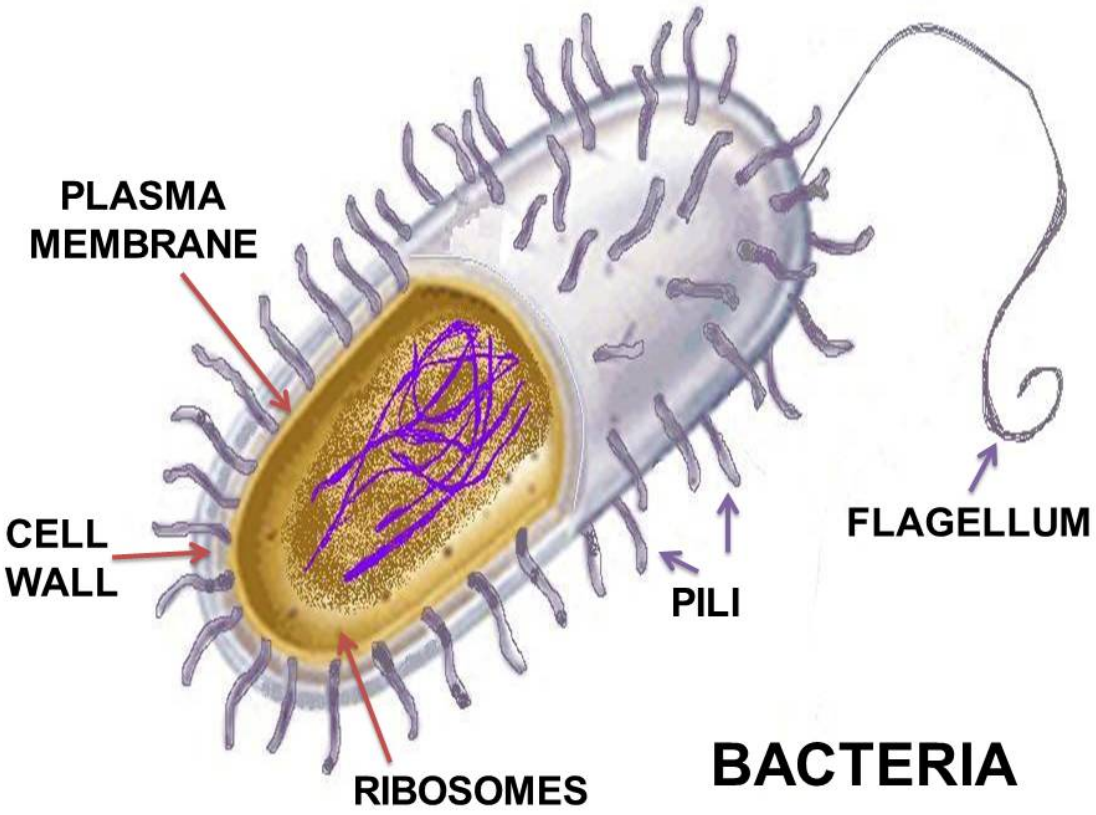
Waterborne Diseases

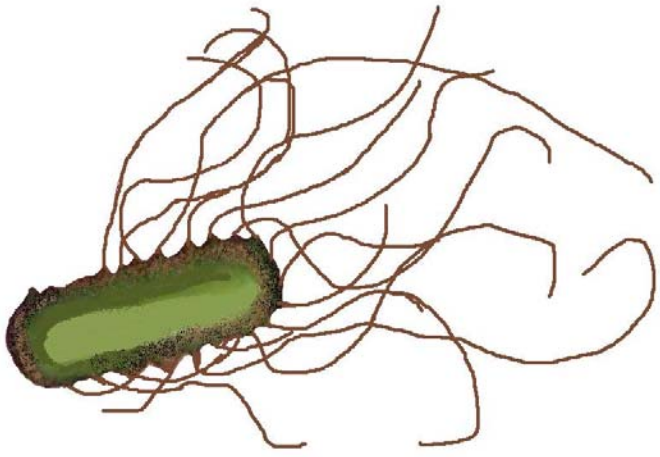
Name	Causative organism	Source of organism	Disease
Viral gastroenteritis	**Rotavirus** (mostly in young children)	Human feces	Diarrhea or vomiting
Norwalk Agent	**Noroviruses** (genus *Norovirus*, family *Caliciviridae*) [*1]	Human feces; also, shellfish; lives in polluted waters	Diarrhea and vomiting
Salmonellosis	***Salmonella*** (bacterium)	Animal or human feces	Diarrhea or vomiting
Gastroenteritis Escherichia *coli*	-- ***E. coli O1* 57:H7** (bacterium): Other *E. coli* organisms:	Human feces	Symptoms vary with type caused
Typhoid	***Salmonella typhi*** (bacterium)	Human feces, urine	Inflamed intestine, enlarged spleen, high temperature-sometimes fatal
Shigellosis	***Shigella*** (bacterium)	Human feces	Diarrhea
Cholera	***Vibrio choleras*** (bacterium)	Human feces; also, shellfish; lives in many coastal waters	Vomiting, severe diarrhea, rapid dehydration, mineral loss-high mortality
Hepatitis A	**Hepatitis A** virus	Human feces; shellfish grown in polluted waters	Yellowed skin, enlarged liver, fever, vomiting, weight loss, abdominal pain-low mortality, lasts up to four months
Amebiasis	***Entamoeba histolytica*** (protozoan)	Human feces	Mild diarrhea, dysentery, extra intestinal infection
Giardiasis	***Giardia lamblia*** (protozoan)	Animal or human feces	Diarrhea, cramps, nausea, and general weakness — lasts one week to months
Cryptosporidiosis	***Cryptosporidium parvum***	Animal or human feces	Diarrhea, stomach pain — lasts (protozoan) days to weeks

Notes:

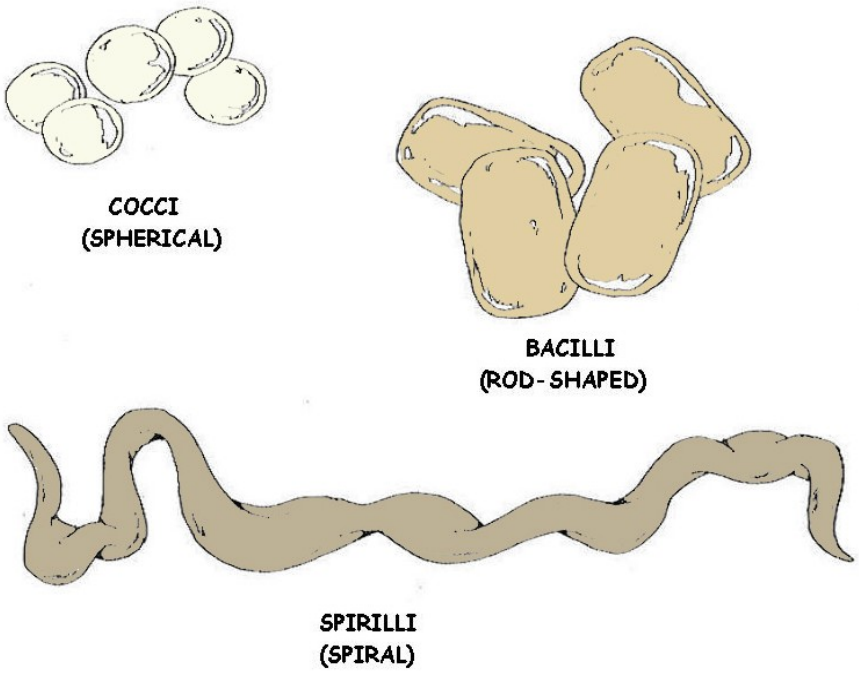
*1 http://www.cdc.gov/ncidod/dvrd/revb/gastro/norovirus.htm
http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5009a1.htm

Bacteria Section





Peritrichous Bacteria



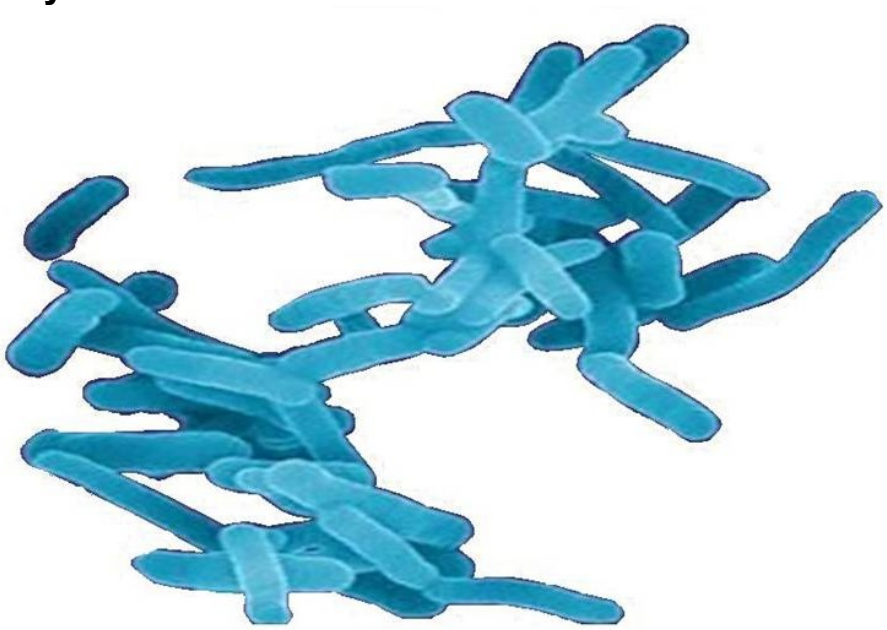
BACTERIA TYPES

Microbiologists broadly classify bacteria according to their shape: spherical, rod-shaped, and spiral-shaped. Pleomorphic bacteria can assume a variety of shapes. Bacteria may be further classified according to whether they require oxygen (aerobic or anaerobic) and how they react to a test with Gram's stain. Bacteria in which alcohol washes away Gram's stain are called gram-negative, while bacteria in which alcohol causes the bacteria's walls to absorb the stain are called gram-positive.



COLORLESS FILAMENTOUS
SULFUR BACTERIA

Shigella dysenteriae



SHIGELLA DYSENTERIAE

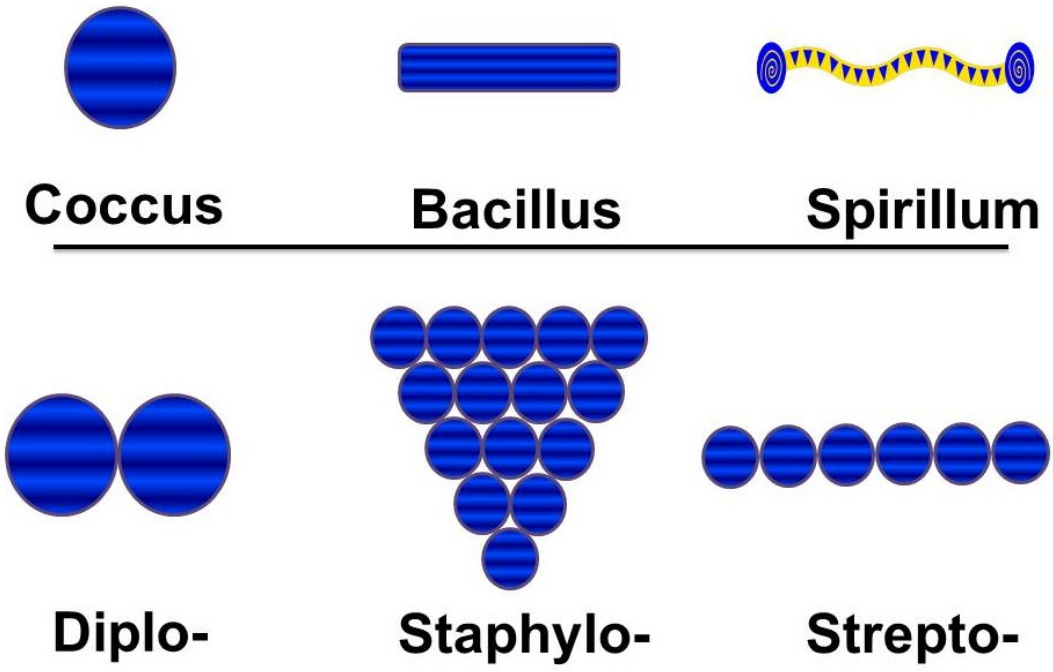
Shigella dysenteriae is a species of the rod-shaped bacterial genus Shigella. Shigella can cause shigellosis (bacillary dysentery). Shigellae are Gram-negative, non-spore-forming, facultatively anaerobic, non-motile bacteria.

S. dysenteriae, spread by contaminated water and food, causes the most severe dysentery because of its potent and deadly Shiga toxin, but other species may also be dysentery agents. Shigella infection is typically via ingestion (fecal–oral contamination); depending on age and condition of the host as few as ten bacterial cells can be enough to cause an infection. Shigella causes dysentery that result in the destruction of the epithelial cells of the intestinal mucosa in the cecum and rectum. Some strains produce enterotoxin and Shiga toxin, similar to the verotoxin of E. coli O157:H7. Both Shiga toxin and verotoxin are associated with causing hemolytic uremic syndrome.

Shigella invades the host through epithelial cells of the large intestine. Using a Type III secretion system acting as a biological syringe, the bacterium injects IpaD protein into cell, triggering bacterial invasion and the subsequent lysis of vacuolar membranes using IpaB and IpaC proteins. It utilizes a mechanism for its motility by which its IcsA protein triggers actin polymerization in the host cell (via N-WASP recruitment of Arp2/3 complexes) in a "rocket" propulsion fashion for cell-to-cell spread.

The most common symptoms are diarrhea, fever, nausea, vomiting, stomach cramps, and straining to have a bowel movement. The stool may contain blood, mucus, or pus (e.g. dysentery). In rare cases, young children may have seizures. Symptoms can take as long as a week to show up, but most often begin two to four days after ingestion. Symptoms usually last for several days, but can last for weeks. Shigella is implicated as one of the pathogenic causes of reactive arthritis worldwide.

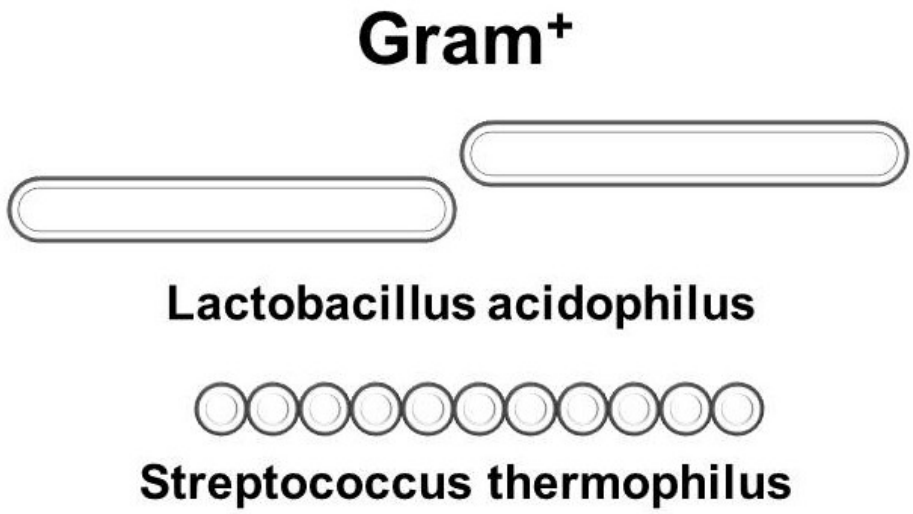


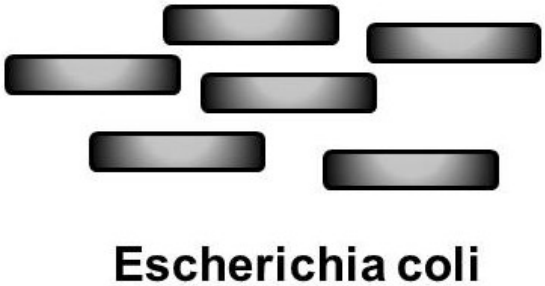


Bacteria Types

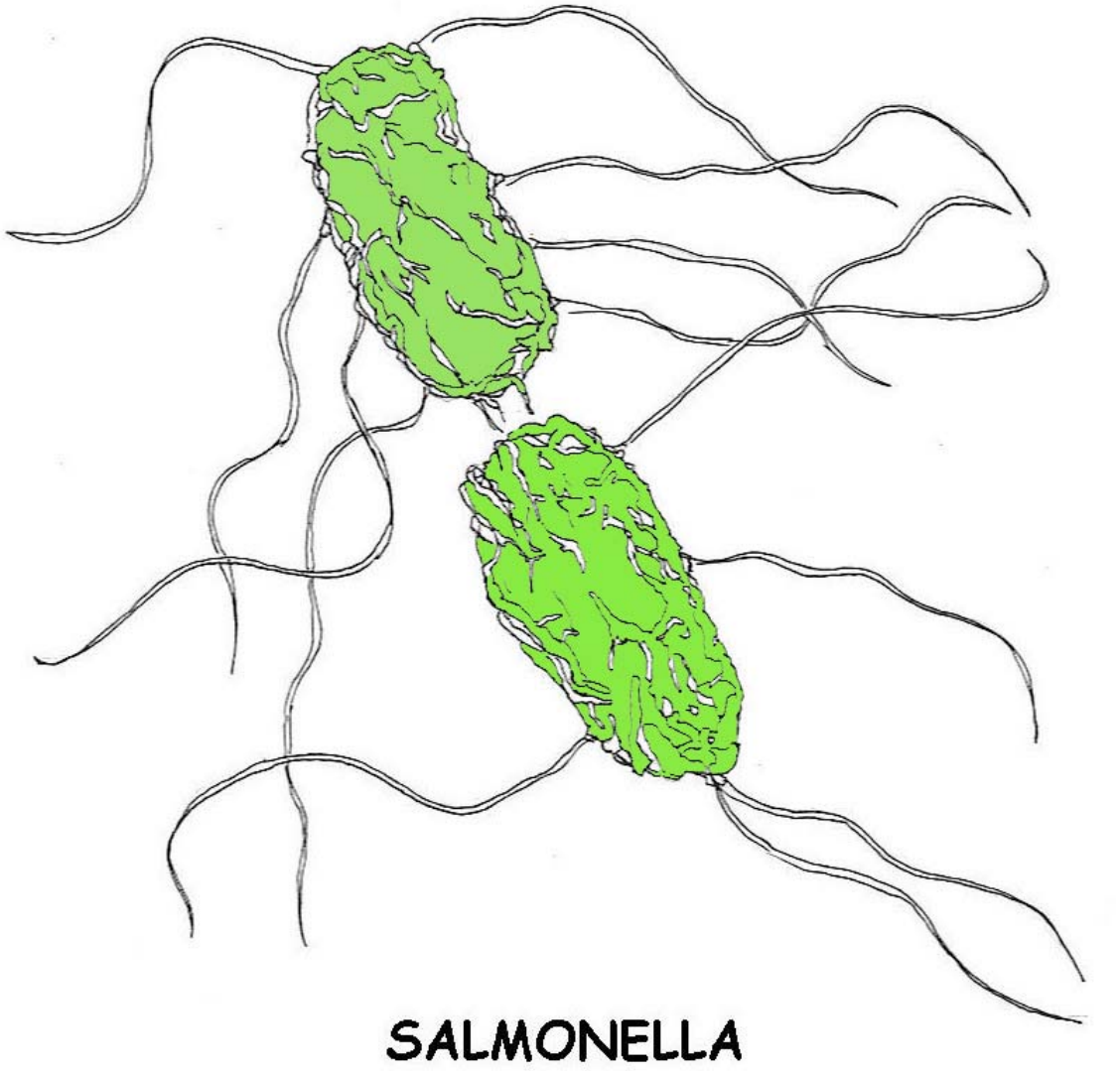
Type	Characteristics
Acetic acid	Rod-shaped, gram-negative, aerobic; highly tolerant of acidic conditions; generate organic acids
Actinomycete	Rod-shaped or filamentous, gram-positive, aerobic; common in soils; essential to growth of many plants; source of much of original antibiotic production in pharmaceutical industry
Coccoid	Spherical, sometimes in clusters or strings, gram-positive, aerobic and anaerobic; resistant to drying and high-salt conditions; *Staphylococcus* species common on human skin, certain strains associated with toxic shock syndrome
Coryneform	Rod-shaped, form club or V shapes, gram-positive, aerobic; found in wide variety of habitats, particularly soils; highly resistant to drying; include *Arthrobacter,* among most common forms of life on earth
Endospore-forming	Usually rod-shaped, can be gram-positive or gram-negative; have highly adaptable, heat-resistant spores that can go dormant for long periods, possibly thousands of years; include *Clostridium* (anaerobic) and *Bacillus* (aerobic)
Enteric	Rod-shaped, gram-negative, aerobic but can live in certain anaerobic conditions; produce nitrite from nitrate, acids from glucose; include *Escherichia coli, Salmonella* (over 1000 types), and *Shigella*
Gliding	Rod-shaped, gram-negative, mostly aerobic; glide on secreted slimy substances; form colonies, frequently with complex fruiting structures
Lactic acid	Gram-positive, anaerobic; produce lactic acid through fermentation; include *Lactobacillus*, essential in dairy product formation, and *Streptococcus*, common in humans
Mycobacterium	Pleomorphic, spherical or rod-shaped, frequently branching, no gram stain, aerobic; commonly form yellow pigments; include *Mycobacterium tuberculosis,* cause of tuberculosis
Mycoplasma	Spherical, commonly forming branching chains, no gram stain, aerobic but can live in certain anaerobic conditions; without cell walls yet structurally resistant to lysis; among smallest of bacteria; named for superficial resemblance to fungal hyphae (*myco-* means 'fungus')
Nitrogen-fixing	Rod-shaped, gram-negative, aerobic; convert atmospheric nitrogen gas to ammonium in soil; include *Azotobacter,* a common genus
Propionic acid	Rod-shaped, pleomorphic, gram-positive, anaerobic; ferment lactic acid; fermentation produces holes in Swiss cheese from the production of carbon dioxide
Pseudomonad	Rod-shaped (straight or curved) with polar flagella, gram-negative, aerobic; can use up to 100 different compounds for carbon and energy
Rickettsia	Spherical or rod-shaped, gram-negative, aerobic; cause Rocky Mountain spotted fever and typhus; closely related to *Agrobacterium,* a common gall-causing plant bacterium
Sheathed	Filamentous, gram-negative, aerobic; 'swarmer' (colonizing) cells form and break out of a sheath; sometimes coated with metals from environment

Spirillum	Spiral-shaped, gram-negative, aerobic; include *Bdellovibrio,* predatory on other bacteria
Spirochete	Spiral-shaped, gram-negative, mostly anaerobic; common in moist environments, from mammalian gums to coastal mudflats; complex internal structures convey rapid movement; include *Treponemapallidum,* cause of syphilis
Sulfate- and Sulfur-reducing	Commonly rod-shaped, mostly gram-negative, anaerobic; include *Desulfovibrio,* ecologically important in marshes
Sulfur- and iron-oxidizing	Commonly rod-shaped, frequently with polar flagella, gram-negative, mostly anaerobic; most live in neutral (nonacidic) environment
Vibrio	Rod- or comma-shaped, gram-negative, aerobic; commonly with a single flagellum; include *Vibrio cholerae,* cause of cholera, and luminescent forms symbiotic with deep-water fishes and squids





Salmonella



Salmonella is a Gram-negative bacterium. It is found in many turtles and other reptiles. In clinical laboratories, it is usually isolated on MacConkey agar, XLD agar, XLT agar, DCA agar, or Önöz agar. Because they cause intestinal infections and are greatly outnumbered by the bacteria normally found in the healthy bowel, primary isolation requires the use of a selective medium, so use of a relatively non-selective medium such as CLED agar is not often practiced. Numbers of salmonella may be so low in clinical samples that stools are routinely also subjected to "enrichment culture", where a small volume of stool is incubated in a selective broth medium, such as selenite broth or Rappaport Vassiliadis soya peptone broth, overnight. These media are inhibitory to the growth of the microbes normally found in the healthy human bowel, while allowing salmonellae to become enriched in numbers. Salmonellae may then be recovered by inoculating the enrichment broth on one or more of the primary selective media. On blood agar, they form moist colonies about 2 to 3 mm in diameter.

When the cells are grown for a prolonged time at a range of 25—28°C, some strains produce a biofilm, which is a matrix of complex carbohydrates, cellulose and proteins. The ability to produce biofilm (a.k.a. "rugose", "lacy", or "wrinkled") can be an indicator of dimorphism, which is the ability of a single genome to produce multiple phenotypes in response to environmental conditions. Salmonellae usually do not ferment lactose; most of them produce hydrogen sulfide which, in media containing ferric ammonium citrate, reacts to form a black spot in the centre of the creamy colonies.

Classification

Salmonella taxonomy is complicated. As of December 7, 2005, there are two species within the genus: S. bongori (previously subspecies V) and S. enterica (formerly called S. choleraesuis), which is divided into six subspecies:

* I—enterica
* II—salamae
* IIIa—arizonae
* IIIb—diarizonae
* IV—houtenae
* V—obsolete (now designated S. bongori)
* VI—indica



There are also numerous (over 2500) serovars within both species, which are found in a disparate variety of environments and which are associated with many different diseases. The vast majority of human isolates (>99.5%) are subspecies S. enterica. For the sake of simplicity, the CDC recommends that Salmonella species be referred to only by their genus and serovar, e.g.

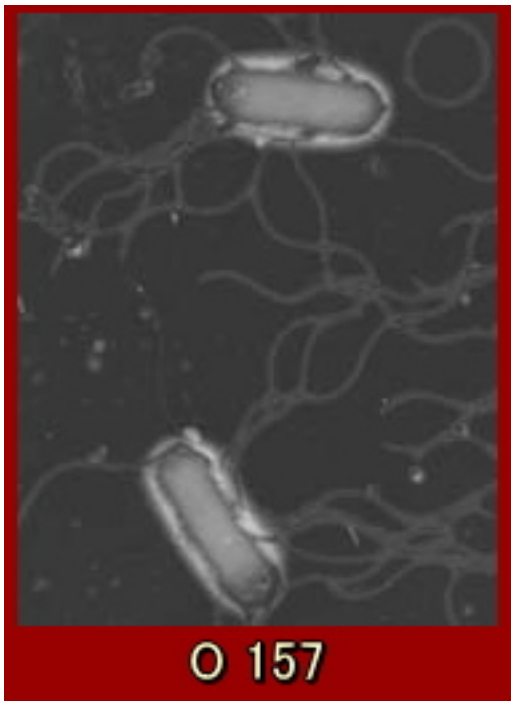
Salmonella Typhi instead of the more technically correct designation, Salmonella enterica subspecies enterica serovar Typhi.

Escherichia Coli Section

Fecal Coliform Bacteria
Fecal coliform bacteria are microscopic organisms that live in the intestines of warm-blooded animals. They also live in the waste material, or feces, excreted from the intestinal tract. When fecal coliform bacteria are present in high numbers in a water sample, it means that the water has received fecal matter from one source or another. Although not necessarily agents of disease, fecal coliform bacteria may indicate the presence of disease-carrying organisms, which live in the same environment as the fecal coliform bacteria.

Reasons for Natural Variation
Unlike the other conventional water quality parameters, fecal coliform bacteria are living organisms. They do not simply mix with the water and float straight downstream. Instead they multiply quickly when conditions are favorable for growth, or die in large numbers when conditions are not. Because bacterial concentrations are dependent on specific conditions for growth, and these conditions change quickly, fecal coliform bacteria counts are not easy to predict. For example, although winter rains may wash more fecal matter from urban areas into a stream, cool water temperatures may cause a major die-off. Exposure to sunlight (with its ultraviolet disinfection properties) may have the same effect, even in the warmer water of summertime.

Expected Impact of Pollution
The primary sources of fecal coliform bacteria to fresh water are wastewater treatment plant discharges, failing septic systems, and animal waste. Bacteria levels do not necessarily decrease as a watershed develops from rural to urban. Instead, urbanization usually generates new sources of bacteria. Farm animal manure and septic systems are replaced by domestic pets and leaking sanitary sewers. In fact, stormwater runoff in urbanized areas has been found to be surprisingly high in fecal coliform bacteria concentrations.



The presence of old, disintegrating storm and sanitary sewers, misplaced sewer pipes, and good breeding conditions are common explanations for the high levels measured.

Coliform Standards (in colonies/100ml)
Drinking water.....1FC
Total body contact (swimming).....200FC
Partial body contact (boating).....1000FC
Threatened sewage effluent .....not to exceed 200 FC

*Total coliform (TC) includes bacteria from cold-blooded animals and various soil organisms. According to recent literature, total coliform counts are normally about 10 times higher than fecal coliform (FC) counts.

Indicator Connection Varies
General coliforms, E. Coli, and Enterococcus bacteria are the "indicator" organisms generally measured to assess microbiological quality of water. However, these aren't generally what get people sick. Other bacteria, viruses, and parasites are what we are actually worried about.

Because it is so much more expensive and tedious to do so, actual pathogens are virtually never tested for. Over the course of a professional lifetime pouring over indicator tests, in a context where all standards are based on indicators, water workers tend to forget that the indicators are not the things we actually care about.

What are these indicators? More information in the Laboratory section.

- **General coliforms** indicate that the water has come in contact with plant or animal life. General coliforms are universally present, including in pristine spring water. They are of little concern at low levels, except to indicate the effectiveness of disinfection. Chlorinated water and water from perfectly sealed tube wells is the only water I've tested which had zero general coliforms. At very high levels they indicate there is what amounts to a lot of compost in the water, which could easily include pathogens (Ten thousand general coliform bacteria will get you a beach closure, compared to two or four hundred fecal coliforms, or fifty enterococcus).
- **Fecal coliforms**, particularly E. coli, indicate that there are mammal or bird feces in the water.
- **Enterococcus bacteria** also indicate that there are feces from warm blooded animals in the water. Enterococcus are a type of fecal streptococci. They are another valuable indicator for determining the amount of fecal contamination of water.
  According to studies conducted by the EPA, enterococci have a greater correlation with swimming-associated gastrointestinal illness in both marine and fresh waters than other bacterial indicator organisms, and are less likely to "die off" in saltwater.

