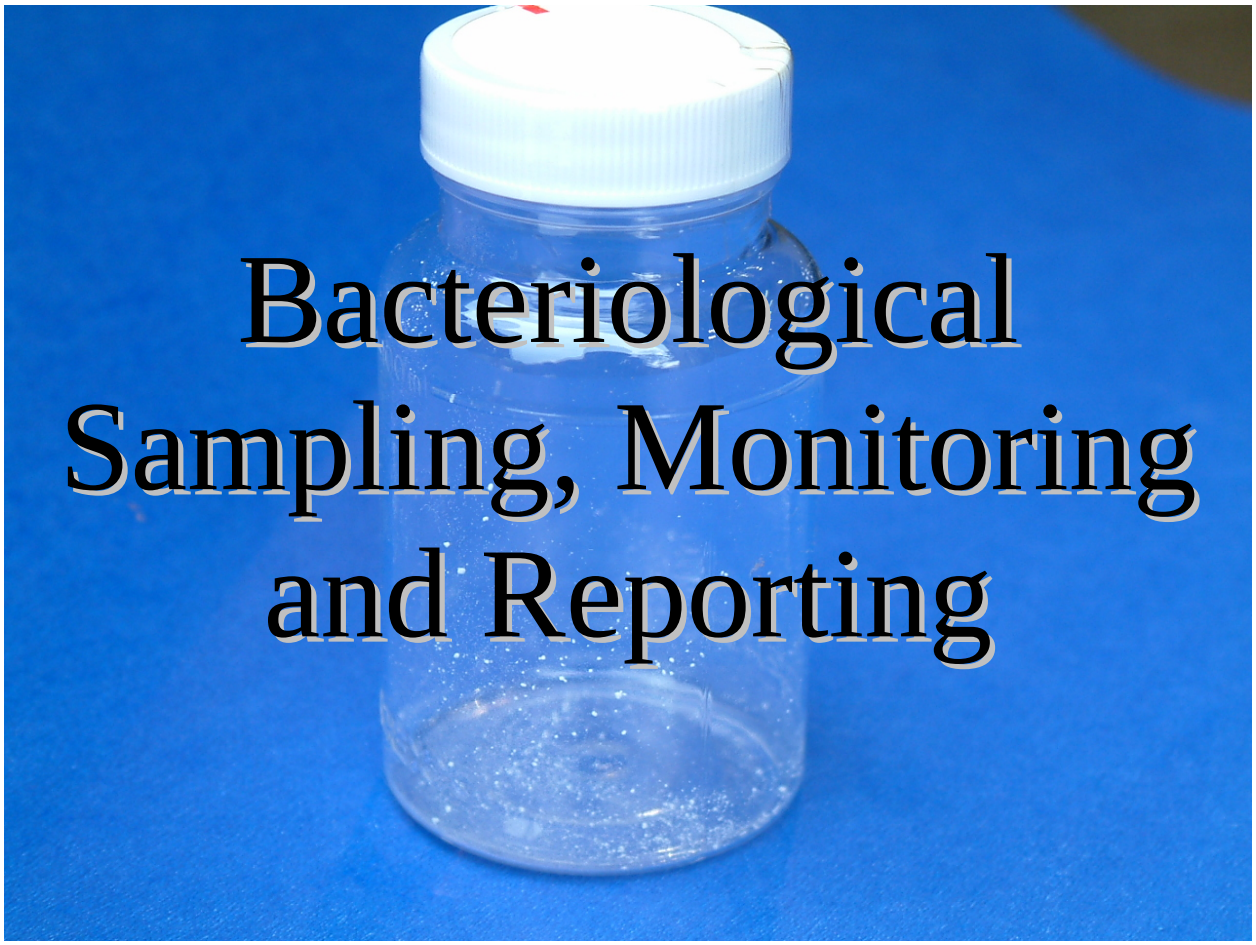

Bacteriological Sampling, Monitoring and Reporting

Prepared and presented by the

Tennessee Association of Utility Districts

SE-TAC

Southeastern Regional Small Public Water Systems Technical Assistance Center

This course is funded by a grant from the Mississippi Water Resources Research Institute through the Southeastern Regional Small Public Water Systems Technical Assistance Center (SE-TAC) at Mississippi State University. SE-TAC provides technical assistance and instructional support to small drinking water systems in the southeastern U.S.

Bacteriological Sampling, Monitoring, and Reporting

Prepared and presented by the
Tennessee Association of Utility Districts
Murfreesboro, Tennessee

This course is funded by a grant from the Mississippi Water Resources Research Institute through the Southeastern Regional Small Public Water Systems Technical Assistance Center (SETAC) at Mississippi State University. SETAC provides technical and instructional support to small drinking water systems in the southeastern U. S.

This training course was developed under Sub-Assistance Agreement No. X 83211101-4 awarded by the Southeastern Regional Small Public Water Systems Technical Assistance Center (SE-TAC) through U.S. Environmental Protection Agency. It has not been formally reviewed by EPA. The views expressed in this document are solely those of TAUD and neither SE-TAC nor EPA endorses any products or commercial services mentioned in this publication.

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Bacteriological Sampling, Monitoring, and Reporting

Course Description

The course is designed to familiarize the student with the knowledge, necessary skills, and requirements to develop and operate a state certified laboratory for the conduct of routine bacteriological (BACT) tests. The course covers a summary presentation of state regulatory rules governing BACT sampling, monitoring, and reporting. Certain microbiological characteristics of drinking water are also presented in conjunction with a presentation on common waterborne diseases, focusing on the latest pathogenic concerns with *Cryptosporidium parvum* and *Giardia lamblia.*
Proper BACT sampling procedures are developed in a student participation exercise followed by actual water sampling for BACT using one of the EPA approved presence-absence methods. Test results are read on the second day to provide the required 24 hr incubation period. EPA approved test methods for BACT are presented in a slide presentation.
Students work in teams to develop a BACT Sampling Plan for a hypothetical water system.
All requirements for developing a state certified laboratory are presented.
The course includes a presentation of cause-effect, control measures, and biological activity reaction tests (BART) for iron bacteria and sulfate reducing bacteria.
Course review is accomplished by a Jeopardy game with the class competing in teams of 3 to 4 students.

Course Objectives

The course has several specific objectives. At the completion of the course, each student should:

- Be familiar with the BACT sampling, monitoring, and reporting rules;
- Have a working knowledge of microbiological aspects of drinking water with respect to coliform bacteria, other heterotrophs, parasitic protozoans, and viruses and a knowledge of waterborne diseases of concern;
- Have developed a proper procedure for BACT sampling;
- Know the rationale, rules, and methodologies required to develop an approvable BACT sampling plan;
- Have a working knowledge of approved BACT test methods;
- Have performed a hands-on BACT sampling procedure and a presence-absence BACT test and properly documented the results;
- Be familiar with the causes, effects, control measures, and test methods for iron bacteria and sulfate reducing bacteria; and
- Know all of the rules and requirements necessary to develop and operate a state certified BACT laboratory for routine drinking water analysis.

Course Outline

The following is a suggested course outline. The instructor should designate a table in the classroom or an adjacent room for setup of an incubator which is turned on and set to a temperature of 35 degrees C before the class starts. Adjacent to the incubator, a UV lamp should be set up. The course requires a supply (one per student) of approved sample bottles and labels, pre-packaged enzyme substrate media packets, and BART test vials.

- Introductory remarks as to the subject of the course and the course objectives. Slides 3-7 can be modified and displayed as appropriate for the course.
- Pretest- intended to gauge the knowledge level of the class as a whole. Test is closed book and students do not put their name on the test paper. Test papers are traded for grading and answers are given without discussion. Grades are recorded and an average grade calculated.
- Slide presentation/lecture (Slides 8-12). A copy of the Tennessee Rules governing BACT monitoring and analytical requirements is included in the student text. **Note: Obviously, this section would be replaced with actual rules and summary slides pertaining to the state in which the course is given.**
- Slide presentation/lecture (Slides 13-41).
- BACT Sampling Procedure Exercise (Slide 42). The exercise is a jumbled BACT sampling procedure. Students are given 20-30 minutes to number the procedural steps in the correct order and x out the steps that do not belong in the procedure. When all students have completed the exercise, the class is queried as to the steps that do not belong and why. The class is then queried to give the proper order, one step at a time with discussion as to why the step is necessary and important for proper BACT sampling. It is likely that this exercise will generate some debate and lively discussion as actual field procedures vary and state guidelines vary as well.
- Approved sample bottles and labels are distributed (one per student). The proper procedure for holding and opening the bottle is demonstrated. Students are then taken in pairs to pre-designated potable water taps and each student is observed while BACT samples are collected and labeled by each student. Labeled samples are carried by the student back to their seat.
- Slide presentation/lecture (Slides 43-52) on coliform bacteria and the approved methods for analysis. Focus is on presence absence methods for drinking water and particular focus on the Colitag method used in this course. **Note: Other approved presence absence methods may be substituted or added at the discretion of the instructor.**
- Test media packets are distributed. Students are instructed on the proper procedure for opening the packet. Each student inoculates their sample, mixes the contents thoroughly, and places the sample in the incubator. A test record sheet (copy in the text) is placed next to the incubator and is completed by

each student as the bottles are placed in the incubator. This should complete Day 1 of the course.

- Day 2 begins with a short lecture on the rules and rationale utilized in the development of a BACT Sampling Plan. Two different sampling plan templates (Hometown and Anytown) are distributed. Students work in pairs on a single sampling plan. Students follow the instructions and complete the plan and prepare to present their plan to the class. This exercise requires about 2-2.5 hours.
- Slide presentation/lecture (separate Power Point file) on the Requirements for Laboratory Certification followed by a question/answer period. **Note: This presentation will vary for different states. In this course, the text includes a sample Laboratory Quality Assurance Plan, regulatory forms for recordkeeping, checklist for lab audits, and a copy of the rules governing laboratory certification. The presenter reviews these documents with the class.**
- Slide presentation/lecture (Slides 53-69) on iron bacteria. Distribute an IRB-BART vial to each student to take home and use for testing of suspect water in their home system.
- Slide presentation/lecture (70-73) on sulfate reducing bacteria. Distribute a SLYM-BART vial to each student to take home and use for testing of suspect water in their home system.
- Review BACT reporting requirements from the rules provided in the text. **Note: This presentation will vary according to different state rules.**
- In pairs, students retrieve their BACT samples from the incubator. Results are read and recorded by each student. Pre-prepared positive coliform and positive E. coli standards are shown to the students.
- As a course review, students are divided into teams of 3-4 to compete in Jeopardy. A hard copy of the game answers/questions in each category is in the trainer text. The game is displayed on a separate Power Point file. The score is kept and the winning team is awarded a door prize.
- Post-Test- Test is closed book and students do not put their name on the test. After ~10 min, test papers are traded and graded as the answers are given and briefly discussed. The class test grade average is calculated and compared to the class grade on the Pre-test given at the beginning of the class.
- Students are thanked for their attendance and work/participation and class is adjourned.

Bacteriological Sampling, Monitoring, and Reporting

Course Agenda

Day 1

8:00 Registration
8:30 Introduction
Course Objectives
Pretest
Rules and Regulations
Microbiology
BACT Sampling Procedures
BACT Sampling Exercise
BACT Testing Procedures
BACT Testing Exercise
3:30 Adjourn

Day 2

8:30 BACT Sampling Plan
Laboratory Certification Requirements
Laboratory QA Plan
Iron Bacteria
Slime Bacteria
Coliform Reading Exercise
BACT Reporting
Jeopardy
Post Test
3:30 Adjourn

BACT Post Test

Name: Unnecessary
Class Location: _____

Your Score:
Highest Possible Score: 11

Multiple Choice: For each of the following questions, circle the letter of the answer that best answers the question.

1. Which of the following waterborne diseases has been largely eliminated in the US?

   A. Cryptosporidiosis
   B. Gastroenteritis
   C. Cholera
   D. Tuberculosis

2. Which organism is responsible for the largest number of disease outbreaks in the US in the last 20 years?

   A. Escherichia coli
   B. Giardia lamblia
   C. Salmonella typhi
   D. Fecal coliform

True or False: For each statement, circle True or False.

True	False	3. Protozoans are generally more resistant to chlorination than coliform.
True	False	4. Cryptosporidium parvum is an infectious bacteria.
True	False	5. Typhoid fever is caused by the bacteria, Salmonella typhi.
True	False	6. Viruses cannot be transmitted in water.
True	False	7. Proper BACT sampling requires the use of sterile sample bottles, a dechlorination preservative, and proper handling without introducing contamination.

