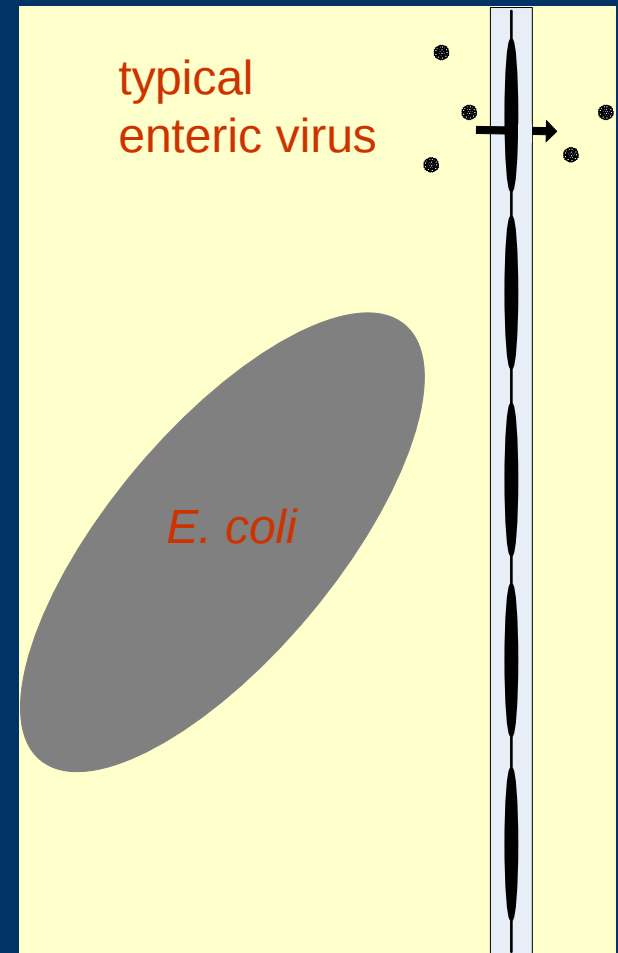

Viruses in water

John Scott Meschke

Email: jmeschke@u.washington.edu

Viruses

- Small in size (0.02-0.3 micrometers diameter)
- Spherical (icosahedral) or rod-shaped (helical)
- No biological activity outside of host cells/or host organisms
 - Obligate intracellular parasites; recruit host cell to make new viruses, often destroying the cell
- Non-enveloped viruses tend to be the most persistent in the environment (particularly in aqueous systems)
 - Protein coat confers stability
- Enteric viruses are most relevant for waterborne exposures
 - Although viruses, spread by other routes, may be present in water samples



0.45 μ m Filter

Virion Composition

Nucleic acid:

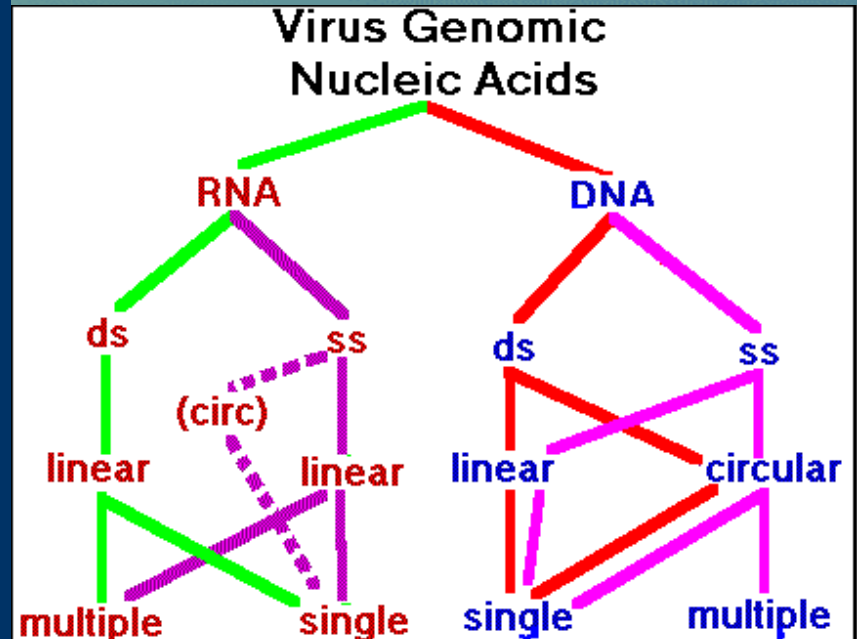
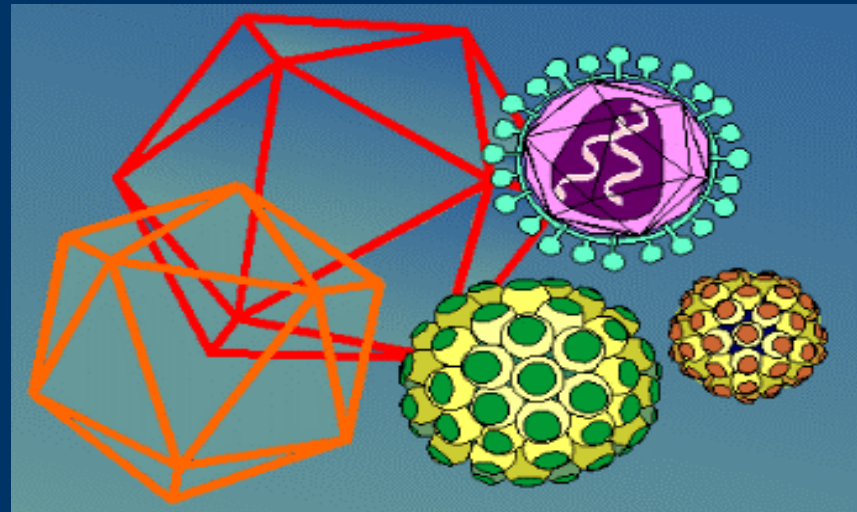
- DNA or RNA
- single or double-stranded
- 1 or several segments