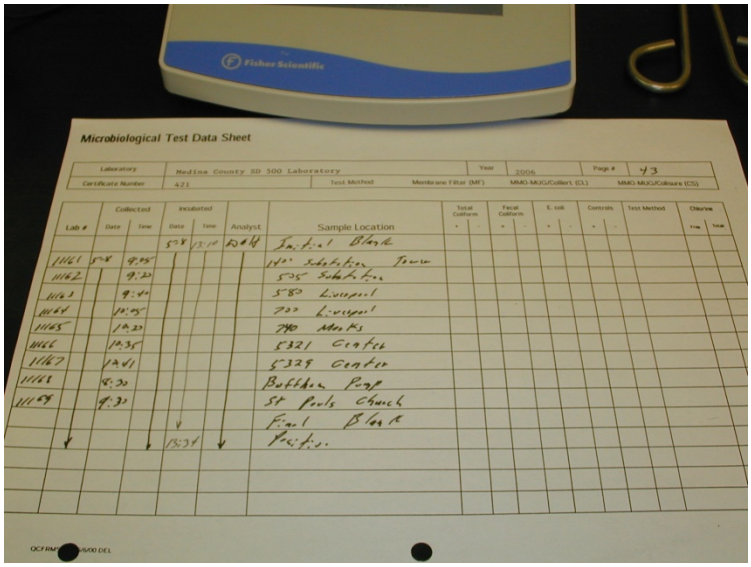
The more closely related the animal, the more likely pathogens excreted with their feces can infect us. Human feces are the biggest concern, because anything which infects one human could infect another. There isn't currently a quantitative method for measuring specifically human fecal bacteria (expensive genetic studies can give a presence/absence result). Ingesting a human stranger's feces via contaminated water supply is a classic means for infections to spread rapidly. The more pathogens an individual carries, the more hazardous their feces. Ingesting feces from someone who is not carrying any pathogens may gross you out, but it can't infect you. Infection rates are around 5% in the US, and approach 100% in areas with poor hygiene and contaminated water supplies. Keep in the back of your mind that **the ratio of indicators to actual pathogens is not fixed**. It will always be different, sometimes very different. Whenever you are trying to form a mental map of reality based on water tests, you should include in the application of your water intuition an adjustment factor for your best guess of the ratio between indicators and actual pathogens.

Membrane Filter Total Coliform Technique

The membrane filter total Coliform technique is used at Medina County for drinking water quality testing. The following is a summary of this test. A sampling procedure sheet is given to all sample takers by Medina County.

The samples are taken in sterile 100 mL containers. These containers, when used for chlorinated water samples, have a sodium thiosulfate pill or solution to dechlorinate the sample.

The sample is placed in cold storage after proper sample taking procedures are followed. (See sample procedures below)



The samples are taken to the laboratory with a chain of custody to assure no tampering of samples can occur.

These samples are logged in at the laboratory.

No longer than 30 hours can lapse between the time of sampling and time of test incubation. (8 hours for heterotrophic, nonpotable 6 hours, others not longer than 24 hours)

All equipment is sterilized by oven and autoclave.
Glassware in oven at 170°C ± 10°C with foil (or other suitable wrap) loosely fitting and secured immediately after sterilization.

Filtration units in autoclave at 121°C for 30 minutes.



Use sterile petri dishes, grid, and pads bought from a reliable company – certified, quality assured - test for satisfactory known positive amounts.

Incubators – 35°C ± .5°C (60% relative humidity)
M-endo medium is prepared and heated to near boiling removed from heat cooled to 45°C pH adjusted to 7.2 ± .2 and immediately dispensed 8ml to plates. Keep refrigerated and discard after 2 weeks.

Plates can be stored in a dated box with expiration date and discarded if not used. No denatured alcohol should be used. Everclear or 95% proof alcohol or absolute methyl may be used for sterilizing forceps by flame.

Procedure:
Counters are alcohol wiped.
Bench sheets are filled out.
Samples are removed from refrigeration.
Sterile wrapped utensils are placed on counters.
Filtration units are placed onto sterile membrane filters by aseptic technique using sterile forceps.
Sterile petri dishes are labeled.
The samples closures are clipped.
The sample is shaken 25 times 1 foot in length within 7 seconds.
100 mL is filtered and rinsed with sterile distilled water 3 times.
The membrane filter is aseptically removed from filter holder.
A sterile padded petri dish is used and the membrane filter is rolled onto the pad making sure no air bubbles form.
The sterile labeled lid is placed on the petri dish.
2 blanks and a known is run with each series of samples.
The samples are placed in the 35^{o}C $\pm$ .5^{o}C incubator stacked no higher than 3 for 22 – 24 hours (Humidity can be maintained by saturated paper towels placed under containers holding petri dishes.)
After 22- 24 hours view the petri dishes under a 10 –15 power magnification with cool white fluorescent light.
Count all colonies that appear pink to dark red with a metallic surface sheen – the sheen may vary in size from a pin head to complete coverage.
Report as Total Coliform per 100 mL.
If no colonies are present report as <1 coliform/100mL.

Anything greater than 1 is over the limit for drinking water for 2 samples taken 24 hours apart. Further investigation may be necessary – follow Standard Methods accordingly.



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Aran™ Aqua Analytical Laboratory Director.

Escherichia coli EPEC

Two types of pathogenic Escherichia coli, enteropathogenic E. coli (EPEC) and enterohemorrhagic E. coli (EHEC), cause diarrheal disease by disrupting the intestinal environment through the intimate attachment of the bacteria to the intestinal epithelium.

E. coli O157:H7
E. coli O157:H7 (bacterium) found in human feces. Symptoms vary with type caused gastroenteritis.

Escherichia coli O157:H7 is an emerging cause of foodborne illness. An estimated 73,000 cases of infection and 61 deaths occur in the United States each year. Infection often leads to bloody diarrhea, and occasionally to kidney failure. Most illnesses have been associated with eating undercooked, contaminated ground beef. Person-to-person contact in families and child care centers is also an important mode of transmission. Infection can also occur after drinking raw milk and after swimming in or drinking sewage-contaminated water.

Consumers can prevent *E. coli* O157:H7 infection by thoroughly cooking ground beef, avoiding unpasteurized milk, and washing hands carefully. Because the organism lives in the intestines of healthy cattle, preventive measures on cattle farms and during meat processing are being investigated.

What is *Escherichia coli* O157:H7?

E. coli O157:H7 is one of hundreds of strains of the bacterium Escherichia coli. Although most strains are harmless and live in the intestines of healthy humans and animals, this strain produces a powerful toxin and can cause severe illness.

E. coli O157:H7 was first recognized as a cause of illness in 1982 during an outbreak of severe bloody diarrhea; the outbreak was traced to contaminated hamburgers. Since then, most infections have come from eating undercooked ground beef.

The combination of letters and numbers in the name of the bacterium refers to the specific markers found on its surface and distinguishes it from other types of *E. coli.*

Currently, there are four recognized classes of enterovirulent *E. coli* (collectively referred to as the EEC group) that cause gastroenteritis in humans. Among these is the enterohemorrhagic (EHEC) strain designated *E. coli* O157:H7. *E. coli* is a normal inhabitant of the intestines of all animals, including humans. When aerobic culture methods are used, *E. coli* is the dominant species found in feces.

Normally *E. coli* serves a useful function in the body by suppressing the growth of harmful bacterial species and by synthesizing appreciable amounts of vitamins. A minority of *E. coli* strains are capable of causing human illness by several different mechanisms. *E. coli* serotype O157:H7 is a rare variety of *E. coli* that produces large quantities of one or more related, potent toxins that cause severe damage to the lining of the intestine. These toxins [verotoxin (VT), shiga-like toxin] are closely related or identical to the toxin produced by *Shigella dysenteriae*.

How does *E. coli* or other fecal coliforms get in the water?
E. coli comes from human and animal wastes. During rainfalls, snow melts, or other types of precipitation, *E. coli* may be washed into creeks, rivers, streams, lakes, or groundwater. When these waters are used as sources of drinking water and the water is not treated or inadequately treated, *E. coli* may end up in drinking water.

How is water treated to protect me from *E. coli*?
The water can be treated using chlorine, ultra-violet light, or ozone, all of which act to kill or inactivate *E. coli.* Systems using surface water sources are required to disinfect to ensure that all bacterial contamination such as *E. coli*. is inactivated. Systems using ground water sources are not required to disinfect, although many of them do.

How does the U.S. Environmental Protection Agency regulate *E. coli*?
According to EPA regulations, a system that operates at least 60 days per year, and serves 25 people or more or has 15 or more service connections, is regulated as a public water system under the Safe Drinking Water Act. If a system is not a public water system as defined by EPA regulations, it is not regulated under the Safe Drinking Water Act, although it may be regulated by state or local authorities.

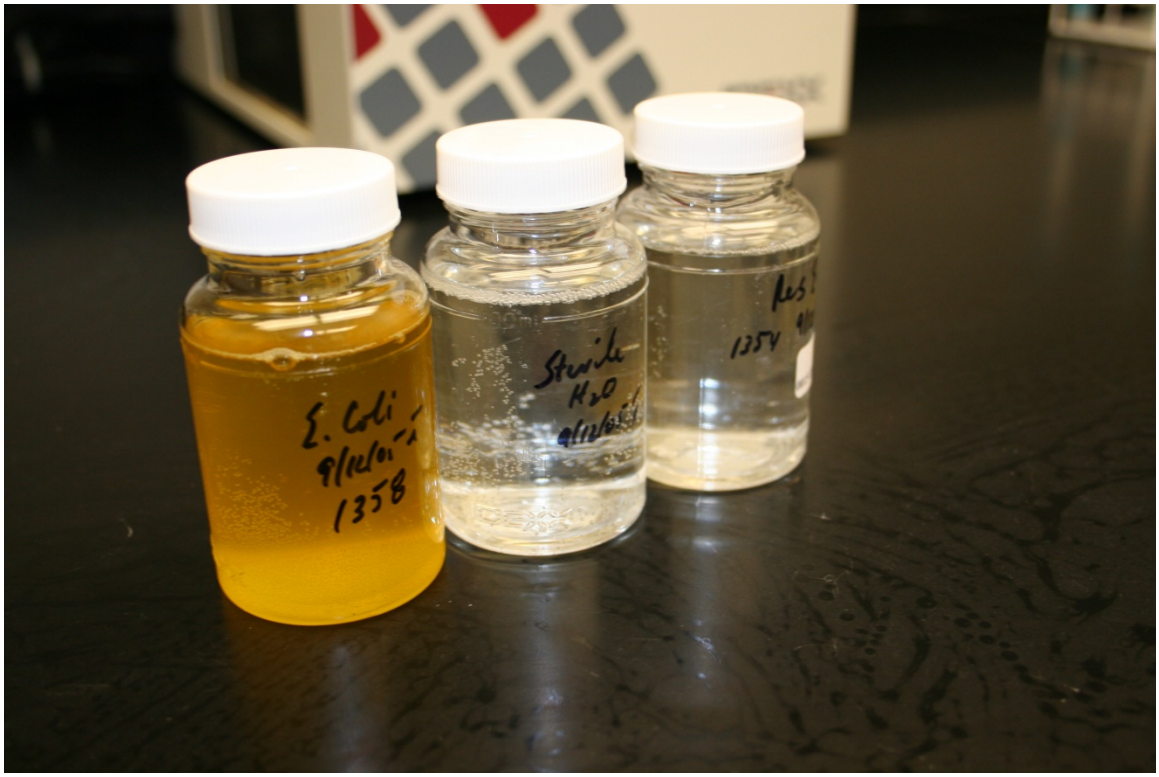
Under the Safe Drinking Water Act, the EPA requires public water systems to monitor for coliform bacteria. Systems analyze first for total coliform, because this test is faster to produce results. Any time that a sample is positive for total coliform, the same sample must be analyzed for either fecal coliform or *E. coli*. Both are indicators of contamination with animal waste or human sewage.

The largest public water systems (serving millions of people) must take at least 480 samples per month. Smaller systems must take at least five samples a month unless the state has conducted a sanitary survey – a survey in which a state inspector examines system components and ensures they will protect public health – at the system within the last five years.

Systems serving 25 to 1,000 people typically take one sample per month. Some states reduce this frequency to quarterly for ground water systems if a recent sanitary survey shows that the system is free of sanitary defects. Some types of systems can qualify for annual monitoring. Systems using surface water, rather than ground water, are required to take extra steps to protect against bacterial contamination because surface water sources are more vulnerable to such contamination. At a minimum, all systems using surface waters must disinfect. Disinfection will kill *E. coli* O157:H7.

What can I do to protect myself from *E. coli* O157:H7 in drinking water?
Approximately 89 percent of Americans are receiving water from community water systems that meet all health-based standards. Your public water system is required to notify you if, for any reason, your drinking water is not safe. If you wish to take extra precautions, you can boil your water for one minute at a rolling boil, longer at higher altitudes. To find out more information about your water, see the Consumer Confidence Report from your local water supplier or contact your local water supplier directly. You can also obtain information about your local water system on the EPA's website at www.epa.gov/safewater/dwinfo.htm.

Positive Tests
If you draw water from a private well, you can contact your state health department to obtain information on how to have your well tested for total coliforms, and *E. coli* contamination. If your well tests positive for *E. coli*, there are several steps that you should take: (1) begin boiling all water intended for consumption, (2) disinfect the well according to procedures recommended by your local health department, and (3) monitor your water quality to make certain that the problem does not recur. If the contamination is a recurring problem, you should investigate the feasibility of drilling a new well or install a point-of-entry disinfection unit, which can use chlorine, ultraviolet light, or ozone.



How is *E. coli* O157:H7 spread?
The organism can be found on a small number of cattle farms and can live in the intestines of healthy cattle. Meat can become contaminated during slaughter, and organisms can be thoroughly mixed into beef when it is ground. Bacteria present on a cow's udders or on equipment may get into raw milk. Eating meat, especially ground beef that has not been cooked sufficiently to kill *E. coli* O157:H7 can cause infection. Contaminated meat looks and smells normal. Although the number of organisms required to cause disease is not known, it is suspected to be very small.

Among other known sources of infection are consumption of sprouts, lettuce, salami, unpasteurized milk and juice, and swimming in or drinking sewage-contaminated water. Bacteria in diarrheal stools of infected persons can be passed from one person to another if hygiene or hand washing habits are inadequate. This is particularly likely among toddlers who are not toilet trained. Family members and playmates of these children are at high risk of becoming infected. Young children typically shed the organism in their feces for a week or two after their illness resolves. Older children rarely carry the organism without symptoms.

What illness does *E. coli* O157:H7 cause?
E. coli O157:H7 infection often causes severe bloody diarrhea and abdominal cramps; sometimes the infection causes non-bloody diarrhea or no symptoms. Usually little or no fever is present, and the illness resolves in 5 to 10 days. Hemorrhagic colitis is the name of the acute disease caused by *E. coli* O157:H7.

In some persons, particularly children under 5 years of age and the elderly, the infection can also cause a complication called hemolytic uremic syndrome, in which the red blood cells are destroyed and the kidneys fail. About 2%-7% of infections lead to this complication. In the United States, hemolytic uremic syndrome is the principal cause of acute kidney failure in children, and most cases of hemolytic uremic syndrome are caused by *E. coli* O157:H7.



How is *E. coli* O157:H7 infection diagnosed?
Infection with *E. coli* O157:H7 is diagnosed by detecting the bacterium in the stool. Most laboratories that culture stool do not test for *E. coli* O157:H7, so it is important to request that the stool specimen be tested on sorbitol-MacConkey (SMAC) agar for this organism. All persons who suddenly have diarrhea with blood should get their stool tested for *E. coli* O157:H7.

How is the illness treated?
Most persons recover without antibiotics or other specific treatment in 5-10 days. There is no evidence that antibiotics improve the course of disease, and it is thought that treatment with some antibiotics may precipitate kidney complications. Antidiarrheal agents, such as loperamide (Imodium), should also be avoided. Hemolytic uremic syndrome is a life-threatening condition usually treated in an intensive care unit. Blood transfusions and kidney dialysis are often required. With intensive care, the death rate for hemolytic uremic syndrome is 3%-5%.

Legionnaires' Disease Legionella Section

Introduction **Genus: *Legionella* Species: *pneumophila***

The first discovery of bacteria from genus Legionella came in 1976 when an outbreak of pneumonia at an American Legion convention led to 29 deaths. The causative agent, what would come to be known as Legionella pneumophila, was isolated and given its own genus. The organisms classified in this genus are Gram-negative bacteria that are considered intracellular parasites. The disease has two distinct forms:



- Legionnaires' disease, the more severe form of infection which includes pneumonia, and
- Pontiac fever, a milder illness.

What have been the water sources for Legionnaires' disease?

The major source is water distribution systems of large buildings, including hotels and hospitals. Cooling towers have long been thought to be a major source for *Legionella*, but new data suggest that this is an overemphasized mode of transmission. Other sources include mist machines, humidifiers, whirlpool spas, and hot springs. Air conditioners are not a source for Legionnaires' disease. They were suspected to be the source in the original American Legion outbreak in a Philadelphia hotel, but new data now suggests that the water in the hotel was the actual culprit.



Legionnaire's disease is caused most commonly by the inhalation of small droplets of water or fine aerosol containing Legionella bacteria. Legionella bacteria are naturally found in environmental water sources such as rivers, lakes and ponds and may colonize man-made water systems that include air conditioning systems, humidifiers, cooling tower waters, hot water systems, spas and pools.

How do people contract Legionella?

The most popular theory is that the organism is aerosolized in water and people inhale the droplets containing *Legionella*. However, new evidence suggests that another way of contracting *Legionella* is more common. "Aspiration" is the most common way that bacteria enter into the lungs to cause pneumonia. Aspiration means choking such that secretions in the mouth get past the choking reflexes and instead of going into the esophagus and stomach, mistakenly, enter the lung. The protective mechanisms to prevent aspiration is defective in patients who smoke or have lung disease. Aspiration now appears to be the most common mode of transmission.

Legionella may multiply to high numbers in cooling towers, evaporative condensers, air washers, humidifiers, hot water heaters, spas, fountains, and plumbing fixtures. Within one month, Legionella can multiply, in warm water-containing systems, from less than 10 per milliliter to over 1,000 per milliliter of water. Once high numbers of Legionella have been found, a relatively simple procedure for disinfecting water systems with chlorine

and detergent is available. This procedure is not part of a routine maintenance program because equipment may become corroded. Property owners have been sued for the spread of Legionella, resulting in expensive settlements. Regular monitoring with a battery of DFA monoclonal antibodies for several serogroups and species of Legionella morphologically intact bacteria provides a means for exercising 'reasonable care' to deter potential litigation.

Currently, there are no United States government regulations concerning permissible numbers of legionella in water systems and there are no federal or state certification programs for laboratories that perform legionella testing of environmental samples.

Epifluorescence Microscopy DFA Method
The epifluorescence microscopy DFA method that most labs use was published in the British Journal, Water Research 19:839-848, 1985 "Disinfection of circulating water systems by ultraviolet light and halogenation", R. Gilpin, et al. so we can count viable-but-nonculturable (VBNC) legionella.

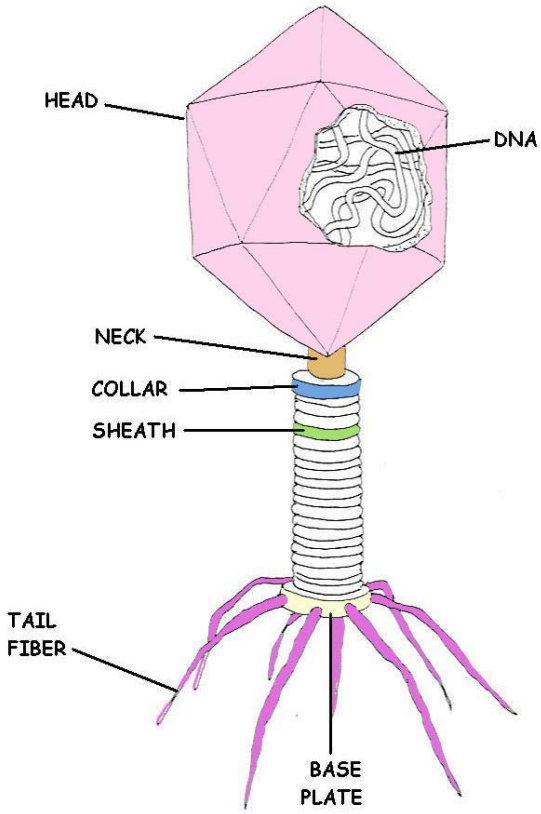
Most labs will provide a quantitative epifluorescence microscopic analysis of your cooling tower and potable water samples for 14 serogroups of Legionella pneumophila and 15 other Legionella species (listed below).

Legionella anisa	*Legionella bozemanii* sg 1 & 2
Legionella dumoffi	*Legionella feeleii* sg 1 & 2
Legionella gormanii	*Legionella hackeliae* sg 1 & 2
Legionella jordanis	*Legionella longbeachae* sg 1& 2
Legionella maceachernii	*Legionella micdadei*
Legionella oakridgensis	*Legionella parisiensis*
Legionella pneumophila sg 1-14	*Legionella sainthelensi*
Legionella santicrucis	*Legionella wadsworthii*

Heterotrophic bacterial CFU are often inversely proportional to numbers of Legionella in cooling tower samples, in our experience. Routine biocide treatments will not eradicate Legionella bacteria in the environment, only in laboratory studies. Culture methods are good during outbreaks for bio-typing; but culture methods lack sensitivity for routine, quantitative monitoring. Many factors will inhibit growth or identification of legionella on BCYE with or without antimicrobial agents, heat or acid treatment.

Culture methods will not identify non-culturable legionella that can still cause outbreaks (non-culturable, viable legionella have been reported in several peer-reviewed journals). Only DFA tests performed by trained laboratory personnel can identify these legionella. Direct fluorescent antibody (DFA) tests using a battery of monoclonal antibodies provide more useful routine monitoring information than culture methods. Legionella species of bacteria cause Legionnaire's disease. They are gram negative (but stain poorly), strictly aerobic rods.

The U.S. Environmental Protection Agency and the U.S. Occupational Safety and Health Administration recommend routine maintenance of water-containing equipment. Most State health departments recommend monthly testing for Legionella as part of a routine maintenance program.



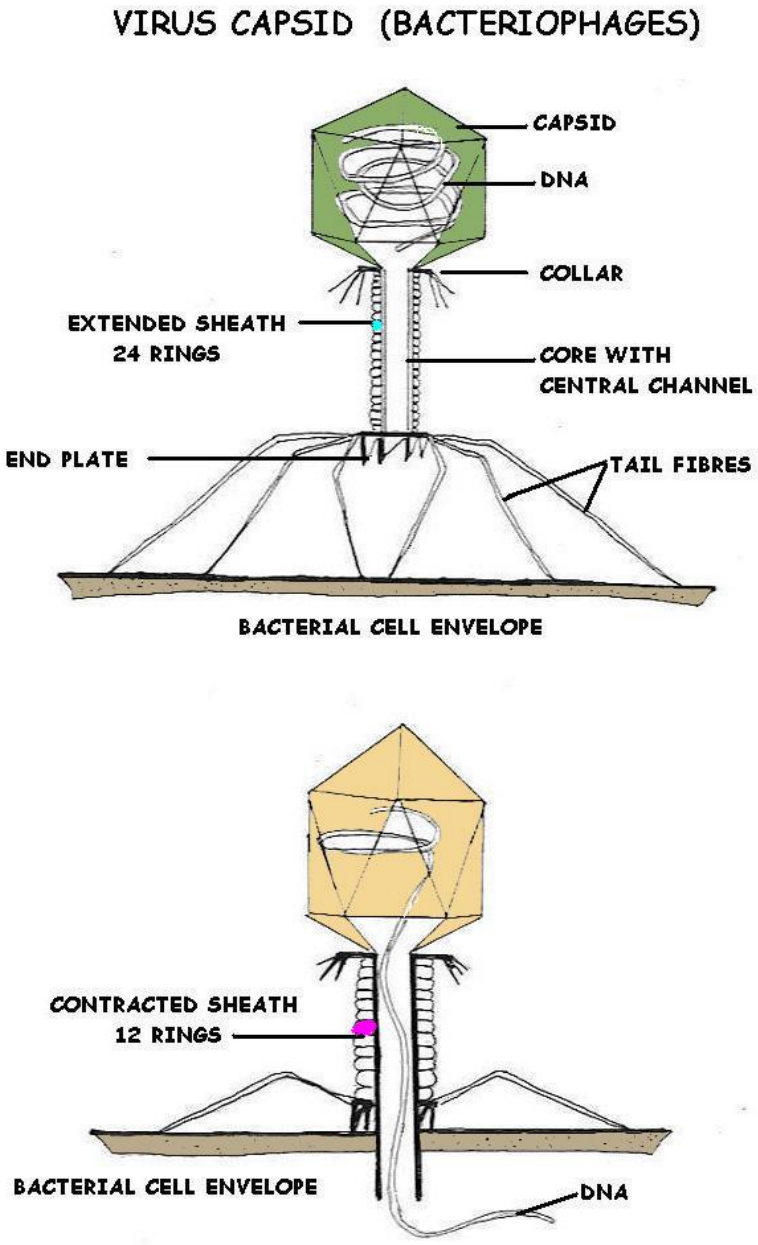
BACTERIOPHAGE

Bacteriophage

A bacteriophage (from 'bacteria' and Greek phagein, 'to eat') is any one of a number of viruses that infect bacteria. The term is commonly used in its shortened form, phage.

Typically, bacteriophages consist of an outer protein hull enclosing genetic material. The genetic material can be ssRNA (single stranded RNA), dsRNA, ssDNA, or dsDNA between 5 and 500 kilo base pairs long with either circular or linear arrangement. Bacteriophages are much smaller than the bacteria they destroy - usually between 20 and 200 nm in size.

Phages are estimated to be the most widely distributed and diverse entities in the biosphere. Phages are ubiquitous and can be found in all reservoirs populated by bacterial hosts, such as soil or the intestine of animals. One of the densest natural sources for phages and other viruses is sea water, where up to 9×108 virions per milliliter have been found in microbial mats at the surface, and up to 70% of marine bacteria may be infected by phages.

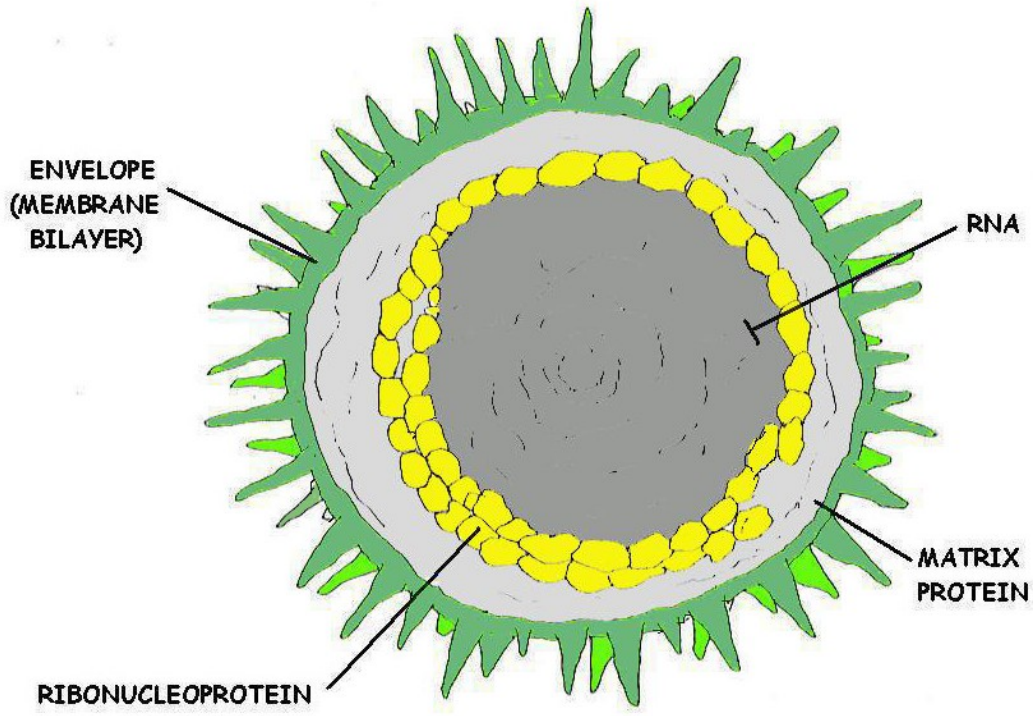


Release of Virions

Phages may be released via cell lysis or by host cell secretion. In the case of the T4 phage, in just over twenty minutes after injection upwards of three hundred phages will be released via lysis within a certain timescale. This is achieved by an enzyme called endolysin which attacks and breaks down the peptidoglycan. In contrast, "lysogenic" phages do not kill the host but rather become long-term parasites and make the host cell continually secrete more new virus particles. The new virions bud off the plasma membrane, taking a portion of it with them to become enveloped viruses possessing a viral envelope. All released virions are capable of infecting a new bacterium.

Viruses

Viruses are acellular microorganisms. They are made up of only genetic material and a protein coat. Viruses depend on the energy and metabolic machinery of the host cell to reproduce. A virus is an infectious agent found in virtually all life forms, including humans, animals, plants, fungi, and bacteria. Viruses consist of genetic material—either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA)—surrounded by a protective coating of protein, called a capsid, with or without an outer lipid envelope. Viruses are between 20 and 100 times smaller than bacteria and hence are too small to be seen by light microscopy.



CROSS SECTIONAL VIEW

Viruses vary in size from the largest poxviruses of about 450 nanometers (about 0.000014 in) in length to the smallest polioviruses of about 30 nanometers (about 0.000001 in). Viruses are not considered free-living, since they cannot reproduce outside of a living cell; they have evolved to transmit their genetic information from one cell to another for the purpose of replication. Viruses often damage or kill the cells that they infect, causing disease in infected organisms. A few viruses stimulate cells to grow uncontrollably and produce cancers. Although many infectious diseases, such as the common cold, are caused by viruses, there are no cures for these illnesses. The difficulty in developing antiviral therapies stems from the large number of variant viruses that can cause the same disease, as well as the inability of drugs to disable a virus without disabling healthy cells. However, the development of antiviral agents is a major focus of current research, and the study of viruses has led to many discoveries important to human health.

Virions
Individual viruses, or virus particles, also called virions, contain genetic material, or genomes, in one of several forms. Unlike cellular organisms, in which the genes always are made up of DNA, viral genes may consist of either DNA or RNA. Like cell DNA, almost all viral DNA is double-stranded, and it can have either a circular or a linear arrangement. Almost all viral RNA is single-stranded; it is usually linear, and it may be either segmented (with different genes on different RNA molecules) or non-segmented (with all genes on a single piece of RNA).

Capsids
The viral protective shell, or capsid, can be either helical (spiral-shaped) or icosahedral (having 20 triangular sides). Capsids are composed of repeating units of one or a few different proteins. These units are called protomers or capsomers. The proteins that make up the virus particle are called structural proteins. Viruses also carry genes for making proteins that are never incorporated into the virus particle and are found only in infected cells. These viral proteins are called nonstructural proteins; they include factors required for the replication of the viral genome and the production of the virus particle.

Capsids and the genetic material (DNA or RNA) they contain are together referred to as nucleocapsids. Some virus particles consist only of nucleocapsids, while others contain additional structures.