Fill in the Blank: Into each sentence below, copy a term from the word bank that correctly completes the sentence.

Membrane Filter Technique	Typhoid fever	Oxygen Uptake test	Total coliform	Chlorination
Fecal coliform	Hepatitis B	35 degrees C	Giardia lamblia	Colitag
Hydrogen peroxide	Cryptosporidium parvum	Multiple Tube Fermentation	E. coli	25 degrees C

8. A number of test methods are EPA approved for testing of total coliform in drinking water including _____, _____, and _____.

9. The MCL for _____ in drinking water is zero colonies per 100 ml.

10. Parasitic protozoans of significant concern at the present time include _____ and _____.

11. The proper incubation temperature for the total coliform test is _____.

12. Essay Question: Describe at least three things that water suppliers can and should do to protect customers from waterborne disease.

WELCOME

- Find your favorite seat
- Complete your registration form
- Provide your SSN and license if you want 12 CEUs
- Get a cup of coffee or a soft drink

Bacteriological Sampling, Monitoring, and Reporting

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Tennessee Association of Utility Districts

This course is funded by a grant from the Mississippi Water Resources Research Institute through the Southeastern Regional Small Public Water Systems Technical Assistance Center (SETAC) at Mississippi State University. SETAC provides technical and instructional support to small drinking water systems in the southeastern U. S.

Course Objectives

What are your objectives and expectations for this course?

Do you have specific issues with BACT monitoring and testing in your system?

Do you have interest in developing your own certified lab for BACT testing?

Specific Objectives

- Learn the BACT sampling, testing, and reporting rules
- Learn some microbiology of drinking water
- Learn proper BACT sampling procedures
- Develop a BACT sample siting plan
- Learn BACT testing procedures
- Conduct BACT sampling and testing
- Learn methods to control iron bacteria
- Learn methods to control slime-producing bacteria
- Learn how to get your lab certified to conduct your own BACT tests

Pretest

- Closed book
- No names
- 10 minutes
- Do your best

Rules Summary

- Systems must monitor for total coliform with 100 ml samples
- Must utilize one of the following test methods:

  Multiple Tube Fermentation

  Membrane Filter

  Presence-Absence Coliform Test

  Minimal Medium ONPG-MUG Test

Rules Summary

- If total coliform results are positive, must test for fecal coliform
- E. coli may be tested for in lieu of fecal coliform
- Coliform samples must be taken at regular intervals and in numbers proportional to population served (ranging from 1/mo-480/mo)

Rules Summary

- Systems must have written sample siting plans
- Routine sampling must be conducted in accordance with a TDEC approved sampling plan
- Routine samples shall be collected at sites representative of water throughout the system

Rules Summary

- Systems must collect repeat samples within 24 hrs of being notified of a positive coliform test result
- Must collect either 3 or 4 repeat samples for each coliform-positive routine sample (depending on routine monitoring frequency)

Rules Summary

- Repeat samples must be collected on the same day; and from the original sample site and at least one sample within 5 services upstream and 5 services downstream
- If any repeat sample is coliform-positive, repeat sampling must be re-conducted
- Sample results can be invalidated because of certain factors
- Sanitary survey findings may result in changes to a system's BACT sampling frequency

Waterborne Disease by Pathogens

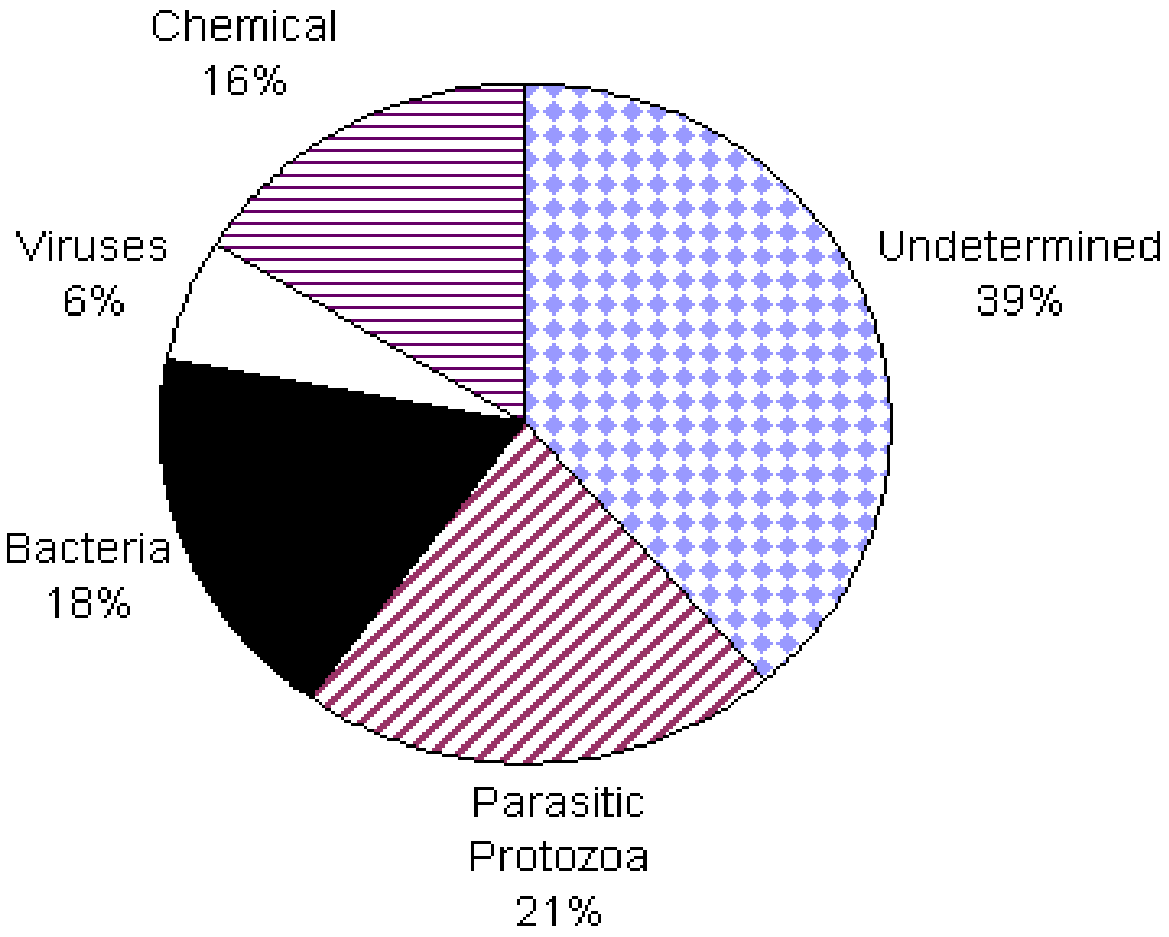
Filtration and disinfection have largely eliminated many deadly waterborne diseases of the past.

1. Cholera (essentially gone since 1960), *V. cholerae*
2. Typhoid fever (few cases since 1971), *Salmonella typhi*
3. Hepatitis A (only 4% of cases since 1971), virus

Salmonella typhi

Etiological Agent	Community Water Systems[2]		Noncommunity Water Systems[3]		Individual Water Systems[4]		All Systems	
	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases
Giardia	11	2,073	5	167	6	16	22	2,256
Cryptosporidium[1]	7	407,642	2	578	2	39	11	408,259
Campylobacter	1	172	3	66	1	102	5	340
Salmonellae, nontyphoid	2	749	0	0	1	84	3	833
E. coli	3	208	3	39	3	12	9	259
E. coli O157:H7/C. jeuni	0	0	1	781	0	0	1	781
Shigella	1	83	5	484	2	38	8	605
Plesiomonas shigelloides	0	0	1	60	0	0	1	60
Non-01 V. cholerae	1	11	0	0	0	0	1	11
Hepatitis A virus	0	0	1	46	1	10	2	56
Norwalk-like viruses	1	594	4	1806	0	0	3	2400
Small, round-structured virus	1	148	1	70	0	0	2	218
Chemical	18	522	0	0	7	9	25	531

Causes of Waterborne Disease Outbreaks in the USA, 1991-2000

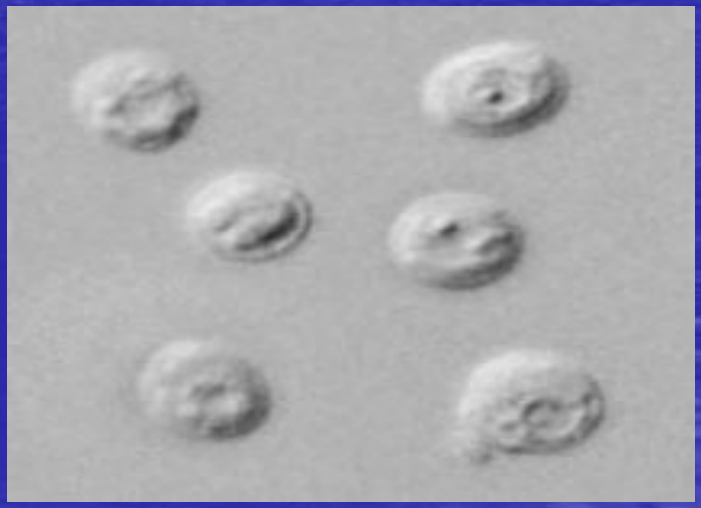


Waterborne Disease by Pathogens

Problem Protozoans

1. *Giardia lamblia*

2. *Cryptosporidium parvum*



Waterborne Disease by Pathogens

Newly identified waterborne pathogens of emerging concern :

- Norwalk-like virus
- Rotavirus
- Yersinia- bacteria
- Campylobacter- bacteria
- E. coli 0157:H7- bacteria
- Cyclospora cayetanensis- protozoan
- Aeromonas- bacteria

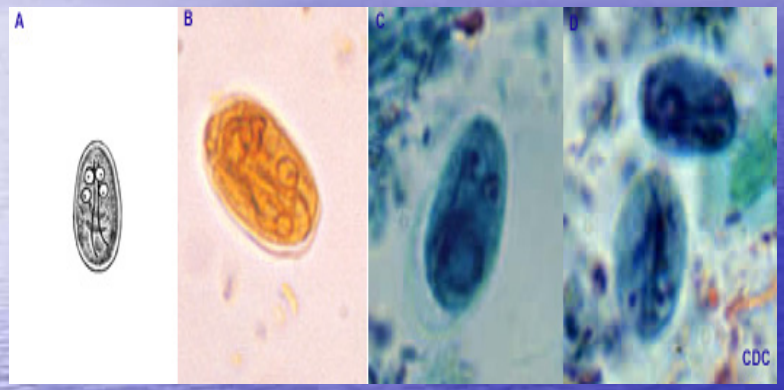
Giardia lamblia

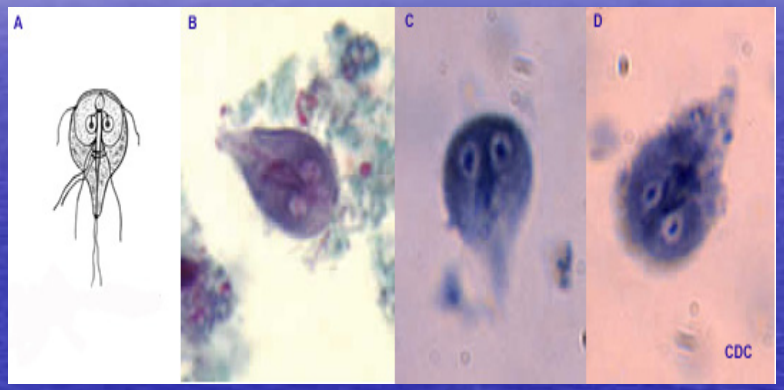
- Protozoan that is more resistant to disinfection than coliform organisms
- Cause of more outbreaks in the US than any other singly identified agent between 1971 and 1983
- A number of different animal feces are implicated as the carriers, e.g. dogs, cats, sheep, pigs, goats, and cattle
- Primary prevention method is protection of the raw water source from contaminated runoff, both urban and rural