Capsid (protein coat):

- multiple copies of 1 or more proteins in an array

Envelope:

- lipid bilayer membrane + glycoproteins)
- typically acquired from host cell membranes



Viral Gastroenteritis

- It is thought that viruses are responsible for up to 3/4 of all infective diarrhoeas.
- Viral gastroenteritis is the second most common viral illness after upper respiratory tract infection.
- In developing countries, viral gastroenteritis is a major killer of infants who are undernourished. Rotaviruses are responsible for half a million deaths a year.
- Many different types of viruses are found in the gut but only some are associated with gastroenteritis

Viruses found in the gut (1)

A. Associated with gastroenteritis

- Rotaviruses
- Adenoviruses 40 41
- Caliciviruses
- Norwalk like viruses or SRSV (Small Round Structured Viruses)
- Astroviruses
- SRV (Small Round Viruses)
- Coronaviruses
- Toroviruses

Viruses found in the gut (2)

B. Found in the gut, not normally associated with gastroenteritis

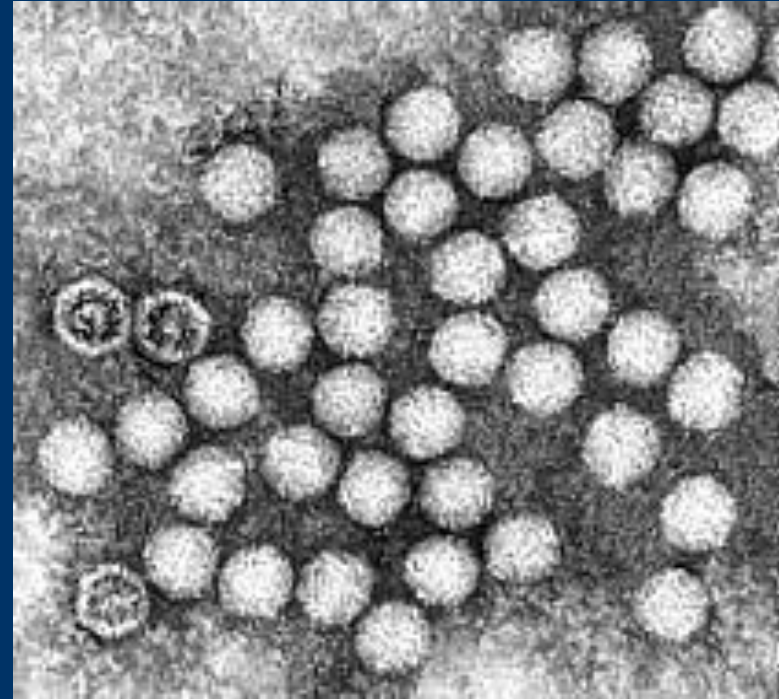
- Polio
- Coxsackie A
- Coxsackie B
- Echo
- Enteroviruses 68-71
- Hepatitis A
- Hepatitis E
- Adenoviruses 1-39
- Reoviruses