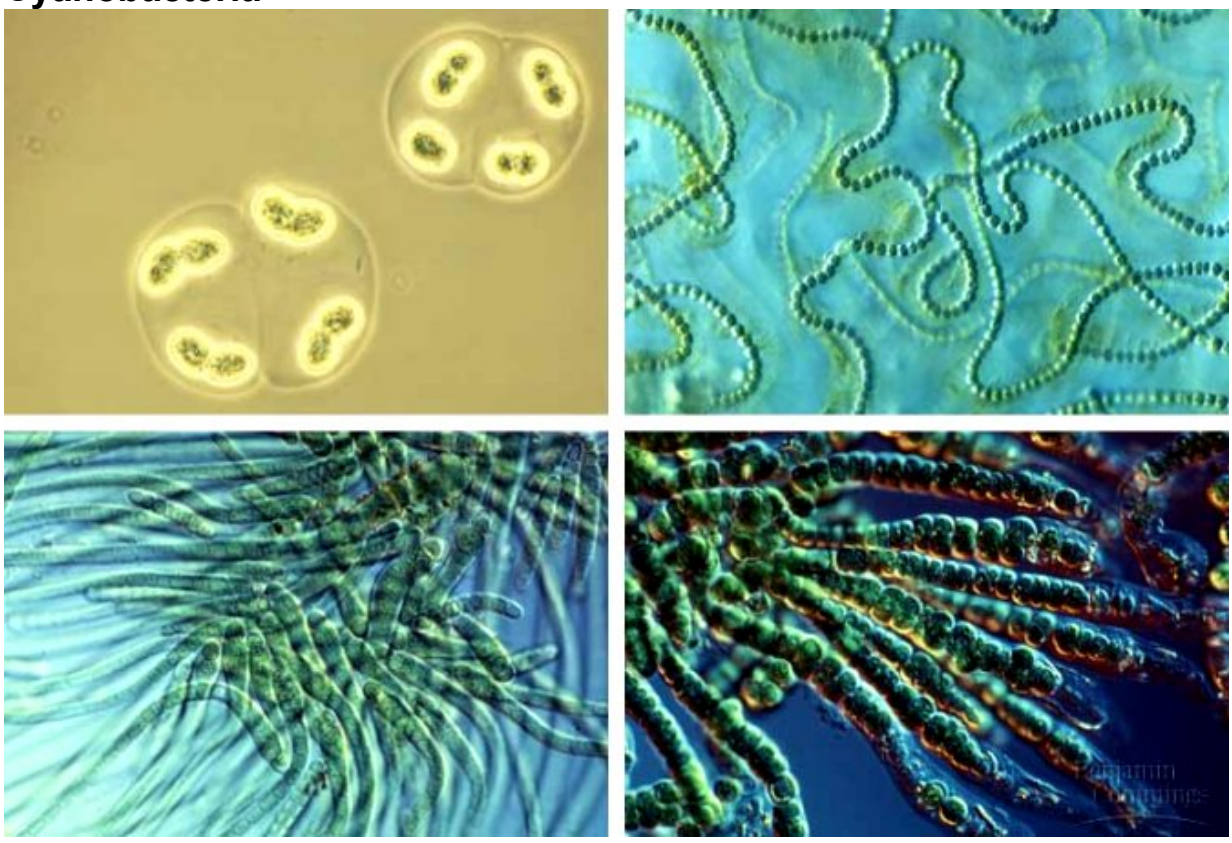
Some icosahedral and helical animal viruses are enclosed in a lipid envelope acquired when the virus buds through host-cell membranes. Inserted into this envelope are glycoproteins that the viral genome directs the cell to make; these molecules bind virus particles to susceptible host cells.

Bacteriophages
The most elaborate viruses are the bacteriophages, which use bacteria as their hosts. Some bacteriophages resemble an insect with an icosahedral head attached to a tubular sheath. From the base of the sheath extend several long tail fibers that help the virus attach to the bacterium and inject its DNA to be replicated, direct capsid production, and virus particle assembly inside the cell.

Viroids and Prions
Viroids and prions are smaller than viruses, but they are similarly associated with disease. Viroids are plant pathogens that consist only of a circular, independently replicating RNA molecule. The single-stranded RNA circle collapses on itself to form a rod-like structure. The only known mammalian pathogen that resembles plant viroids is the deltavirus (hepatitis D), which requires hepatitis B virus proteins to package its RNA into virus particles. Co-infection with hepatitis B and D can produce more severe disease than can infection with hepatitis B alone. Prions are mutated forms of a normal protein found on the surface of certain animal cells.

Virus Classification
Viruses are classified according to their type of genetic material, their strategy of replication, and their structure. The International Committee on Nomenclature of Viruses (ICNV), established in 1966, devised a scheme to group viruses into families, subfamilies, genera, and species. The ICNV report published in 1995 assigned more than 4000 viruses into 71 virus families. Hundreds of other viruses remain unclassified because of the lack of sufficient information.

Cyanobacteria



Cyanobacteria

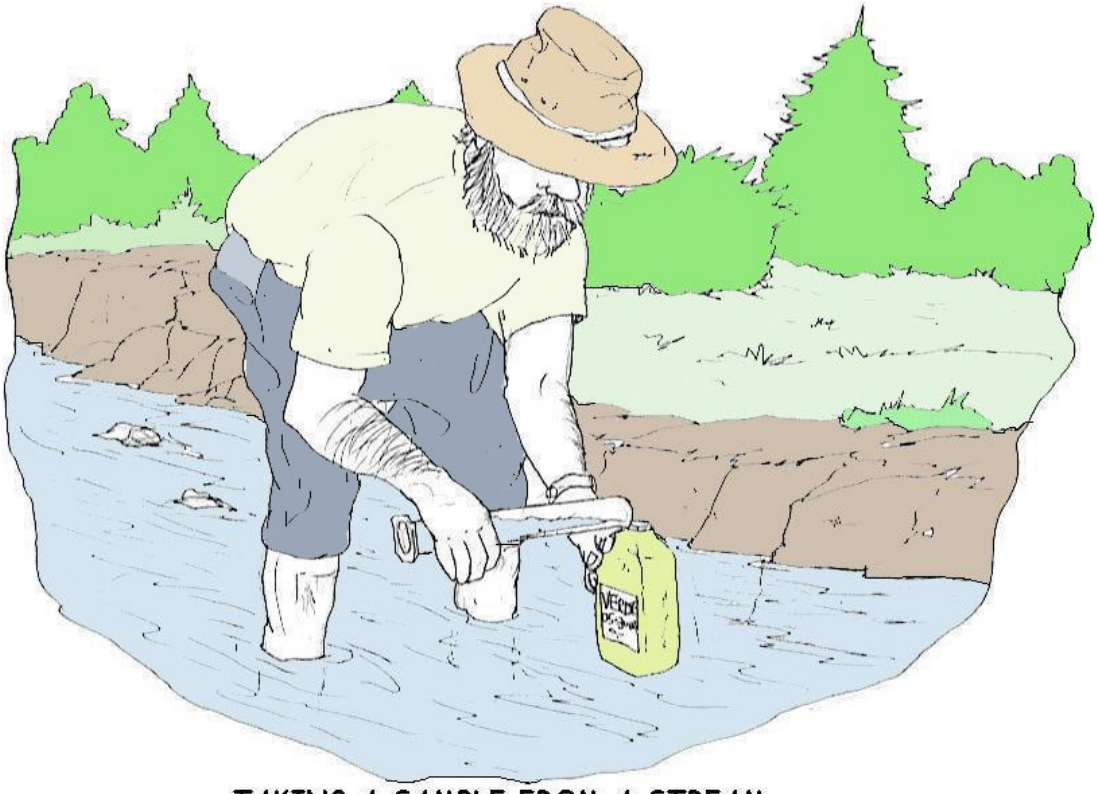
Cyanobacteria, also known as blue-green algae, blue-green bacteria or Cyanophyta, is a phylum of bacteria that obtain their energy through photosynthesis. The name "cyanobacteria" comes from the color of the bacteria (Greek: kyanós = blue). They are a significant component of the marine nitrogen cycle and an important primary producer in many areas of the ocean, but are also found on land.

Cyanobacteria include unicellular and colonial species. Colonies may form filaments, sheets or even hollow balls. Some filamentous colonies show the ability to differentiate into several different cell types: vegetative cells, the normal, photosynthetic cells that are formed under favorable growing conditions; akinetes, the climate-resistant spores that may form when environmental conditions become harsh; and thick-walled heterocysts, which contain the enzyme nitrogenase, vital for nitrogen fixation. Heterocysts may also form under the appropriate environmental conditions (anoxic) wherever nitrogen is necessary. Heterocyst-forming species are specialized for nitrogen fixation and are able to fix nitrogen gas, which cannot be used by plants, into ammonia (NH_3), nitrites (NO_2) or nitrates (NO_3), which can be absorbed by plants and converted to protein and nucleic acids.

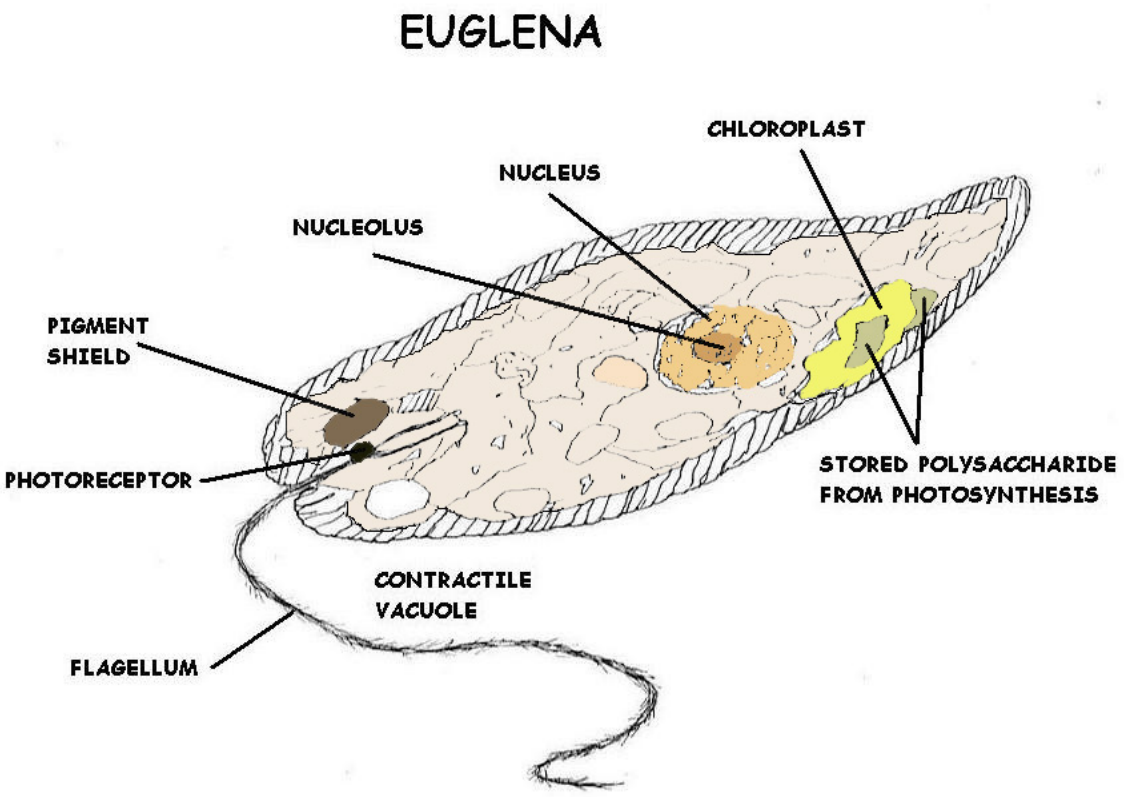
The rice paddies of Asia, which produce about 75% of the world's rice, could not do so were it not for healthy populations of nitrogen-fixing cyanobacteria in the rice paddy fertilizer too.

Many cyanobacteria also form motile filaments, called hormogonia, that travel away from the main biomass to bud and form new colonies elsewhere. The cells in a hormogonium are often thinner than in the vegetative state, and the cells on either end of the motile chain may be tapered. In order to break away from the parent colony, a hormogonium often must tear apart a weaker cell in a filament, called a necridium.

Each individual cell of a cyanobacterium typically has a thick, gelatinous cell wall. They differ from other gram-negative bacteria in that the quorum sensing molecules autoinducer-2[4] and acyl-homoserine lactones are absent. They lack flagella, but hormogonia and some unicellular species may move about by gliding along surfaces. In water columns some cyanobacteria float by forming gas vesicles, like in archaea.



TAKING A SAMPLE FROM A STREAM



Euglena

Euglenas are common protists, of the class Euglenoidea of the phylum Euglenophyta. Currently, over 1000 species of Euglena have been described. Marin et al. (2003) revised the genus so, and including several species without chloroplasts, formerly classified as Astasia and Khawkinea. Euglena sometimes can be considered to have both plant and animal features.

Euglena gracilis has a long hair-like thing that stretches from its body. You need a very powerful microscope to see it. This is called a flagellum, and the euglena uses it to swim. It also has a red eyespot. Euglena gracilis uses its eyespot to locate light. Without light, it cannot use its chloroplasts to make itself food. In order for Euglena gracilis to make more Euglena gracilis it will complete a process called mitosis. That means it can split itself in half and become two Euglena gracilis. It can only do this if it is well-fed and if the temperature is right. Euglena gracilis can reproduce better in warm temperatures.

Euglena gracilis, and other euglena, are harmless to people, but they are often signs that water is polluted, since they do well where there is a lot of green algae to eat. Green algae does well where there is a lot of nitrogen (comes from waste) in the water. If you don't clean your swimming pool, leaves and twigs get in the water and turn into waste. Then algae and euglena show up.

KINGDOM: Protist, PHYLUM: Euglenophyta, CLASS: Euglenophyceae, ORDER: Euglenales, FAMILY: Euglenidae, GENUS: Euglena, SPECIES: Euglena gracilis

Peptidoglycan

Peptidoglycan, also known as murein, is a polymer consisting of sugars and amino acids that forms a mesh-like layer outside the plasma membrane of eubacteria. The sugar component consists of alternating residues of β-(1,4) linked N-acetylglucosamine and N-acetylmuramic acid residues. Attached to the N-acetylmuramic acid is a peptide chain of three to five amino acids. The peptide chain can be cross-linked to the peptide chain of another strand forming the 3D mesh-like layer.



Hepatitis



There are five types of hepatitis -- A through E -- all of which cause inflammation of the liver. Type D affects only those who also have hepatitis B, and hepatitis E is extremely rare in the United States.

- Type A hepatitis is contracted through anal-oral contact, by coming in contact with the feces of someone with hepatitis A, or by eating or drinking hepatitis A contaminated food or water.
- Type B hepatitis can be contracted from infected blood, seminal fluid, vaginal secretions, or contaminated drug needles, including tattoo or body-piercing equipment. It can also be spread from a mother to her newborn.
- Type C hepatitis is not easily spread through sex. You're more likely to get it through contact with infected blood, contaminated razors, needles, tattoo and body-piercing equipment, or manicure or pedicure tools that haven't been properly sanitized, and a mother can pass it to her baby during delivery.
- Type D hepatitis can be passed through contact with infected blood, contaminated needles, or by sexual contact with an HIV-infected person.
- Type E hepatitis is most likely to be transmitted in feces, through oral contact, or in water that's been contaminated.

Sampling Glossary

ABIOGENESIS: The concept of spontaneous generation (that life can come from non-life). This idea was refuted by Pasteur.

ABIOTIC: The non-living components of an organism's environment. The term abiotic is also used to denote a process which is not facilitated by living organisms.

ABORAL: Pertaining to the region of the body opposite that of the mouth. Normally used to describe radially symmetrical animals.

ABSCISIC ACID (ABA): A plant hormone that generally acts to inhibit growth, promote dormancy, and help the plant withstand stressful conditions.

ABSENCE OF OXYGEN: The complete absence of oxygen in water described as Anaerobic.

ABSORPTION SPECTRUM: The range of a material's ability to absorb various wavelengths of light. The absorption spectrum is studied to evaluate the function of photosynthetic pigments.

ACCURACY: How closely an instrument measures the true or actual value.

ACCESSORY PIGMENT: A photosynthetic pigment which absorbs light and transfers energy to chlorophylls during photosynthesis. Because accessory pigments have different absorption optima than chlorophylls, presence of accessory pigments allows photosynthetic systems to absorb light more efficiently than would be possible otherwise.

ACELLULAR: Not within cells. Sometimes used as a synonym for unicellular (but multinucleate). Unicellular also pertains to single: celled organisms.

ACETYL COA: Acetyl CoenzymeA is the entry compound for the Krebs cycle in cellular respiration; formed from a fragment of pyruvic acid attached to a coenzyme.

ACETYLCHOLINE: A neurotransmitter substance that carries information across vertebrate neuromuscular junctions and some other synapses.

ACID: Slowly add the acid to water while stirring. An operator should not mix acid and water or acid to a strong base.

ACID AND BASE ARE MIXED: When an acid and a base are mixed, an explosive reaction occurs and decomposition products are created under certain conditions.

ACID RAIN: Rain that is excessively acidic due to the presence of acid: causing pollutants in the atmosphere. Pollutants include nitrogen and sulfur oxides due to burning of coal and oil.

ACIDOSIS: A condition whereby the hydrogen ion concentration of the tissues is increased (and pH decreased). Respiratory acidosis is due to the retention of CO_2; metabolic acidosis by retention of acids due either to kidney failure or diarrhea.

ACOELOMATE: Lacking a coelom.

ACQUIRED IMMUNITY: Results from exposure to foreign substances or microbes (also called natural immunity).

ACROSOME: An organelle at the tip of a sperm cell that helps the sperm penetrate the egg.

ACTH (adrenocorticotrophic hormone): A proteineinaceous hormone from the anterior pituitary that stimulates the adrenal cortex. Used to stimulate the production of cortisol.

ACTIN: A globular protein that links into chains, two of which twist helically about each other, forming microfilaments in muscle and other contractile elements in cells.

ACTION POTENTIAL: The stimulus- triggered change in the membrane potential of an excitable cell, caused by selective opening and closing of ion channels.

ACTION SPECTRUM: A graph which illustrates the relationship between some biological activity and wavelength of light.

ACTIVATING ENZYME: An enzyme that couples a low-energy compound with ATP to yield a high-energy derivative.

ACTIVATION ENERGY: In a chemical reaction, the initial investment required to energize the bonds of the reactants to an unstable transition state that precedes the formation of the products.

ACTIVE SITE: That specific portion of an enzyme that attaches to the substrate by means of weak chemical bonds.

ACTIVE TRANSPORT: The movement of a substance across a biological membrane against its concentration or electrochemical gradient with the help of energy input and specific transport proteins.

ACTIVATED SLUDGE: The biologically active solids in an activated sludge process wastewater treatment plant.

ACTIVATED SLUDGE PROCESS: A biological wastewater treatment process in which a mixture of wastewater and biologically enriched sludge is mixed and aerated to facilitate aerobic decomposition by microbes.

ADAPTATION: Any genetically controlled characteristic that increases an organism's fitness, usually by helping the organism to survive and reproduce in the environment it inhabits.

ADAPTIVE RADIATION: This refers to the rapid evolution of one or a few forms into many different species that occupy different habitats within a new geographical area.

ADHESION: In chemistry, the phenomenon whereby one substance tends to cling to another substance. Water molecules exhibit adhesion, especially toward charged surfaces.

ADP (Adenosine diphosphate): A doubly phosphorylated organic compound that can be further phosphorylated to form ATP.

ADRENAL GLAND: An endocrine gland located adjacent to the kidney in mammals. It is composed of an outer cortex, and a central medulla, each involved in different hormone: mediated phenomena.

ADRENALIN: A hormone produced by the pituitary that stimulates the adrenal cortex.

ADSORB: Hold on a surface.

ADSORPTION: *Not to be confused with absorption.* Adsorption is a process that occurs when a gas or liquid solute accumulates on the surface of a solid or a liquid (adsorbent), forming a film of molecules or atoms (the adsorbate). It is different from absorption, in which a substance diffuses into a liquid or solid to form a solution. The term sorption encompasses both processes, while desorption is the reverse process. Adsorption is present in many natural physical, biological, and chemical systems, and is widely used in industrial applications such as activated charcoal, synthetic resins, and water purification. Adsorption, ion exchange, and chromatography are sorption processes in which certain adsorbates are selectively transferred from the fluid phase to the surface of insoluble, rigid particles suspended in a vessel or packed in a column. Similar to surface tension, adsorption is a consequence of surface energy. In a bulk material, all the bonding requirements (be they ionic, covalent, or metallic) of the constituent atoms of the material are filled by other atoms in the material. However, atoms on the surface of the adsorbent are not wholly surrounded by other adsorbent atoms, and therefore can attract adsorbates. The exact nature of the bonding depends on the details of the species involved, but the adsorption process is generally classified as physisorption (characteristic of weak van der Waals forces) or chemisorption (characteristic of covalent bonding).

ADVANCED: New, unlike the ancestral condition.

AERATION: The addition of air or oxygen to water or wastewater, usually by mechanical means, to increase dissolved oxygen levels and maintains aerobic conditions.

AEROBIC: The condition of requiring oxygen; an aerobe is an organism which can live and grow only in the presence of oxygen.

AEROBIC DIGESTION: Sludge stabilization process involving direct oxidation of biodegradable matter and oxidation of microbial cellular material.

AGE STRUCTURE: The relative numbers of individuals of each age in a population.

AGNATHAN: A member of a jawless class of vertebrates represented today by the lampreys and hagfishes.

AGONISTIC BEHAVIOR: A type of behavior involving a contest of some kind that determines which competitor gains access to some resource, such as food or mates.

AIDS (acquired immune deficiency syndrome): A condition in which the body's helper T lymphocytes are destroyed, leaving the victim subject to opportunistic diseases.

AIR GAP SEPARATION: A physical separation space that is present between the discharge vessel and the receiving vessel; for an example, a kitchen faucet.

AIR ENTRAINMENT: The dissolution or inclusion of air bubbles into water.

ALCOHOL: Any of a class of organic compounds in which one or more - OH groups are attached to a carbon compound.

ALDEHYDE: An organic molecule with a carbonyl group located at the end of the carbon skeleton.

ALGAE: Microscopic plants that are free-living and usually live in water. They occur as single cells floating in water, or as multicellular plants like seaweed or strands of algae that attach to rocks.

ALPHA AND BETA RADIOACTIVITY: Represent two common forms of radioactive decay. Radioactive elements have atomic nuclei so heavy that the nucleus will break apart, or disintegrate spontaneously. When decay occurs, high-energy particles are released. These high-energy particles are called radioactivity. Although radioactivity from refined radioactive elements can be dangerous, it is rare to find dangerous levels of radioactivity in natural waters. An alpha particle is a doubly-charged helium nucleus comprised of two protons, two neutrons, and no electrons. A beta particle is a high-speed electron. Alpha particles do not penetrate matter easily, and are stopped by a piece of paper. Beta particles are much more penetrating and can pass through a millimeter of lead.

ALKALINE: Having a pH of more than 7. Alkaline solutions are also said to be basic.

ALKALINITY: Alkalinity or AT is a measure of the ability of a solution to neutralize acids to the equivalence point of carbonate or bicarbonate. Alkalinity is closely related to the acid neutralizing capacity (ANC) of a solution and ANC is often incorrectly used to refer to alkalinity. However, the acid neutralizing capacity refers to the combination of the solution and solids present (e.g., suspended matter, or aquifer solids), and the contribution of solids can dominate the ANC (see carbonate minerals below). The alkalinity is equal to the stoichiometric sum of the bases in solution. In the natural environment carbonate alkalinity tends to make up most of the total alkalinity due to the common occurrence and dissolution of carbonate rocks and presence of carbon dioxide in the atmosphere. Other common natural components that can contribute to alkalinity include borate, hydroxide, phosphate, silicate, nitrate, dissolved ammonia, the conjugate bases of some organic acids and sulfide. Solutions produced in a laboratory may contain a virtually limitless number of bases that contribute to alkalinity. Alkalinity is usually given in the unit mEq/L (milliequivalent per liter). Commercially, as in the pool industry, alkalinity might also be given in the unit ppm or parts per million. Alkalinity is sometimes incorrectly used interchangeably with basicity. For example, the pH of a solution can be lowered by the addition of CO_2. This will reduce the basicity; however, the alkalinity will remain unchanged.

ALLANTOIS: One of the four extraembryonic membranes found associated with developing vertebrates; it serves in gas exchange and as a repository for the embryo's nitrogenous waste. In humans, the allantois is involved in early blood formation and development of the urinary bladder.

ALLELE: Alternate forms of a gene which may be found at a given location (locus) on members of a homologous set of chromosomes. Structural variations between alleles may lead to different phenotypes for a given trait.

ALLOMETRIC: The variation in the relative rates of growth of various parts of the body, which helps shape the organism.

ALLOPATRIC SPECIATION: A type of speciation which occurs when a population becomes segregated into two populations by some sort of geographic barrier (also called geographic speciation). This phenomenon is presumed to have been the mechanism whereby many species of organisms evolved.

ALLOPOLYPLOID: A common type of polyploid species resulting from two different species interbreeding and combining their chromosomes.

ALL-OR-NONE: (event) An action that occurs either completely or not at all, such as the generation of an action potential by a neuron.

ALLOSTERIC ENZYME: An enzyme that can exist in two or more conformations.

ALLOSTERIC SITE: A receptor on an enzyme molecule which is remote from the active site. Binding of the appropriate molecule to the allosteric site changes the conformation of the active site, making it either more or less receptive to the substrate.

ALPHA HELIX: A spiral shape constituting one form of the secondary structure of proteins, arising from a specific hydrogen: bonding structure.

ALTERNATION OF GENERATIONS: Occurrences of a multicellular diploid form, the sporophyte, with a multicellular haploid form, the gametophyte.

ALTERNATIVE DISINFECTANTS: Disinfectants - other than chlorination (halogens) - used to treat water, e.g. ozone, ultraviolet radiation, chlorine dioxide, and chloramine. There is limited experience and scientific knowledge about the by-products and risks associated with the use of alternatives.

ALTRUISM: The willingness of an individual to sacrifice its fitness for the benefit of another.

ALUMINUM SULFATE: The chemical name for Alum. The molecular formula of Alum is $Al_2(SO_4)3{\sim}14H_2O$. It is a cationic polymer.

ALVEOLUS: One of the dead-end, multilobed air sacs that constitute the gas exchange surface of the lungs.

AMINO ACID: An organic molecule possessing a carboxyl (COOH) and amino group. Amino acids serve as the monomers of polypeptides and proteins.

AMINO GROUP: A functional group consisting of a nitrogen atom bonded to two hydrogens; can act as a base in solution, accepting a hydrogen ion and acquiring a charge of +1.

AMINOACYL: tRNA synthetases- A family of enzymes, at least one for each amino acid, that catalyze the attachment of an amino acid to its specific tRNA molecule.

AMOEBA: Amoeba (sometimes amœba or ameba, plural amoebae) is a genus of protozoa that moves by means of pseudopods, and is well-known as a representative unicellular organism. The word amoeba or ameba is variously used to refer to it and its close relatives, now grouped as the Amoebozoa, or to all protozoa that move using pseudopods, otherwise termed amoeboids.

AMOEBOID: (cell) A cell which has the tendency to change shape by protoplasmic flow. (movement) A streaming locomotion characteristic of Amoeba and other protists, as well as some individual cells, such as white blood cells, in animals.

AMMONIA: A chemical made with Nitrogen and Hydrogen and used with chlorine to disinfect water. Most ammonia in water is present as the ammonium ion rather than as ammonia.

AMP (Adenosine monophosphate): A singly phosphorylated organic compound that can be further phosphorylated to form ADP.

AMYLASE: A starch-digesting enzyme.

ANABOLISM: A metabolic pathway of biosynthesis that consumes energy to build a large molecule from simpler ones.

ANAEROBIC: Without oxygen. An organism which lives in the absence of oxygen is called an anaerobe. An abnormal condition in which color and odor problems are most likely to occur.

ANAEROBIC CONDITIONS: When anaerobic conditions exist in either the metalimnion or hypolimnion of a stratified lake or reservoir, water quality problems may make the water unappealing for domestic use without costly water treatment procedures. Most of these problems are associated with Reduction in the stratified waters.

ANAEROBIC DIGESTION: Sludge stabilization process where the organic material in biological sludges are converted to methane and carbon dioxide in an airtight reactor.

ANAGENESIS: A pattern of evolutionary change involving the transformation of an entire population, sometimes to a state different enough from the ancestral population to justify renaming it as a separate species; also called phyletic.

ANALOGOUS: Characteristics of organisms which are similar in function (and often in structure) but different in embryological and/or evolutionary origins.

ANALYST: The analyst must have at least 2 years of college lecture and laboratory course work in microbiology or a closely related field. The analyst also must have at least 6 months of continuous bench experience with environmental protozoa detection techniques and IFA microscopy, and must have successfully analyzed at least 50 water and/or wastewater samples for *Cryptosporidium* and *Giardia*. Six months of additional experience in the above areas may be substituted for two years of college.

ANCESTRAL TRAIT: Trait shared by a group of organisms as a result of descent from a common ancestor.

ANEUPLOIDY: A chromosomal aberration in which certain chromosomes are present in extra copies or are deficient in number.

ANION: A negatively charged ion.

ANISOGAMOUS: Reproducing by the fusion of gametes that differ only in size, as opposed to gametes that are produced by oogamous species. Gametes of oogamous species, such as egg cells and sperm, are highly differentiated.

ANNUAL: A plant that completes its entire life cycle in a single year or growing season.

ANOXIC: A biological environment that is deficient in molecular oxygen, but may contain chemically bound oxygen, such as nitrates and nitrites.

ANTERIOR: Referring to the head end of a bilaterally symmetrical animal.

ANTHROPOMORPHISM: Attributing a human characteristic to an inanimate object or a species other than a human.

ANTIBIOTIC: A chemical that kills or inhibits the growth of bacteria, often via transcriptional or translational regulation.

ANTIBODY: A protein, produced by the B lymphocytes of the immune system that binds to a particular antigen.

ANTICODON: The specialized base triplet on one end of a tRNA molecule that associates with a particular complementary codon on an mRNA molecule during protein synthesis.

ANTIDIURETIC HORMONE: A hormone important in osmoregulation (it acts to reduce the elimination of water from the body.

ANTIGEN: A foreign macromolecule that does not belong to the host organism and that elicits an immune response.

APOMORPHIC CHARACTER: A derived phenotypic character, or homology, that evolved after a branch diverged from a phylogenetic tree.

APOSEMATIC COLORATION: Serving as a warning, with reference particularly to colors and structures that signal possession of defensive device.

AQUEOUS SOLUTION: A solution in which water is the solvent.

ARCHAEBACTERIA: A lineage of prokaryotes, represented today by a few groups of bacteria inhabiting extreme environments. Some taxonomists place archaebacteria in their own kingdom, separate from the other bacteria.

ARCHENTERON: The endoderm-lined cavity formed during the gastrulation process that develops into the digestive tract of the animal.

ARISTOTLE: A Greek philosopher often credited as the first to use empirical and deductive methods in logic.

ARTIFICIAL SELECTION: The selective breeding of domesticated plants and animals to encourage the occurrence of desirable traits.

AS: The chemical symbol of Arsenic.

AS NITROGEN: An expression that tells how the concentration of a chemical is expressed mathematically. The chemical formula for the nitrate ion is NO3 , with a mass of 62. The concentration of nitrate can be expressed either in terms of the nitrate ion or in terms of the principal element, nitrogen. The mass of the nitrogen atom is 14. The ratio of the nitrate ion mass to the nitrogen atom mass is 4.43. Thus a concentration of 10 mg/L nitrate expressed as nitrogen would be equivalent to a concentration of 44.3 mg/L nitrate expressed as nitrate ion. When dealing with nitrate numbers it is very important to know how numeric values are expressed.

ASCUS: The elongate spore sac of a fungus of the Ascomycota group.

ASEXUAL: A type of reproduction involving only one parent that produces genetically identical offspring by budding or division of a single cell or the entire organism into two or more parts.

ASSORTATIVE MATING: A type of nonrandom mating in which mating partners resemble each other in certain phenotypic characters.

ASYMMETRIC CARBON: A carbon atom covalently bonded to four different atoms or groups of atoms.