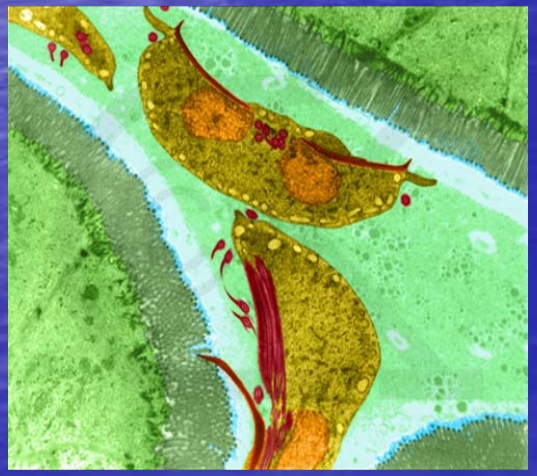
Giardia lamblia

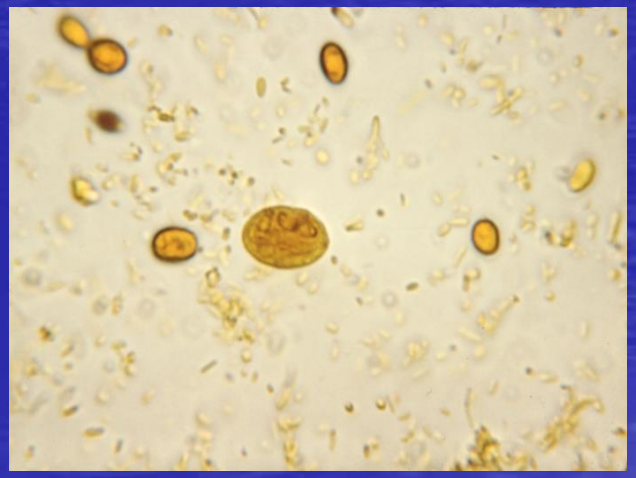
Round to oval, 8-18um long by 5-16um wide

Cyst containing 2-4 nuclei









Giardiasis

- Symptoms

  Diarrhea

  Fatigue

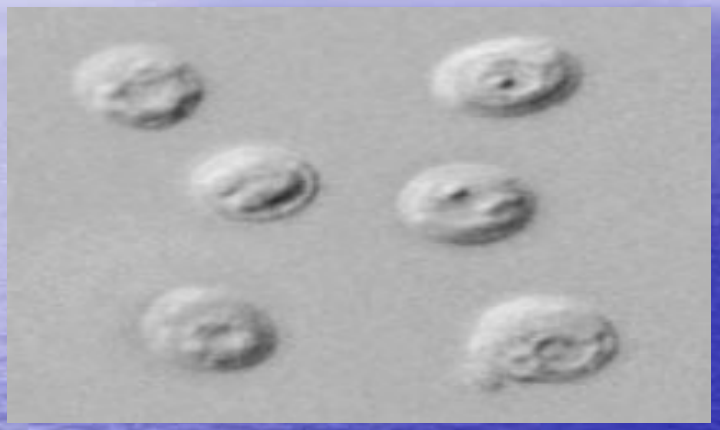
  Abdominal cramps
- USEPA concedes that the present sampling and analytical methodology is too difficult and it is impossible to distinguish between viable and nonviable cysts

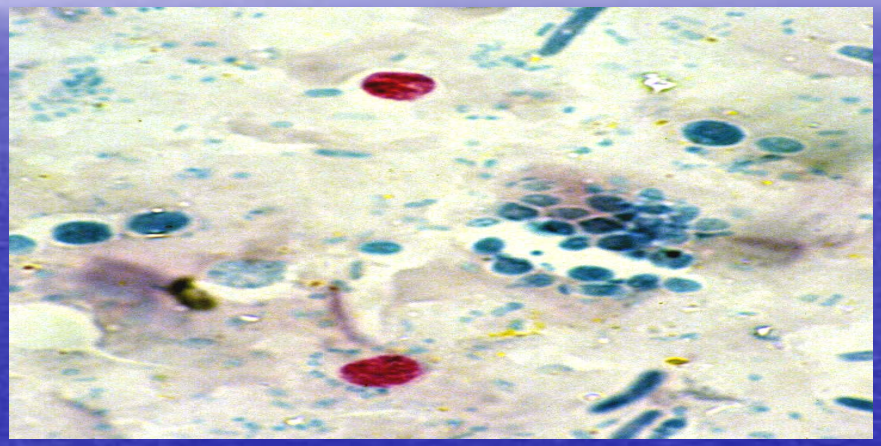
Cryptosporidium parvum

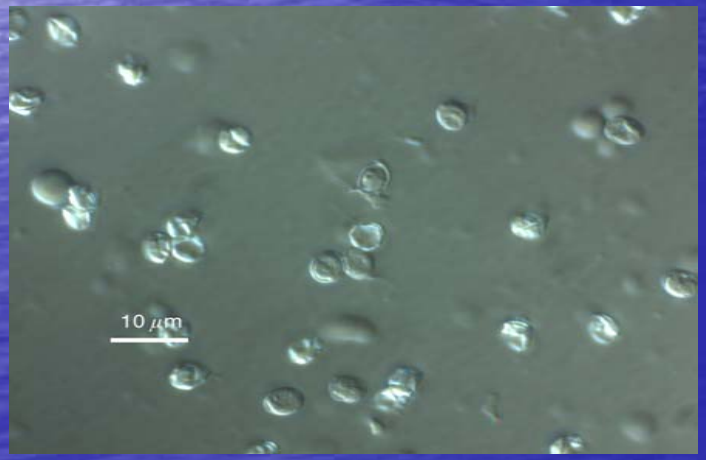
- Parasitic protozoan that infects humans and many animals
- Recognized as a pathogen since 1976
- Forced EPA to enact ESWTR in 1994 and now LT2ESWTR
- Has likely resulted in more changes to drinking water regulations than any singular factor in 50 years

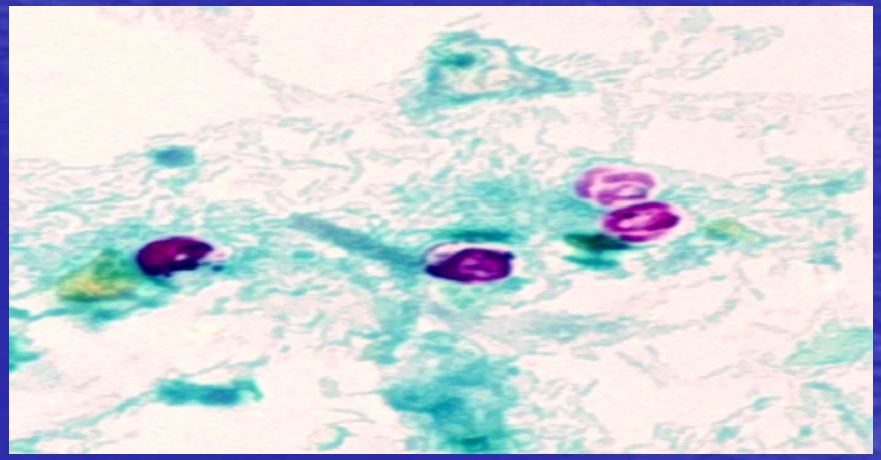
Cryptosporidium parvum

Spherical, thick-walled, 4-6 um cyst containing 4 sporozoites









Cryptosporidium parvum

- When oocysts are ingested, they lodge in the small intestine, split open, and release sporozoites which create new oocysts. The infection persists unless the oocysts are excreted.
- Infections have been found in cattle, dogs, cats, deer, and rabbits, and unfortunately,

  .....people

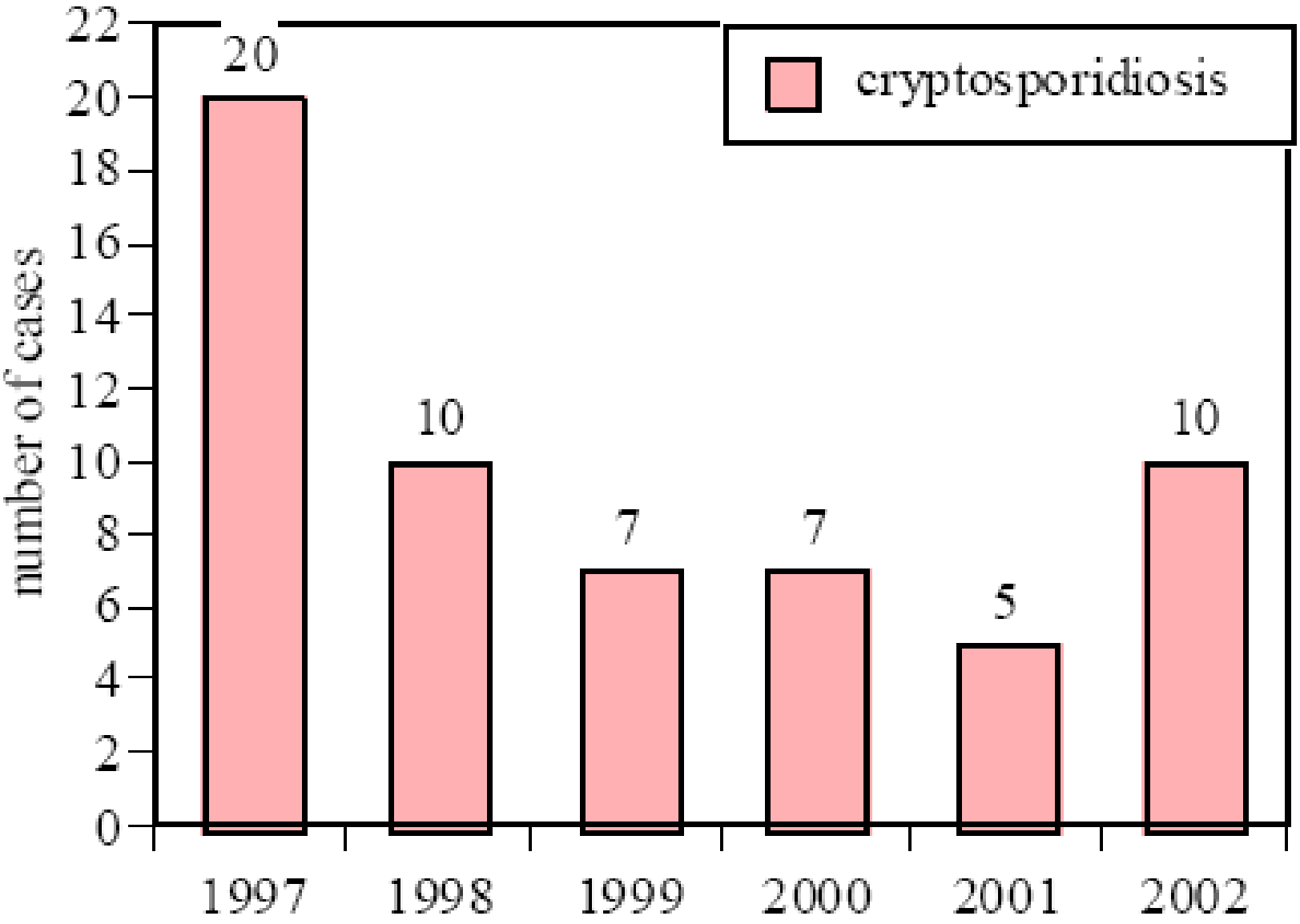
Cryptosporidiosis

- First significant outbreak in Milwaukee in April., 1993
- 400,000 people became ill
- 100 deaths were registered

What did we learn about Crypto from this outbreak?

- It is prevalent in the aquatic environment
- Humans can be carriers without symptoms
- Median dose of infection is ~130 oocysts
- No special drug or treatment cure
- Persons with weakened immune systems are especially vulnerable

Cryptosporidiosis Cases in Kentucky



What should be done to better protect the public?

- Protect watersheds from human and animal waste
- Consider a disinfection substitute for chlorine such as ozone or hydrogen peroxide
- Increase monitoring for coliform, fecal coliform, and Crypto
- Upgrade treatment to achieve a target turbidity of < 0.1 NTU
- Curtail the practice of recycling backwash water

So..... new tougher rules were enacted in stages to provide greater protection.