C. Found in the gut as opportunistic infection

- CMV
- HSV
- VZV
- HIV

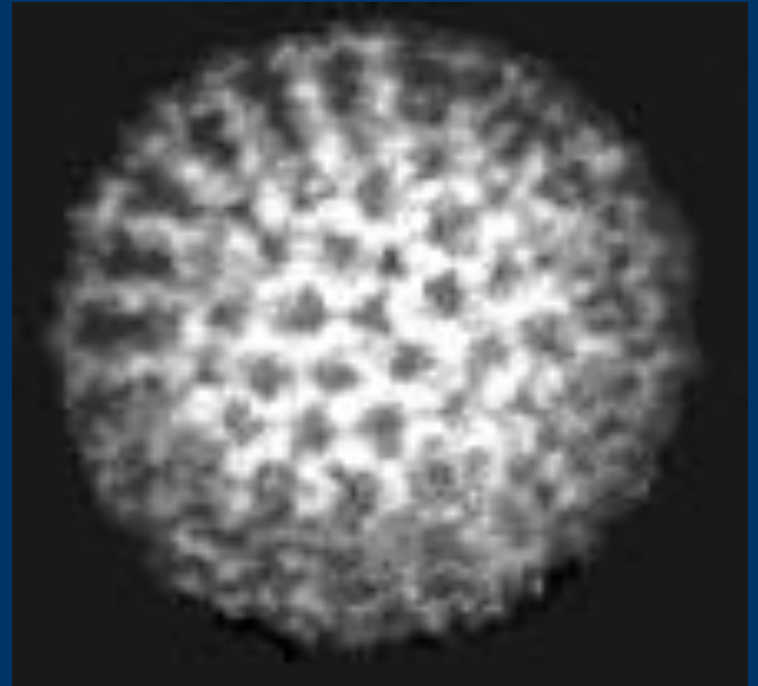
Genus: *Enterovirus*

- Icosahedral shape, ~27-30 nm diameter
- single-stranded +sense RNA
 - about 7,500 nucleotides
- icosahedral protein coat (capsid)
 - 4 capsid proteins: VP1, VP2, VP3, VP4 (all cleaved from VP0)
- 10 viral species
 - *Human enterovirus A*
 - *Human enterovirus B*
 - *Human enterovirus C* (Polioviruses)
 - *Human enterovirus D*
 - (*Human rhinovirus A*)
 - (*Human rhinovirus B*)
 - *Bovine enterovirus*
 - *Porcine enterovirus A*
 - *Porcine enterovirus B*
 - *Simian enterovirus A*



Genus: *Rotavirus*

- ~spherical; icosahedral
- ~75-80 nm diameter
- double-layered capsid
- nucleic acid:
 - double-stranded RNA
 - 11 segments (rotavirus)
 - electropherotypes
- 5 Species
 - Human rotavirus A
 - Human rotavirus B
 - Human rotavirus C
 - Human rotavirus D
 - Human rotavirus E
- Other viruses in *Reoviridae*



Rotaviruses

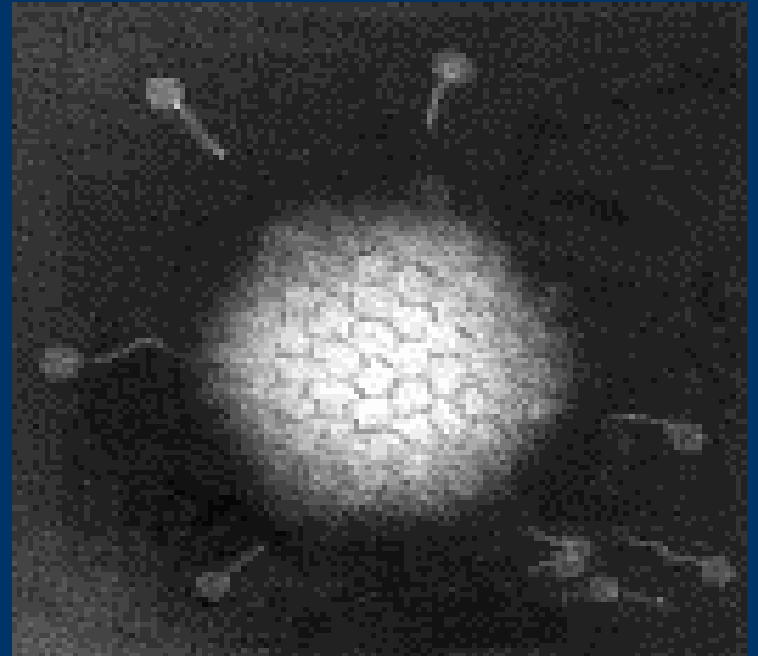
- account for 50-80% of all cases of viral gastroenteritis
- usually endemic, but responsible for occasional outbreaks
- causes disease in all age groups but most severe symptoms in neonates and young children. Asymptomatic infections common in adults and older children. Symptomatic infections again common in people over 60
- up to 30% mortality rate in malnourished children, responsible for up to half a million deaths per year