ATOM: The general definition of an ion is an atom with a positive or negative charge. Electron is the name of a negatively charged atomic particle.

ATOMIC NUMBER: The number of protons in the nucleus of an atom, unique for each element.

ATOMIC THEORY: The physical theory of the structure, properties and behavior of the atom.

ATOMIC WEIGHT: The total atomic mass, which is the mass in grams of one mole of the atom (relative to that of 12C, which is designated as 12).

ATP (Adenosine triphosphate): A triply phosphorylated organic compound that functions as "energy currency" for organisms, thus allowing life forms to do work; it can be hydrolyzed in two steps (first to ADP and then to AMP) to liberate 7.3 Kcal of energy per mole during each hydrolysis.

ATPASE: An enzyme that functions in producing or using ATP.

AUTOGENOUS MODEL: A hypothesis which suggests that the first eukaryotic cells evolved by the specialization of internal membranes originally derived from prokaryotic plasma membranes.

AUTOIMMUNE DISEASE: An immunological disorder in which the immune system goes awry and turns against itself.

AUTONOMIC NERVOUS SYSTEM: A subdivision of the motor nervous system of vertebrates that regulates the internal environment; consists of the sympathetic and parasympathetic subdivisions.

AUTOPOLYPLOID: A type of polyploid species resulting from one species doubling its chromosome number to become tetraploids, which may self-fertilize or mate with other tetraploids.

AUTOSOME: Chromosomes that are not directly involved in determining sex.

AUTOTROPH: An organism which is able to make organic molecules from inorganic ones either by using energy from the sun or by oxidizing inorganic substances.

AUXIN: One of several hormone compounds in plants that have a variety of effects, such as phototropic response through stimulation of cell elongation, stimulation of secondary growth, and development of leaf traces and fruit.

AUXOTROPH: A nutritional mutant that is unable to synthesize and that cannot grow on media lacking certain essential molecules normally synthesized by wild-type strains of the same species.

AXON: A typically long outgrowth, or process, from a neuron that carries nerve impulses away from the cell body toward target cells.

AXONEME: An internal flagellar structure that occurs in some protozoa, such as *Giardia*, *Spironucleous*, and *Trichonmonas*.

B-CELL LYMPHOCYTE: A type of lymphocyte that develops in the bone marrow and later produces antibodies, which mediate humoral immunity.

BACKFLOW: To reverse the natural and normal directional flow of a liquid, gases, or solid substances back in to the public potable (drinking) water supply. This is normally an undesirable effect.

BACKFLOW PREVENTION: To stop or prevent the occurrence of, the unnatural act of reversing the normal direction of the flow of liquid, gases, or solid substances back in to the public potable (drinking) water supply. See Cross-connection control.

BACKSIPHONAGE: A liquid substance that is carried over a higher point. It is the method by which the liquid substance may be forced by excess pressure over or into a higher point.

BACTERIA: Small, one-celled animals too small to be seen by the naked eye. Bacteria are found everywhere, including on and in the human body. Humans would be unable to live without the bacteria that inhabit the intestines and assist in digesting food. Only a small percentage of bacteria cause disease in normal, healthy humans. Other bacteria can cause infections if they get into a cut or wound. Bacteria are the principal concern in evaluating the microbiological quality of drinking water, because some of the bacteria-caused diseases that can be transmitted by drinking water are potentially life-threatening.

BACTERIOPHAGE: A bacteriophage (from 'bacteria' and Greek phagein, 'to eat') is any one of a number of viruses that infect bacteria. The term is commonly used in its shortened form, phage. Typically, bacteriophages consist of an outer protein hull enclosing genetic material. The genetic material can be ssRNA (single stranded RNA), dsRNA, ssDNA, or dsDNA between 5 and 500 kilo base pairs long with either circular or linear arrangement. Bacteriophages are much smaller than the bacteria they destroy - usually between 20 and 200 nm in size.

BACTERIUM: A unicellular microorganism of the Kingdom Monera. Bacteria are prokaryotes; their cells have no true nucleus. Bacteria are classified into two groups based on a difference in cell walls, as determined by Gram staining.

BALANCED POLYMORPHISM: A type of polymorphism in which the frequencies of the coexisting forms do not change noticeably over many generations.

BARR BODY: The dense object that lies along the inside of the nuclear envelope in cells of female mammals, representing the one inactivated X chromosome.

BASAL BODY: A cell structure identical to a centriole that organizes and anchors the microtubule assembly of a cilium or flagellum.

BASE PAIRING: Complementary base pairing refers to the chemical affinities between specific base pairs in a nucleic acid: adenine always pairs with thymine, and guanine always pairs with cytosine. In pairing between DNA and RNA, the uracil of RNA always pairs with adenine. Complementary base pairing is not only responsible for the DNA double helix, but it is also essential for various in vitro techniques such as PCR (polymerase chain reaction). Complementary base pairing is also known as Watson-Crick pairing.

BASE: A substance that reduces the hydrogen ion concentration in a solution.

BASEMENT MEMBRANE: The floor of an epithelial membrane on which the basal cells rest.

BASIDIUM: The spore-bearing structure of Basidiomycota.

BATESIAN MIMICRY: A type of mimicry in which a harmless species looks like a different species that is poisonous or otherwise harmful to predators.

BEHAVIORAL ECOLOGY: A heuristic approach based on the expectation that Darwinian fitness (reproductive success) is improved by optimal behavior.

BELT PRESS: A dewatering device utilizing two opposing synthetic fabric belts, revolving over a series of rollers to “squeeze” water from the sludge.

BENIGN TUMOR: A noncancerous abnormal growth composed of cells that multiply excessively but remain at their place of origin in the body.

BENTHIC: Pertaining to the bottom region of an aquatic environment.

BENCH TEST: A small-scale test or study used to determine whether a technology is suitable for a particular application.

BEST AVAILABLE TECHNOLOGY ECONOMICALLY ACHIEVABLE (BAT): A level of technology based on the best existing control and treatment measures that are economically achievable within the given industrial category or subcategory.

BEST MANAGEMENT PRACTICES (BMPs): Schedules of activities, prohibitions of practices, maintenance procedures, and other management practices to prevent or reduce the pollution of waters of the U.S. BMPs also include treatment requirements, operating procedures and practices to control plant site runoff, spillage or leaks, sludge or waste disposal, or drainage from raw material storage.

BEST PRACTICABLE CONTROL TECHNOLOGY CURRENTLY AVAILABLE (BPT):
A level of technology represented by the average of the best existing wastewater treatment performance levels within an industrial category or subcategory.

BEST PROFESSIONAL JUDGMENT (BPJ): The method used by a permit writer to develop technology-based limitations on a case-by-case basis using all reasonably available and relevant data.

BETA PLEATED SHEET: A zigzag shape, constituting one form of the secondary structure of proteins formed of hydrogen bonds between polypeptide segments running in opposite directions.

BILATERAL SYMMETRY: The property of having two similar sides, with definite upper and lower surfaces and anterior and posterior ends. The Bilateria are members of the branch of Eumetazoa (Kingdom Animalia) which possess bilateral symmetry.

BILE: A mixture of substances containing bile salts, which emulsify fats and aid in their digestion and absorption.

BINARY FISSION: The kind of cell division found in prokaryotes, in which dividing daughter cells each receive a copy of the single parental chromosome.

BINOMIAL NOMENCLATURE: Consisting of two names. In biology, each organism is given a *genus* name and a species name (i.e., the human is Homo sapiens.

BIOCHEMICAL OXYGEN DEMAND (BOD): The BOD test is used to measure the strength of wastewater. The BOD of wastewater determines the milligrams per liter of oxygen required during stabilization of decomposable organic matter by aerobic bacteria action. Also, the total milligrams of oxygen required over a five-day test period to biologically assimilate the organic contaminants in one liter of wastewater maintained at 20 degrees Centigrade.

BIOGENESIS: A central concept of biology, that living organisms are derived from other living organisms (contrasts to the concept of abiogenesis, or spontaneous generation, which held that life could be derived from inanimate material).

BIOGEOCHEMICAL CYCLE: A circuit whereby a nutrient moves between both biotic and abiotic components of ecosystems.

BIOGEOGRAPHY: The study of the past and present distribution of species.

BIOLOGICAL MAGNIFICATION: Increasing concentration of relatively stable chemicals as they are passed up a food chain from initial consumers to top predators.

BIOLOGICAL SPECIES: A population or group of populations whose members have the potential to interbreed. This concept was introduced by Ernst Mayr.

BIOMASS: The total weight of all the organisms, or of a designated group of organisms, in a given area

BIOME: A large climatic region with characteristic sorts of plants and animals.

BIOSOLIDS: Solid organic matter recovered from municipal wastewater treatment that can be beneficially used, especially as a fertilizer. “Biosolids” are solids that have been stabilized within the treatment process, whereas “sludge” has not.

BIOSPHERE: The region on and surrounding the earth which is capable of supporting life. Theoretically, the concept may be ultimately expanded to include other regions of the universe.

BMR: The basal metabolic rate is the minimal energy (in kcal) required by a homeotherm to fuel itself for a given time. Measured within the thermoneutral zone for a postabsorptive animal at rest.

BODY FEED: Coating or bulking material added to the influent of material to be treated. This adds “body” to the material during filtration cycle.

BREAK POINT CHLORINATION: The process of chlorinating the water with significant quantities of chlorine to oxidize all contaminants and organic wastes and leave all remaining chlorine as free chlorine.

BROMINE: Chemical disinfectant (HALOGEN) that kills bacteria and algae. This chemical disinfectant has been used only on a very limited scale for water treatment because of its handling difficulties. This chemical causes skin burns on contact, and a residual is difficult to obtain.

BUFFER: Chemical that resists pH change, e.g. sodium bicarbonate

BULKING SLUDGE: A poor or slow settling activated sludge that results from the prevalence of filamentous organisms.

BULKING SLUDGE: A phenomenon that occurs in activated sludge plants whereby the sludge occupies excessive volumes and will not concentrate readily. This condition refers to a decrease in the ability of the sludge to settle and consequent loss over the settling tank weir. Bulking in activated sludge aeration tanks is caused mainly by excess suspended solids (SS) content. Sludge bulking in the final settling tank of an activated sludge plant may be caused by improper balance of the BOD load, SS concentration in the mixed liquor, or the amount of air used in aeration.

Ca: The chemical symbol for calcium.

CADMIUM: A contaminant that is usually not found naturally in water or in very small amounts.

CAKE: Dewatered sludge material with a satisfactory solids concentration to allow handling as a solid material.

CALCIUM HARDNESS: A measure of the calcium salts dissolved in water.

CALCIUM ION: Is divalent because it has a valence of +2.

CALCIUM, MAGNESIUM AND IRON: The three elements that cause hardness in water.

$CaOCl_2.4H_2O$: The molecular formula of Calcium hypochlorite.

CARBON DIOXIDE GAS: The pH will decrease and alkalinity will change as measured by the Langelier index after pumping carbon dioxide gas into water.

CARBONATE HARDNESS: Carbonate hardness is the measure of Calcium and Magnesium and other hard ions associated with carbonate ($CO_3$2-) and bicarbonate (HCO_3-) ions contained in a solution, usually water. It is usually expressed either as parts per million (ppm or mg/L), or in degrees (KH - from the German "Karbonathärte"). One German degree of carbonate hardness is equivalent to about 17.8575 mg/L.

Both measurements (mg/L or KH) are usually expressed "as $CaCO_3$" – meaning the amount of hardness expressed as if calcium carbonate was the sole source of hardness. Every bicarbonate ion only counts for half as much carbonate hardness as a carbonate ion does. If a solution contained 1 liter of water and 50 mg $NaHCO_3$ (baking soda), it would have a carbonate hardness of about 18 mg/L as $CaCO_3$. If you had a liter of water containing 50 mg of Na_2CO_3, it would have a carbonate hardness of about 29 mg/L as $CaCO_3$. Carbonate hardness supplements non-carbonate (a.k.a. "permanent") hardness where hard ions are associated with anions such as Chloride that do not precipitate out of solution when heated. Carbonate hardness is removed from water through the process of softening. Softening can be achieved by adding lime in the form of $Ca(OH)_2$, which reacts first with CO_2 to form calcium carbonate precipitate, reacts next with multi-valent cations to remove carbonate hardness, then reacts with anions to replace the non-carbonate hardness due to multi-valent cations with non-carbonate hardness due to calcium. The process requires recarbonation through the addition of carbon-dioxide to lower the pH which is raised during the initial softening process.

CARBONATE, BICARBONATE AND HYDROXIDE: Chemicals that are responsible for the alkalinity of water.

CAROLUS LINNAEUS: Swedish botanist and originator of the binomial nomenclature system of taxonomic classification

CATHODIC PROTECTION: An operator should protect against corrosion of the anode and/or the cathode by painting the copper cathode. Cathodic protection interrupts corrosion by supplying an electrical current to overcome the corrosion-producing mechanism. Guards against stray current corrosion.
CAUSTIC: NaOH (also called Sodium Hydroxide) is a strong chemical used in the treatment process to neutralize acidity, increase alkalinity or raise the pH value.

CAUSTIC SODA: Also known as sodium hydroxide and is used to raise pH.

CENTRATE: The liquid remaining after solids have been removed in a centrifuge.

CENTRIFUGE: A dewatering device relying on centrifugal force to separate particles of varying density such as water and solids.

CENTRIFUGAL FORCE: That force when a ball is whirled on a string that pulls the ball outward. On a centrifugal pump, it is that force which throws water from a spinning impeller.

CENTRIFUGAL PUMP: A pump consisting of an impeller fixed on a rotating shaft and enclosed in a casing, having an inlet and a discharge connection. The rotating impeller creates pressure in the liquid by the velocity derived from centrifugal force.

CHAIN OF CUSTODY (COC): A record of each person involved in the possession of a sample from the person who collects the sample to the person who analyzes the sample in the laboratory.

CHECK VALVE: Allows water to flow in only one direction.

CHELATION: A chemical process used to control scale formation in which a chelating agent "captures" scale-causing ions and holds them in solution.

CHEMICAL FEED RATE: Chemicals are added to the water in order to improve the subsequent treatment processes. These may include pH adjusters and coagulants. Coagulants are chemicals, such as alum, that neutralize positive or negative charges on small particles, allowing them to stick together and form larger particles that are more easily removed by sedimentation (settling) or filtration. A variety of devices, such as baffles, static mixers, impellers and in-line sprays, can be used to mix the water and distribute the chemicals evenly.

CHEMICAL OXIDIZER: KMnO4 is used for taste and odor control because it is a strong oxidizer which eliminates many organic compounds.

CHEMICAL REATION RATE: In general, when the temperature decreases, the chemical reaction rate also decreases. The opposite is true for when the temperature increases.

CHEMICAL SLUDGE: Sludge resulting from chemical treatment processes of inorganic wastes that are not biologically active.

CHEMICAL OXYGEN DEMAND (COD): The milligrams of oxygen required to chemically oxidize the organic contaminants in one liter of wastewater.

CHLORAMINATION: Treating drinking water by applying chlorine before or after ammonia. This creates a persistent disinfectant residual called chloramines.

CHLORAMINES: A group of chlorine ammonia compounds formed when chlorine combines with organic wastes in the water. Chloramines are not effective as disinfectants and are responsible for eye and skin irritation as well as strong chlorine odors (also known as Combined Chlorine).

CHLORINATION: The process in water treatment of adding chlorine (gas or solid hypochlorite) for purposes of disinfection.

CHLORINE: A chemical used to disinfect water. Chlorine is extremely reactive, and when it comes in contact with microorganisms in water it kills them. Chlorine is added to swimming pools to keep the water safe for swimming. Chlorine is available as solid tablets for swimming pools. Some public water system's drinking water treatment plants use chlorine in a gas form because of the large volumes required. Chlorine is very effective against algae, bacteria and viruses. Protozoa are resistant to chlorine because they have thick coats; protozoa are removed from drinking water by filtration.

CHLORINE DEMAND: Amount of chlorine required to react on various water impurities before a residual is obtained. Also, means the amount of chlorine required to produce a free chlorine residual of 0.1 mg/l after a contact time of fifteen minutes as measured by iodmetic method of a sample at a temperature of twenty degrees in conformance with Standard methods.

CHLORINE FEED: Chlorine may be delivered by vacuum-controlled solution feed chlorinators. The chlorine gas is controlled, metered, introduced into a stream of injector water and then conducted as a solution to the point of application.

CHLORINE, FREE: Chlorine available to kill bacteria or algae. The amount of chlorine available for sanitization after the chlorine demand has been met. Also known as chlorine residual.

CIRCULATION: The continual flow of drilling fluid from injection to recovery and recirculation at the surface.

ClO_2: The molecular formula of Chlorine dioxide.

CLARIFIER: A settling tank used to remove suspended solids by gravity settling. Commonly referred to as sedimentation or settling basins, they are usually equipped with a motor driven chain and flight or rake mechanism to collect settled sludge and move it to a final removal point.

COMPOSITE SAMPLE: To have significant meaning, samples for laboratory tests on wastewater should be representative of the wastewater. The best method of sampling is proportional composite sampling over several hours during the day. Composite samples are collected because the flow and characteristics of the wastewater are continually changing. A composite sample will give a representative analysis of the wastewater conditions.

COAGULATION: The best pH range for coagulation is between 5 and 7. Mixing is an important part of the coagulation process you want to complete the coagulation process as quickly as possible. A chemical added to initially destabilize, aggregate, and bind together colloids and emulsions to improve settleability, filterability, or drainability.

COLIFORM: Bacteria normally found in the intestines of warm-blooded animals. Coliform bacteria are present in high numbers in animal feces. They are an indicator of potential contamination of water. Adequate and appropriate disinfection effectively destroys coliform bacteria. Public water systems are required to deliver safe and reliable drinking water to their customers 24 hours a day, 365 days a year. If the water supply becomes contaminated, consumers can become seriously ill. Fortunately, public water systems take many steps to ensure that the public has safe, reliable drinking water. One of the most important steps is to regularly test the water for coliform bacteria. Coliform bacteria are organisms that are present in the environment and in the feces of all warm-blooded animals and humans. Coliform bacteria will not likely cause illness. However, their presence in drinking water indicates that disease-causing organisms (pathogens) could be in the water system. Most pathogens that can contaminate water supplies come from the feces of humans or animals. Testing drinking water for all possible pathogens is complex, time-consuming, and expensive. It is relatively easy and inexpensive to test for coliform bacteria. If coliform bacteria are found in a water sample, water system operators work to find the source of contamination and restore safe drinking water. There are three different groups of coliform bacteria; each has a different level of risk.

COLIFORM TESTING: The effectiveness of disinfection is usually determined by Coliform bacteria testing. A positive sample is a bad thing and indicates that you have bacteria contamination.

COLLOIDAL SUSPENSIONS: Because both iron and manganese react with dissolved oxygen to form insoluble compounds, they are not found in high concentrations in waters containing dissolved oxygen except as colloidal suspensions of the oxide.

COLORIMETRIC MEASUREMENT: A means of measuring an unknown chemical concentration in water by measuring a sample's color intensity.

CHRONIC: A stimulus that lingers or continues for a relatively long period of time, often one-tenth of the life span or more. Chronic should be considered a relative term depending on the life span of an organism. The measurement of chronic effect can be reduced growth, reduced reproduction, etc., in addition to lethality.

COMBINED CHLORINE: The reaction product of chlorine with ammonia or other pollutants, also known as chloramines.

COMPOSITE SAMPLE: A water sample that is a combination of a group of samples collected at various intervals during the day. A combination of individual samples of water or wastewater taken at predetermined intervals to minimize the effect of variability of individual samples.

COMPOSTING: Stabilization process relying on the aerobic decomposition of organic matter in sludge by bacteria and fungi.

CONDENSATION: The process that changes water vapor to tiny droplets or ice crystals.

CONTACT STABILIZATION PROCESS: Modification of the activated sludge process where raw wastewater is aerated with activated sludge for a short time prior to solids removal and continued aeration in a stabilization tank.

CONTACT TIME: If the water temperature decreases from 70°F (21°C) to 40°F (4°C). The operator needs to increase the detention time to maintain good disinfection of the water.

CONTAINS THE ELEMENT CARBON: A simple definition of an organic compound.

CONTAMINANT: Any natural or man-made physical, chemical, biological, or radiological substance or matter in water, which is at a level that may have an adverse effect on public health, and which is known or anticipated to occur in public water systems.

CONTAMINATE:
1. To make impure or unclean by contact or mixture.
2. To expose to or permeate with radioactivity.

CONTAMINATION: A degradation in the quality of groundwater in result of the it's becoming polluted with unnatural or previously non-existent constituents.

COPPER: The chemical name for the symbol Cu.

CORROSION: The removal of metal from copper, other metal surfaces and concrete surfaces in a destructive manner. Corrosion is caused by improperly balanced water or excessive water velocity through piping or heat exchangers.

CORROSIVITY: The Langelier Index measures corrosivity.

CROSS-CONNECTION: A physical connection between a public water system and any source of water or other substance that may lead to contamination of the water provided by the public water system through backflow. Might be the source of an organic substance causing taste and odor problems in a water distribution system.

CROSS-CONTAMINATION: The mixing of two unlike qualities of water. For example, the mixing of good water with a polluting substance like a chemical.

CRYPTOSPORIDIUM: A disease-causing parasite, resistant to chlorine disinfection. It may be found in fecal matter or contaminated drinking water. Cryptosporidium is a protozoan pathogen of the Phylum Apicomplexa and causes a diarrheal illness called cryptosporidiosis. Other apicomplexan pathogens include the malaria parasite Plasmodium, and Toxoplasma, the causative agent of toxoplasmosis. Unlike Plasmodium, which transmits via a mosquito vector, Cryptosporidium does not utilize an insect vector and is capable of completing its life cycle within a single host, resulting in cyst stages which are excreted in feces and are capable of transmission to a new host.

CYANURIC ACID: Chemical used to prevent the decomposition of chlorine by ultraviolet (UV) light.

CYANOBACTERIA: Cyanobacteria, also known as blue-green algae, blue-green bacteria or Cyanophyta, is a phylum of bacteria that obtain their energy through photosynthesis. The name "cyanobacteria" comes from the color of the bacteria (Greek: kyanós = blue). They are a significant component of the marine nitrogen cycle and an important primary producer in many areas of the ocean, but are also found on land.

CYST: A phase or a form of an organism produced either in response to environmental conditions or as a normal part of the life cycle of the organism. It is characterized by a thick and environmentally resistant cell wall.

DAILY MAXIMUM LIMITATIONS: The maximum allowable discharge of pollutants during a 24 hour period. Where daily maximum limitations are expressed in units of mass, the daily discharge is the total mass discharged over the course of the day. Where daily maximum limitations are expressed in terms of a concentration, the daily discharge is the arithmetic average measurement of the pollutant concentration derived from all measurements taken that day.

DANGEROUS CHEMICALS: The most suitable protection when working with a chemical that produces dangerous fumes is to work under an air hood.

DECANT: Separation of a liquid from settled solids by removing the upper layer of liquid after the solids have settled.

DECIBELS: The unit of measurement for sound.

DECOMPOSE: To decay or rot.

DECOMPOSTION OF ORGANIC MATERIAL: The decomposition of organic material in water produces taste and odors.

DEMINERALIZATION PROCESS: Mineral concentration of the feed water is the most important consideration in the selection of a demineralization process. Acid feed is the most common method of scale control in a membrane demineralization treatment system.

DENITRIFICATION: A biological process by which nitrate is converted to nitrogen gas.

DEPOLARIZATION: The removal of hydrogen from a cathode.

DESICCANT: When shutting down equipment which may be damaged by moisture, the unit may be protected by sealing it in a tight container. This container should contain a desiccant.

DESORPTION: Desorption is a phenomenon whereby a substance is released from or through a surface. The process is the opposite of sorption (that is, adsorption and absorption). This occurs in a system being in the state of sorption equilibrium between bulk phase (fluid, i.e. gas or liquid solution) and an adsorbing surface (solid or boundary separating two fluids). When the concentration (or pressure) of substance in the bulk phase is lowered, some of the sorbed substance changes to the bulk state. In chemistry, especially chromatography, desorption is the ability for a chemical to move with the mobile phase. The more a chemical desorbs, the less likely it will adsorb, thus instead of sticking to the stationary phase, the chemical moves up with the solvent front. In chemical separation processes, stripping is also referred to as desorption as one component of a liquid stream moves by mass transfer into a vapor phase through the liquid-vapor interface.

DIATOMACEOUS EARTH: A fine silica material containing the skeletal remains of algae.

DIGESTER: A tank or vessel used for sludge digestion.

DIGESTION: The biological decomposition of organic matter in sludge resulting in partial gasification, liquefaction, and mineralization of putrescible and offensive solids.

DIRECT CURRENT: A source of direct current (**DC**) may be used for standby lighting in a water treatment facility. The electrical current used in a DC system may come from a battery.

DISINFECT: The application of a chemical to kill most, but not all, microorganisms that may be present. Chlorine is added to public water drinking systems drinking water for disinfection. Depending on your state rule, drinking water must contain a minimum of 0.2 mg/L free chlorine. Disinfection makes drinking water safe to consume from the standpoint of killing pathogenic microorganisms including bacteria and viruses. Disinfection does not remove all bacteria from drinking water, but the bacteria that can survive disinfection with chlorine are not pathogenic bacteria that can cause disease in normal healthy humans.

DISINFECTION: The treatment of water to inactivate, destroy, and/or remove pathogenic bacteria, viruses, protozoa, and other parasites.

DISSOLVED OXYGEN: Can be added to zones within a lake or reservoir that would normally become anaerobic during periods of thermal stratification.

DISSOLVED SOLIDS: Solids in solution that cannot be removed by filtration with a 0.45 micron filter.

DISTILLATION, REVERSE OSMOSIS AND FREEZING: Processes that can be used to remove minerals from the water.

DRY ACID: A granular chemical used to lower pH and or total alkalinity.

E. COLI, Escherichia coli: A bacterium commonly found in the human intestine. For water quality analyses purposes, it is considered an indicator organism. These are considered evidence of water contamination. Indicator organisms may be accompanied by pathogens, but do not necessarily cause disease themselves.

ECDYSONE: A steroid hormone that triggers molting in arthropods.

ECOLOGICAL EFFICIENCY: The ratio of net productivity at one trophic level to net productivity at the next lower level.

ECOLOGICAL NICHE: The sum total of an organism's utilization of the biotic and abiotic resources of its environment. The fundamental niche represents the theoretical capabilities and the realized niche represents the actual role.

ECOLOGY: The study of how organisms interact with their environments.

ECOSYSTEM: The sum of physical features and organisms occurring in a given area.

ECTODERM: The outermost tissue layer of an animal embryo. Also, tissue derived from an embryonic ectoderm.

ECTOTHERM: An organism that uses environmental heat and behavior to regulate its body temperature.

EDWARD JENNER: A pioneer of vaccination; used vaccination with material from cowpox lesions to protect people against smallpox.

EFFECTIVENESS OF CHLORINE: The factors which influence the effectiveness of chlorination the most are pH, turbidity and temperature. Effectiveness of Chlorine decreases occurs during disinfection in source water with excessive turbidity.

EFFECTOR: The part of an organism that produces a response to a stimulus.

EFFLUENT: Partially or completely treated water or wastewater flowing out of a basin or treatment plant.

ELECTRICAL SYNAPSE: A junction between two neurons separated only by a gap junction, in which the local currents sparking the action potential pass directly between the cells.

ELECTROCARDIOGRAM: A plot of electrical activity of the heart over the cardiac cycle; measured via multiple skin electrodes.

ELECTROCHEMICAL GRADIENT: Combined electrostatic and osmotic-concentration gradient, such as the chemiosmotic gradient of mitochondria and chloroplasts.

ELECTROGENIC PUMP: An ion transport protein generating voltage across a membrane.

ELECTROMAGNETIC SPECTRUM: The entire spectrum of radiation; ranges in wavelength from less than a nanometer to more than a kilometer.

ELECTRON: The name of a negatively charged atomic particle. A negatively charged subatomic particle of an atom or ion. In atoms, the number of electrons present is equal to the number of positively charged protons present. Hence, atoms are electrically neutral.

ELECTRON MICROSCOPE: A microscope that focuses an electron beam through a specimen, resulting in resolving power a thousandfold greater that of a light microscope. A transmission EM is used to study the internal structure of thin sections of cells; a scanning EM is used to study the ultrastructure of surfaces.

ELECTRON TRANSPORT CHAIN: A series of enzymes found in the inner membranes of mitochondria and chloroplasts. These are involved in transport of protons and electrons either across the membrane during ATP synthesis.

ELECTRONEGATIVITY: A property exhibited by some atoms whereby the nucleus has a tendency to pull electrons toward itself.

ELECTRONIC CHARGE UNIT: The charge of one electron (1.6021 x 10e - 19 coulomb).

ELECTROSTATIC FORCE: The attraction between particles with opposite charges.

ELECTROSTATIC GRADIENT: The free-energy gradient created by a difference in charge between two points, generally the two sides of a membrane.

ELEMENT: Any substance that cannot be broken down into another substance by ordinary chemical means.

ELIMINATION: The release of unabsorbed wastes from the digestive tract.

EMERGENT PROGERTY: A property exhibited at one level of biological organization but not exhibited at a lower level. For example, a population exhibits a birth rate, an organism does not.

EMULSION: A suspension, usually as fine droplets of one liquid in another. A mixture made up of dissimilar elements, usually of two or more mutually insoluble liquids that would normally separate into layers based on the specific gravity of each liquid.

ENDERGONIC: A phenomenon which involves uptake of energy.

ENDOCRINE: A phenomenon which relates to the presence of ductless glands of the type typically found in vertebrates. The endocrine system involves hormones, the glands which secrete them, the molecular hormone receptors of target cells, and interactions between hormones and the nervous system.

ENDOCYTOSIS: A process by which liquids or solid particles are taken up by a cell through invagination of the plasma membrane.

ENDODERM: The innermost germ layer of an animal embryo.

ENDODERMIS: A plant tissue, especially prominent in roots, that surrounds the vascular cylinder; all endodermal cells have Casparian strips.

ENDOMEMBRANE SYSTEM: The system of membranes inside a eukaryotic cell, including the membranous vesicles which associate with membrane sheets and/or tubes.

ENDOMETRIUM: The inner lining of the uterus, which is richly supplied with blood vessels that provide the maternal part of the placenta and nourish the developing embryo.

ENDONUCLEASE: An enzyme that breaks bonds within nucleic acids. A restriction endonuclease is an enzyme that breaks bonds only within a specific sequence of bases.

ENDOPLASMIC RETICULUM: A system of membrane-bounded tubes and flattened sacs, often continuous with the nuclear envelope, found in the cytoplasm of eukaryotes. Exists as rough ER, studded with ribosomes, and smooth ER, lacking ribosomes.

ENDORPHIN: A hormone produced in the brain and anterior pituitary that inhibits pain perception.

ENDOSKELETON: An internal skeleton.

ENDOSPERM: A nutritive material in plant seeds which is triploid (3n) and results from the fusion of three nuclei during double fertilization.

ENDOSYMBIOTIC: 1) An association in which the symbiont lives within the host 2) A widely accepted hypothesis concerning the evolution of the eukaryotic cell: the idea that eukaryotes evolved as a result of symbiotic associations between prokaryote cells. Aerobic symbionts ultimately evolved into mitochondria; photosynthetic symbionts became chloroplasts.