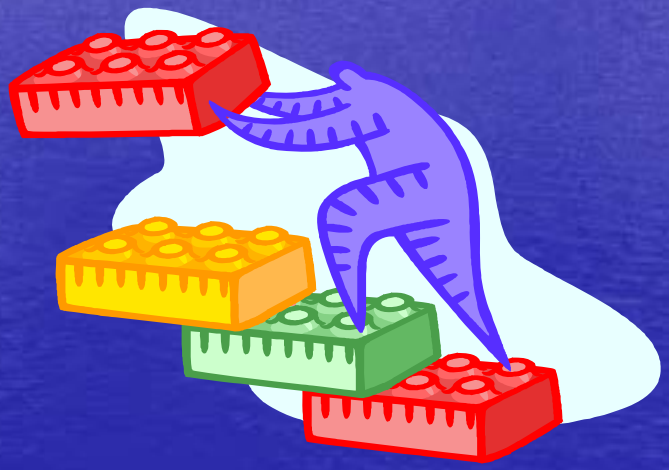
- Started with SWTR in June, 1989
- Continued with ESWTR in July, 1994
- And now..... LT2ESWTR beginning October, 2006 and extending until 2016

LT2ESWTR Overview

- Source water monitoring
- Screening procedure for small systems
- Target treatment for highest risk systems
- Crypto inactivation at all unfiltered systems
- Cover or treat uncovered storage facilities

Review of SWTRs

- The LT2ESWTR builds on and complements previous SWTRs
- Offers flexibility to systems and states
- Focuses protection on the systems that need it most



SWTRs Summary:

Ongoing Requirements for All Systems

- Residual disinfectant continuously at entry point
  - ≥ 0.2 mg/L
  - Detectable in distribution system
  - Small system may take grab samples
- Distribution system residual measurements
  - Measure at same time & place as TCR
  - Never undetectable in >5% samples for 2 consecutive months
- Removal and/or inactivation
  - *Giardia*: 3-log
  - Viruses: 4-log
- Removal
  - *Crypto*: 2-log; or watershed protection if unfiltered

SWTRs Summary: ≥ 10,000 pop.
Conventional & Direct Filtration

ONGOING

- Monitoring:
  - CFE every 4 hours
  - 95% ≤ 0.3 NTU
  - Max = 1 NTU
- IFE continuously

ADDITIONAL

- Monthly for source water:
  - *Crypto*
  - *E. coli*
  - Turbidity
- Monitor for 24 months
- Possible additional removal and/or inactivation

SWTRs Summary: < 10,000 (Sched.1-3) Conventional & Direct Filtration

ONGOING

- CFE every 4 hours
  - 95% ≤ 0.3 NTU
  - Max = 1 NTU
  - 1/day for < 500 with state approval
- IFE continuously

OR

- CFE continuously if no more than 2 filters

ADDITIONAL

- Monthly for source water:
  - *Crypto*
  - *E. coli*
  - Turbidity
- Monitor for 24 months
- Possible additional removal and/or inactivation

SWTRs Summary: ≥ 10,000
Slow Sand, Diatomaceous Earth, & Alternative

ONGOING

- CFE every 4 hours
  - 95% ≤ 1 NTU
  - Max = 5 NTU
  - 1/day with state approval for:
    - Slow sand
    - Alternative

ADDITIONAL

- Monthly source water:
  - *Crypto*
  - *E. coli*
  - Turbidity
- Monitor for 24 months
- Possible additional removal and/or inactivation

SWTRs Summary: < 10,000 (Sched.1-3) Slow Sand, Diatomaceous Earth, & Alternative

ONGOING

- CFE every 4 hours
  - 95% ≤ 1 NTU
  - Max = 5 NTU
  - 1/day (with state approval) for:
    - < 501
    - slow sand
    - alternative

ADDITIONAL

- Monthly source water:
  - *Crypto*
  - *E. coli*
  - Turbidity
- Monitor for 24 months
- Possible additional removal and/or inactivation

SWTRs Summary: ≥ 10,000 Unfiltered

ONGOING

- Source water coliform
  - Fecal ≤ 20/100 mL

  OR
  - Total ≤ 100/100 mL
  - 1-5/week
- Turbidity
  - At least every 4 hours
  - 100% ≤ 5 NTU
- Daily water quality parameters
- Other requirements

ADDITIONAL

- Monthly source water:
  - *Crypto*
- Monitor for 24 months
- Possible additional log inactivation

SWTRs Summary: < 10,000 (Sched.1-3) Unfiltered

ONGOING

- Source water coliform
  - Fecal ≤ 20/100 mL

  OR
  - Total ≤ 100/100 mL
  - 1-5/week
- Turbidity
  - At least every 4 hours
  - 100% ≤ 5 NTU
- Daily water quality parameters
- Other requirements

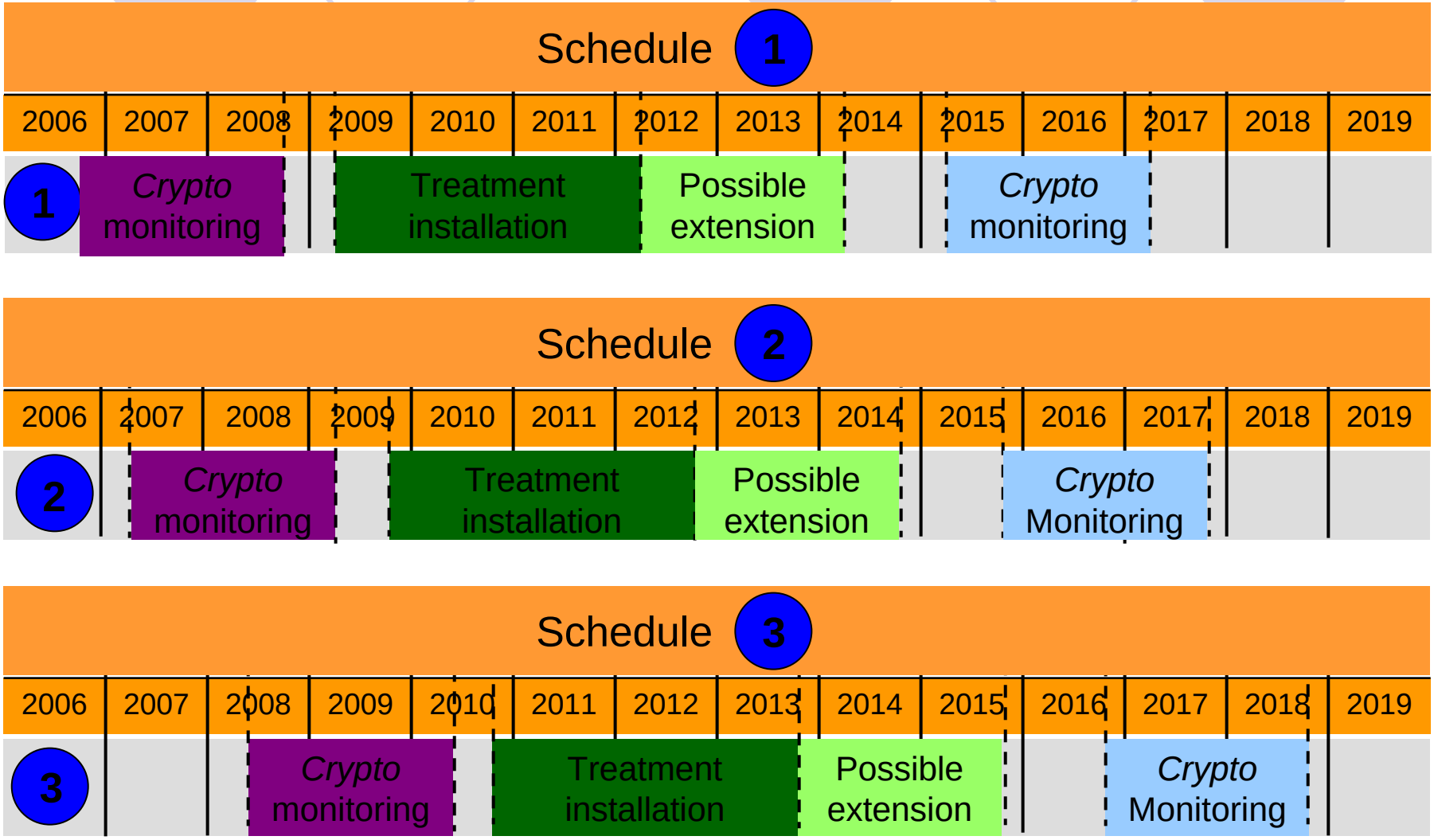
ADDITIONAL

- Monthly source water:
  - *Crypto*
- Monitor for 24 months
- Possible additional log inactivation

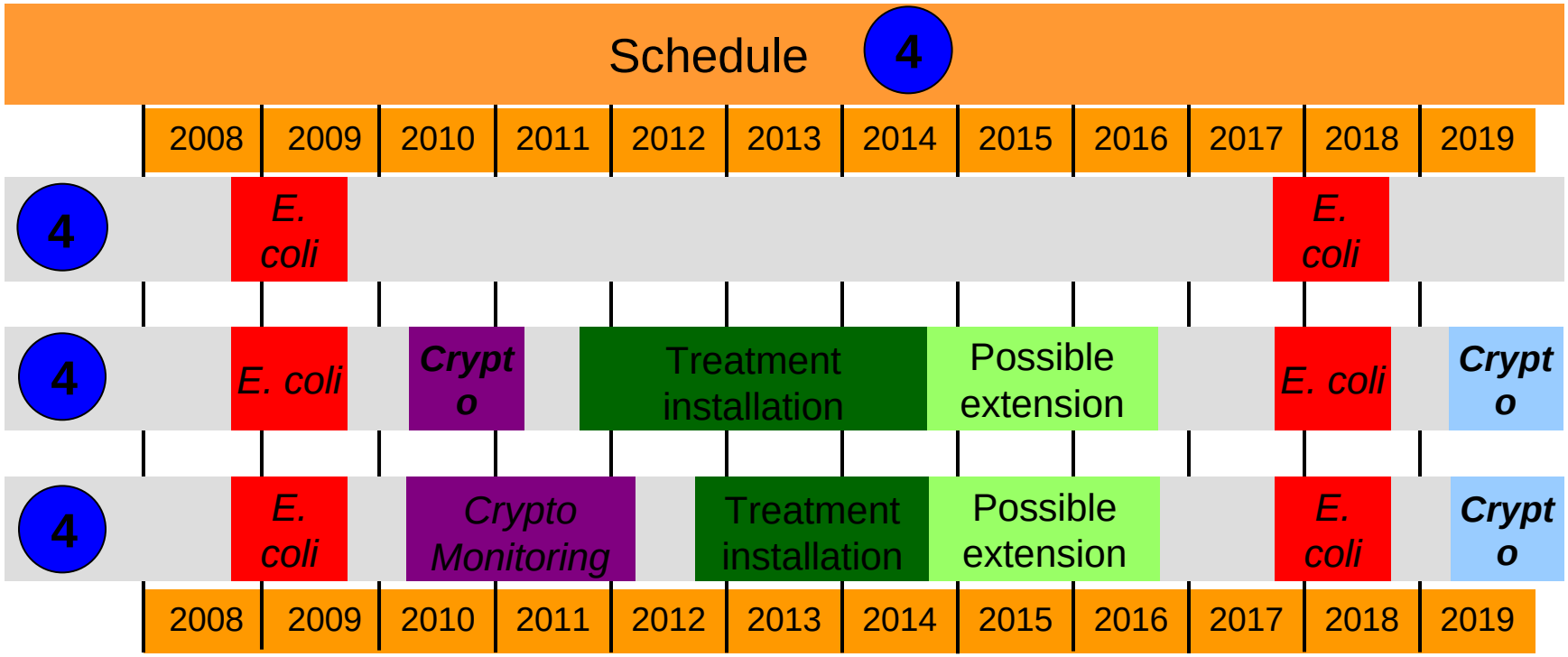
Contact Information

- TDEC
- Safe Drinking Water Hotline
  - 800-426-4791
  - Hotline-sdwa@epa.gov
  - http://www.epa/gov/safewater/
- EPA's M-DBP team
  - stage2mdbp@epa.gov

Implementation Timeline



Implementation Timeline (cont.)



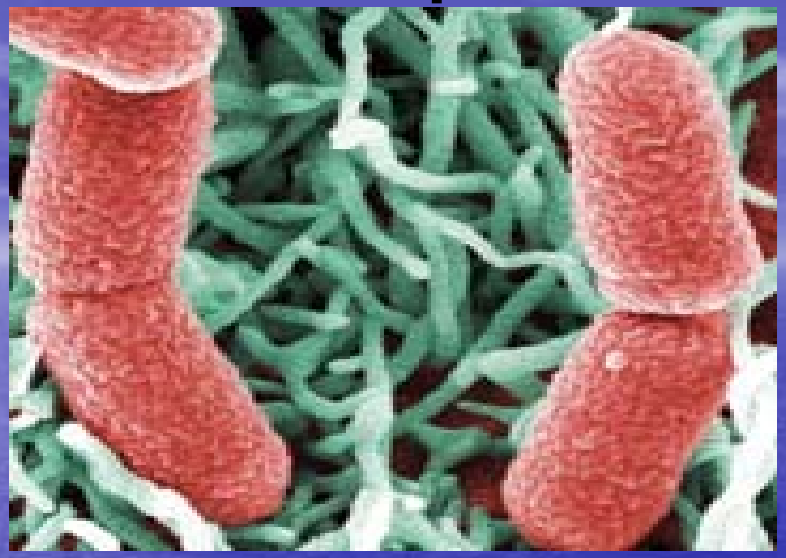
The Bottom Line

- Waterborne pathogens continue to emerge and evolve
- Disease outbreaks continue
- Water suppliers must be diligent to protect their customers
- Proper sampling and testing techniques for bacteriological quality are vital in this diligence

BACT Sampling

- Procedure Exercise
- Questions about procedures
- Collect and label BACT samples

Coliform Group of Bacteria



- Short definition- Lactose fermenters
- Long Definition- All facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with gas and acid formation within 48 hrs at 35 degrees C.