Rotaviruses

- 80% of the population have antibody against rotavirus by the age of 3
- more frequent during the winter
- faecal-oral spread. ? respiratory droplets
- 24-48 hr incubation period followed by an abrupt onset of vomiting and diarrhoea, a low grade fever may be present.
- Live attenuated vaccines now available for use in children

Genus: *Mastadenovirus*:

- icosahedral
- ~80 nm diameter
- double-stranded, linear DNA
- protein coat contains at least 10 proteins
- 6 species (*Human adenovirus A-F*)
- >50 human adenoviruses
 - mostly respiratory
 - but may be fecally shed
 - types 40 and 41 are enteric
- Often the most prevalent viruses in treated sewage
 - resistance to treatment?
- Distinct animal adenoviruses

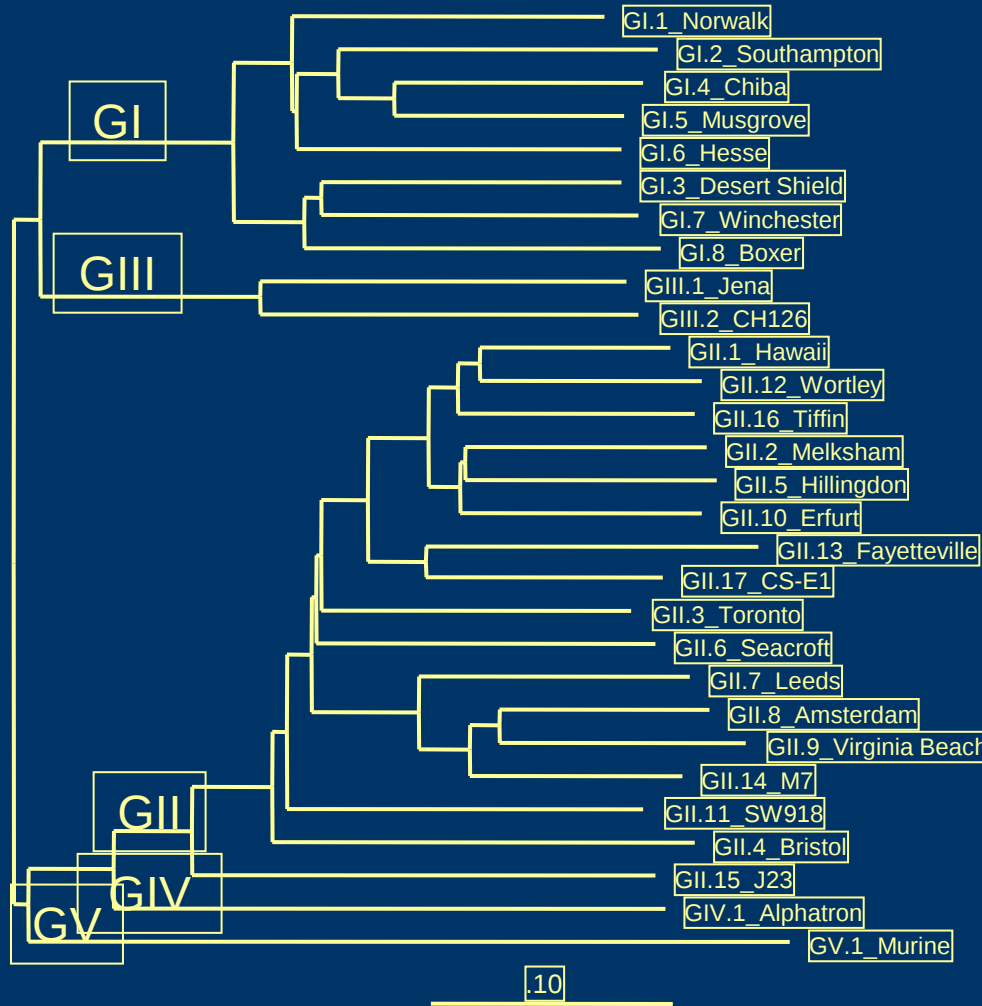


Genus: *Norovirus*

- Icosahedral
- “structured”; cup-like surface morphology
- 27-35 nm diameter
- ss(+) RNA, ~7.7 KB
- 1 major capsid polypeptide, ~60 kD
- minor protein, ~30 kD
- 5 major Norovirus groups,
- No culture (except in humans)
- Distinct animal Noroviruses