ENDOTHELIUM: The innermost, simple squamous layer of cells lining the blood vessels; the only constituent structure of capillaries.

ENDOTHERMIC: In chemistry, a phenomenon in which energy is absorbed by the reactants. In physiology, this term concerns organisms whose thermal relationship with the environment is dependent substantially on internal production of heat.

ENDOTOXIN: A component of the outer membranes of certain gram-negative bacteria responsible for generalized symptoms of fever and ache.

ENERGY: The capacity to do work by moving matter against an opposing force.

ENHANCER: A DNA sequence that recognizes certain transcription factors that can stimulate transcription of nearby genes.

ENTAMOEBA HISTOLYTICA: Entamoeba histolytica, another water-borne pathogen, can cause diarrhea or a more serious invasive liver abscess. When in contact with human cells, these amoebae are cytotoxic. There is a rapid influx of calcium into the contacted cell, it quickly stops all membrane movement save for some surface blebbing. Internal organization is disrupted, organelles lyse, and the cell dies. The ameba may eat the dead cell or just absorb nutrients released from the cell.

ENTERIC: Rod-shaped, gram-negative, aerobic but can live in certain anaerobic conditions; produce nitrite from nitrate, acids from glucose; include Escherichia coli, Salmonella (over 1000 types), and Shigella.

ENTEROVIRUS: A virus whose presence may indicate contaminated water; a virus that may infect the gastrointestinal tract of humans.

ENTROPY: A type of energy which is not biologically useful to do work (in contrast to free energy).

ENVELOPE: 1) (nuclear) The surface, consisting of two layers of membrane, that encloses the nucleus of eukaryotic cells. 2) (virus) A structure which is present on the outside of some viruses (exterior to the capsid).

ENVIRONMENT: Water, air, and land, and the interrelationship that exists among and between water, air and land and all living things. The total living and nonliving aspects of an organism's internal and external surroundings.

ENZYME: A protein, on the surface of which are chemical groups so arranged as to make the enzyme a catalyst for a chemical reaction.

EPICOTYL: A portion of the axis of a plant embryo above the point of attachment of the cotyledons; forms most of the shoot.

EPIDERMIS: The outermost portion of the skin or body wall of an animal.

EPINEPHRINE: A hormone produced as a response to stress; also called adrenaline.

EPIPHYTE: A plant that nourishes itself but grows on the surface of another plant for support, usually on the branches or trunks of tropical trees.

EPISOME: Genetic element at times free in the cytoplasm, at other times integrated into a chromosome.

EPISTASIS: A phenomenon in which one gene alters the expression of another gene that is independently inherited.

EPITHELIUM: An animal tissue that forms the covering or lining of all free body surfaces, both external and internal.

EPITOPE: A localized region on the surface of an antigen that is chemically recognized by antibodies; also called antigenic determinant.

EQUATION: A precise representation of the outcome of a chemical reaction, showing the reactants and products, as well as the proportions of each.

EQUILIBRIUM: In a reversible reaction, the point at which the rate of the forward reaction equals that of the reverse reaction. (constant) At equilibrium, the ratio of products to reactants. (potential) The membrane potential for a given ion at which the voltage exactly balances the chemical diffusion gradient for that ion.

ERNST MAYR: Formulated the biological species concept.

ERYTHROCYTE: A red blood corpuscle.

ESOPHAGUS: An anterior part of the digestive tract; in mammals it leads from the pharynx to the stomach.

ESSENTIAL: 1) An amino or fatty acid which is required in the diet of an animal because it cannot be synthesized. 2) A chemical element required for a plant to grow from a seed and complete the life cycle.

ESTIVATION: A physiological state characterized by slow metabolism and inactivity, which permits survival during long periods of elevated temperature and diminished water supplies.

ESTRADIOL: 1,3,5(10)-estratriene- 3,17 beta-diol C18H24O2. This is the natural hormone - present in pure form in the urine of pregnant mares and in the ovaries of pigs.

ESTROGEN: Any of a group of vertebrate female sex hormones.

ESTROUS CYCLE: In female mammals, the higher primates excepted, a recurrent series of physiological and behavioral changes connected with reproduction.

ESTRUS: The limited period of heat or sexual receptivity that occurs around ovulation in female mammals having estrous cycles.

ESTUARY: That portion of a river that is close enough to the sea to be influenced by marine tides.

ETHYLENE: The only gaseous plant hormone, responsible for fruit ripening, growth inhibition, leaf abscission, and aging.

EUBACTERIA: The lineage of prokaryotes that includes the cyanobacteria and all other contemporary bacteria except archaebacteria.

EUCHROMATIN: The more open, unraveled form of eukaryotic chromatin, which is available for transcription.

EUCOELOMATE: An animal whose body cavity is completely lined by mesoderm, the layers of which connect dorsally and ventrally to form mesenteries.

EUGLENA: Euglena are common protists, of the class Euglenoidea of the phylum Euglenophyta. Currently, over 1000 species of Euglena have been described. Marin et al. (2003) revised the genus so and including several species without chloroplasts, formerly classified as Astasia and Khawkinea. Euglena sometimes can be considered to have both plant and animal features. Euglena gracilis has a long hair-like thing that stretches from its body. You need a very powerful microscope to see it. This is called a flagellum, and the euglena uses it to swim. It also has a red eyespot. Euglena gracilis uses its eyespot to locate light. Without light, it cannot use its chloroplasts to make itself food.

EUKARYOTE: A life form comprised of one or more cells containing a nucleus and membrane - bound organelles. Included are members of the Kingdoms Protista, Fungi, Plantae and Animalia.

EUMETAZOA: Members of the subkingdom that includes all animals except sponges.

EUTROPHIC: A highly productive condition in aquatic environments which owes to excessive concentrations of nutrients which support the growth of primary producers.

EVAGINATED: Folded or protruding outward.

EVAPORATIVE COOLING: The property of a liquid whereby the surface becomes cooler during evaporation, owing to the loss of highly kinetic molecules to the gaseous state.

EVERSIBLE: Capable of being turned inside out.

EVOLUTION: A theory that all of the changes that have transformed life on earth from its earliest beginnings to the diversity that characterizes it today. As used in biology, the term evolution means descent with change. See Intelligent Design.

EXCITABLE CELLS: A cell, such as a neuron or a muscle cell that can use changes in its membrane potential to conduct signals.

EXCITATORY POSTSYNAPTIC POTENTIAL: An electrical change (depolarization) in the membrane of a postsynaptic neuron caused by the binding of an excitatory neurotransmitter from a presynaptic cell to a postsynaptic receptor. This phenomenon facilitates generation of an action potential in the PSP.

EXCRETION: Release of materials which arise in the body due to metabolism (e.g., CO_2, NH_3, H_20).

EXERGONIC: A phenomenon which involves the release of energy.

EXOCYTOSIS: A process by which a vesicle within a cell fuses with the plasma membrane and releases its contents to the outside.

EXON: A part of a primary transcript (and the corresponding part of a gene) that is ultimately either translated (in the case of mRNA) or utilized in a final product, such as tRNA.

EXOSKELETON: An external skeleton, characteristic of members of the phylum, Arthropoda.

EXOTHERMIC: A process or reaction that is accompanied by the creation of heat.

EXOTOXIN: A toxic protein secreted by a bacterial cell that produces specific symptoms even in the absence of the bacterium.

EXPONENTIAL: (population growth) The geometric increase of a population as it grows in an ideal, unlimited environment.

EXTRAEMBRYONIC MEMBRANES: Four membranes (yolk sac, amnion, chorion, allantois) that support the developing embryo in reptiles, birds, and mammals.

EXTRINSIC: External to, not a basic part of; as in extrinsic isolating mechanism.

F: The chemical symbol of Fluorine.

F PLASMID: The fertility factor in bacteria, a plasmid that confers the ability to form pili for conjugation and associated functions required for transfer of DNA from donor to recipient.

F1 GENERATION: The first filial or hybrid offspring in a genetic cross-fertilization.

F2 GENERATION: Offspring resulting from interbreeding of the hybrid F1 generation.

FACILITATED DIFFUSION: Passive movement through a membrane involving a specific carrier protein; does not proceed against a concentration gradient.

FACULTATIVE: An organism which exhibits the capability of changing from one habit or metabolic pathway to another, when conditions warrant. (anaerobe) An organism that makes ATP by aerobic respiration if oxygen is present but that switches to fermentation under anaerobic conditions.

FAT: A biological compound consisting of three fatty acids linked to one glycerol molecule.

FATE MAP: A means of tracing the fates of cells during embryonic development.

FATTY ACID: A long carbon chain carboxylic acid. Fatty acids vary in length and in the number and location of double bonds; three fatty acids linked to a glycerol molecule form fat.

FAUNA: The animals of a given area or period.

FEATURE DETECTOR: A circuit in the nervous system that responds to a specific type of feature, such as a vertically moving spot or a particular auditory time delay.

FECAL COLIFORM: A group of bacteria that may indicate the presence of human or animal fecal matter in water. Total coliform, fecal coliform, and E. coli are all indicators of drinking water quality. The total coliform group is a large collection of different kinds of bacteria. Fecal coliforms are types of total coliform that mostly exist in feces. E. coli is a sub-group of fecal coliform. When a water sample is sent to a lab, it is tested for total coliform. If total coliform is present, the sample will also be tested for either fecal coliform or E. coli, depending on the lab testing method.

FECES: Indigestible wastes discharged from the digestive tract.

FEEDBACK: The process by which a control mechanism is regulated through the very effects it brings about. Positive feedback is when the effect is amplified; negative feedback is when the effect tends toward restoration of the original condition. Feedback inhibition is a method of metabolic control in which the end-product of a metabolic pathway acts as an inhibitor of an enzyme within that pathway.

FERMENTATION: Anaerobic production of alcohol, lactic acid or similar compounds from carbohydrate resulting from glycolysis.

FERRIC CHLORIDE: An iron salt commonly used as a coagulant. Chemical formula is FeCl3.

FIBRIN: The activated form of the blood: clotting protein fibrinogen, which aggregates into threads that form the fabric of the clot.

FIBROBLAST: A type of cell in loose connective tissue that secretes the protein ingredients of the extracellular fibers.

FIBRONECTINS: A family of extracellular glycoproteins that helps embryonic cells adhere to their substrate as they migrate.

FILTER: A device utilizing a granular material, woven cloth or other medium to remove pollutants from water, wastewater or air.

FILTER AID: A polymer or other material added to improve the effectiveness of the filtration process.

FILTER CAKE: The layer of solids that is retained on the surface of a filter.

FILTER CLOGGING: An inability to meet demand may occur when filters are clogging.

FILTER PRESS: A dewatering device where sludge is pumped onto a filtering medium and water is forced out of the sludge, resulting in a “cake”.

FILTRATE: Liquid remaining after removal of solids with filtration.

FILTRATION RATE: A measurement of the volume of water applied to a filter per unit of surface area in a given period of time.

FITNESS: The extent to which an individual passes on its genes to the next generation. Relative fitness is the number of offspring of an individual compared to the mean.

FIXATION: 1) Conversion of a substance into a biologically more usable form, for example, CO_2 fixation during photosynthesis and N_2 fixation. 2) Process of treating living tissue for microscopic examination.

FIXED ACTION PATTERN (FAP): A highly: stereotyped behavior that is innate and must be carried to completion once initiated.

FLACCID: Limp; walled cells are flaccid in isotonic surroundings, where there is no tendency for water to enter.

FLAGELLIN: The protein from which prokaryotic flagella are constructed.

FLAGELLUM: A long whip-like appendage that propels cells during locomotion in liquid solutions. The prokaryote flagellum is comprised of a protein, flagellin. The eukaryote flagellum is longer than a cilium, but as a similar internal structure of microtubules in a"9 + 2" arrangement.

FLAME CELL: A flagellated cell associated with the simplest tubular excretory system, present in flatworms: it acts to directly regulate the contents of the extracellular fluid.

FLOCCULATION: The process of bringing together destabilized or coagulated particles to form larger masses that can be settled and/or filtered out of the water being treated. Conventional coagulation–flocculation-sedimentation practices are essential pretreatments for many water purification systems—especially filtration treatments. These processes agglomerate suspended solids together into larger bodies so that physical filtration processes can more easily remove them. Particulate removal by these methods makes later filtering processes far more effective. The process is often followed by gravity separation (sedimentation or flotation) and is always followed by filtration. A chemical coagulant, such as iron salts, aluminum salts, or polymers, is added to source water to facilitate bonding among particulates. Coagulants work by creating a chemical reaction and eliminating the negative charges that cause particles to repel each other. The coagulant-source water mixture is then slowly stirred in a process known as flocculation. This water churning induces particles to collide and clump together into larger and more easily removable clots, or “flocs.” The process requires chemical knowledge of source water characteristics to ensure that an effective coagulant mix is employed. Improper coagulants make these treatment methods ineffective. The ultimate effectiveness of coagulation/flocculation is also determined by the efficiency of the filtering process with which it is paired.

FLOCCULANTS: Flocculants, or flocculating agents, are chemicals that promote flocculation by causing colloids and other suspended particles in liquids to aggregate, forming a floc. Flocculants are used in water treatment processes to improve the sedimentation or filterability of small particles. For example, a flocculant may be used in swimming pool or drinking water filtration to aid removal of microscopic particles which would otherwise cause the water to be cloudy and which would be difficult or impossible to remove by filtration alone. Many flocculants are multivalent cations such as aluminum, iron, calcium or magnesium. These positively charged molecules interact with negatively charged particles and molecules to reduce the barriers to aggregation. In addition, many of these chemicals, under appropriate pH and other conditions such as temperature and salinity, react with water to form insoluble hydroxides which, upon precipitating, link together to form long chains or meshes, physically trapping small particles into the larger floc. Long-chain polymer flocculants, such as modified polyacrylamides, are manufactured and sold by the flocculant producing business. These can be supplied in dry or liquid form for use in the flocculation process. The most common liquid polyacrylamide is supplied as an emulsion with 10-40 % actives and the rest is a carrier fluid, surfactants and latex. Emulsion polymers require activation to invert the emulsion and allow the electrolyte groups to be exposed.
The following chemicals are used as flocculants:
* alum
* aluminum chlorohydrate
* aluminum sulfate
* calcium oxide
* iron(III) chloride
* iron(II) sulfate
* polyacrylamide
* sodium aluminate
* sodium silicate

FLOC SHEARING: Likely to happen to large floc particles when they reach the flocculation process.

FLOCCULATION BASIN: A compartmentalized basin with a reduction of speed in each compartment. This set-up or basin will give the best overall results.

FLOOD RIM: The point of an object where the water would run over the edge of something and begin to cause a flood.

FLORA: The plants of a given area or period.

FLOW CYTOMETER: A particle-sorting instrument capable of counting protozoa.

FLOW MUST BE MEASURED: A recorder that measures flow is most likely to be located in a central location.

FLUID FEEDER: An animal that lives by sucking nutrient-rich fluids from another living organism.

FLUID MOSAIC MODEL: The currently accepted model of cell membrane structure, which envisions the membrane as a mosaic of individually inserted protein molecules drifting laterally in a fluid bilayer of phospholipids.

FLUX: The term flux describes the rate of water flow through a semipermeable membrane. When the water flux decreases through a semipermeable membrane, it means that the mineral concentration of the water is increasing.

FLY ASH: The noncombustible particles in flue gas. Often used as a body feed or solidification chemical.

FOLLICLE STIMULATING HORMONE (FSH): A gonadotropic hormone of the anterior pituitary that stimulates growth of follicles in the ovaries of females and function of the seminiferous tubules in males.

FOLLICLE: A jacket of cells around an egg cell in an ovary.

FOOD CHAIN: Sequence of organisms, including producers, consumers, and decomposers, through which energy and materials may move in a community.

FOOD WEB: The elaborate, interconnected feeding relationships in an ecosystem.

FOOT CANDLE: Unit of illumination; the illumination of a surface produced by one standard candle at a distance of one foot.

FORMULA: A precise representation of the structure of a molecule or ion, showing the proportion of atoms which comprise the material.

FOUNDER EFFECT: The difference between the gene pool of a population as a whole and that of a newly isolated population of the same species.

FRACTIONATION: An experimental technique which involves separation of parts of living tissue from one another using centrifugation.

FRAGMENTATION: A mechanism of asexual reproduction in which the parent plant or animal separates into parts that reform whole organisms.

FRAMESHIFT MUTATION: A mutation occurring when the number of nucleotides inserted or deleted is not a multiple of 3, thus resulting in improper grouping into codons.

FREE CHLORINE: In disinfection, chlorine is used in the form of free chlorine or as hypochlorite ion.

FREE CHLORINE RESIDUAL: Regardless of whether pre-chloration is practiced or not, a free chlorine residual of at least 10 mg/L should be maintained in the clear well or distribution reservoir immediately downstream from the point of post-chlorination. The reason for chlorinating past the breakpoint is to provide protection in case of backflow.

FREE ENERGY OF ACTIVATION: See Activation energy.

FREE ENERGY: Usable energy in a chemical system; energy available for producing change.

FREE OIL: Non-emulsified oil that separates from water, in a given period of time.

FREQUENCY DEPENDENT SELECTION: A decline in the reproductive success of a morph resulting from the morph's phenotype becoming too common in a population; a cause of balanced polymorphism in populations.

FUNCTIONAL GROUP: One of several groups of atoms commonly found in organic molecules. A functional group contributes somewhat predictable properties to the molecules which possess them.

FUNDAMENTAL NICHE: The total resources an organism is theoretically capable of utilizing.

G: (protein) A membrane protein that serves as an intermediary between hormone receptors and the enzyme adenylate cyclase, which converts ATP to cAMP in the second messenger system in non-steroid hormone action. Depending on the system, G proteins either increase or decrease cAMP production.

G1 PHASE: The first growth phase of the cell cycle, consisting of the portion of interphase before DNA synthesis is initiated.

G2 PHASE: The second growth phase of the cell cycle, consisting of the portion of interphase after DNA synthesis but before mitosis.

GAIA HYPOTHESIS: An idea, first formulated by James E. Lovelock in 1979, which suggests that the biosphere of the earth exists as a "superorganism" which exhibits homeostatic self- regulation of the environment-biota global system.

GAMETANGIUM: The reproductive organ of bryophytes, consisting of the male antheridium and female archegonium; a multi-chambered jacket of sterile cells in which gametes are formed.

GAMETE: A sexual reproductive cell that must usually fuse with another such cell before development begins; an egg or sperm.

GAMETOPHYTE: A haploid plant that can produce gametes.

GANGLION: A structure containing a group of cell bodies of neurons.

GAP JUNCTION: A narrow gap between plasma membranes of two animal cells, spanned by protein channels. They allow chemical substances or electrical signals to pass from cell to cell.

GASTRULA: A two-layered, later three-layered, animal embryonic stage.

GASTRULATION: The process by which a blastula develops into a gastrula, usually by an involution of cells.

GATED ION CHANNEL: A membrane channel that can open or close in response to a signal, generally a change in the electrostatic gradient or the binding of a hormone, transmitter, or other molecular signal.

GEL ELECTROPHORESIS: In general, electrophoresis is a laboratory technique used to separate macromolecules on the basis of electric charge and size; the technique involves application of an electric field to a population of macromolecules which disperse according to their electric mobilities. In gel electrophoresis, the porous medium through which the macromolecules move is a gel.

GEL: Colloid in which the suspended particles form a relatively orderly arrangement.

GENE: The hereditary determinant of a specified characteristic of an individual; specific sequences of nucleotides in DNA.

GENE AMPLIFICATION: Any of the strategies that give rise to multiple copies of certain genes, thus facilitating the rapid synthesis of a product (such as rRna for ribosomes) for which the demand is great.

GENE CLONING: Formation by a bacterium, carrying foreign genes in a recombinant plasmid, of a clone of identical cells containing the replicated foreign genes.

GENE DELIVERY: This is a general term for the introduction of new genetic elements into the genomes of living cells. The delivery problem is essentially conditioned by the fact that the new genetic elements are usually large, and by the presence of the outer cell membrane and the nuclear membrane acting as barriers to incorporation of the new DNA into the genome already present in the nucleus. Viruses possess various natural biochemical methods for achieving gene delivery; artificial gene delivery is one of the essential problems of "genetic engineering". The most important barrier is apparently the outer cell membrane, which is essentially a lipid barrier, and introduction of any large complex into the cell requires a fusion of one kind or another with this membrane. Liposomes, which consist of lipid membranes themselves, and which can fuse with outer cell membranes, are thus potential vehicles for delivery of many substances, including DNA.

GENE FLOW: The movement of genes from one part of a population to another, or from one population to another, via gametes.

GENE POOL: The sum total of all the genes of all the individuals in a population.

GENE REGULATION: Any of the strategies by which the rate of expression of a gene can be regulated, as by controlling the rate of transcription.

GENETIC DRIFT: Change in the gene pool as a result of chance and not as a result of selection, mutation, or migration.

GENETIC RECOMBINATION: The general term for the production of offspring that combine traits of the two parents.

GENETICS: The science of heredity; the study of heritable information.

GENOME: The cell's total complement of DNA.

GENOMIC EQUIVALENCE: The presence of all of an organism's genes in all of its cells.

GENOMIC IMPRINTING: The parental effect on gene expression. Identical alleles may have different effects on offspring depending on whether they arrive in the zygote via the ovum or via the sperm.

GENOMIC LIBRARY: A set of thousands of DNA segments from a genome, each carried by a plasmid or phage.

GENOTYPE: The particular combination of genes present in the cells of an individual.

GENUS: A taxonomic category above the species level, designated by the first word of a species' binomial Latin name.

GIARDIA LAMLIA: Giardia lamblia (synonymous with Lamblia intestinalis and Giardia duodenalis) is a flagellated protozoan parasite that colonizes and reproduces in the small intestine, causing giardiasis. The giardia parasite attaches to the epithelium by a ventral adhesive disc, and reproduces via binary fission. Giardiasis does not spread via the bloodstream, nor does it spread to other parts of the gastro-intestinal tract, but remains confined to the lumen of the small intestine. Giardia trophozoites absorb their nutrients from the lumen of the small intestine, and are anaerobes.

GIARDIASAS, HEPATITIS OR TYHOID: Diseases that may be transmitted through the contamination of a water supply, but not AIDS.

GIS – GRAPHIC INFORMATION SYSTEM: Detailed information about the physical locations of structures such as pipes, valves, and manholes within geographic areas with the use of satellites.

GLIAL CELL: A non-conducting cell of the nervous system that provides support, insulation, and protection for the neurons.

GLIDING: Rod-shaped, gram-negative, mostly aerobic; glide on secreted slimy substances; form colonies, frequently with complex fruiting structures.

GLOMERULUS: A capillary bed within Bowman's capsule of the nephron; the site of ultrafiltration.

GLUCOSE: A six carbon sugar which plays a central role in cellular metabolism.

GLYCOCALYX: The layer of protein and carbohydrates just outside the plasma membrane of an animal cell; in general, the proteins are anchored in the membrane, and the carbohydrates are bound to the proteins.

GLYCOGEN: A long, branched polymer of glucose subunits that is stored in the muscles and liver of animals and is metabolized as a source of energy.

GLYCOLYSIS: A metabolic pathway which occurs in the cytoplasm of cells and during which glucose is oxidized anaerobically to form pyruvic acid.

GLYCOPROTEIN: A protein with covalently linked sugar residues. The sugars may be bound to OH side chains of the polypeptide (O: linked) or the amide nitrogen of asparagine side chains (N: linked).

GLYCOSIDIC: A type of bond which links monosaccharide subunits together in di- or polysaccharides.

GLYOXYSOME: A type of microbody found in plants, in which stored lipids are converted to carbohydrates.

GOLGI APPARATUS: A system of concentrically folded membranes found in the cytoplasm of eukaryotic cells. Plays a role in the production and release of secretory materials such as the digestive enzymes manufactured in the pancreas.

GONADOTROPIN: Refers to a member of a group of hormones capable of promoting growth and function of the gonads. Includes hormones such as follicle stimulating hormone (FSH) and luteinizing hormone (LH) which are stimulatory to the gonads.

GOOD CONTACT TIME, pH and LOW TURBIDITY: These are factors that are important in providing good disinfection when using chlorine.

GPM: Gallons per minute.

GRAB SAMPLE: A sample which is taken from a water or wastestream on a one-time basis with no regard to the flow of the water or wastestream and without consideration of time. A single grab sample should be taken over a period of time not to exceed 15 minutes.

GRAB SAMPLE: A single water or wastewater sample taken at a time and place representative of total discharge.

GRADED POTENTIAL: A local voltage change in a neuron membrane induced by stimulation of a neuron, with strength proportional to the strength of the stimulus and lasting about a millisecond.

GRANUM: A stack-like grouping of photosynthetic membranes in a chloroplast

GRAVITY BELT THICKENER: A sludge dewatering device utilizing a filter belt to promote gravity drainage of water. Usually precedes additional dewatering treatment.

GRAVITY FILTER: A filter that operates at atmospheric pressure.

GRAVITY THICKENING: A sedimentation basin designed to operate at high solids loading rates.

GRAVITROPISM: A response of a plant or animal in response to gravity.

GREENHOUS EFFECT: The warming of the Earth due to atmospheric accumulation of carbon dioxide which absorbs infrared radiation and slows its escape from the irradiated Earth.

GREGOR MENDEL: The first to make quantitative observations of the patterns of inheritance and proposing plausible explanations for them.

GROWTH FACTOR: A protein that must be present in a cell's environment for its normal growth and development.

GT: Represents (Detention time) x (mixing intensity) in flocculation.

GUARD CELL: A specialized epidermal cell that regulates the size of stoma of a leaf.

GYMNOSPERM: A vascular plant that bears naked seeds not enclosed in any specialized chambers.

H2SO4: The molecular formula of Sulfuric acid.

HABIT: In biology, the characteristic form or mode of growth of an organism.

HABITAT: The kind of place where a given organism normally lives.

HABITUATION: The process that results in a long-lasting decline in the receptiveness of interneurons to the input from sensory neurons or other interneurons (sensitization, adaptation).

HALF: The average amount of time it takes for one-half of a specified quantity of a substance to decay or disappear.

HALIDES: A halide is a binary compound, of which one part is a halogen atom and the other part is an element or radical that is less electronegative than the halogen, to make a fluoride, chloride, bromide, iodide, or astatide compound. Many salts are halides. All Group 1 metals form halides with the halogens and they are white solids. A halide ion is a halogen atom bearing a negative charge. The halide anions are fluoride (F), chloride (Cl), bromide (Br), iodide (I) and astatide (At). Such ions are present in all ionic halide salts.

HALOACETIC ACIDS: Haloacetic acids are carboxylic acids in which a halogen atom takes the place of a hydrogen atom in acetic acid. Thus, in a monohaloacetic acid, a single halogen would replace a hydrogen atom. For example, chloroacetic acid would have the structural formula CH_2ClCO_2H. In the same manner, in dichloroacetic acid two chlorine atoms would take the place of two hydrogen atoms ($CHCl_2CO_2H$).

HAPLOID: The condition of having only one kind of a given type of chromosome.

HARD WATER: Hard water causes a buildup of scale in household hot water heaters. Hard water is a type of water that has high mineral content (in contrast with soft water). Hard water primarily consists of calcium (Ca2+), and magnesium (Mg2+) metal cations, and sometimes other dissolved compounds such as bicarbonates and sulfates. Calcium usually enters the water as either calcium carbonate ($CaCO_3$), in the form of limestone and chalk, or calcium sulfate ($CaSO_4$), in the form of other mineral deposits. The predominant source of magnesium is dolomite ($CaMg(CO_3)2$). Hard water is generally not harmful. The simplest way to determine the hardness of water is the lather/froth test: soap or toothpaste, when agitated, lathers easily in soft water but not in hard water. More exact measurements of hardness can be obtained through a wet titration. The total water 'hardness' (including both Ca2+ and Mg2+ ions) is read as parts per million or weight/volume (mg/L) of calcium carbonate (CaCO3) in the water. Although water hardness usually only measures the total concentrations of calcium and magnesium (the two most prevalent, divalent metal ions), iron, aluminum, and manganese may also be present at elevated levels in some geographical locations.

HARDNESS: A measure of the amount of calcium and magnesium salts in water. More calcium and magnesium lead to greater hardness. The term "hardness" comes from the fact that it is hard to get soap suds from soap or detergents in hard water. This happens because calcium and magnesium react strongly with negatively-charged chemicals like soap to form insoluble compounds.

HARDY-WEINBERG THEOREM: An axiom maintaining that the sexual shuffling of genes alone cannot alter the overall genetic makeup of a population.

HAUSTORIUM: In parasitic fungi, a nutrient-absorbing hyphal tip that penetrates the tissues of the host but remains outside the host cell membranes.

HAVERSIAN SYSTEM: One of many structural units of vertebrate bone, consisting of concentric layers of mineralize bone matrix surrounding lacunae, which contain osteocytes, and a central canal, which contains blood vessels and nerves.

HAZARDS OF POLYMERS: Slippery and difficult to clean-up are the most common hazards associated with the use of polymers in a water treatment plant.

HEAD: The measure of the pressure of water expressed in feet of height of water. 1 PSI = 2.31 feet of water or 1 foot of head equals about a half a pound of pressure or .433 PSI. There are various types of heads of water depending upon what is being measured. Static (water at rest) and Residual (water at flow conditions).

HEADWORKS: The facility at the "**head**" of the water source where water is first treated and routed into the distribution system.

HEALTH ADVISORY: An EPA document that provides guidance and information on contaminants that can affect human health and that may occur in drinking water, but which the EPA does not currently regulate in drinking water.

HEAT OF VAPORIZATION: The amount of energy absorbed by a substance when it changes state to a gas. Water absorbs approximately 580 calories per gram when it changes from liquid water to water vapor.

HEAT: The total amount of kinetic energy due to molecular motion in a body of matter. Heat is energy in its most random form.

HELPER T CELL: A type of T cell that is required by some B cells to help them make antibodies or that helps other T cells respond to antigens or secrete lymphokines or interleukins.

HEMAGGLUTININ: A surface antigen on influenza viruses which controls infectivity by associating with receptors on host erythrocytes or other cells.

HEMATOPOIESIS: The formation of blood.

HEMATOPOIETIC STEM CELLS: Cells found in the bone marrow of adult mammals which give rise to erythroid stem cells, lymphoid stem cells, and myeloid stem cells. Such cells give rise to erythrocytes and a variety of types of lymphocytes and leucocytes.

HEMOGLOBIN: An iron-containing respiratory pigment found in many organisms.

HEMOLYMPH: In invertebrates with open circulatory systems, the body fluid that bathes tissues.

HEMOPHILIA: A genetic disease resulting from an abnormal sex-linked recessive gene, characterized by excessive bleeding following injury.

HEPATIC: Pertaining to the liver.

HEREDITY: A biological phenomenon whereby characteristics are transmitted from one generation to another by virtue of chemicals (i.e. DNA) transferred during sexual or asexual reproduction.

HERPESVIRUS: A double stranded DNA virus with an enveloped, icosahedral capsid.

HERTZ: The term used to describe the frequency of cycles in an alternating current (AC) circuit. A unit of frequency equal to one cycle per second.

HETEROCHROMATIN: Non-transcribed eukaryotic chromatin that is so highly compacted that it is visible with a light microscope during interphase.

HETEROCHRONY: Evolutionary changes in the timing or rate of development.

HETEROCYST: A specialized cell that engages in nitrogen fixation on some filamentous cyanobacteria.