Total Coliform Testing

- Multiple Tube Fermentation Technique (MTF)
- Membrane Filter Technique (MF)
- Presence/Absence Test - pre-packaged MTF method suitable for potable water testing
- Multiple patented Presence/Absence test methods including Colilert, Colisure, Colitag, Readycult, Ecolite, etc.

All above methods are EPA approved and described in *Standard Methods for the Examination of Water and Wastewater*

Multiple Tube Fermentation (MTF)

- Necessary procedure for non-potable waters due to the expected density of organisms
- Precision depends on the number of tubes used
- For non-potable waters, multiple dilutions and tubes are used
- For potable water, use either:
  - 10 tubes @ 10 ml of sample each,
  - 5 tubes @ 20 ml of sample each, or
  - 1 tube @ 100 ml of sample
- Inoculate each tube with Lauryl tryptose broth

Multiple Tube Fermentation (MTF)

Lauryl Tryptose Broth

In one liter of reagent grade water, add:

Tryptose 20 gm

Lactose 5 gm

Dipotassium hydrogen phosphate 2.75 gm

Potassium dihydrogen phosphate 2.75 gm

Sodium chloride 5 gm

Sodium lauryl sulfate 0.1 gm

Bromcresol purple 0.01 gm

Multiple Tube Fermentation (MTF)

- Add the dehydrated broth to the water sample
- Mix by shaking thoroughly
- Incubate at 35 degrees C for 24-48 hrs
- Read the results

Multiple Tube Fermentation (MTF)

- If coliform group bacteria are present in the water sample, they will feed off of the lactose using it as substrate for energy.
- As the bacteria degrade the lactose anaerobically, volatile acids are generated.
- If coliform are absent, there will be no acid generation and therefore no color change. This is a ***negative*** result, and your test is complete.
- If coliform are present, the acids will react with the **bromcresol purple** indicator and turn the solution *yellow* in color. This is a ***positive presumptive*** reaction.

Multiple Tube Fermentation (MTF)

- If positive, this tells you that you have coliform bacteria. Now you can confirm if the coliform are fecal coliform.
- The E. coli bacteria is a member of the fecal coliform group and can be assayed.
- The occurrence of E. coli is a specific indicator of fecal contamination and the possible presence of enteric pathogens.

Membrane Filter Technique (MF)

- Method is extensively used, particularly for waters of relatively low turbidity
- Requires significant lab apparatus and sterile supplies (filters, agar, plates) and a microscope
- Is a quicker test than MTF
- Provides for better quantitative and qualitative analysis of multiple organisms including coliform

Presence/Absence Test (Colitag)

- Colitag is an EPA approved procedure for determining coliform presence or absence without fully quantifying the results.
- Pre-packaged sterile media packets
- Same biochemistry as MTF technique
- 100 ml sample in a single bottle
- Culture media is triple strength

Colitag P/A Water Test Kit

Procedure

1. Aseptically add Colitag to 100 ml water sample. Agitate to begin dissolution.
2. Incubate the sample at 35 degrees C for 24 hrs

Interpretation

1. Visually check the sample for yellow color. If the sample is yellow, the coliform bacteria are present.
2. Examine the solution for fluorescence using a long wavelength UV lamp. If bright blue fluorescence is observed, E. coli is present.
3. If no yellow color in the test sample is observed after 24 hr incubation, the sample is coliform negative.

Iron Bacteria

- Diverse group of microorganisms in nature
- Not pathogens
- Capable of transforming (precipitating) iron
- Precipitated iron fouls wells, lines, and treatment plants

Iron Bacteria

- Converts soluble iron (+2) to insoluble iron (+3)
- Insoluble iron plus bacterial polymers (acts like glue) accumulates around the bacterial cells
- Multiple bacteria can do this

Iron Bacteria

- *Leptothrix*
- *Clonothrix*
- *Hyphomicrobium*
- *Caulobacter*
- *Gallionella*
- *Thiobacillus* (can oxidize sulfur as well)

Iron Bacteria

Bug loves organic food

OH-C-C-C-OH

Fe

$$C_3H_5O_2Fe + 4O_2 \longrightarrow 3CO_2 + H_2O + \underline{Fe(OH)_3}\downarrow$$

Bug eats the iron-organic complex and spits out the iron

Iron Bacteria

Why worry about it?

Taste and odor problems
Frothing
Color
Increased turbidity
Can mask the presence of pathogens
Reduced disinfection efficiency
Increased disinfectant demand

But it gets worse!

Iron Bacteria

- Biofilms act as a media for the growth of corrosion producing bacteria
- Iron bacteria multiply rapidly
- Once established, biofilms are unaffected by normal chlorine residuals

Microbially Induced Fouling

- Accumulation (MIA)
- Gassing (MGG)
- Corrosion (MIC)
- Relocation (MIR)

Iron Bacteria

Left alone, biofilm buildup destroys the system by blockage and corrosion

Prevention is the key

You cannot prevent it unless you first identify it and then control it

Identification of Iron Bacteria

- A number of culture methods available
- Provides early warning so that control strategies can be implemented before your system is destroyed by blockage and corrosion
- Biological Activity Reaction Test (BART) is a presence/absence test
- For iron-related bacteria (IRB-BART)

IRB-BART Test

- Brown slime formation at surface and bottom indicates iron-bacteria presence
- Formation after:

BAD
- 2 days ~140,000 cfu/ml population
- 4 days ~9000 cfu/ml
- 5 days ~2300 cfu/ml

OK
- 6 days ~500 cfu/ml
- 8 days ~25 cfu/ml

GOOD
- 9 days negligible

Control Strategies

You must have three things for iron bacteria fouling to occur:

1. Presence of iron bacteria
2. Presence of dissolved or complexed iron in the source water
3. An environment that encourages bacterial survival and growth

Presence of iron bacteria

- Not a lot can be done because most of the bacteria are coming in with the source water (deep wells and reservoirs are the most common problem sources)
- Can also be introduced from system design features:

  - cascading water

  - use of corrodible metals in well/pumps

Presence of dissolved iron in the source water

Common Pretreatment Processes

Aeration

Chlorination

Potassium Permanganate

pH Adjustment

Aeration

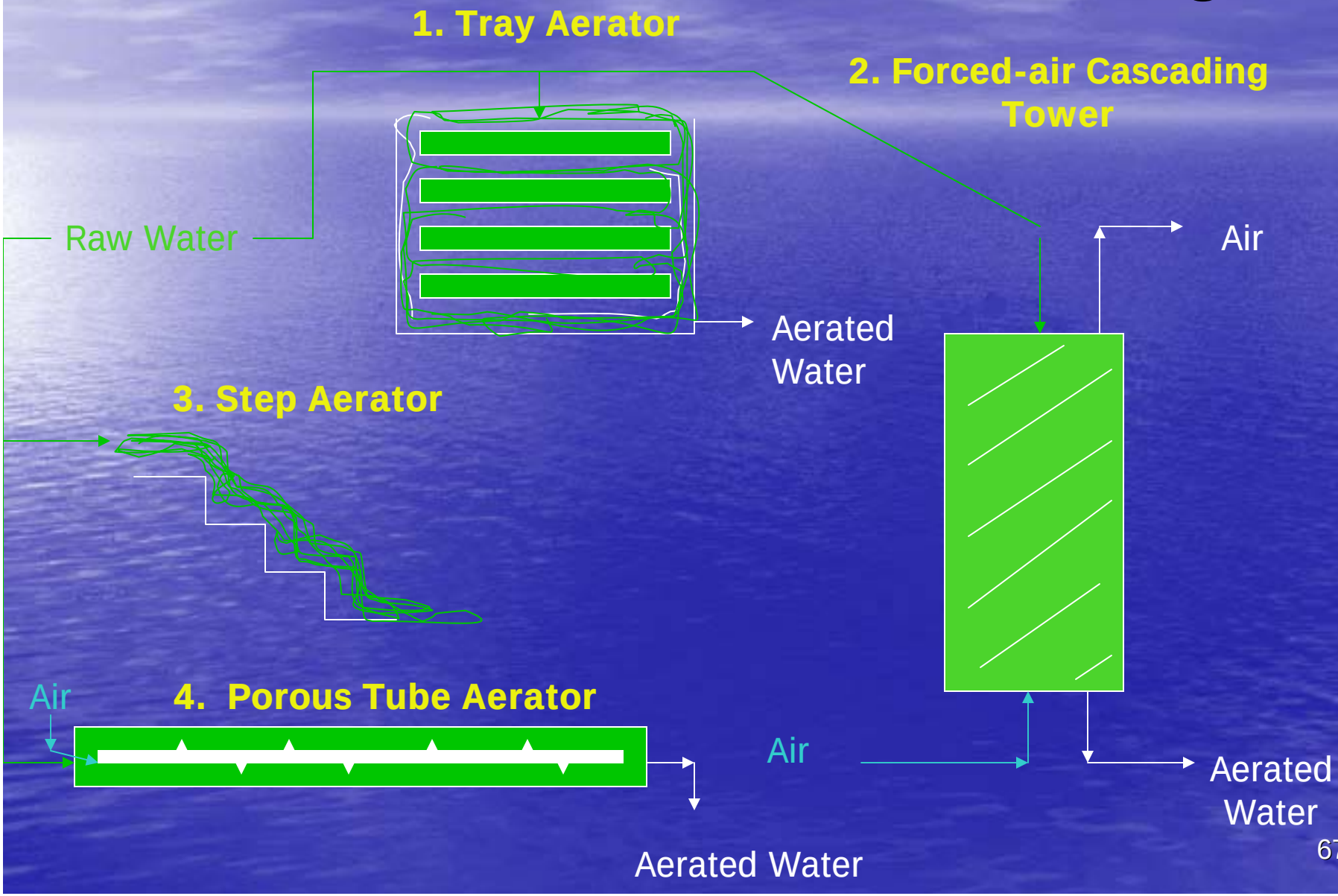
Advantages

- Readily oxidizes/precipitates soluble, non-complexed iron
- Iron hydroxide precipitate is a large, heavy floc and easily filterable
- Easy and usually cost effective
- Also strips out unwanted gases such as hydrogen sulfide

Disadvantages

- Potential organic interferences
- Not as effective in cold weather
- May need to adjust pH if <6.5
- Doesn't work with manganese at pH <9.5

Common Aeration Technologies



Prechlorination

Advantages

- Rapid reaction rate
- Works well with low pH water
- Oxidizes interfering organic compounds
- Will react with iron before organics
- Keeps the plant cleaner
- Chlorine is usually available already

Disadvantages

- Potential THM/HAA5 formation
- Produces a calcium carbonate precipitate in hard water
- Requires extra chlorine dose for demand of other constituents
- Chemical cost
- Less desirable byproducts

Potassium Permanganate

Advantages

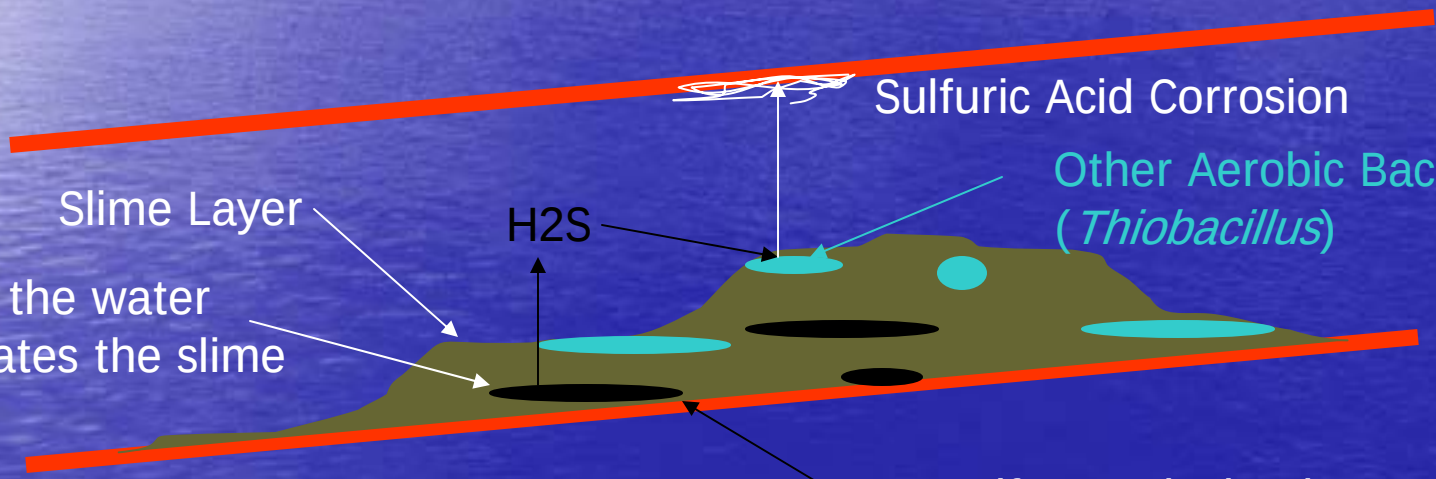
- Oxidizes iron and manganese
- No harmful byproducts of reaction

Disadvantages

- Must be careful with storage
- Reacts vigorously with organic materials
- Comes in a solid so it must be mixed into a solution
- Solubility in cold water is difficult
- If it gets damp, it cakes
- Best results are with two tank (mix/decant) method
- Very pH dependent
- Chemical cost

Sulfate Reducing Bacteria (SRB)

SRB protected by a cohabiting slime layer with other bacteria. Oxygen cannot penetrate the slime layer. SRB reduce sulfate to sulfide, forming entrapped H2S gas. Disturbance of the slime layer by flow surge or swabbing releases the gas and the rotten egg smell.