Genotypes: ex. 29 Clusters of Noroviruses

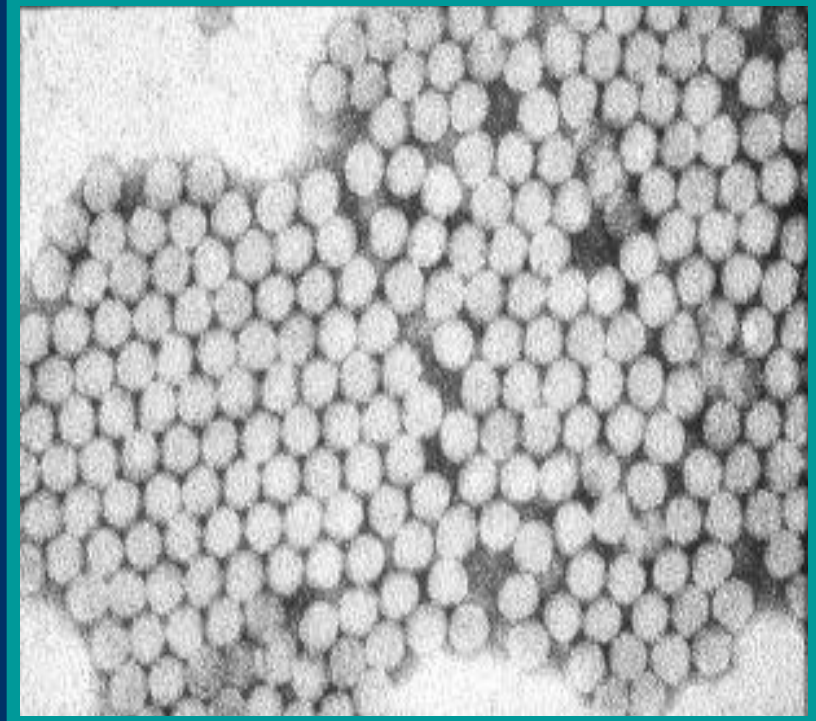


Clusters differ by $\geq 20\%$ amino acid pairwise distance

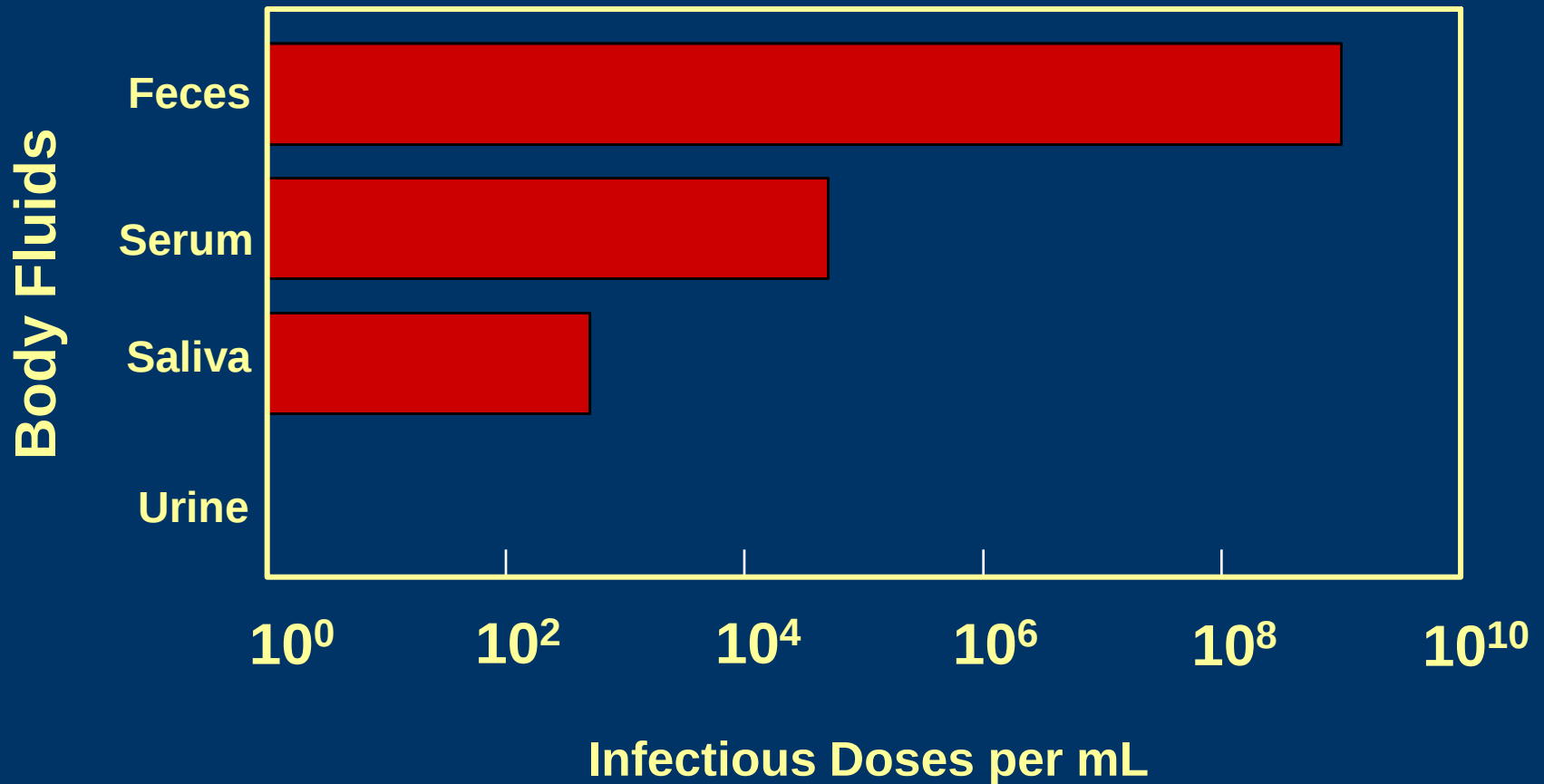
Genogroups differ by 44-55% amino acid pairwise distance

Genus: *Hepatovirus*

- 1 species, *Hepatitis A virus*