HETEROGAMY: The condition of producing gametes of two different types (contrast with isogamy).

HETEROMORPHIC: A condition in the life cycle of all modern plants in which the sporophyte and gametophyte generations differ in morphology.

HETEROSPOROUS: Referring to plants in which the sporophyte produces two kinds of spores that develop into unisexual gametophytes, either male or female.

HETEROTROPH: An organism dependent on external sources of organic compounds as a means of obtaining energy and/or materials. Such an organism requires carbon ("food") from its environment in an organic form. (synonym-organotroph).

HETEROTROPHIC PLATE COUNT: A test performed on drinking water to determine the total number of all types of bacteria in the water.

HETEROZYGOTE ADVANTAGE: A mechanism that preserves variation in eukaryotic gene pools by conferring greater reproductive success on heterozygotes over individuals homozygous for any one of the associated alleles.

HETEROZYGOUS: The condition whereby two different alleles of the gene are present within the same cell.

HF: The molecular formula of Hydrofluoric acid.

HIGH TURBIDITY CAUSING INCREASED CHLORINE DEMAND: May occur or be caused by the inadequate disinfection of water.

HISTAMINE: A substance released by injured cells that causes blood vessels to dilate during an inflammatory response.

HISTOLOGY: The study of tissues.

HISTONE: A type of protein characteristically associated with the chromosomes of eukaryotes.

HIV-1: Acute human immunodeficiency virus type 1 is the subtype of HIV (human immune deficiency virus) that causes most cases of AIDS in the Western Hemisphere, Europe, and Central, South, and East Africa. HIV is a retrovirus (subclass lentivirus), and retroviruses are single: stranded RNA viruses that have an enzyme called reverse transcriptase. With this enzyme the viral RNA is used as a template to produce viral DNA from cellular material. This DNA is then incorporated into the host cell's genome, where it codes for the synthesis of viral components. An HIV-1 infection should be distinguished from AIDS. Acquired immunodeficiency syndrome (AIDS) is a secondary immunodeficiency syndrome resulting from HIV infection and characterized by opportunistic infections, malignancies, neurologic dysfunction, and a variety of other syndromes.

HOLOBLASTIC: A type of cleavage in which there is complete division of the egg, as in eggs having little yolk (sea urchin) or a moderate amount of yolk (frog).

HOME RANGE: An area within which an animal tends to confine all or nearly all its activities for a long period of time.

HOMEOBOX: Specific sequences of DNA that regulate patterns of differentiation during development of an organism.

HOMEOSTASIS: A phenomenon whereby a state or process (for example, within an organism) is regulated automatically despite the tendency for fluctuations to occur.

HOMEOTHEMIC: Capable of regulation of constancy with respect to temperature.

HOMEOTIC GENES: Genes that control the overall body plan of animals by controlling the developmental fate of groups of cells.

HOMEOTIC: (mutation) A mutation in genes regulated by positional information that results in the abnormal substitution of one type of body part in place of another.

HOMOLOGOUS CHROMOSOMES: Chromosomes bearing genes for the same characters.

HOMOLOGOUS STRUCTURES: Characters in different species which were inherited from a common ancestor and thus share a similar ontogenetic pattern.

HOMOLOGY: Similarity in characteristics resulting from a shared ancestry.

HOMOPLASY: The presence in several species of a trait not present in their most common ancestor. Can result from convergent evolution, reverse evolution, or parallel evolution.

HOMOSPOROUS: Referring to plants in which a single type of spore develops into a bisexual gametophyte having both male and female sex organs.

HOMOZYGOUS: Having two copies of the same allele of a given gene.

HORMONE: A control chemical secreted in one part of the body that affects other parts of the body.

HOST RANGE: The limited number of host species, tissues, or cells that a parasite (including viruses and bacteria) can infect.

HUMORAL IMMUNITY: The type of immunity that fights bacteria and viruses in body fluids with antibodies that circulate in blood plasma and lymph, fluids formerly called humors.

HYBIRD VIGOR: Increased vitality (compared to that of either parent stock) in the hybrid offspring of two different, inbred parents.

HYBIRD: In evolutionary biology, a cross between two species. In genetics, a cross between two genetic types.

HYBIRDIZATION: The process whereby a hybrid results from interbreeding two species; 2) DNA hybridization is the comparison of whole genomes of two species by estimating the extent of hydrogen bonding that occurs between single-stranded DNA obtained from the two species.

HYBRIDOMA: A hybrid cell that produces monoclonal antibodies in culture, formed by the fusion of a myeloma cell with a normal antibody-producing lymphocyte.

HYDRATED LIME: The calcium hydroxide product that results from mixing quicklime with water. Chemical formula is CaOH2.

HYDRATION SHELL: A "covering" of water molecules which surrounds polar or charged substances in aqueous solutions. The association is due to the charged regions of the polar water molecules themselves.

HYDRIDES: Hydride is the name given to the negative ion of hydrogen, H. Although this ion does not exist except in extraordinary conditions, the term hydride is widely applied to describe compounds of hydrogen with other elements, particularly those of groups 1–16. The variety of compounds formed by hydrogen is vast, arguably greater than that of any other element. Various metal hydrides are currently being studied for use as a means of hydrogen storage in fuel cell-powered electric cars and batteries. They also have important uses in organic chemistry as powerful reducing agents, and many promising uses in hydrogen economy.
Every element of the periodic table (except some noble gases) forms one or more hydrides. These compounds may be classified into three main types by the predominant nature of their bonding:
* Saline hydrides, which have significant ionic character,
* Covalent hydrides, which include the hydrocarbons and many other compounds, and
* Interstitial hydrides, which may be described as having metallic bonding.

HYDROCARBON: Any compound made of only carbon and hydrogen.

HYDROCHLORIC AND HYPOCHLOROUS ACIDS: The compounds that are formed in water when chlorine gas is introduced.

HYDROFLUOSILIC ACID: (H_2SiF_6) a clear, fuming corrosive liquid with a pH ranging from 1 to 1.5. Used in water treatment to fluoridate drinking water.

HYDROGEN BOND: A type of bond formed when the partially positive hydrogen atom of a polar covalent bond in one molecule is attracted to the partially negative atom of a polar covalent bond in another.

HYDROGEN SULFIDE OR CHLORINE GAS: These chemicals can cause olfactory fatigue.

HYDROPHOBIC: Does not mix readily with water.

HYDROGEN ION: A single proton with a charge of +1. The dissociation of a water molecule (H2O) leads to the generation of a hydroxide ion (OH-) and a hydrogen ion (H+).

HYDROGEN SULFIDE: A toxic gas formed by the anaerobic decomposition of organic matter. Chemical formula is H2S.

HYDROLYSIS: The chemical reaction that breaks a covalent bond through the addition of hydrogen (from a water molecule) to the atom forming one side of the original bond, and a hydroxyl group to the atom on the other side.

HYDROPHILIC: Having an affinity for water.

HYDROPHOBIC INTERACTION: A type of weak chemical bond formed when molecules that do not mix with water coalesce to exclude the water.

HYDROPHOBIC: The physicochemical property whereby a substance or region of a molecule resists association with water molecules.

HYDROSTATIC: Pertaining to the pressure and equilibrium of fluids. A hydrostatic skeleton is a skeletal system composed of fluid held under pressure in a closed body compartment; the main skeleton of most cnidarians, flatworms, nematodes, and annelids.

HYDROXYL GROUP: A functional group consisting of a hydrogen atom joined to an oxygen atom by a polar covalent bond. Molecules possessing this group are soluble in water and are called alcohols.

HYDROXYL ION: The OH- ion.

HYPEROSMOTIC: A solution with a greater solute concentration than another, a hypoosmotic solution. If the two solutions are separated from one another by a membrane permeable to water, water would tend to move from the hypo- to the hyperosmotic side.

HYPERPOLARIZATION: An electrical state whereby the inside of the cell is made more negative relative to the outside than was the case at resting potential. A neuron membrane is hyperpolarized if the voltage is increased from the resting potential of about -70 mV, reducing the chance that a nerve impulse will be transmitted.

HYPERTROPHY: Abnormal enlargement, excessive growth.

HYPHA: A fungal filament.

HYPOCHLORITE AND ORGANIC MATERIALS: Heat and possibly fire may occur when hypochlorite is brought into contact with an organic material.

HYPOCOTYL: The portion of the axis of a plant embryo below the point of attachment of the cotyledons; forms the base of the shoot and the root.

HYPOOSMOTIC SOLUTION: A solution with a lesser solute concentration than another, a hyperosmotic solution. If the two solutions are separated from one another by a membrane permeable to water, water would tend to move from the hypo- to the hyperosmotic side.

HYPOTHESIS: A formal statement of supposition offered to explain observations. Note that a hypothesis is only useful if it can be tested. Even if correct, it is not scientifically useful if untestable.

HYPOTHETICO-DEDUCTIVE: A method used to test hypotheses. If deductions formulated from the hypothesis are tested and proven false, the hypothesis is rejected.

IMAGINAL DISK: An island of undifferentiated cells in an insect larva, which are committed (determined) to form a particular organ during metamorphosis to the adult.

IMBIBITION: The soaking of water into a porous material that is hydrophilic.

IMMUNE RESPONSE: 1) A primary immune response is the initial response to an antigen, which appears after a lag of a few days. 2) A secondary immune response is the response elicited when the animal encounters the same antigen at a later time. The secondary response is normally more rapid, of greater magnitude and of longer duration than the primary response.

IMMUNOGLOBULINE: The class of proteins comprising the antibodies.

IMMUNOLOGICAL: 1) Immunological distance is the amount of difference between two proteins as measured by the strength of the antigen: antibody reaction between them. 2) Immunological tolerance is a mechanism by which an animal does not mount an immune response to the antigenic determinants of its own macromolecules.

IMMUNOMAGNETIC SEPARATION (IMS): A purification procedure that uses microscopic, magnetically responsive particles coated with an antibodies targeted to react with a specific pathogen in a fluid stream. Pathogens are selectively removed from other debris using a magnetic field.

IMPELLERS: The semi-open or closed props or blades of a turbine pump that when rotated generate the pumping force.

IMPERVIOUS: Not allowing, or allowing only with great difficulty, the movement of water.

IMPRINTING: A type of learned behavior with a significant innate component, acquired during a limited critical period.

IN SERIES: Several components being connected one to the other without a bypass, requiring each component to work dependent on the one before it.

IN SITU: Treatment or disposal methods that do not require movement of contaminated material.

IN VITRO FERTILIZATION: Fertilization of ova in laboratory containers followed by artificial implantation of the early embryo in the mother's uterus.

INCINERATION: The process of reducing the volume of a material by burning and reducing to ash if possible.

INCLINED PLATE SEPARATOR: A series of parallel inclined plates that can be used to increase the efficiency of clarifiers and gravity thickeners.

INCOMPLETE DOMINANCE: A type of inheritance in which F1 hybrids have an appearance that is intermediate between the phenotypes of the parental varieties.

INDETERMINATE: 1) A type of cleavage exhibited during the embryonic development in deuterostomes, in which each cell produced by early cleavage divisions retains the capacity to develop into a complete embryo; 2) A type of growth exhibited by plants: they continue to grow as long as they live, because they always retain meristematic cells capable of undergoing mitosis.

INDIRECT REUSE: The beneficial use of reclaimed water into natural surface waters or groundwater.

INDUCED FIT: The change in shape of the active site of an enzyme so that it binds more snugly to the substrate, induced by entry of the substrate.

INDUCTION: 1) The ability of one group of embryonic cells to influence the development of another. 2) A method in logic which proceeds from the specific to general and develops a general statement which explains all of the observations. Commonly used to formulate scientific hypotheses.

INDUSTRIAL MELANISM: Melanism which has resulted from blackening of environmental surfaces (tree bark, etc.) by industrial pollution. This favors survival of melanic forms such as moths which rest on tree bark and are less likely to be seen by predators.

INDUSTRIAL WASTEWATER: Liquid wastes resulting from industrial processes.

INFECTIOUS: 1) An infectious disease is a disease caused by an infectious microbial or parasitic agent. 2) Infectious hepatitis is the former name for hepatitis A. 3) Infectious mononucleosis is an acute disease that affects many systems, caused by the Epstein: Barr virus.

INFECTIOUS PATHOGENS/MICROBES/GERMS: are considered disease-producing bacteria, viruses and other microorganisms.

INFLAMMATORY RESPONSE: A line of defense triggered by penetration of the skin or mucous membranes, in which small blood vessels in the vicinity of an injury dilate and become leakier, enhancing infiltration of leukocytes; may also be widespread in the body.

INFLUENT: Water or wastewater flowing into a basin or treatment plant.

INGESTION: A heterotrophic mode of nutrition in which other organisms or detritus are eaten whole or in pieces.

INHIBITORY POSTSYNAPTIC POTENTIAL: An electrical charge (hyperpolarization) in the membrane of a postsynaptic neuron caused by the binding of an inhibitory neurotransmitter from a presynaptic cell to a postsynaptic receptor.

INITIAL PRECISION AND RECOVERY (IPR): Four aliquots of spiking suspension analyzed to establish the ability to generate acceptable precision and accuracy. An IPR is performed prior to the first time this method is used and any time the method or instrumentation is modified.

INNER CELL MASS: A cluster of cells in a mammalian blastocyst that protrudes into one end of the cavity and subsequently develops into the embryo proper and some of the extraembryonic membranes.

INORGANIC COMPOUND: Compounds that contain no carbon or contain only carbon bound to elements other than hydrogen.

INORGANIC CONTAMINANTS: Mineral-based compounds such as metals, nitrates, and asbestos. These contaminants are naturally-occurring in some water, but can also get into water through farming, chemical manufacturing, and other human activities. EPA has set legal limits on 15 inorganic contaminants.

INORGANIC IONS: Present in all waters. Inorganic ions are essential for human health in small quantities, but in larger quantities they can cause unpleasant taste and odor or even illness. Most community water systems will commonly test for the concentrations of seven inorganic ions: nitrate, nitrite, fluoride, phosphate, sulfate, chloride, and bromide. Nitrate and nitrite can cause an illness in infants called methemoglobinemia. Fluoride is actually added to the drinking water in some public water systems to promote dental health. Phosphate, sulfate, chloride, and bromide have little direct effect on health, but high concentrations of inorganic ions can give water a salty or briny taste.

INSOLUBLE COMPOUNDS: are types of compounds cannot be dissolved. When iron or manganese reacts with dissolved oxygen (**DO**) insoluble compound are formed.

INTAKE FACILITIES: One of the more important considerations in the construction of intake facilities is the ease of operation and maintenance over the expected lifetime of the facility. Every intake structure must be constructed with consideration for operator safety and for cathodic protection.

INOSITOL TRIPHOSPHATE: The second messenger, which functions as an intermediate between certain non-steroid hormones and the third messenger, a rise in cytoplasmic Ca++ concentration.

INSERTION: A mutation involving the addition of one or more nucleotide pairs to a gene.

INSIGHT LEARNING: The ability of an animal to perform a correct or appropriate behavior on the first attempt in a situation with which it has had no prior experience.

INSULIN: The vertebrate hormone that lowers blood sugar levels by promoting the uptake of glucose by most body cells and promoting the synthesis and storage of glycogen in the liver; also stimulates protein and fat synthesis; secreted by endocrine cells of the pancreas called islets of Langerhans.

INTEGRAL PROTEIN: A protein of biological membranes that penetrates into or spans the membrane.

INTERBREED: To breed with another kind or species; hybridize.

INTERFERON: A chemical messenger of the immune system, produced by virus: infected cells and capable of helping other cells resist the virus.

INTERLEUKIN: 1: A chemical regulator (cytokine) secreted by macrophages that have ingested a pathogen or foreign molecule and have bound with a helper T cell; stimulates T cells to grow and divide and elevates body temperature. Interleukin: 2, secreted by activated T cells, stimulates helper T cells to proliferate more rapidly.

INTERMEDIATE FILAMENT: A component of the cytoskeleton that includes all filaments intermediate in size between microtubules and microfilaments.

INTERNEURON: An association neuron; a nerve cell within the central nervous system that forms synapses with sensory and motor neurons and integrates sensory input and motor output.

INTERNODE: The segment of a plant stem between the points where leaves are attached.

INTERSTITIAL CELLS: Cells scattered among the seminiferous tubules of the vertebrate testis that secrete testosterone and other androgens, the male sex hormones.

INTERSTITIAL FLUID: The internal environment of vertebrates consisting of the fluid filling the spaces between cells.

INTERTIDAL ZONE: The shallow zone of the ocean where land meets water.

INTRINSIC RATE OF INCREASE: The difference between number of births and number of deaths, symbolized as rmax; maximum population growth rate.

INTROGRESSION: Transplantation of genes between species resulting from fertile hybrids mating successfully with one of the parent species.

INTRON: The noncoding, intervening sequence of coding region (exon) in eukaryotic genes.

INVAGINATION: The buckling inward of a cell layer, caused by rearrangements of microfilaments and microtubules; an important phenomenon in embryonic development.

INVERSION: 1) An aberration in chromosome structure resulting from an error in meiosis or from mutagens; reattachment in a reverse orientation of a chromosomal fragment to the chromosome from which the fragment originated. 2) A phenomenon which occurs during early development of sponges at which time the external ciliated cells become inward-directed.

INVERTEBRATE: An animal without a backbone; invertebrates make up about 95% of animal species.

ION: A charged chemical formed when an atom or group of atoms has more or less electrons than protons (rather than an equal number).

ION EXCHANGE: An effective treatment process used to remove iron and manganese in a water supply. The hardness of the source water affects the amount of water an ion exchange softener may treat before the bed requires regeneration.

IONIC BOND: A chemical bond due to attraction between oppositely charged ions.

IRON: The elements iron and manganese are undesirable in water because they cause stains and promote the growth of iron bacteria.

IRON AND MANGANESE: In water they can usually be detected by observing the color of the inside walls of filters and the filter media. If the raw water is pre-chlorinated, there will be black stains on the walls below the water level and a black coating over the top portion of the sand filter bed. When significant levels of dissolved oxygen are present, iron and manganese exist in an oxidized state and normally precipitate into the reservoir bottom sediments. The presence of iron and manganese in water promote the growth of Iron bacteria. Only when a water sample has been acidified then you can perform the analysis beyond the 48

hour holding time. Iron and Manganese in water may be detected by observing the color of the of the filter media. Maintaining a free chlorine residual and regular flushing of water mains may control the growth of iron bacteria in a water distribution system.

IRRUPTION: A rapid increase in population density often followed by a mass emigration.

ISOGAMY: A condition in which male and female gametes are morphologically indistinguishable.

ISOMER: Molecules consisting of the same numbers and kinds of atoms, but differing in the way in which the atoms are combined.

ISOSMOTIC: Solutions of equal concentration with respect to osmotic pressure.

ISOTOPE: An atomic form of an element, containing a different number of neutrons than another isotope. Isotopes vary from one another with respect to atomic mass.

JUXTAGLOMERULAR APPARATUS (JGA): Specialized tissue located near the afferent arteriole that supplies blood to the kidney glomerulus; JGA raises blood pressure by producing renin, which activates angiotensin.

K- SELECTION: The concept that life history of the population is centered upon producing relatively few offspring that have a good chance of survival.

KARYOGAMY: The fusion of nuclei of two cells, as part of syngamy.

KARYOTYPE: A method of classifying the chromosomes of a cell in relation to number, size and type.

KEYSTONE PREDATOR: A species that maintains species richness in a community through predation of the best competitors in the community, thereby maintaining populations of less competitive species.

KILOCALORIE: A thousand calories; the amount of heat energy required to raise the temperature of 1 kilogram of water by primary C.

KILL = C X T: Where other factors are constant, the disinfecting action may be represented by: Kill=C x T.

KIN SELECTION: A phenomenon of inclusive fitness, used to explain altruistic behavior between related individuals.

KINESIS: A change in activity rate in response to a stimulus.

KINETIC ENERGY: The ability of an object to do work by virtue of its motion. The energy terms that are used to describe the operation of a pump are pressure and head. The energy of motion. Moving matter does work by transferring some of its kinetic energy to other matter.

KINETOCHORE: A specialized region on the centromere that links each sister chromatid to the mitotic spindle.

KINGDOM: A taxonomic category, the second broadest after domain.

KREBS CYCLE: A chemical cycle involving eight steps that completes the metabolic breakdown of glucose molecules to carbon dioxide; occurs within the mitochondrion; the second major stage in cellular respiration. Also called citric acid cycle or tricarboxylic acid (TCA) cycle.

LABORATORY BLANK: See Method blank

LABORATORY CONTROL SAMPLE (LCS): See Ongoing precision and recovery (OPR) standard

LACTEAL: A tiny lymph vessel extending into the core of the intestinal villus and serving as the destination for absorbed chylomicrons.

LACTIC ACID: Gram-positive, anaerobic; produce lactic acid through fermentation; include Lactobacillus, essential in dairy product formation, and Streptococcus, common in humans.

LAGGING STRAND: A discontinuously synthesized DNA strand that elongates in a direction away from the replication fork.

LAMARCK: Proposed, in the early 1800s, that evolutionary change may occur via the inheritance of acquired characteristics. This idea, which has since been discredited, holds that the changes in characteristics which occur during an individual's life can be passed on to its offspring.

LAND APPLICATION: The disposal of wastewater or municipal solids onto land under controlled conditions.

LAND DISPOSAL: Application of municipal wastewater solids to the soil without production of usable agricultural products.

LANDFILL: A land disposal site that employs an engineering method of solid waste disposal to minimize environmental hazards and protect the quality of surface and subsurface waters.

LANGELIER INDEX: A measurement of Corrosivity. The water is becoming corrosive in the distribution system causing rusty water if the Langelier index indicates that the pH has decreased from the equilibrium point. Mathematically derived factor obtained from the values of calcium hardness, total alkalinity, and pH at a given temperature. A Langelier index of zero indicates perfect water balance (i.e., neither corroding nor scaling). The Langelier Saturation Index (sometimes Langelier Stability Index) is a calculated number used to predict the calcium carbonate stability of water. It indicates whether the water will precipitate, dissolve, or be in equilibrium with calcium carbonate. Langelier developed a method for predicting the pH at which water is saturated in calcium carbonate (called pHs). The LSI is expressed as the difference between the actual system pH and the saturation pH.
LSI = pH - pHs
If the actual pH of the water is below the calculated saturation pH, the LSI is negative and the water has a very limited scaling potential. If the actual pH exceeds pHs, the LSI is positive, and being supersaturated with CaCO3, the water has a tendency to form scale. At increasing positive index values, the scaling potential increases.
Langelier saturation index is defined as:
LSI = pH (measured) - pHs
* For LSI > 0, water is super saturated and tends to precipitate a scale layer of $CaCO_3$
* For LSI = 0, water is saturated (in equilibrium) with $CaCO_3$. A scale layer of $CaCO_3$ is neither precipitated nor dissolved
* For LSI < 0, water is under saturated and tends to dissolve solid $CaCO_3$
In practice, water with an LSI between -0.5 and +0.5 will not display enhanced mineral dissolving or scale forming properties. Water with an LSI below -0.5 tends to exhibit noticeably increased dissolving abilities while water with an LSI above +0.5 tends to exhibit noticeably increased scale forming properties.

It is also worth noting that the LSI is temperature sensitive. The LSI becomes more positive as the water temperature increases. This has particular implications in situations where well water is used. The temperature of the water when it first exits the well is often significantly lower than the temperature inside the building served by the well or at the laboratory where the LSI measurement is made.

LARVA (pl. larvae): A free-living, sexually immature form in some animal life cycles that may differ from the adult in morphology, nutrition, and habitat.

LATERAL LINE SYSTEM: A mechanoreceptor system consisting of a series of pores and receptor units (neuromasts) along the sides of the body of fishes and aquatic amphibians; detects water movements made by an animal itself and by other moving objects.

LATERAL MERISTEMS: The vascular and cork cambia, cylinders of dividing cells that run most of the length of stems and roots and are responsible for secondary growth.

LAW OF INDEPENDENT ASSORTMENT: Mendel's second law, stating that each allele pair segregates independently during gamete formation; applies when genes for two traits are located on different pairs of homologous chromosomes.

LAW OF SEGREGATION: Mendel's first law, stating that allele pairs separate during gamete formation, and then randomly re-form pairs during the fusion of gametes at fertilization.

LEACHATE: Fluid that trickles through solid materials or wastes and contains suspended or dissolved materials or products of the solids.

LEACHING: A chemical reaction between water and metals that allows for removal of soluble materials.

LEADING STRAND: The new continuously complementary DNA strand synthesized along the template strand in the 5' --- > 3' direction.

LEUKOCYTE: A white blood cell; typically functions in immunity, such as phagocytosis or antibody production.

LEVELS OF ORGANIZATION: A basic concept in biology is that organization is based on a hierarchy of structural levels, with each level building on the levels below it.

LICHEN: An organism formed by the symbiotic association between a fungus and a photosynthetic alga.

LIFE: (table) A table of data summarizing mortality in a population.

LIGAMENT: A type of fibrous connective tissue that joins bones together at joints.

LIGAND: A ligand is a molecule that binds specifically to a receptor site of another molecule. A ligase is an enzyme which catalyzes such a reaction. For example, a DNA ligase is an enzyme which catalyzes the covalent bonding of the 3' end of a new DNA fragment to the 5' end of a growing chain.

LIGASE: Ligases are enzymes that catalyze the "stitching together" of polymer fragments. DNA ligase, for example, catalyzes phosphodiester bond formation between two DNA fragments, and this enzyme is involved in normal DNA replication, repair of damaged chromosomes, and various in vitro techniques in genetic engineering that involve linking DNA fragments.

LIGNIN: A hard material embedded in the cellulose matrix of vascular plant cell walls that functions as an important adaptation for support in terrestrial species.

LIMBIC SYSTEM: A group of nuclei (clusters of nerve cell bodies) in the lower part of the mammalian forebrain that interact with the cerebral cortex in determining emotions; includes the hippocampus and the amygdala.

LIME: The term generally used to describe ground limestone (calcium carbonate), hydrated lime (calcium hydroxide), or burned lime (calcium oxide).

LIME SOFTENING: Lime softening is primarily used to “soften” water—that is to remove calcium and magnesium mineral salts. But it also removes harmful toxins like radon and arsenic. Though there is no consensus, some studies have even suggested that lime softening is effective at removal of Giardia. Hard water is a common condition responsible for numerous problems. Users often recognize hard water because it prevents their soap from lathering properly. However, it can also cause buildup (“scale”) in hot water heaters, boilers, and hot water pipes. Because of these inconveniences, many treatment facilities use lime softening to soften hard water for consumer use. Before lime softening can be used, managers must determine the softening chemistry required. This is a relatively easy task for groundwater sources, which remain more constant in their composition. Surface waters, however, fluctuate widely in quality and may require frequent changes to the softening chemical mix. In lime softening, lime and sometimes sodium carbonate are added to the water as it enters a combination solids contact clarifier. This raises the pH (i.e., increases alkalinity) and leads to the precipitation of calcium carbonate. Later, the pH of the effluent from the clarifier is reduced again, and the water is then filtered through a granular media filter. The water chemistry requirements of these systems require knowledgeable operators, which may make lime softening an economic challenge for some very small systems.

LIME STABILIZATION: The addition of lime to untreated sludge to raise the pH to 12 for a minimum of 2 hours to chemically inactivate microorganisms.

LINKED GENES: Genes that are located on the same chromosomes.

LIPID: One of a family of compounds, including fats, phospholipids, and steroids, that are insoluble in water.

LIPOPROTEIN: A protein bonded to a lipid; includes the low-density lipoproteins (LDLS) and high-density lipoproteins (HDLS) that transport fats and cholesterol in the blood.

LIPOSOME: Liposomes are vesicles (spherules) in which the lipid molecules are spontaneously arranged into bilayers with hydrophilic groups exposed to water molecules both outside the vesicle and in the core.

LISTED HAZARDOUS WASTE: The designation for a waste material that appears on an EPA list of specific hazardous wastes or hazardous waste categories.

LOCUS: A particular place along the length of a certain chromosome where a specified allele is located.

LOGISTIC POPULATION GROWTH: A model describing population growth that levels off as population size approaches carrying capacity.

L.O.T.O.: If a piece of equipment is locked out, the key to the lock-out device the key should be held by the person who is working on the equipment. The tag is an identification device and the lock is a physical restraint.

LYMPHOCYTE: Lymphocytes (lymph cells, lympho- leukocytes) are a type of leukocyte (white blood cell) responsible for the immune response. There are two classes of lymphocytes: 1) the B- cells, when presented with a foreign chemical entity (antigen), change into antibody producing plasma cells; and, 2) the T- cells interact directly with foreign invaders such as bacteria and viruses. The T- cells express various surface marker macromolecules. For example, CD4+ is the notation for a specific expressed T- cell surface marker that can be identified by assay.

LYSIS: The destruction of a cell by rupture of the plasma membrane.

LYSOGENIC CYCLE: A type of viral replication cycle in which the viral genome becomes incorporated into the bacterial host chromosome as a prophage.

LYSOSOME: A membrane-bounded organelle found in eukaryotic cells (other than plants). Lysosomes contain a mixture of enzymes that can digest most of the macromolecules found in the rest of the cell.

LYSOZYME: An enzyme in perspiration, tears, and saliva that attacks bacterial cell walls.

LYTIC CYCLE: A type of viral replication cycle resulting in the release of new phages by death or lysis of the host cell.

M-ENDO BROTH: The coliform group are used as indicators of fecal pollution in water, for assessing the effectiveness of water treatment and disinfection, and for monitoring water quality. m-Endo Broth is used for selectively isolating coliform bacteria from water and other specimens using the membrane filtration technique. m-Endo Broth is prepared according to the formula of Fifield and Schaufus.1 It is recommended by the American Public Health Association in standard total coliform membrane filtration procedure for testing water, wastewater, and foods.2,3 The US EPA specifies using m-Endo Broth in the total coliform methods for testing water using single-step, two-step, and delayed incubation membrane filtration methods.

M PHASE: The mitotic phase of the cell cycle, which includes mitosis and cytokinesis.

MACROEVOLUTION: Evolutionary change on a grand scale, encompassing the origin of novel designs, evolutionary trends, adaptive radiation, and mass extinction.

MACROMOLECULE: A giant molecule of living matter formed by the joining of smaller molecules, usually by condensation synthesis. Polysaccharides, proteins, and nucleic acids are macromolecules.

MACROPHAGE: An amoeboid cell that moves through tissue fibers, engulfing bacteria and dead cells by phagocytosis.

MAGNESIUM HARDNESS: Measure of the magnesium salts dissolved in water – it is not a factor in water balance.

MAGNETIC STARTER: Is a type of motor starter should be used in an integrated circuit to control flow automatically.

MAJOR HISTOCOMPATIBILITY COMPLEX: A large set of cell surface antigens encoded by a family of genes. Foreign MHC markers trigger T-cell responses that may lead to rejection of transplanted tissues and organs.

MAKEUP WATER: Fluid introduced in a recirculating stream to maintain an equilibrium of temperature, solids concentration or other parameters. Also refers to the quantity of water required to make a solution.

MALIGNANT TUMOR: A cancerous growth; an abnormal growth whose cells multiply excessively, have altered surfaces, and may have unusual numbers of chromosomes and/or aberrant metabolic processes.

MALPHIGHIAN TUBULE: A unique excretory organ of insects that empties into the digestive tract, removes nitrogenous wastes from the blood, and functions in osmoregulation.