Aerobic sulfur-oxidizing bacteria (*Beggiotoa and Thiothrix)* in the surface slime layer oxidize the H2S to sulfuric acid which attacks the pipe wall causing corrosion.

Sulfate Reducing Bacteria (SRB)

Primary indicators of SRB problems include:

- Odors
- Black slimes
- Corrosion of metal pipes and equipment

Sulfate Reducing Bacteria (SRB)

SLYM-BART test provides an easy test to measure presence and rough quantification of SRB population.

Sulfate Reducing Bacteria (SRB)

Control Techniques

- Keep the water moving
- Flushing program
- Periodic cleaning of problem lines
- Monitor dissolved oxygen content and note dramatic declines

TAUD Jeopardy

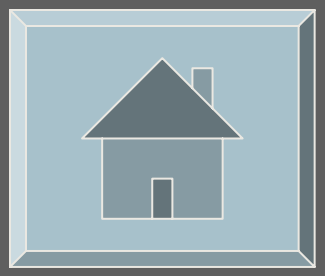
By TAUD Staff

Jeopardy

Play by the Rules	Bugs & Such	Sample Platter	Genie in a bottle	Stain Master	Potluck
100	100	100	100	100	100
200	200	200	200	200	200
300	300	300	300	300	300
400	400	400	400	400	400
500	500	500	500	500	500

Credits

- That’s all folks.



Exit

Play by the rules $100

- Total Coliform

Play by the Rules $100

- What BACT test is required for every system?

Play by the Rules $200

- TDEC-approved sampling plan.

Play by the Rules $200

- What must every system have?

Play by the Rules $300

- 3 or 4 repeat samples

Play by the Rules $300

- What must be collected for every positive coliform test result?

Play by the Rules $400

- E. coli or fecal coliform

Play by the Rules $400

- What must be tested for if total coliform is positive?

Play by the Rules $500

- 1 per month to 480 per month

Play by the Rules $500

- What is the range of sampling frequencies based on service population?

Bugs & Stuff $100

- Cholera, Typhoid fever & Hepatitis A

Bugs & Stuff $100

- What three waterborne diseases have been largely eliminated in the US?

Bugs & Stuff $200

- Problem protozoans

Bugs & Stuff $200

- What are Giardia lamblia & Crytosporidium?

Bugs & Stuff $300

- Resistant to disinfection by chlorination.

Bugs & Stuff $300

- What are protozoans?

Bugs & Stuff $400

- Killed 100 people in Milwaukee in 1993

Bugs & Stuff $400

- What is Cryptosporidiosis?

Bugs & Stuff $500

- Rules beginning in October 2006

Bugs & Stuff $500

- What is LT2ESWTR?

Sample Platter $100

- Sample location, date & time.

Sample Platter $100

- What information should be recorded on a sample bottle?

Sample Platter $200

- Within 48 hours of sample collection

Sample Platter $200

- When should be the total coliform test be done?

Sample Platter $300

- Let it run for 3 to 5 minutes

Sample Platter $300

- What is the cold water tap?

Sample Platter $400

- Sodium Thiosulfate

Sample Platter $400

- What dechlorination chemical is in the sample bottle?

Sample Platter $500

- Strainers/aerators or other attachments.

Sample Platter $500

- What should be removed before sampling?

Genie in a Bottle $100

- Lactose fermenters

Genie in a Bottle $100

- What are the coliform group of bacteria?

Genie in a Bottle $200

- Three EPA approved coliform test methods

Genie in a Bottle $200

- What are the Multiple Tube Fermentation, Membrane Filter, & Present/absence (Colitag)?

Genie in a Bottle $300

- Bromcresol Purple

Genie in a Bottle $300

- What is the color indicator in th MTF & Coitag method broth media?

Genie in a Bottle $400

- 100 ml

Genie in a Bottle $400

- How many ml of sample are required for the Presence/Absence test method?

Genie in a Bottle $500

- 35 degrees C

Genie in a Bottle $500

- What is the incubation temperature for total coliform test?

Stain Master $100

- Capable of precipitating iron.

Stain Master $100

- What are iron bacteria?

Stain Master $200

- Deep wells & reservoirs.

Stain Master $200

- What are the primary problem water sources for iron bacteria?

Stain Master $300

- Aeration

Stain Master $300

- What is the most common treatment process for iron removal?

Stain Master $400

- *DAILY DOUBLE*
- Primary disadvantage for iron oxidation with chlorine.

Stain Master $400

- What is THM/HAA5 formation?

Stain Master $500

- Brown slime formation & a 140,000 cfu/ml population.

Stain Master $500

- What is a bad IRB-BART test result?

Potluck $100

- Characterized by black slimes, odor, & corrosion.

Potluck $100

- What are sulfate reducing bacteria?

Potluck $200

- The microorganism responsible for most waterborne disease outbreaks in the US between 1971-1983.

Potluck $200

- What are Giardia lamblia.

Potluck $300

- Must be representative of water throughout the system.

Potluck $300

- What are routine Bact samples.

Potluck $400

- Process that will not precipitate manganese at pH less than 9.5.

Potluck $400

- What is aeration?

Potluck $500

- Periodic & regular line cleaning & flushing.

Potluck $500

- What are the most effective ways to control the growth of sulfate-producing bacteria?

Operator Training

Tennessee Drinking Water Laboratory Certification Program

Division of Water Supply
Troy Taubert
E-mail troy.taubert@state.tn.us

Tennessee Department of Environment and Conservation

Laboratory Certification Personnel

Certification Authority – Robert L Foster, Jr.

Certification Officers - Troy Taubert (931-432-7643)

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Amy Francis (423-634-5720)

USEPA

Manual for the Certification of Laboratories Analyzing Drinking Water

Fifth Edition

2005

Download the manual in PDF format at:

http://www.epa.gov/safewater/labcert/labindex.html

RULES OF THE TENNESSEE DEPARTMENT OF ENVIRONMENT AND CONSERVATION

DIVISION OF WATER SUPPLY

1200-5-1-.14 Laboratory Certification

Download these Rules at:

http://www.state.tn.us/sos/rules/1200/1200-05/1200-05.htm

What must I do to get certified

- Request certification
- Must have a QA plan which addresses items listed in 1200-5-1-.14(2)(a)
- Complete PE samples as described in 1200-5-1-.14(9)
- Pass an On-site Audit at least every three years
- Pay certification fees annually

Lab Certification Rules
1200-5-1-.14

- New Rules will be effective October 14, 2006
- Written Application indicating which groups of contaminants a lab is seeking certification. Call, email, or a letter will be accepted. .14(1)(c)
- Laboratories must have an on-site audit every three years .14(1)(d)
- Record keeping requirements further specified and increased to six years .14(1)(g)

- EPA Certification manual included in Rules by reference .14(1)(h)
- Public Water Systems are exempted from Lab fees .14(2)(c)
- Ranking of Labs: Certified, Provisionally Certified, Not Certified, or Interim certification .14(3)
- Labs may be downgraded to Provisionally Certified for item listed in 1200-5-1-.14(4)
- Labs may have certification revoked for any of the items listed in .14(5)

PE Samples

- Laboratories must successfully analyze Proficiency Evaluation (PE) samples annually for all analytes and by all methods used for compliance samples .14(9)
- Micro Labs using enzyme substrate methods must successfully analyze 9 of 10 PE samples correctly with no false negatives (cert manual chap V 7.2)
- PE samples must be from an approved vendor http://www.a2la.org/dirsearch/nelacptproviders.cfm
- Will need an EPA lab # before running PE samples EPA contact: Charles Feldmann 513-569-7671 or by FAX at 513-569-7191

QA plan 1200-5-1-.14(2)(a) and Cert Manual Chapter III Sec 11

1. Laboratory organization and responsibility
2. Process used to identify clients' Data Quality Objectives
3. SOPs with dates of last revision
4. Field sampling procedures
5. Laboratory sample receipt and handling procedures
6. Instrument calibration procedures
7. Analytical procedures
8. Data reduction, validation, reporting and verification

QA plan

9. Type of quality control (QC) checks and the frequency of their use
10. List schedules of internal and external system and data quality audits and inter laboratory comparisons
11. Preventive maintenance procedures and schedules
12. Corrective action contingencies
13. Record keeping procedures

Documentation for many of the listed QA plan items may be made by reference

Microbiology Requirements focus on Enzyme substrate methods

- Lab supervisor must ensure all personnel have demonstrated their ability satisfactorily perform all analysis assigned to them
- Thermometers graduated in 0.5° C and calibrated against a N.I.S.T. traceable thermometer
- Stable Incubator with temperature reading twice a day separated by at least 4 hours
- Sample bottles must have at least 120ml capacity
- Sample analyzed must be 100ml
- Samples must be shaken vigorously at least 25 times

Microbiology

- Bottle sterility check performed on each batch of sample bottles
- Bottles and media must be checked to ensure they do not fluoresce
- Sterile water must have documentation or verified by 50ml water and 50ml non-selective broth and incubated. Check growth after 24 and 48 hours
- Positive and Negative controls must be run on commercially prepared media quarterly
- Samples which produces an atypical color changes must be invalidated

Microbiology

- Sample volume for drinking water samples must be 100ml
- Samples less than 100ml must be rejected
- Samples greater than 100ml must be poured into a larger sterile container, stirred, and accurately transferred to a sterile vessel for incubation

Questions?

BACT SAMPLING EXERCISE

Develop a BACT sampling procedure by selecting and numbering each step in the proper order.

___ a. Label the bottle with sample location, date, and time of sampling
___ b. Allow the hot water tap to run for 5 min before collecting the sample
___ c. Collect the sample from a non-swivel faucet, if available
___ d. Rinse the sample bottle thoroughly before sampling
___ e. Allow the cold water tap to flow at a moderate rate for 3-5 minutes before sampling
___ f. Use only sterile sample bottles provided by the lab specifically for BACT testing and containing sodium thiosulfate
___ g. If a faucet has a strainer, aerator or any other attachment, remove them before sampling
___ h. When filling the bottle, hold the bottle so as to avoid water contacting the hand and running into the bottle
___ i. Keep the sample at a temperature of 4 degrees C.
___ j. Do not open the sample bottle until you are ready to collect the sample
___ k. Wipe the faucet clean before sampling
___ l. Replace all attachments after sampling is completed
___ m. Thoroughly clean the sample tap with either a dilute bleach spray, rubbing alcohol, or flame it with a propane or butane torch
___ n. Adjust the water flow to a moderate rate to prevent splashing during sampling
___ o. Inspect sample bottles for damage and secured cap without opening it
___ p. Fill the bottle to overflow and cap it without any air space
___ q. Ship or carry the samples to the lab in a refrigerated, insulated container
___ r. Taste the water before sampling
___ s. Immediately cap the bottle
___ t. Fill the sample container to the 100 ml line
___ u. Measure the chlorine residual before sampling
___ v. Package the sample(s) for delivery to the lab for testing
___ w. Ensure that the BACT test can be done within 30 hrs of sample collection
___ x. Preserve each sample bottle with sulfuric acid
___ y. Avoid sampling from hydrants, taps from water softeners, and leaking faucets
___ z. If the sample spills while filling, rinse the bottle and re-sample

Bacteriological Sampling Plan

Instructions

1. Your team is to develop a Bacteriological Sampling Plan for Anytown, TN.
2. You are to follow and fill in the blanks on the attached plan template.
3. Anytown has a household factor of 2.3 and 15200 service connections.
4. The Industrial Park includes four manufacturing plants including an electric motors plant (45 employees), a Gatorade plant (75 employees), a Sara Lee Foods plant (240 employees), and a Good Year tire plant (300 employees).
5. State College has 200 full time faculty and staff and 2200 students.
6. Main St. and 5th Avenue are heavily commercialized.
7. The shopping mall is 300,000 SF and includes 25 stores and 4 restaurants.
8. Schools are located at the corner of 5th and Elm, east end of 11th Ave, west end of 6th Ave., south end of Randolph Lane, corner of 1st and Lewis St., corner of 4th and Smith St., west end 10th Ave., and north end of Fir St.
9. Anytown is serviced by three wells, each with a chlorination system for disinfection.
10. Divide the town into sampling zones and delineate your sampling locations on the system map.
11. Make a table similar to the following example.