 - Single serotype worldwide
 - Acute disease and asymptomatic infection
- No chronic infection
 - Protective antibodies develop in response to infection - confers lifelong immunity



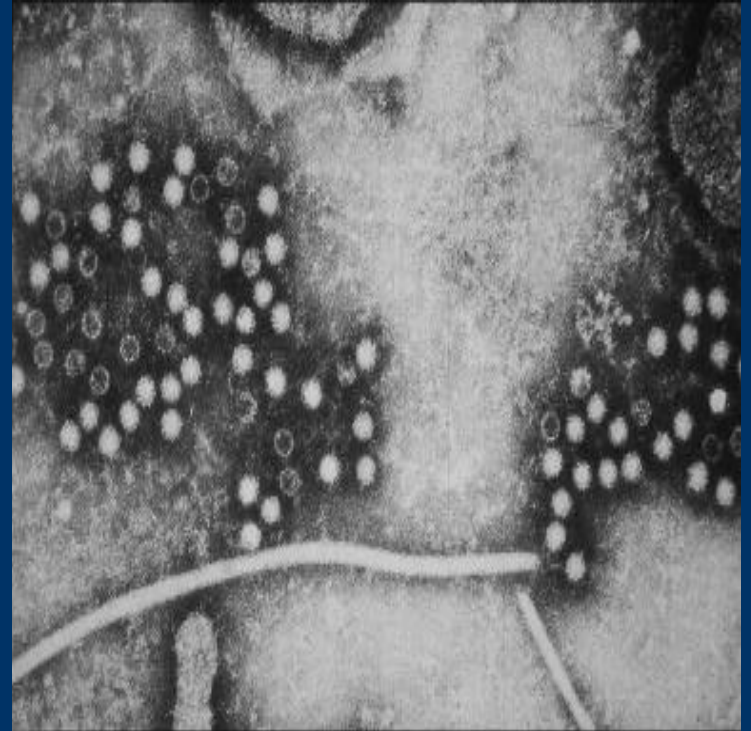
Concentration of Hepatitis A Virus in Various Body Fluids