MANTLE: A heavy fold of tissue in mollusks that drapes over the visceral mass and may secrete a shell.

MARBLE AND LANGELIER TESTS: Are used to measure or determine the corrosiveness of a water source.

MASS NUMBER: The sum of the number of protons plus the number of neutrons in the nucleus of an atom; unique for each element and designated by a superscript to the left of the elemental symbol.

MATRIX SPIKE (MS): A sample prepared by adding a known quantity of organisms to a specified amount of sample matrix for which an independent estimate of target analyte concentration is available. A matrix spike is used to determine the effect of the matrix on a method's recovery efficiency.

MATRIX: The nonliving component of connective tissue, consisting of a web of fibers embedded in homogeneous ground substance that may be liquid, jellylike, or solid.

MATTER: Anything that takes up space and has mass.

MAXIMUM CONTAMINANT LEVEL (MCLs): The maximum allowable level of a contaminant

MECHANICAL SEAL: A mechanical device used to control leakage from the stuffing box of a pump. Usually made of two flat surfaces, one of which rotates on the shaft. The two flat surfaces are of such tolerances as to prevent the passage of water between them. Held in place with spring pressure.

MECHANORECEPTOR: A sensory receptor that detects physical deformations in the body environment associated with pressure, touch, stretch, motion, and sound.

MEDIAN BODIES: Prominent, dark-staining, paired organelles consisting of microtubules and found in the posterior half of *Giardia*. In *G. intestinalis* (from humans), these structures often have a claw-hammer shape, while in *G. muris* (from mice), the median bodies are round.

MEDIUM WATER SYSTEM: More than 3,300 persons and 50,000 or fewer persons.

MEDULLA OBLONGATA: The lowest part of the vertebrate brain; a swelling of the hindbrain dorsal to the anterior spinal cord that controls autonomic, homeostatic functions, including breathing, heart and blood vessel activity, swallowing, digestion, and vomiting.

MEDUSA: The floating, flattened, mouth-down version of the cnidarian body plan. The alternate form is the polyp.

MEGAPASCAL: A unit of pressure equivalent to 10 atmospheres of pressure.

MEGGER: Used to test the insulation resistance on a motor.

MEIOSIS: A two-stage type of cell division in sexually reproducing organisms that results in gametes with half the chromosome number of the original cell.

MEMBRANE: A thin barrier that permits passage of particles of a certain size or of particular physical or chemical properties.

MEMBRANE POTENTIAL: The charge difference between the cytoplasm and extracellular fluid in all cells, due to the differential distribution of ions. Membrane potential affects the activity of excitable cells and the transmembrane movement of all charged substances.

MESENTERIES: Membranes that suspend many of the organs of vertebrates inside fluid- filled body cavities.

MESODERM: The middle primary germ layer of an early embryo that develops into the notochord, the lining of the coelom, muscles, skeleton, gonads, kidneys and most of the circulatory system.

MESOSOME: A localized infolding of the plasma membrane of a bacterium.

MESSENGER: (RNA) A type of RNA synthesized from DNA in the genetic material that attaches to ribosomes in the cytoplasm and specifies the primary structure of a protein.

METABOLISM: The sum total of the chemical and physical changes constantly taking place in living substances.

METALLOID: Metalloid is a term used in chemistry when classifying the chemical elements. On the basis of their general physical and chemical properties, nearly every element in the periodic table can be termed either a metal or a nonmetal. A few elements with intermediate properties are, however, referred to as metalloids. (In Greek metallon = metal and eidos = sort) There is no rigorous definition of the term, but the following properties are usually considered characteristic of metalloids:
* metalloids often form amphoteric oxides.
* metalloids often behave as semiconductors (B,Si,Ge) to semimetals (e.g. Sb).
The concepts of metalloid and semiconductor should not be confused. Metalloid refers to the properties of certain elements in relation to the periodic table. Semiconductor refers to the physical properties of materials (including alloys, compounds) and there is only partial overlap between the two. The following elements are generally considered metalloids:
* Boron (B)
* Silicon (Si)
* Germanium (Ge)
* Arsenic (As)
* Antimony (Sb)
* Tellurium (Te)

METAMORPHOSIS: The resurgence of development in an animal larva that transforms it into a sexually mature adult.

METANEPHRIDIUM: A type of excretory tubule in annelid worms that has internal openings called nephrostomes that collect body fluids and external openings called nephridiopores.

METASTASIS: The spread of cancer cells beyond their original site.

METAZOAN: A multicellular animal. Among important distinguishing characteristics of metazoa are cell differentiation and intercellular communication. For certain multicellular colonial entities such as sponges, some biologists prefer the term "parazoa".

METHANE: Methane is a chemical compound with the molecular formula CH_4. It is the simplest alkane, and the principal component of natural gas. Methane's bond angles are 109.5 degrees. Burning methane in the presence of oxygen produces carbon dioxide and water. The relative abundance of methane and its clean burning process makes it a very attractive fuel. However, because it is a gas at normal temperature and pressure, methane is difficult to transport from its source. In its natural gas form, it is generally transported in bulk by pipeline or LNG carriers; few countries still transport it by truck. Methane is a relatively potent greenhouse gas with a high global warming potential of 72 (averaged over 20 years) or 25 (averaged over 100 years).[1] Methane in the atmosphere is eventually oxidized, producing carbon dioxide and water. As a result, methane in the atmosphere has a half-life of seven years (if no methane was added, then every seven years, the amount of methane would halve). The abundance of methane in the Earth's atmosphere in 1998 was 1745 parts per billion, up from 700 ppb in 1750. In the same time period, CO_2 increased from 278 to 365 parts per million. The radiative forcing effect due to this increase in methane abundance is about one-third of that of the CO_2 increase. In addition, there is a large, but unknown, amount of methane in methane clathrates in the ocean floors. Global warming could release this methane, which could cause a further

sharp rise in global temperatures. Such releases of methane may have been a major factor in previous major extinction events. The Earth's crust also contains huge amounts of methane. Large amounts of methane are produced anaerobically by methanogenesis. Other sources include mud volcanoes which are connected with deep geological faults.

METHOD BLANK: An aliquot of reagent water that is treated exactly as a sample, including exposure to all glassware, equipment, solvents, and procedures that are used with samples. The method blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

Mg/L: Stands for "**milligrams per liter**." A common unit of chemical concentration. It expresses the mass of a chemical that is present in a given volume of water. A milligram (one one-thousandth of a gram) is equivalent to about 18 grains of table salt. A liter is equivalent to about one quart.

MICROBE OR MICROBIAL: Any minute, simple, single-celled form of life, especially one that causes disease.

MICROBIAL CONTAMINANTS: Microscopic organisms present in untreated water that can cause waterborne diseases.

MICROBIOLOGICAL: Is a type of analysis in which a composite sample unacceptable.

MICROBODY: A small organelle, bounded by a single membrane and possessing a granular interior. Peroxisomes and glyoxysomes are types of microbodies.

MICROEVOLUTION: A change in the gene pool of a population over a succession of generations.

MICROFILAMENT: Minute fibrous structure generally composed of actin found in the cytoplasm of eukaryotic cells. They play a role in motion within cells.

MICROFILTRATION: A low pressure membrane filtration process that removes suspended solids and colloids generally larger than 0.1 micron diameter.

MICROORGANISMS: Very small animals and plants that are too small to be seen by the naked eye and must be observed using a microscope. Microorganisms in water include algae, bacteria, viruses, and protozoa. Algae growing in surface waters can cause off-taste and odor by producing the chemicals MIB and geosmin. Certain types of bacteria, viruses, and protozoa can cause disease in humans. Bacteria are the most common microorganisms found in treated drinking water. The great majority of bacteria are not harmful. In fact, humans would not be able to live without the bacteria that inhabit the intestines. However, certain types of bacteria called coliform bacteria can signal the presence of possible drinking water contamination.

MICROSCOPE: An instrument which magnifies images either by using lenses in an optical system to bend light (light microscope) or electromagnets to direct the movement of electrons (electron microscope).

MICROTUBULE: A minute tubular structure found in centrioles, spindle apparati, cilia, flagella, and other places in the cytoplasm of eukaryotic cells. Microtubules play a role in movement and maintenance of shape.

MICROVILLUS: Collectively, fine, fingerlike projections of the epithelial cells in the lumen of the small intestine that increase its surface area.

MILLIGRAMS PER LITER: (mg/L) A common unit of measurement of the concentration of a material in solution.

MILLILITER: One one-thousandth of a liter; A liter is a little more than a quart. A milliliter is about two drops from an eye dropper.

MIMICRY: A phenomenon in which one species benefits by a superficial resemblance to an unrelated species. A predator or species of prey may gain a significant advantage through mimicry.

MISCIBLE: Capable of being mixed together.

MISSENSE: (mutation) The most common type of mutation involving a base- pair substitution within a gene that changes a codon, but the new codon makes sense, in that it still codes for an amino acid.

MITOCHONDRIAL MATRIX: The compartment of the mitochondrion enclosed by the inner membrane and containing enzymes and substrates for the Krebs cycle.

MITOCHONDRION: An organelle that occurs in eukaryotic cells and contains the enzymes of the citric acid cycle, the respiratory chain, and oxidative phosphorylation. A mitochondrion is bounded by a double membrane.

MITOSIS: A process of cell division in eukaryotic cells conventionally divided into the growth period (interphase) and four stages: prophase, metaphase, anaphase, and telophase. The stages conserve chromosome number by equally allocating replicated chromosomes to each of the daughter cells.

MIXED LIQUOR SUSPENDED SOLIDS: Suspended solids in the mixture of wastewater and activated sludge undergoing aeration in the aeration basin.

MODEM SYNTHESIS: A comprehensive theory of evolution emphasizing natural selection, gradualism, and populations as the fundamental units of evolutionary change; also called Neo-Darwinism.

MOISTURE: If a material is hygroscopic, it must it be protected from water.

MOISTURE AND POTASSIUM PERMANGANATE: The combination of moisture and potassium permanganate produces heat.

MOLARITY: A common measure of solute concentration, referring to the number of moles of solute in 1 L of solution.

MOLD: A rapidly growing, asexually reproducing fungus.

MOLE: The number of grams of a substance that equals its molecular weight in daltons and contains Avogadro's number of molecules.

MOLECULAR FORMULA: A type of molecular notation indicating only the quantity of the constituent atoms.

MOLECULAR WEIGHT: The molecular mass (abbreviated Mr) of a substance, formerly also called molecular weight and abbreviated as MW, is the mass of one molecule of that substance, relative to the unified atomic mass unit u (equal to 1/12 the mass of one atom of carbon-12). This is distinct from the relative molecular mass of a molecule, which is the ratio of the mass of that molecule to 1/12 of the mass of carbon 12 and is a dimensionless number. Relative molecular mass is abbreviated to Mr.

MOLECULE: Two or more atoms of one or more elements held together by ionic or covalent chemical bonds.

MOLTING: A process in arthropods in which the exoskeleton is shed at intervals to allow growth by secretion of a larger exoskeleton.

MONERA: The kingdom of life forms that includes all of the bacteria.

MONOCLONAL ANTIBODY: A defensive protein produced by cells descended from a single cell; an antibody that is secreted by a clone of cells and, consequently, is specific for a single antigenic determinant.

MONOECIOUS: Referring to an organism having the capacity of producing both sperm and eggs.

MONOHYBRID CROSS: A breeding experiment that employs parental varieties differing in a single character.

MONOMER: A small molecule, two or more of which can be combined to form oligomers (consisting of a few monomers) or polymers (consisting of many monomers).

MONOPHYLETIC: A term used to describe any taxon derived from a single ancestral form that gave rise to no species in other taxa.

MONOSACCHARIDE: A simple sugar; a monomer.

MONOZYGOTIC TWINS: Monozygotic twins are genetically identical, derived from the division and autonomous development of a single zygote (fertilized egg).

MORPHOGENESIS: The development of body shape and organization during ontogeny.

MORPHOSPECIES: Species defined by their anatomical features.

MOSAIC EVOLUTION: The evolution of different features of an organism at different rates.

MOSAIC: A pattern of development, such as that of a mollusk, in which the early blastomeres each give rise to a specific part of the embryo. In some animals, the fate of the blastomeres is established in the zygote.

MOTOR NERVOUS SYSTEM: In vertebrates, the component of the peripheral nervous system that transmits signals from the central nervous system to effector cells.

MPF: M: phase promoting factor: A protein complex required for a cell to progress from late interphase to mitosis; the active form consists of cyclin and cdc2, a protein kinase.

M.S.D.S.: A safety document must an employer provide to an operator upon request.

MUCOSA: Refers to the mucous tissue lining various tubular structures in the body.

MUD BALLS IN FILTER MEDIA: Is a possible result of an ineffective or inadequate filter backwash.

MULLERIAN MIMICRY: A mutual mimicry by two unpalatable species.

MULTIGENE FAMILY: A collection of genes with similar or identical sequences, presumably of common origin.

MUNICIPAL WASTE: The combined solid and liquid waste from residential, commercial and industrial sources.

MUNICIPAL WASTEWATER TREATMENT PLANT (MWTP): Treatment works designed to treat municipal wastewater.

MURIATIC ACID: An acid used to reduce pH and alkalinity. Also used to remove stain and scale.

MUST: This action, activity, or procedural step is required.

MUTAGEN: A chemical or physical agent that interacts with DNA and causes a mutation.

MUTAGENESIS: The creation of mutations.

MUTATION: A spontaneous or induced change in a gene's or chromosome's structure or number. The resulting individual is termed a mutant.

MUTUALISM: A symbiotic relationship in which both the host and the symbiont benefit.

MYCELIUM: The densely branched network of hyphae in a fungus.

MYCOBACTERIUM: Pleomorphic spherical or rod-shaped, frequently branching, no gram stain, aerobic; commonly form yellow pigments; include Mycobacterium tuberculosis, cause of tuberculosis.

MYCOPLASMA: Spherical, commonly forming branching chains, no gram stain, aerobic but can live in certain anaerobic conditions; without cell walls yet structurally resistant to lysis; among smallest of bacteria; named for superficial resemblance to fungal hyphae (myco-means “fungus’).

MYELIN SHEATH: An insulating coat of cell membrane from Schwann cells that is interrupted by nodes of Ranvier where saltatory conduction occurs.

MYOFIBRILS: Fibrils arranged in longitudinal bundles in muscle cells (fibers); composed of thin filaments of actin and a regulatory protein and thick filaments of myosin.

MYOGLOBIN: An oxygen-storing, pigmented protein in muscle cells.

MYOSIN: A type of protein filament that interacts with actin filaments to cause cell movement, such as contraction in muscle cells.

NAD+: Nicatinamide adenine dinucleotide (oxidized); a coenzyme present in all cells that assists enzymes in transferring electrons during the redox reactions of metabolism.

NANO-FILTRATION: A specialty membrane filtration process that rejects solutes larger than approximately one nanometer (10 angstroms) in size.

NANOMETER: A unit of measure (length). 1 nm is equal to 1 x 10: 9 m, or 1/1,000,000 mm.

NaOCI: Is the molecular formula of Sodium hypochlorite.

NaOH: Is the molecular formula of Sodium hydroxide.

NATURAL ORGANIC MATTER: Organic matter present in natural waters.

NEGATIVE CONTROL: See Method blank.

NEGATIVE FEEDBACK: A primary mechanism of homeostasis, whereby a change in a physiological variable that is being monitored triggers a response that counteracts the initial fluctuation.

NEURAMINIDASE: A surface enzyme possessed by some influenza viruses which help the virus penetrate the mucus layer protecting the respiratory epithelium and also plays a role in budding of new virus particles from infected cells.

NEUTRALIZATION: The chemical process that produces a solution that is neither acidic nor alkaline. Usually with a pH between 6 and 8.

NEURON: A nerve cell; the fundamental unit of the nervous system, having structure and properties that allow it to conduct signals by taking advantage of the electrical charge across its cell membrane.

NEUROSECRETORY CELLS: Cells that receive signals from other nerve cells, but instead of signaling to an adjacent nerve cell or muscle, release hormones into the blood stream.

NEUROTRANSMITTER: The chemical messenger released from the synaptic terminals of a neuron at a chemical synapse that diffuses across the synaptic cleft and binds to and stimulates the postsynaptic cell.

NEUTRAL VARIATION: Genetic diversity that confers no apparent selective advantage.

NEUTRALIZATION REACTIONS: Chemical reactions between acids and bases where water is an end product.

NEUTRON: An uncharged subatomic particle of about the same size and mass as a proton.

NH3: The molecular formula of Ammonia.

NH4+: The molecular formula of the Ammonium ion.

NITRATES: A dissolved form of nitrogen found in fertilizers and sewage by-products that may leach into groundwater and other water sources. Nitrates may also occur naturally in some waters. Over time, nitrates can accumulate in aquifers and contaminate groundwater.

NITROGEN: Nitrogen is a nonmetal, with an electronegativity of 3.0. It has five electrons in its outer shell and is therefore trivalent in most compounds. The triple bond in molecular nitrogen (N2) is one of the strongest in nature. The resulting difficulty of converting (N_2) into other compounds, and the ease (and associated high energy release) of converting nitrogen compounds into elemental N2, have dominated the role of nitrogen in both nature and human economic activities. At atmospheric pressure molecular nitrogen condenses (liquefies) at 77 K (-195.8 °C) and freezes at 63 K (-210.0 °C) into the beta hexagonal close-packed crystal allotropic form. Below 35.4 K (-237.6 °C) nitrogen assumes the alpha cubic crystal allotropic form. Liquid nitrogen, a fluid resembling water, but with 80.8% of the density, is a common cryogen. Unstable allotropes of nitrogen consisting of more than two nitrogen atoms have been produced in the laboratory, like N3 and N4.[1] Under extremely high pressures (1.1 million atm) and high temperatures (2000 K), as produced under diamond anvil conditions, nitrogen polymerizes into the single bonded diamond crystal structure, an allotrope nicknamed "nitrogen diamond."

NITROGEN AND PHOSPHORUS: Pairs of elements and major plant nutrients that cause algae to grow.

NITROGEN-FIXING: Rod-shaped, gram-negative, aerobic; convert atmospheric nitrogen gas to ammonium in soil; include Azotobacter, a common genus.

NO3-: The molecular formula of the Nitrate ion.

NOMENCLATURE: The method of assigning names in the classification of organisms.

NON-CARBONATE HARDNESS: The portion of the total hardness in excess of the alkalinity.

NON-CARBONATE IONS: Water contains non-carbonate ions if it cannot be softened to a desired level through the use of lime only.

NON-POINT SOURCE POLLUTION: Air pollution may leave contaminants on highway surfaces. This non-point source pollution adversely impacts reservoir water and groundwater quality.

NONCOMPETITIVE INHIBITOR: A substance that reduces the activity of an enzyme by binding to a location remote from the active site, changing its conformation so that it no longer binds to the substrate.

NONCYCLIC ELECTRON FLOW: A route of electron flow during the light reactions of photosynthesis that involves both photosystems and produces ATP, NADPH, and oxygen; the net electron flow is from water to NADP+.

NONCYCLIC PHOTOPHOSPHORYLATION: The production of ATP by noncyclic electron flow.

NONDISJUNCTION: An accident of meiosis or mitosis, in which both members of a pair of homologous chromosomes or both sister chromatids fail to separate normally.

NONPOLAR: Electrically symmetrical. For example, in many molecules with covalent bonds, the electrons are shared equally; the poles are electrically neutral.

NONSENSE MUTATION: A mutation that changes an amino acid codon to one of the three stop codons, resulting in a shorter and usually nonfunctional protein.

NORM OF REACTION: The range of phenotypic possibilities for a single genotpe, as influenced by the environment.

NORMALITY: It is the number of equivalent weights of solute per liter of solution. Normality highlights the chemical nature of salts: in solution, salts dissociate into distinct reactive species (ions such as H+, Fe3+, or Cl-). Normality accounts for any discrepancy between the concentrations of the various ionic species in a solution. For example, in a salt such as MgCl2, there are two moles of Cl- for every mole of Mg2+, so the concentration of Cl- as well as of Mg2+ is said to be 2 N (read: "two normal"). Further examples are given below. A normal is one gram equivalent of a solute per liter of solution. The definition of a gram equivalent varies depending on the type of chemical reaction that is discussed - it can refer to acids, bases, redox species, and ions that will precipitate. It is critical to note that normality measures a single ion which takes part in an overall solute. For example, one could determine the normality of hydroxide or sodium in an aqueous solution of sodium hydroxide, but the normality of sodium hydroxide itself has no meaning.

Nevertheless it is often used to describe solutions of acids or bases, in those cases it is implied that the normality refers to the H+ or OH- ion.

NTU: (Nephelometric turbidity unit)**:** A measure of the clarity or cloudiness of water.

NUCLEAR: 1) (envelope) The surface, consisting of two layers of membrane, that encloses the nucleus of eukaryotic cells. 2) (pore) An opening of the nuclear envelope which allows for the movement of materials between the nucleus and surrounding cytoplasm.

NUCLEASE: This term refers to any enzyme that acts on nucleic acids, e.g., Dnase, Rnase, endonuclease, etc.

NUCLEIC: (acid) A polymer composed of nucleotides that are joined by covalent bonds (phosphodiester linkages) between the phosphate of one nucleotide and the sugar of the next nucleotide.

NUCLEOID: The region that harbors the chromosome of a prokaryotic cell. Unlike the eukaryotic nucleus, it is not bounded by a membrane.

NUCLEOLUS (pl. nucleoli): A specialized structure in the nucleus, formed from various chromosomes and active in the synthesis of ribosomes.

NUCLELUS: A small, generally spherical body found within the nucleus of eukaryotic cells. The site of ribosomal RNA synthesis.

NUCLEOSIDE: An organic molecule consisting of a nitrogenous base joined to a five- carbon sugar.

NUCLEOSOME: The basic, beadlike unit of DNA packaging in eukaryotes, consisting of a segment of DNA wound around a protein core composed of two copies of each of four types of histone.

NUCLEOTIDE: The basic chemical unit (monomer) of a nucleic acid. A nucleotide in RNA consists of one of four nitrogenous bases linked to ribose, which in turn is linked to phosphate. In DNA, deoxyribose is present instead of ribose.

NUCLEUS: A membrane-bound organelle containing genetic material. Nuclei are a prominent internal structure seen both in *Cryptosporidium* oocysts and *Giardia* cysts. In *Cryptosporidium* oocysts, there is one nucleus per sporozoite. One to four nuclei can be seen in *Giardia* cysts.

NUCLEUS: The membrane bound organelle of eukaryotic cells that contains the cell's genetic material. Also the central region of an atom composed of protons and neutrons.

NULL: In the scientific method, the hypothesis which one attempts to falsify.

$O^{3:}$ The molecular formula of ozone.

OLIGOTROPHIC: A reservoir that is nutrient-poor and contains little plant or animal life. An oligotrophic ecosystem or environment is one that offers little to sustain life. The term is commonly utilized to describe bodies of water or soils with very low nutrient levels. It derives etymologically from the Greek oligo (small, little, few) and trophe (nutrients, food). Oligotrophic environments are of special interest for the alternative energy sources and survival strategies upon which life could rely.

ONGOING PRECISION AND RECOVERY (OPR) STANDARD: A method blank spiked with known quantities of analytes. The OPR is analyzed exactly like a sample. Its purpose is to assure that the results produced by the laboratory remain within the limits specified in this method for precision and recovery.

OOCYST AND CYST SPIKING SUSPENSION: See Spiking suspension.

OOCYST AND CYST STOCK SUSPENSION: See Stock suspension.

OOCYST: The encysted zygote of some sporozoa; e.g., *Cryptosporidium*. The oocyst is a phase or form of the organism produced as a normal part of the life cycle of the organism. It is characterized by a thick and environmentally resistant outer wall.

ORGANIC: Relating to, or derived from, a living thing. A description of a substance that contains carbon atoms linked together by carbon-carbon bonds.

ORGANIC MATTER: Substances containing carbon compounds, usually of animal or vegetable origin.

ORGANIC PRESURSORS: Natural or man-made compounds with chemical structures based upon carbon that, upon combination with chlorine, leading to trihalomethane formation.

OSMOSIS: Osmosis is the process by which water moves across a semi permeable membrane from a low concentration solute to a high concentration solute to satisfy the pressure differences caused by the solute.

OVER-RANGE PROTECTION DEVICES: Mechanical dampers, snubbers and an air cushion chamber are examples of surging and overrange protection devices.

OXIDE: An oxide is a chemical compound containing at least one oxygen atom as well as at least one other element. Most of the Earth's crust consists of oxides. Oxides result when elements are oxidized by oxygen in air. Combustion of hydrocarbons affords the two principal oxides of carbon, carbon monoxide and carbon dioxide. Even materials that are considered to be pure elements often contain a coating of oxides. For example, aluminum foil has a thin skin of Al2O3 that protects the foil from further corrosion. Virtually all elements burn in an atmosphere of oxygen. In the presence of water and oxygen (or simply air), some elements - lithium, sodium, potassium, rubidium, caesium, strontium and barium - react rapidly, even dangerously to give the hydroxides. In part for this reason, alkali and alkaline earth metals are not found in nature in their metallic, i.e., native, form. Caesium is so reactive with oxygen that it is used as a getter in vacuum tubes, and solutions of potassium and sodium, so called NaK are used to deoxygenate and dehydrate some organic solvents. The surface of most metals consists of oxides and hydroxides in the presence of air. A well-known example is aluminum foil, which is coated with a thin film of aluminum oxide that passivates the metal, slowing further corrosion. The aluminum oxide layer can be built to greater thickness by the process of electrolytic anodizing. Although solid magnesium and aluminum react slowly with oxygen at STP, they, like most metals, will burn in air, generating very high temperatures. As a consequence, finely divided powders of most metals can be dangerously explosive in air.

OXIDIZED:
1. to convert (an element) into an oxide; combine with oxygen.
2. to cover with a coating of oxide or rust.
3. to take away hydrogen, as by the action of oxygen; add oxygen or any nonmetal.
4. to remove electrons from (an atom or molecule), thereby increasing the valence. Compare REDUCE (def. 12).

–verb (used without object)

5. to become oxidized.
6. (esp. of white wine) to lose freshness after prolonged exposure to air and often to darken in color.

OXIDIZING: The process of breaking down organic wastes into simpler elemental forms or by products. Also used to separate combined chlorine and convert it into free chlorine.

OXYGEN DEFICIENT ENVIRONMENT: One of the most dangerous threats to an operator upon entering a manhole.

OZONE: Ozone or trioxygen (O3) is a triatomic molecule, consisting of three oxygen atoms. It is an allotrope of oxygen that is much less stable than the diatomic O2. Ground-level ozone is an air pollutant with harmful effects on the respiratory systems of animals. Ozone in the upper atmosphere filters potentially damaging ultraviolet light from reaching the Earth's surface. It is present in low concentrations throughout the Earth's atmosphere. It has many industrial and consumer applications. Ozone, the first allotrope of a chemical element to be recognized by science, was proposed as a distinct chemical compound by Christian Friedrich Schönbein in 1840, who named it after the Greek word for smell (ozein), from the peculiar odor in lightning storms.[The formula for ozone, O3, was not determined until 1865 by Jacques-Louis Soret and confirmed by Schönbein in 1867. Ozone is a powerful oxidizing agent, far better than dioxygen. It is also unstable at high concentrations, decaying to ordinary diatomic oxygen (in about half an hour in atmospheric conditions):
2 O3 = 3 O2
This reaction proceeds more rapidly with increasing temperature and decreasing pressure. Deflagration of ozone can be triggered by a spark, and can occur in ozone concentrations of 10 wt% or higher.

PACKING: Material, usually of woven fiber, placed in rings around the shaft of a pump and used to control the leakage from the stuffing box.

PAINT FILTER TEST: Test to determine free water content of sludge or dewatered solids sample. Usually used as the criteria for admission to a landfill.

PARAMECIUM: Paramecia are a group of unicellular ciliate protozoa formerly known as slipper animalcules from their slipper shape. They are commonly studied as a representative of the ciliate group. Simple cilia cover the body which allows the cell to move with a synchronous motion (like a caterpilla). There is also a deep oral groove containing inconspicuous compound oral cilia (as found in other peniculids) that is used to draw food inside. They generally feed upon bacteria and other small cells. Osmoregulation is carried out by a pair of contractile vacuoles, which actively expel water absorbed by osmosis from their surroundings. Paramecia are widespread in freshwater environments, and are especially common in scums. Paramecia are attracted by acidic conditions. Certain single-celled eukaryotes, such as Paramecium, are examples for exceptions to the universality of the genetic code (translation systems where a few codons differ from the standard ones).

PARTS PER MILLION (PPM): A common unit of measure used to express the number of parts of a substance contained within a million parts of a liquid, solid, or gas.

PASTEURIZATION: A process for killing pathogenic organisms by applying heat for a specific period of time.

PATHOGENS: Disease-causing pathogens; waterborne pathogens A pathogen may contaminate water and cause waterborne disease.

Pb: The chemical symbol of Lead.

PCE: abbr. perchloroethylene. Known also as perc or tetrachloroethylene, perchloroethylene is a clear, colorless liquid with a distinctive, somewhat ether-like odor. It is non-flammable, having no measurable flashpoint or flammable limits in air. Effective over a wide range of applications, perchloroethylene is supported by closed loop transfer systems, stabilizers and employee exposure monitoring.

pCi/L: Picocuries per liter A curie is the amount of radiation released by a set amount of a certain compound. A picocurie is one quadrillionth of a curie.

PEAK DEMAND: The maximum momentary load placed on a water treatment plant, pumping station or distribution system.

PERKINESIS: The aggregation resulting from random thermal motion of fluid molecules.

PERMEATE: The term for water which has passed through the membrane of a reverse osmosis unit. The liquid that passes through a membrane.

pH: A unit of measure which describes the degree of acidity or alkalinity of a solution. The pH scale runs from 0 to 14 with 7 being the mid-point or neutral. A pH of less than 7 is on the acid side of the scale with 0 as the point of greatest acid activity. A pH of more than 7 is on the basic (alkaline) side of the scale with 14 as the point of greatest basic activity. The term pH is derived from “p”, the mathematical symbol of the negative logarithm, and “H”, the chemical symbol of Hydrogen. The definition of pH is the negative logarithm of the Hydrogen ion activity. $pH=-\log[H^+]$.

pH OF SATURATION: The ideal pH for perfect water balance in relation to a particular total alkalinity level and a particular calcium hardness level, at a particular temperature. The pH where the Langelier Index equals zero.

PHENOLPHTHALEIN/TOTAL ALKALINITY: The relationship between the alkalinity constituent’s bicarbonate, carbonate, and hydroxide can be based on the P and T alkalinity measurement.

PHENOL RED: Chemical reagent used for testing pH in the range of 6.8 - 8.4.

PHOSPHATE, NITRATE AND ORGANIC NITROGEN: Nutrients in a domestic water supply reservoir may cause water quality problems if they occur in moderate or large quantities.

PHYSICAL CHEMICAL TREATMENT: Treatment processes that are non-biological in nature.

PICOCURIE: A unit of radioactivity. "Pico" is a metric prefix that means one one-millionth of one one-millionth. A picocurie is one one-millionth of one one-millionth of a Curie. A Curie is that quantity of any radioactive substance that undergoes 37 billion nuclear disintegrations per second. Thus a picocurie is that quantity of any radioactive substance that undergoes 0.037 nuclear disintegrations per second.