Sampling Zone	Sampling Site	Location	Sampling Day of the Week	Sampling Time	Rationale
A	1	100 Bill St	Monday	9am	Low usage
	2	200 John St	Monday	9:30am	Storage tank
B	1	12 Dan Dr	Monday	1pm	Res.
	2	44 Steve Ln.	Monday	1:30pm	Res.
C	1	22 Tonia Dr.	Monday	10 am	Com.
	2	400 Beth St	Monday	10:30am	Dead end
	3	100 Carol Ave	Monday	11am	Res.

Bacteriological Sampling Plan

Instructions

1. Your team is to develop a Bacteriological Sampling Plan for Hometown, TN.
2. You are to follow and fill in the blanks on the attached plan template.
3. Hometown has a household factor of 2.25 and 11200 service connections.
4. The Industrial Park includes three manufacturing plants including a clothing plant (45 employees), a pork&beans plant (75 employees), and an International paper box plant (250 employees).
5. State College has 175 full time faculty and staff and 2000 students.
6. College St. and Smallhouse Road are heavily commercialized.
7. Normal Dr. is developing commercial with restaurants, motels, and truck stops.
8. Schools are located at the corner of 16th and State, east end of Magnolia Ave., west end of Elm St., south end of 16th St., corner of 36th St and Broad St., corner of 20th and Main St., west end of Virginia St., and north end of Smallhouse Road.
9. Hometown is serviced by a WTP withdrawing from surface water and employs conventional coagulation/flocculation/sedimentation/filtration/chlorination treatment.
10. Divide the town into sampling zones and delineate your sampling locations on the system map.
11. Make a table similar to the following example.

Sampling Zone	Sampling Site	Location	Sampling Day of the Week	Sampling Time	Rationale
A	1	100 Tony St	Monday	9am	Res
	2	200 Larry St	Monday	9:30am	Com.
B	1	12 Greg Dr	Monday	1pm	Low usage
	2	44 Steve Ln.	Monday	1:30pm	Storage tank
C	1	900 Doug Pl.	Monday	10 am	Dead end
	2	400 Roger St	Monday	10:30am	Res
	3	100 John Ave	Monday	11am	Ind. Park

BACT Post Test

Name: Unnecessary
Class Location: _____

Your Score:
Highest Possible Score: 11

Multiple Choice: For each of the following questions, circle the letter of the answer that best answers the question.

1. Which of the following waterborne diseases has been largely eliminated in the US?

   A. Cryptosporidiosis
   B. Gastroenteritis
   C. Cholera
   D. Tuberculosis

2. Which organism is responsible for the largest number of disease outbreaks in the US in the last 20 years?

   A. Escherichia coli
   B. Giardia lamblia
   C. Salmonella typhi
   D. Fecal coliform

True or False: For each statement, circle True or False.

True	False	3. Protozoans are generally more resistant to chlorination than coliform.
True	False	4. Cryptosporidium parvum is an infectious bacteria.
True	False	5. Typhoid fever is caused by the bacteria, Salmonella typhi.
True	False	6. Viruses cannot be transmitted in water.
True	False	7. Proper BACT sampling requires the use of sterile sample bottles, a dechlorination preservative, and proper handling without introducing contamination.

Fill in the Blank: Into each sentence below, copy a term from the word bank that correctly completes the sentence.

Membrane Filter Technique	Typhoid fever	Oxygen Uptake test	Total coliform	Chlorination
Fecal coliform	Hepatitis B	35 degrees C	Giardia lamblia	Colitag
Hydrogen peroxide	Cryptosporidium parvum	Multiple Tube Fermentation	E. coli	25 degrees C

8. A number of test methods are EPA approved for testing of total coliform in drinking water including _____, _____, and _____.

9. The MCL for _____ in drinking water is zero colonies per 100 ml.

10. Parasitic protozoans of significant concern at the present time include _____ and _____.

11. The proper incubation temperature for the total coliform test is _____.

12. Essay Question: Describe at least three things that water suppliers can and should do to protect customers from waterborne disease.

Course References

1. ***Waterborne Pathogens,*** **AWWA Manual of Water Supply Practices M48, First Edition, 1999.**
2. ***Problem Organisms in Water: Identification and Treatment,*** **AWWA Manual of Water Supply Practices M7, Second Edition, 1995.**
3. ***Handbook of Drinking Water Quality,*** **DeZuane, J., John Wiley & Sons, Inc., Second Edition, 1997.**
4. ***Integrated Design and Operation of Water Treatment Facilities,*** **Kawamura, S., John Wiley & Sons, Inc., Second Edition, 2000.**
5. ***Standard Methods for the Examination of Water and Wastewater,*** **APHA, AWWA, WEF, 20th Edition, 1998.**
6. ***Water Treatment, Principles and Practices of Water Supply Operations,*** **AWWA, Second Edition, 1995.**
7. ***Simplified Procedures for Water Examination,*** **AWWA Manual of Water Supply Practices M12.Fifth Edition, 2002.**
8. ***Iron and Manganese Removal Handbook,*** **Sommerfield, Elmer O., AWWA, 1999.**
9. ***Stage 2 DBPR and LT2ESWTR Train the Trainer,*** **Nashville, TN, USEPA, January, 2006.**

Approved Methods for Total Coliform Analysis

T.Coliform Method	Format	Confirmation	Fecal Coliform Method	E. coli Method
Membrane Filter	1@100ml	LT and BGB	EC broth w/filter transfer or EC broth w/swab inoc.	Nutrient agar+MUGw/fil Nutrient agar+MUGw/swab
MTF	1@100ml 5@20ml 10@10ml	BGB	EC broth w/loop inoc.	EC broth+MUG w/ loop inoc.
P-A	1@100ml	BGB	EC broth w/loop inoc.	EC broth+MUG w/ loop inoc.
Colilert	Variable	None	NA	UV light or EC broth+MUGw/ pipet inoc
Colisure	1@100ml	None	NA	UV light
Colitag	1@100ml	None	NA	UV light

LT- Lauryl tryptose broth
BGB- Brilliant green lactose bile broth
P-A- Presence-Absence
EC- E. coli
MUG- *name is too long to type*
NA- Not applicable

Operator Training

Tennessee Drinking Water Laboratory Certification Program

Division of Water Supply
Troy Taubert
E-mail troy.taubert@state.tn.us

Tennessee Department of Environment and Conservation

Laboratory Certification Personnel

Certification Authority – Robert L Foster, Jr.

Certification Officers - Troy Taubert (931-432-7643)

Charlie Mickle (615-532-0178)

Craig Lafever (615-532-0181)

Prasad Subbanna (865-594-5557)

Amy Francis (423-634-5720)

USEPA

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Fifth Edition

2005

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DIVISION OF WATER SUPPLY

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PE Samples

- Laboratories must successfully analyze Proficiency Evaluation (PE) samples annually for all analytes and by all methods used for compliance samples .14(9)
- Micro Labs using enzyme substrate methods must successfully analyze 9 of 10 PE samples correctly with no false negatives (cert manual chap V 7.2)
- PE samples must be from an approved vendor http://www.a2la.org/dirsearch/nelacptproviders.cfm
- Will need an EPA lab # before running PE samples EPA contact: Charles Feldmann 513-569-7671 or by FAX at 513-569-7191

QA plan 1200-5-1-.14(2)(a) and Cert Manual Chapter III Sec 11

1. Laboratory organization and responsibility
2. Process used to identify clients' Data Quality Objectives
3. SOPs with dates of last revision
4. Field sampling procedures
5. Laboratory sample receipt and handling procedures
6. Instrument calibration procedures
7. Analytical procedures
8. Data reduction, validation, reporting and verification

QA plan

9. Type of quality control (QC) checks and the frequency of their use
10. List schedules of internal and external system and data quality audits and inter laboratory comparisons
11. Preventive maintenance procedures and schedules
12. Corrective action contingencies
13. Record keeping procedures

Documentation for many of the listed QA plan items may be made by reference

Microbiology Requirements focus on Enzyme substrate methods

- Lab supervisor must ensure all personnel have demonstrated their ability satisfactorily perform all analysis assigned to them
- Thermometers graduated in 0.5° C and calibrated against a N.I.S.T. traceable thermometer
- Stable Incubator with temperature reading twice a day separated by at least 4 hours
- Sample bottles must have at least 120ml capacity
- Sample analyzed must be 100ml
- Samples must be shaken vigorously at least 25 times

Microbiology

- Bottle sterility check performed on each batch of sample bottles
- Bottles and media must be checked to ensure they do not fluoresce
- Sterile water must have documentation or verified by 50ml water and 50ml non-selective broth and incubated. Check growth after 24 and 48 hours
- Positive and Negative controls must be run on commercially prepared media quarterly
- Samples which produces an atypical color changes must be invalidated

Microbiology

- Sample volume for drinking water samples must be 100ml
- Samples less than 100ml must be rejected
- Samples greater than 100ml must be poured into a larger sterile container, stirred, and accurately transferred to a sterile vessel for incubation

Questions?

Utilities Water Laboratory
Quality Assurance Plan

Revised July 3, 2006

Laboratory Quality Assurance Plan

SECTION TITLE PAGE NO.

1.0-INTRODUCTION

1.1 The organization of the laboratory includes the lab technician and operators who report to the chief operator/supervisor.

1.2 _(Name)_____ Chief Operator/Supervisor

(Name) Lab Technician/QA Manager

(Name) Operator

(Name) Operator

1.3 Lab technician is responsible for ensuring that all SOPs are maintained and followed, data accuracy, meeting all required quality assurance criteria and regulatory requirements, performing analyses, and reporting results.

All of the personnel share the responsibility of the operational aspects of the quality assurance activities. Assignments are made to ensure the involvement of all personnel in quality assurance and to maintain control of quality assurance data. All analysts are required to familiarize themselves with the sections of the QA plan that pertain to their individual operations.

The primary goal of the Quality Assurance (QA) program is to ensure that all microbiological data are of known quality. The quality of data is generally known when all components associated with its derivation are thoroughly documented, such documentation being verifiable and defensible. In order to produce data of known quality it is essential that quality assurances become an integral part of all data collection activities.

1.3 Training

Education and training documentation for each laboratory employee is provided in Appendix A.

2.0 QA OBJECTIVES

The characteristics of data that measure accomplishment of a specified purpose can be expressed in terms of representativeness, comparability, precision, accuracy and completeness.

2.1 Representative

Representative expresses the degree to which data accurately and precisely represents a characteristic of a population, parameter variations at a sampling point, a process condition, or an environmental condition.

Appropriate selection of sample site and sampling procedure is critical to obtaining a sample that is representative of the environment in which it is collected.

2.2 Comparability

Comparability expresses the confidence with which one data set can be compared to another. Comparability of data is assured by following standard analytical procedures and calculating and reporting all data in generally accepted units.

2.3 Precision

Precision is a measure of mutual agreement among individual measurements of the same property, usually under prescribed similar conditions. Precision is best expressed in terms of standard deviation. Various measures of precision exist depending upon the "prescribed similar conditions".

2.4 Accuracy

Accuracy is defined as the degree of a measurement (or an average of measurements of the same thing), X, with an accepted reference or true value, T, usually expressed as the difference between the two values, X-T, or the difference as a percentage of the reference or true value, 100 (X-T)/T and sometimes expressed as a ratio, X/T. Accuracy is a measure of the bias in a system.