Source: Viral Hepatitis and Liver Disease 1984;9-22
J Infect Dis 1989;160:887-890

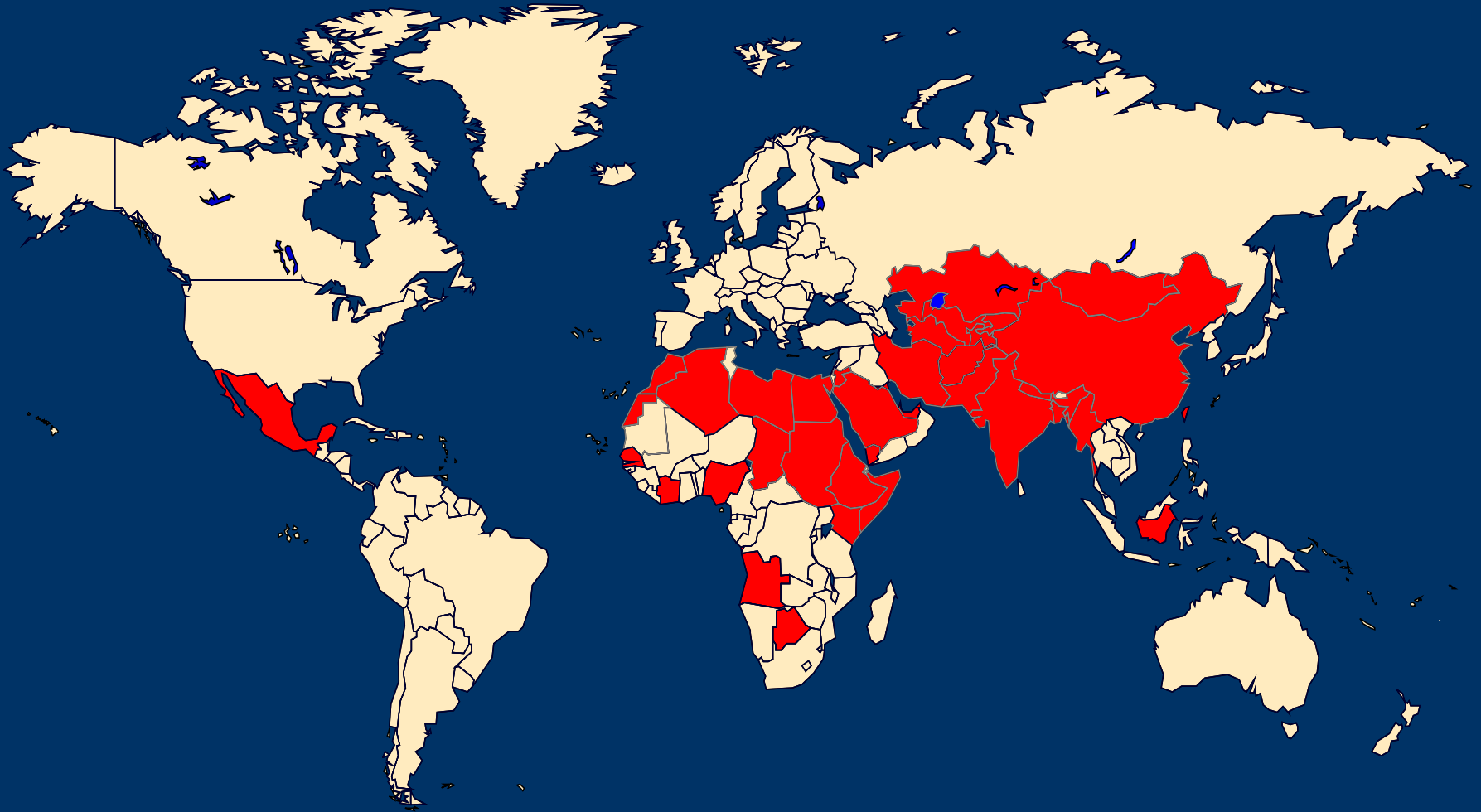
Genus: *Hepevirus*

- 1 species, *Hepatitis E virus*
- Icosahedral
- Incubation period: Average 40 days (Range 15-60 days)
- Case-fatality rate: Overall, 1%-3%; Pregnant women, 15%-25%
- Illness severity: Increased with age
- Chronic sequelae: None identified
- Most outbreaks associated with fecally contaminated drinking water
- U.S. cases usually have history of travel to HEV-endemic areas