PIEZOMETRIC SURFACE: See potentiometric surface.

PIN FLOC: Small flocculated particle size.

PLATE AND FRAME PRESS: A batch process dewatering device in which sludge is pumped under high pressure through a series of parallel plates, in which a chamber is created between the plates. Each plate is fitted with filter cloth and the solids are collected in the chambers and the water is filtered from the sludge.

POINT SOURCE DISCHARGE: A pipe, ditch, channel or other container from which pollutants may be discharged.

POLLUTANT: A substance, organism or energy form present in amounts that impair or threaten an ecosystem to the extent that its current or future uses are prevented.

POLLUTION: To make something unclean or impure. See Contaminated.

POLYMER: A type of chemical when combined with other types of coagulants aid in binding small suspended particles to larger particles to help in the settling and filtering processes. Chemical used for flocculation in dewatering. Also known as a "polyelectrolyte" which is a substance made of giant molecules formed by the union of simple smaller molecules.

POLYPHOSPHATES: Chemicals that may be added to remove low levels of iron and manganese.

PORE SPACE: The interstitial space between sediments and fractures that is capable of storing and transmitting water.

POROSITY: A factor representing a rock, soil, or formations percentage of open space available for the percolation and storage of groundwater.

POSITIVE CONTROL: See Ongoing precision and recovery standard.

POST-CHLORINE: Where the water is chlorinated to make sure it holds a residual in the distribution system.

POST TREATMENT: Treatment of finished water or wastewater to further enhance its quality.

POTABLE: Good water which is safe for drinking or cooking purposes. Non-Potable: A liquid or water that is not approved for drinking.

POTENTIAL ENERGY: The energy that a body has by virtue of its position or state enabling it to do work.

PPM: Abbreviation for parts per million.

PRE-CHLORINE: Where the raw water is dosed with a large concentration of chlorine.

PRE-CHLORINATION: The addition of chlorine before the filtration process will help:
> Control algae and slime growth
> Control mud ball formation
> Improve coagulation
> Precipate iron
The addition of chlorine to the water prior to any other plant treatment processes.

PRECIPITATE: A solid that separates from a solution.

PRECIPTATION: The phenomenon that occurs when a substance held in solution passes out of solution into a solid form.

PRELIMINARY TREATMENT: Treatment steps including comminution, screening, grit removal, pre-aeration, and/or flow equalization that prepares wastewater influent for further treatment.

PRESSURE: Pressure is defined as force per unit area. It is usually more convenient to use pressure rather than force to describe the influences upon fluid behavior. The standard unit for pressure is the Pascal, which is a Newton per square meter. For an object sitting on a surface, the force pressing on the surface is the weight of the object, but in different orientations it might have a different area in contact with the surface and therefore exert a different pressure.

PRESSURE FILTER: Filter unit enclosed in a vessel that may be operated under pressure.

PRESSURE HEAD: The height of a column of water capable of being maintained by pressure. See also Total Head, Total Dynamic Head.

PRESSURE MEASUREMENT: Bourdon tube, Bellows gauge and Diaphragm are commonly used to measure pressure in waterworks systems. A Bellows-type sensor reacts to a change in pressure.

PREVENTION: To take action. Stop something before it happens.

PRIMARY CLARIFIER: Sedimentation basin that precedes secondary wastewater treatment.

PRIMARY SLUDGE: Sludge produced in a primary waste treatment unit.

PRIMARY TREATMENT: Treatment steps including sedimentation and/or fine screening to produce an effluent suitable for biological treatment.

PROCESS WASTEWATER: Wastewater generated during manufacture or production processes.

PROCESS WATER: Water that is used for, or comes in contact with an end product or the materials used in an end product.

PROPIONIC ACID: Rod-shaped, pleomorphic, gram-positive, anaerobic; ferment lactic acid; fermentation produces holes in Swiss cheese from the production of carbon dioxide.

PROTON, NEUTRON AND ELECTRON: Are the 3 fundamental particles of an atom.

PROTOZOA: Microscopic animals that occur as single cells. Some protozoa can cause disease in humans. Protozoa form cysts, which are specialized cells like eggs that are very resistant to chlorine. Cysts can survive the disinfection process, then "hatch" into normal cells that can cause disease. Protozoa must be removed from drinking water by filtration, because they cannot be effectively killed by chlorine.

PSEUDOMONAD: Rod-shaped (straight or curved) with polar flagella, gram-negative, aerobic; can use up to 100 different compounds for carbon and energy.

PUMPING LIFT: The height to which water must be pumped or lifted to, feet of head.

PTFE: Polytetrafluoroethylene.

QUANTITATIVE TRANSFER: The process of transferring a solution from one container to another using a pipette in which as much solution as possible is transferred, followed by rinsing of the walls of the source container with a small volume of rinsing solution (e.g., reagent water, buffer, etc.), followed by transfer of the rinsing solution, followed by a second rinse and transfer.

QUICKLIME: A calcium oxide material produced by calcining limestone to liberate carbon dioxide, also called “calcined lime” or “pebble lime”, commonly used for pH adjustment. Chemical formula is CaO.

RAW TURBIDITY: The turbidity of the water coming to the treatment plant from the raw water source.

REAGENT: A substance used in a chemical reaction to measure, detect, examine, or produce other substances.

REDOX POTENTIAL: Reduction potential (also known as redox potential, oxidation / reduction potential or ORP) is the tendency of a chemical species to acquire electrons and thereby be reduced. Each species has its own intrinsic reduction potential; the more positive the potential, the greater the species' affinity for electrons and tendency to be reduced. In aqueous solutions, the reduction potential is the tendency of the solution to either gain or lose electrons when it is subject to change by introduction of a new species. A solution with a higher (more positive) reduction potential than the new species will have a tendency to gain electrons from the new species (i.e. to be reduced by oxidizing the new species) and a solution with a lower (more negative) reduction potential will have a tendency to lose electrons to the new species (i.e. to be oxidized by reducing the new species). Just as the transfer of hydrogen ions between chemical species determines the pH of an aqueous solution, the transfer of electrons between chemical species determines the reduction potential of an aqueous solution. Like pH, the reduction potential represents an intensity factor. It does not characterize the capacity of the system for oxidation or reduction, in much the same way that pH does not characterize the buffering capacity.

RELAY LOGIC: The name of a popular method of automatically controlling a pump, valve, chemical feeder, and other devices.

RESERVOIR: An impoundment used to store water.

RICKETTSIA: Spherical or rod-shaped, gram-negative, aerobic; cause Rocky Mountain spotted fever and typhus; closely related to Agrobacterium, a common gall-causing plant bacterium.

ROTIFER: Rotifers get their name (derived from Greek and meaning "wheel-bearer"; they have also been called wheel animalcules) from the corona, which is composed of several ciliated tufts around the mouth that in motion resemble a wheel. These create a current that sweeps food into the mouth, where it is chewed up by a characteristic pharynx (called the mastax) containing a tiny, calcified, jaw-like structure called the trophi. The cilia also pull the animal, when unattached, through the water. Most free-living forms have pairs of posterior toes to anchor themselves while feeding. Rotifers have bilateral symmetry and a variety of different shapes. There is a well-developed cuticle which may be thick and rigid, giving the animal a box-like shape, or flexible, giving the animal a worm-like shape; such rotifers are respectively called loricate and illoricate.

RAW SEWAGE: Untreated wastewater and its contents.

RAW SLUDGE: Undigested sludge recently removed from a sedimentation basin.

RAW WATER: Untreated surface or groundwater.

REAGENT WATER BLANK: see Method blank.

REAGENT WATER: Water demonstrated to be free from the analytes of interest and potentially interfering substances at the method detection limit for the analyte.

RECLAIMED WATER: Wastewater that has been treated to a level that allows for its reuse for a beneficial purpose.

RECLAMATION: The process of improving or restoring the condition of land or other material to a better or more useful state.

RECYCLING: The process by which recovered materials are transformed into new products.

RELATIVE STANDARD DEVIATION (RSD): The standard deviation divided by the mean times 100.

RESIDENCE TIME: The period of time that a volume of liquid remains in a tank or system.

RESPIRATION: Intake of oxygen and discharge of carbon dioxide as a result of biological oxidation.

RETURN ACTIVATED SLUDGE: Settled activated sludge that is returned to mix with raw or primary settled wastewater.

ROBERT HOOKE: Coined the term "cell" to describe the structures he saw while examining a piece of cork using a microscope.

ROTARY DRUM SCREEN: Cylindrical screen used to remove floatable and suspended solids.

RSD: See Relative standard deviation.

SANITARY SURVEY: Persons trained in public health engineering and the epidemiology of waterborne diseases should conduct the sanitary survey. The importance of a detailed sanitary survey of a new water source can not be overemphasized. An on-site review of the water sources, facilities, equipment, operation, and maintenance of a public water systems for the purpose of evaluating the adequacy of the facilities for producing and distributing safe drinking water. The purpose of a non-regulatory sanitary survey is to identify possible biological and chemical pollutants which might affect a water supply.

SANITIZER: A disinfectant or chemical which disinfects (kills bacteria), kills algae and oxidizes organic matter.

SATURATION INDEX: See Langelier's Index.

SATURATOR: A device which produces a fluoride solution for the fluoride process. Crystal-grade types of sodium fluoride should be fed with a saturator. Overfeeding must be prevented to protect public health when using a fluoridation system.

SATURATED ZONE: Where an unconfined aquifer becomes saturated beneath the capillary fringe.

SCADA: A remote method of monitoring pumps and equipment. 130 degrees F is the maximum temperature that transmitting equipment is able to with stand. If the level controller may be set with too close a tolerance 45 could be the cause of a control system that is frequently turning a pump on and off.

SCALE: Crust of calcium carbonate, the result of unbalanced water. Hard insoluble minerals deposited (usually calcium bicarbonate) which forms on pool and spa surfaces and clog filters, heaters and pumps. Scale is caused by high calcium hardness and/or high pH. The regular use of stain prevention chemicals can prevent scale.

SCREENINGS PRESS: A mechanical press used to compact and/or dewater material removed from mechanical screening equipment.

SCROLL AND BASKET: The two basic types of centrifuges used in water treatment.

SCRUBBER: A device used to removal particulates or pollutant gases from combustion or chemical process exhaust streams.

SCUM: Floatable materials found on the surface of primary and secondary settling tanks consisting of food wastes, grease, fats, paper, foam, and similar matter.

SECONDARY CLARIFIER: A clarifier following a secondary treatment process, designed for gravity removal of suspended matter.

SECONDARY SLUDGE: The sludge from the secondary clarifier in a wastewater treatment plant.

SECONDARY TREATMENT: The treatment of wastewater through biological oxidation after primary treatment.

SEDIMENTATION: The removal of settleable suspended solids from water or wastewater by gravity in a quiescent basin or clarifier.

SEDIMENTATION BASIN: A quiescent tank used to remove suspended solids by gravity settling. Also called clarifiers or settling tanks, they are usually equipped with a motor driven rake mechanism to collect settled sludge and move it to a central discharge point.

SEDIMENTATION BASIN: Where the thickest and greatest concentration of sludge will be found. Twice a year sedimentation tanks should be drained and cleaned if the sludge buildup interferes with the treatment process.

SEDIMENTATION: The process of suspended solid particles settling out (going to the bottom of the vessel) in water.

SEDIMENT: Grains of soil, sand, gravel, or rock deposited by and generated by water movement.

SENSOR: A float and cable system are commonly found instruments that may be used as a sensor to control the level of liquid in a tank or basin.

SEPTIC: Condition characterized by bacterial decomposition under anaerobic conditions.

SETTLEABILITY: The tendency of suspended solids to settle.

SETTLEABLE SOLIDS: That portion of suspended solids which are of a sufficient size and weight to settle to the bottom of an Imhoff cone in one hour.

SETTLED SLUDGE VOLUME: Volume of settled sludge measured at predetermined time increments for use in process control calculations.

SETTLED SOLIDS: Solids that have been removed from the raw water by the coagulation and settling processes.

SEWAGE: Liquid or waterborne wastes polluted or fouled from households, commercial or industrial operations, along with any surface water, storm water or groundwater infiltration.

SEWER GAS: A gas mixture produced by anaerobic decomposition of organic matter usually containing high percentages of methane and hydrogen sulfide.

SHEATHED: Filamentous, gram-negative, aerobic; “swarmer” (colonizing) cells form and break out of a sheath; sometimes coated with metals from environment.

SHOCK: Also known as superchlorination or break point chlorination. Ridding a water of organic waste through oxidization by the addition of significant quantities of a halogen.

SHOCK LOAD: A sudden hydraulic or organic load to a treatment plant, also descriptive of a change in the material being treated.

SHORT-CIRCUITING: Short Circuiting is a condition that occurs in tanks or basins when some of the water travels faster than the rest of the flowing water. This is usually undesirable since it may result in shorter contact, reaction or settling times in comparison with the presumed detention times.

SHOULD: This action, activity, or procedural step is suggested but not required.

SINGLE PHASE POWER: The type of power used for lighting systems, small motors, appliances, portable power tools and in homes.

SLOP OIL: Separator skimmings and tramp oil generated during refinery startup, shutdown or abnormal operation.

SLUDGE: Accumulated and concentrated solids generated within a treatment process that have not undergone a stabilization process.

SLUDGE BASINS: After cleaning sludge basins and before returning the tanks into service the tanks should be inspected, repaired if necessary, and disinfected.

SLUDGE BLANKET: The accumulated sludge suspended in a clarifier or other enclosed body of water.

SLUDGE DEWATERING: The removal of a portion or majority of the water contained in sludge by means of a filter press, centrifuge or other mechanism.

SLUDGE DRYING BED: A closed area consisting of sand or other porous material upon which sludge is dewatered by gravity drainage and evaporation.

SLUDGE REDUCTION: Organic polymers are used to reduce the quantity of sludge. If a plant produces a large volume of sludge, the sludge could be dewatered, thickened, or conditioned to decrease the volume of sludge. Turbidity of source water, dosage, and type of coagulant used are the most important factors which determine the amount of sludge produced in a treatment of water.

SLURRY: A mixture of a solid and a liquid that facilitates the transfer of the solid into a treatment solution.

SOC: A common way for a synthetic organic chemical such as dioxin to be introduced to a surface water supply is from an industrial discharge, agricultural drainage, or a spill.

SODA ASH: Chemical used to raise pH and total alkalinity (sodium carbonate)

SODIUM BICARBONATE: Commonly used to increase alkalinity of water and stabilize pH.

SODIUM BISULFATE: Chemical used to lower pH and total alkalinity (dry acid).

SODIUM HYDROXIDE: Also known as caustic soda, a by-product chlorine generation and often used to raise pH.

SOFTENING WATER: When the water has a low alkalinity it is advantageous to use soda ash instead of caustic soda for softening water.

SOFTENING: The process that removes the ions which cause hardness in water.

SOLID, LIQUID AND VAPOR: 3 forms of matter.

SOLID WASTE: Garbage, refuse, sludge and other discarded material resulting from community activities or commercial or industrial operations.

SOLUBILITY: The amount of a substance that can dissolve in a solution under a given set of conditions.

SPADNS: The lab reagent called SPADNS solution is used in performing the Fluoride test.

SPIKING SUSPENSION: Diluted stock suspension containing the organism(s) of interest at a concentration appropriate for spiking samples.

SPIRILLUM: Spiral-shaped, gram-negative, aerobic; include Bdellovibrio, predatory on other bacteria.

SPIROCHETE: Spiral-shaped, gram-negative, mostly anaerobic; common in moist environments, from mammalian gums to coastal mudflats; complex internal structures convey rapid movement; include *Treponemapallidum,* cause of syphilis.

SPOROZOITE: A motile, infective stage of certain protozoans; e.g., *Cryptosporidium*. There are four sporozoites in each *Cryptosporidium* oocyst, and they are generally banana-shaped.

SPRAY BOTTLE OF AMMONIA: An operator should use ammonia to test for a chlorine leak around a valve or pipe. You will see white smoke if there is a leak.

SPRING PRESSURE: Is what maintains contact between the two surfaces of a mechanical seal.

STABILIZATION POND: A large shallow basin used for wastewater treatment by natural processes involving the use of algae and bacteria to accomplish biological oxidation of organic matter.

STERILIZED GLASSWARE: The only type of glassware that should be used in testing for coliform bacteria.

STOCK SUSPENSION: A concentrated suspension containing the organism(s) of interest that is obtained from a source that will attest to the host source, purity, authenticity, and viability of the organism(s).

STUFFING BOX: That portion of the pump that houses the packing or mechanical seal.

SUBNATANT: Liquid remaining beneath the surface of floating solids.

SUCCESSION: Transition in the species composition of a biological community, often following ecological disturbance of the community; the establishment of a biological community in an area virtually barren of life.

SULFIDE: The term sulfide refers to several types of chemical compounds containing sulfur in its lowest oxidation number of -2. Formally, "sulfide" is the dianion, S2-, which exists in strongly alkaline aqueous solutions formed from H2S or alkali metal salts such as Li2S, Na2S, and K2S. Sulfide is exceptionally basic and, with a pKa > 14, it does not exist in appreciable concentrations even in highly alkaline water, being undetectable at pH < ~15 (8 M NaOH). Instead, sulfide combines with electrons in hydrogen to form HS, which is variously called hydrogen sulfide ion, hydrosulfide ion, sulfhydryl ion, or bisulfide ion. At still lower pH's (<7), HS- converts to H2S, hydrogen sulfide. Thus, the exact sulfur species obtained upon dissolving sulfide salts depends on the pH of the final solution. Aqueous solutions of transition metals cations react with sulfide sources (H2S, NaSH, Na2S) to precipitate solid sulfides. Such inorganic sulfides typically have very low solubility in water and many are related to minerals. One famous example is the bright yellow species CdS or "cadmium yellow". The black tarnish formed on sterling silver is Ag2S. Such species are sometimes referred to as salts. In fact, the bonding in transition metal sulfides is highly covalent, which gives rise to their semiconductor properties, which in turn is related to the practical applications of many sulfide materials.

SULFATE- AND SULFUR- REDUCING: Commonly rod-shaped, mostly gram-negative, anaerobic; include *Desulfovibrio,* ecologically important in marshes.

SULFUR- AND IRON- OXIDIZING: Commonly rod-shaped, frequently with polar flagella, gram-negative, mostly anaerobic; most live in neutral (nonacidic) environment.

SUPERNATANT: The liquid layer which forms above the sludge in a settling basin.

SURFACE SEAL: The upper portion of a wells construction where surface contaminants are adequately prevented from entering the well, normally consisting of surface casing and neat cement grout.

SURFACTANT: Surfactants reduce the surface tension of water by adsorbing at the liquid-gas interface. They also reduce the interfacial tension between oil and water by adsorbing at the liquid-liquid interface. Many surfactants can also assemble in the bulk solution into aggregates. Examples of such aggregates are vesicles and micelles. The concentration at which surfactants begin to form micelles is known as the critical micelle concentration or CMC. When micelles form in water, their tails form a core that can encapsulate an oil droplet, and their (ionic/polar) heads form an outer shell that maintains favorable contact with water. When surfactants assemble in oil, the aggregate is referred to as a reverse micelle. In a reverse micelle, the heads are in the core and the tails maintain favorable contact with oil. Surfactants are also often classified into four primary groups; anionic, cationic, non-ionic, and zwitterionic (dual charge).

SUSPENDED SOLIDS: Solids captured by filtration through a 0.45 micron filter membrane.

TCE, *trichloroethylene:* A solvent and degreaser used for many purposes; for example dry cleaning, it is a common groundwater contaminant. Trichloroethylene is a colorless liquid which is used as a solvent for cleaning metal parts. Drinking or breathing high levels of trichloroethylene may cause nervous system effects, liver and lung damage, abnormal heartbeat, coma, and possibly death. Trichloroethylene has been found in at least 852 of the 1,430 National Priorities List sites identified by the Environmental Protection Agency (EPA).

TDS-TOTAL DISSOLVED SOLIDS: An expression for the combined content of all inorganic and organic substances contained in a liquid which are present in a molecular, ionized or micro-granular (colloidal sol) suspended form. Generally, the operational definition is that the solids (often abbreviated TDS) must be small enough to survive filtration through a sieve size of two micrometers. Total dissolved solids are normally only discussed for freshwater systems, since salinity comprises some of the ions constituting the definition of TDS. The principal application of TDS is in the study of water quality for streams, rivers and lakes, although TDS is generally considered not as a primary pollutant (e.g. it is not deemed to be associated with health effects), but it is rather used as an indication of aesthetic characteristics of drinking water and as an aggregate indicator of presence of a broad array of chemical contaminants.

TDS: Ion exchange is an effective treatment process used to remove iron and manganese in a water supply. This process is ideal as long as the water does not contain a large amount of TDS. When determining the total dissolved solids, a sample should be filtered before being poured into an evaporating dish and dried. Demineralization may be necessary in a treatment process if the water has a very high value Total Dissolved Solids.

TELEMETERING: The use of a transmission line with remote signaling to monitor a pumping station or motors. Can be used to accomplish accurate and reliable remote monitoring and control over a long distribution system.

TEMPERATURE SAMPLE: This test should be performed immediately in the field, a grab sample.

TERTIARY TREATMENT: The use of physical, chemical, or biological means to improve secondary wastewater effluent quality.

THE RATE DECREASES: In general, when the temperature decreases, the chemical reaction rate decreases also.

THICKENING: A procedure used to increase the solids content of sludge by removing a portion of the liquid.

THICKENING, CONDITIONING AND DEWATERING: Common processes that are utilized to reduce the volume of sludge.

THOMAS MALTHUS: Formulated the concept that population growth proceeds at a geometric rate.

TIME FOR TURBIDITY BREAKTHROUGH AND MAXIMUM HEADLOSS: Are the two factors which determine whether or not a change in filter media size should be made.

TITRATION: A method of testing by adding a reagent of known strength to a water sample until a specific color change indicates the completion of the reaction.

TOTAL ALKALINITY: A measure of the acid-neutralizing capacity of water which indicates its buffering ability, i.e. measure of its resistance to a change in pH. Generally, the higher the total alkalinity, the greater the resistance to pH change.

TOTAL COLIFORM: Total coliform, fecal coliform, and E. coli are all indicators of drinking water quality. The total coliform group is a large collection of different kinds of bacteria. Fecal coliforms are types of total coliform that mostly exist in feces. E. coli is a sub-group of fecal coliform. When a water sample is sent to a lab, it is tested for total coliform. If total coliform is present, the sample will also be tested for either fecal coliform or E. coli, depending on the lab testing method.

TOTAL DISSOLVED SOLIDS (TDS): The accumulated total of all solids that might be dissolved in water. The weight per unit volume of all volatile and non-volatile solids dissolved in a water or wastewater after a sample has been filtered to remove colloidal and suspended solids.

TOTAL DYNAMIC HEAD: The pressure (psi) or equivalent feet of water, required for a pump to lift water to its point of storage overcoming elevation head, friction loss, line pressure, drawdown and pumping lift.

TOTAL SOLIDS: The sum of dissolved and suspended solids in a water or wastewater.

TOTAL SUSPENDED SOLIDS: The measure of particulate matter suspended in a sample of water or wastewater.

TOXIC: Capable of causing an adverse effect on biological tissue following physical contact or absorption.

TREATABILITY STUDY: A study in which a waste is subjected to a treatment process to determine treatment and/or to determine the treatment efficiency or optimal process conditions for treatment.

TRIHALOMETHANES (THM): Four separate compounds including chloroform, dichlorobromomethane, dibromochloromethane, and bromoform. The most common class of disinfection by-products created when chemical disinfectants react with organic matter in water during the disinfection process. See Disinfectant Byproducts.

TUBE SETTLERS: This modification of the conventional process contains many metal tubes that are placed in the sedimentation basin, or clarifier. These tubes are approximately 1 inch deep and 36 inches long, split-hexagonal shape and installed at an angle of 60 degrees or less. These tubes provide for a very large surface area upon which particles may settle as the water flows upward. The slope of the tubes facilitates gravity settling of the solids to the bottom of the basin, where they can be collected and removed. The large surface settling area also means that adequate clarification can be obtained with detention times of 15 minutes or less. As with conventional treatment, this sedimentation step is followed by filtration through mixed media.

TUBERCLES: The creation of this condition is of the most concern regarding corrosive water effects on a water system. Tubercles are formed due to joining dissimilar metals, causing electro-chemical reactions. Like iron to copper pipe. We have all seen these little rust mounds inside cast iron pipe.

TURBIDIMETER: Monitoring the filter effluent turbidity on a continuous basis with an in-line instrument is a recommended practice. Turbidimeter is best suited to perform this measurement.

TURBIDITY: A measure of the cloudiness of water caused by suspended particles. A qualitative measurement of water clarity which results from suspended matter that scatters or otherwise interferes with the passage of light through the water.

ULTRAFILTRATION: A low pressure membrane filtration process which separates solutes up to 0.1 micron size range.

UNDER PRESSURE IN STEEL CONTAINERS: After chlorine gas is manufactured, it is primarily transported in steel containers.

UP FLOW CLARIFIER: Clarifier where flocculated water flows upward through a sludge blanket to obtain floc removal by contact with flocculated solids in the blanket.

U.S. ENVIRONMENTAL PROTECTION AGENCY: In the United States, this agency responsible for setting drinking water standards and for ensuring their enforcement. This agency sets federal regulations which all state and local agencies must enforce.

VANE: That portion of an impeller that throws the water toward the volute.

VAPOR: The gaseous phase of a material that is in the solid or liquid state at standard temperature and pressure.

VARIABLE DISPLACEMENT PUMP: A pump that will produce different volumes of water dependent on the pressure head against it.

VELOCITY HEAD: The vertical distance a liquid must fall to acquire the velocity with which it flows through the piping system. For a given quantity of flow, the velocity head will vary indirectly as the pipe diameter varies.

VENTURI: If water flows through a pipeline at a high velocity, the pressure in the pipeline is reduced. Velocities can be increased to a point that a partial vacuum is created.

VERTICAL TURBINE: A type of variable displacement pump in which the motor or drive head is mounted on the wellhead and rotates a drive shaft connected to the pump impellers.

VIBRIO: Rod- or comma-shaped, gram-negative, aerobic; commonly with a single flagellum; include *Vibrio cholerae,* cause of cholera, and luminescent forms symbiotic with deep-water fishes and squids.

VIRUSES: Very small disease-causing microorganisms that are too small to be seen even with microscopes. Viruses cannot multiply or produce disease outside of a living cell.

VITRIFICATION: Vitrification is a process of converting a material into a glass-like amorphous solid that is free from any crystalline structure, either by the quick removal or addition of heat, or by mixing with an additive. Solidification of a vitreous solid occurs at the glass transition temperature (which is lower than melting temperature, Tm, due to supercooling). When the starting material is solid, vitrification usually involves heating the substances to very high temperatures. Many ceramics are produced in such a manner. Vitrification may also occur naturally when lightning strikes sand, where the extreme and immediate heat can create hollow, branching rootlike structures of glass, called fulgurite. When applied to whiteware ceramics, vitreous means the material has an extremely low permeability to liquids, often but not always water, when determined by a specified test regime. The microstructure of whiteware ceramics frequently contain both amorphous and crystalline phases.

VOLATILE: A substance that evaporates or vaporizes at a relatively low temperature.

VOLATILE ORGANIC COMPOUNDS (VOCs): Solvents used as degreasers or cleaning agents. Improper disposal of VOCs can lead to contamination of natural waters. VOCs tend to evaporate very easily. This characteristic gives VOCs very distinct chemical odors like gasoline, kerosene, lighter fluid, or dry cleaning fluid. Some VOCs are suspected cancer-causing agents. Volatile organic compounds (VOCs) are organic chemical compounds that have high enough vapor pressures under normal conditions to significantly vaporize and enter the atmosphere. A wide range of carbon-based molecules, such as aldehydes, ketones, and other light hydrocarbons are VOCs. The term often is used in a legal or regulatory context and in such cases the precise definition is a matter of law. These definitions can be contradictory and may contain "loopholes"; e.g. exceptions, exemptions, and exclusions. The United States Environmental Protection Agency defines a VOC as any organic compound that participates in a photoreaction; others believe this definition is very broad and vague as organics that are not volatile in the sense that they vaporize under normal conditions can be considered volatile by this EPA definition. The term may refer both to well characterized organic compounds and to mixtures of variable composition.

VOID: An opening, gap, or space within rock or sedimentary formations formed at the time of origin or deposition.

VOLTAGE: Voltage (sometimes also called electric or electrical tension) is the difference of electrical potential between two points of an electrical or electronic circuit, expressed in volts.[1] It measures the potential energy of an electric field to cause an electric current in an electrical conductor. Depending on the difference of electrical potential it is called extra low voltage, low voltage, high voltage or extra high voltage. Specifically Voltage is equal to energy per unit charge.

VOLUTE: The spiral-shaped casing surrounding a pump impeller that collects the liquid discharge by the impeller.

VORTICELLA: Vorticella is a genus of protozoa, with over 100 known species. They are stalked inverted bell-shaped ciliates, placed among the peritrichs. Each cell has a separate stalk anchored onto the substrate, which contains a contracile fibril called a myoneme. When stimulated this shortens, causing the stalk to coil like a spring. Reproduction is by budding, where the cell undergoes longitudinal fission and only one daughter keeps the stalk. Vorticella mainly lives in freshwater ponds and streams - generally anywhere protists are plentiful. Other genera such as Carchesium resemble Vorticella but are branched or colonial.

VORTEX: The helical swirling of water moving towards a pump.

VIRUSES: are very small disease-causing microorganisms that are too small to be seen even with microscopes. Viruses cannot multiply or produce disease outside of a living cell.

VULNERABILITY ASSESSMENT: An evaluation of drinking water source quality and its vulnerability to contamination by pathogens and toxic chemicals.

WAIVERS: Monitoring waivers for nitrate and nitrite are prohibited.

WASTE ACTIVATED SLUDGE: Excess activated sludge that is discharged from an activated sludge treatment process.

WASTEWATER: Liquid or waterborne wastes polluted or fouled from households, commercial or industrial operations, along with any surface water, storm water or groundwater infiltration.

WATER HAMMER: A surge in a pipeline resulting from the rapid increase or decrease in water flow. Water hammer exerts tremendous force on a system and can be highly destructive.

WATER QUALITY CRITERIA: Comprised of both numeric and narrative criteria. Numeric criteria are scientifically derived ambient concentrations developed by EPA or States for various pollutants of concern to protect human health and aquatic life. Narrative criteria are statements that describe the desired water quality goal.

WATER QUALITY STANDARD: A statute or regulation that consists of the beneficial designated use or uses of a waterbody, the numeric and narrative water quality criteria that are necessary to protect the use or uses of that particular waterbody, and an antidegradation statement.

WATERBORNE DISEASE: A disease, caused by a virus, bacterium, protozoan, or other microorganism, capable of being transmitted by water (e.g., typhoid fever, cholera, amoebic dysentery, gastroenteritis).

WATER RECLAMATION: The restoration of wastewater to a state that will allow its beneficial reuse.

WHOLE EFFLUENT TOXICITY: The total toxic effect of an effluent measured directly with a toxicity test.

WPCF: Water Pollution Control Facility

WTP: Water Treatment Plant

WWTP: Wastewater Treatment Plant

ZERO DISCHARGE: A facility that discharges no liquid effluent to the environment.



Glossary References

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