2.5 Completeness

Completeness is a measure of the amount of valid data obtained from a measurement system compared to the amount that was expected to be obtained under correct normal conditions. Completeness of data is dependent upon both field and laboratory personnel. Improper sample collection, sample contamination, and out-of-control analytical procedures can cause the loss of data.

3.0 SOPs WITH DATES OF LAST REVISION

The laboratory maintains SOPs that reflect all phases of current laboratory activities. The SOPs are located in a manual in the laboratory. They are current and reviewed annually. Signature pages are included.

4.0 SAMPLING PROCEDURES - FIELD

Drinking water distribution samples will be collected in accordance with Section 9060A of Standard Methods, 20th Edition, Microbiological Methods for Monitoring the Environment, Water and Wastes, EPA 600/8-78-017, Part II, Section A.5.1 Potable Water Supplies, and the National Primary Drinking Water Regulations.

Select the sampling site from which a sample is to be obtained. Location should be an approved and protected site that accepts the dedicated sampling port to reduce the likelihood of contamination.

Once the line connection is accessible, disinfect the water line connector with several sprays from the 1:10 bleach/water container. Repeat this for the quick-connect end of the sampling port.

Disinfect the sampling port with a volume of the disinfection solution. Close the hose bib valve and pore the solution into the quick-connect end, rotate the port in several directions to insure that the solution contacts all internal surfaces of the port. Open hose bib valve and allow solution to drain. BE CAREFUL NOT TO TOUCH HOSE BIB OUTLET OR QUICK CONNECT OPENING.

Install the sample port onto the line connector and open the line cutoff valve, whatever type it may be. Open hose bib valve and flush sample port for three minutes or until sample line has been flushed completely. Test the free chlorine residual. If not acceptable, continue flushing.

Once acceptable chlorine residual has been established, remove the cellophane seal from around the top of the sterilized IDEXX sample bottle and unscrew the cap. Hold the bottle near the base with one hand and hold the cap in the other hand is such a way that fingertips never touch the inside of the bottle cap or the sample bottle.

Collect 100mL of sample by filling bottle to the line. Do not overfill. Close hose bib valve.

Replace bottle cap and check for leaks by shaking the bottle.

Identify the sample by writing on the label the sample location, time, chlorine residual, and initials and any other required information.

Fill out the sample collection report and the chain of custody form(s) in their entirety.

Place sample on ice in cooler unless the sample is to be taken to the laboratory immediately.

Close the water line cutoff valve and disconnect the sampling port.

Return the cooler containing the sample, sample collection report and chain of custody form to the lab technician. Complete any necessary laboratory paperwork at that time.

An established sampling plan is in place with the state and local authorities. The sampling plan is on file in the laboratory.

4.1 Responsibility

a. To the extent possible, the number and types of samples are determined prior to the actual fieldwork. As few persons as possible should handle the samples.

b. The field sampler is personally responsible for the custody of the samples until they are transferred or properly dispatched.

c. Sample bottles shall be marked and paperwork completed for each sample using waterproof ink.

4.2 Field Forms

The appropriate form must be completed on site at the time of sample collection. In addition to marking sample bottles and paperwork, the lab worksheet must be maintained to provide a daily record of significant events. All entries shall be in waterproof ink, signed and dated. All members use this worksheet, which is kept as a permanent record. In a legal proceeding, such notes, if referred to, are subject to cross-examination and are admissible as evidence.

5.0 SAMPLE RECEIPT AND HANDLING - LABORATORY

5.1 Media

Prepared media commercially manufactured is used. Media is stored in cool dry location and discarded if caked or discolored. Bottles are dated upon receipt and when initially opened.

5.2 Preservation and Storage

Start microbiological examination of water samples promptly after collection to avoid unpredictable changes. If samples cannot be processed within 2 h after collection, use an iced cooler for storage during transport to the laboratory. A bottle of water, or other approved temperature-monitoring device, must be included in the sample container for temperature monitoring. Any SWTR sample that the laboratory determines has exceeded 10º C (50ºF) or frozen must be rejected. Samples must be refrigerated upon arrival at the laboratory until processed. Holding/transit time between sampling and analysis should not exceed 8 hours.

5.3 Integrity

Samples arriving after 6 h since collection shall not be examined. Date and time of sample collection and analysis is to be recorded on microbiology lab worksheet. Integrity is maintained by tracking samples from receipt by laboratory through analysis to disposal. Sampler and lab personnel fill in a chain of custody.

5.4 Sample Custody

Applicable state regulations pertaining to chain of custody are followed. Due to the possible evidentiary nature of samples possession must be traceable from the time the samples are collected until they are introduced as evidence or are officially discarded. The following general procedures are followed to maintain and document sample possession.

A sample is under custody if:

a. It is in your possession, or
b. It is in your view, after being in your possession, or
c. It was in your possession and then locked up by you to prevent tampering, or
d. It is in a designated secure area.

5.5 Transfer of Custody and Shipment

Samples are accompanied by a chain-of-custody record, contained both on the sample bottle and a separate accountable form. When transferring the possession of samples, the individuals relinquishing and receiving shall sign, date, and note the time on the record in the spaces provided and maintained in the laboratory.

5.6 Sample Management and Document Control

The analytical laboratory is the ultimate destination and repository for samples that have been collected for some specific data monitoring, regulatory compliance, or enforcement purpose. To assure that data produced from the analysis of such samples are legally defensible, it is incumbent on laboratory personnel to maintain complete documentation of sample receipt and analytical processing until such time as a final analytical report has been produced.

All records which accompany the sample and those generated within the laboratory should be maintained for a minimum of five years. Samples that are analyzed as supporting evidence for enforcement cases and could go to litigation should be held indefinitely. Some regulations may specify the length of time records must be kept. This may be less than or greater than five years depending on the type of product for which the samples were collected. As a practical matter no records shall be intentionally discarded without the concurrence of the laboratory technician or chief operator.

The date and time of receipt, temperature of the samples, and the name of the person or entity (if common carrier) from whom received, should be recorded in the appropriate spaces. All documents accompanying the samples will be placed in the appropriate files.

5.7 Criteria for Rejection

Holding/transit time between sampling and analysis should not exceed 8 hours. If sampling time is beyond meeting this timeframe, then sample must be invalidated. If sample does not reach the lab in time to be analyzed within two hours of collection then the sample must be chilled, for SWTR samples the temperature must be between 10º and 0º C. The sample bottle must contain a minimum of 100 ml of sample or it will be rejected. Turbid samples or samples with a disinfectant odor will also be invalidated.

6.0-INSTRUMENT CALIBRATION PROCEDURES

6.1 Thermometers

Temperature monitoring devices are calibrated annually by an outside agency.

6.2 Incubator

Fisher Scientific incubator, or equivalent, maintaining temperature of 35.0+/- 0.5 degrees Celsius is calibrated annually by an outside agency. A thermometer graduated in 0.5° C increments with bulb immersed in liquid is placed on shelf of use area.

6.3 Refrigerator

Refrigerator thermometers are calibrated on an annual basis as stated in the temperature monitoring devices. If a problem is found, the refrigerator will be serviced.

7.0-ANALYTICAL PROCEDURES

7.1 Analytical Procedures are in accordance with the following approved methods under the total coliform rule.

1. Minimal Medium ONPG-MUG Test (Colilert) Test Standard Methods, 19th edition, Methods 9223A, 9223B

Consistent with the requirements of the total coliform final rule 40 CFR 141 and 142, June 29, 1989, National Primary Drinking Water Regulations, 40 CFR 141 and 142, December 4, 1994 and the National Primary Drinking Water Regulations 40 CFR 141, January 8, 1991, and National Primary Drinking Water Regulations EPA 816-F-03-016, June 2003.

The laboratory is using the total coliform and E. coli methods specified in the National Primary Drinking Water Regulations and the Final Long Term 2 Enhanced Surface Water Treatment Rule that are current as of the date of the preparation of the QA plan. After promulgation of any additional methods for E. coli by the EPA the analytical procedures section will be changed to reflect any new methodology the laboratory implements.

7.2 Analytical Quality Control Procedures

The MMO-MUG (Colilert) color comparator is used for all P-A bottles where the color is indeterminate.

This section describes the checks and monitoring procedures that should be performed on materials, supplies, instrumentation and the physical facility. These quality control checks should be documented completely and recorded as performed.

Laboratory Facilities

The laboratory has approximately 198 sq. ft. of space. The heating and air conditioning system is equipped to provide a continuous supply of fresh air when laboratory work is in progress. Ambient temperature is controlled to be within 68° F to 72°F range.

Workbenches are cleaned with disinfectant before and after each use.

Laboratory Supplies

Sample Bottles: Pre-sterilized Colilert bottles that are wide mouth, plastic 120 ml bottles or equivalent are used for sample collection. A sterility check is performed on one of each batch of sample bottles, by putting 25 ml of purchased tryptic soy broth solution in the bottle and incubating for forty-eight hours at 35 +/- 0.5 degrees Celsius. Record the results.

Reagents: Culture Media is dated when received and stored in a cool dry place. Media is discarded consistent with manufacturer's expiration date.

Media Quality Control Tests are performed as follows:

Positive and negative performance checks of media are preformed when it is purchased and every 3 months thereafter and are recorded in the media preparation QC log.

Data Handling

Samples received are logged in before tests are run. Included is the date received, sample number and (or) location description, type of sample, the analyses run per sample, and the method used.

A bench sheet is then prepared yielding all pertinent information related to a sample with space for recording test results. This sheet is placed in a loose-leaf bench record book until the final results are obtained. Upon completion of analyses, results are double-checked.

8.0-DATA REDUCTION, VALIDATION, REPORTING, VERIFICATION

Data Reduction

All volumes are converted to standard units and always recorded in similar terms.

Validation

The integrity of the data generated will be validated at several points during the collection and reporting process. The two principal checkpoints are the laboratory analyst and the supervisor who will review the data.

Reporting

The Bacteriological Analysis Detail Form (CN-0800) and the Monthly Microbiological Monitoring Report (CN-0780) with be submitted monthly. Notification of a positive sample will be made to the proper authority the same day the result is obtained.

Verification

All samples analyzed in "out-of-control" situations will be reanalyzed. If this is not possible the data will be invalidated. It is the responsibility of the analyst to note any "out-of-control" situation and to notify the supervisor.

Data Correction

Data should be recorded in ink with any changes lined through such that the original entry is visible. Changes need to be initialed and dated.

9.0-TYPES AND FREQUENCY OF (QC) CHECKS

Positive and Negative Controls

The controlled growth of positive and negative growth organisms is used as a quality check in media preparation. Each new batch of prepared media is checked for suitability.

Sterility Controls

Sterility controls are conducted periodically for quality control. Routine checks are listed below.

Sample containers - 25ml of tryptic soy broth is added to each lot of sample containers and incubated for 48 hrs and checked for growth. Results are recorded.

Performance Evaluation and Quality Control Samples

The microbiology laboratory participates in the U.S. Environmental Protection Agency Water Supply performance evaluation sample program.

10.0-LIST OF SCHEDULES OF INTERNAL AND EXTERNAL SYSTEM AND DATA QUALITY AUDITS AND INTER LABORATORY COMPARISONS

Internal audits are performed monthly on total coliform and E. Coli. Checks between all operators to meet within 10%of the readings.

11.0-PREVENTATIVE MAINTENANCE PROCEDURES AND SCHEDULES

11.1 Availability of Manuals and Spare Parts

Manuals for all instruments are kept on file at the laboratory. This includes original thermometer certification and calibration documents.

11.2 Routine Equipment Maintenance

Incubators and refrigerators are serviced when daily records indicate a malfunction.

12.0-CORRECTIVE ACTION CONTINGENCIES

12.1 Unacceptable Results from QC Checks

Whenever the internal quality control checks or EPA performance audits indicate an out-of-control situation, corrective actions must be taken. The analyst is responsible for detecting out-of-control situations and initiating the corrective actions.

12.2 Responsibility for Corrective Actions

When an out-of-control situation occurs, the analysis must be stopped until the problem has been identified and resolved. The corrective action must be approved by the supervisor and documented.

12.3 Documenting Corrective Action

Results of performance audits and QA problems are reported to management by form of memorandum detailing QA work done and the results by the person carrying out the QA work

13.0-RECORD KEEPING PROCEDURES

13.1 Procedures and Documentation

All distribution, repeat, and special sample results will be reported. Routine records on pH and incubator temperatures will not be reported but are recorded in the laboratory.

13.2 Length of Storage

All files are maintained as per the requirements for the drinking water requirements for the EPA.

Notes:

The Quality Assurance Plan seeks to minimize paperwork while improving dependability and quality of data.