Geographic Distribution of Hepatitis E

Outbreaks or Confirmed Infection in >25% of Sporadic Non-ABC Hepatitis



The Challenge of Environmental Sampling for Viruses

- Variation in virus type and distribution
- Low viral numbers: need to concentrate them
- Non-random distribution and physical state of viruses of interest: aggregated, particle-associated, embedded, etc.
- Volume considerations
- Environmental factors may inhibit or interfere with downstream detection
- Separate them from interfering and excess other material

Detection of Viruses in The Environment

- Three main steps:
- (1) recovery and concentration,
- (2) purification and separation, and
- (3) assay and characterization.

Pathogens in Raw Sewage

- Viruses (10^5 - 10^6)
- Salmonella (5,000-80,000)
- Giardia (9,000-200,000)
- Cryptosporidium (1-4000)
- In Biosolids:
 - Viruses $\sim 10^2$ - 10^4 (primary) $\sim 10^2$ (secondary)
 - Salmonella 10^2 - 10^3 (primary) $\sim 10^2$ (secondary)
 - Giardia $\sim 10^2$ - 10^3 (primary) $\sim 10^2$ - 10^3 (secondary)

Water Concentration

- Distribution of viruses in water necessitates sampling of large volumes of water (1-1000s of liters)
- Filtration is typically used for concentration
- Several formats utilized:
 - Membrane filter, pleated capsule, cartridge, hollowfiber
- Several types of media
 - e.g. cellulose ester, fiberglass, polysulfone, polyether sulfone

Filtration: Viruses

- **Adsorbent filters; VIRADEL**
 - Pore size of filters larger than viruses; viruses retained by adsorption
 - Electrostatic and hydrophobic interactions
 - Negatively charged cellulose esters, fiberglass
 - Positively charged modified cellulose, fiberglass, alumina nanofibers
- **Ultrafiltration: 1,000-100,000 MWCO**
 - Viruses are retained by size exclusion
 - Hollow fiber, spiral cartridge, multiple sheets, flat disks, etc.
 - Polysulfones, cellulose ester, etc.
 - Tangential flow to minimize clogging

Adsorption/Adhesion

- May be reversible or non-reversible
- 3 main forces
 - Electrostatic
 - Hydrophobic
 - Van der Waals forces

Table 4-1. L- α -Amino acids present in proteins.*

Name	Symbol	Structural Formula	pK ₁	pK ₂	pK ₃
With Aliphatic Side Chains			α -COOH	α -NH ₃ ⁺	R Group
Glycine	Gly [G]		2.4	9.8	
Alanine	Ala [A]		2.4	9.9	
Valine	Val [V]		2.2	9.7	
Leucine	Leu [L]		2.3	9.7	
Isoleucine	Ile [I]		2.3	9.8	
With Side Chains Containing Hydroxylic (OH) Groups					
Serine	Ser [S]		2.2	9.2	about 13
Threonine	Thr [T]		2.1	9.1	about 13
Tyrosine	Tyr [Y]	See below.			
With Side Chains Containing Sulfur Atoms					
Cysteine	Cys [C]		1.9	10.8	8.3
Methionine	Met [M]		2.1	9.3	
With Side Chains Containing Acidic Groups or Their Amides					
Aspartic acid	Asp [D]		2.0	9.9	3.9
Asparagine	Asn [N]		2.1	8.8	
Glutamic acid	Glu [E]		2.1	9.5	4.1
Glutamine	Gln [Q]		2.2	9.1	

Table 4-1. L- α -Amino acids present in proteins.* (continued)

Name	Symbol	Structural Formula	pK ₁	pK ₂	pK ₃
With Side Chains Containing Basic Groups			α -COOH	α -NH ₃ ⁺	R Group
Arginine	Arg [R]		1.8	9.0	12.5
Lysine	Lys [K]		2.2	9.2	10.8
Histidine	His [H]		1.8	9.3	6.0
Containing Aromatic Rings					
Histidine	His [H]	See above.			
Phenylalanine	Phe [F]		2.2	9.2	
Tyrosine	Tyr [Y]		2.2	9.1	10.1
Tryptophan	Trp [W]		2.4	9.4	
Imino Acids					
Proline	Pro [P]		2.0	10.6	

Electrophoretic mobility of rNV particles (circles) and MS2 (squares) as a function of solution pH in the presence of 0.01 M NaCl.

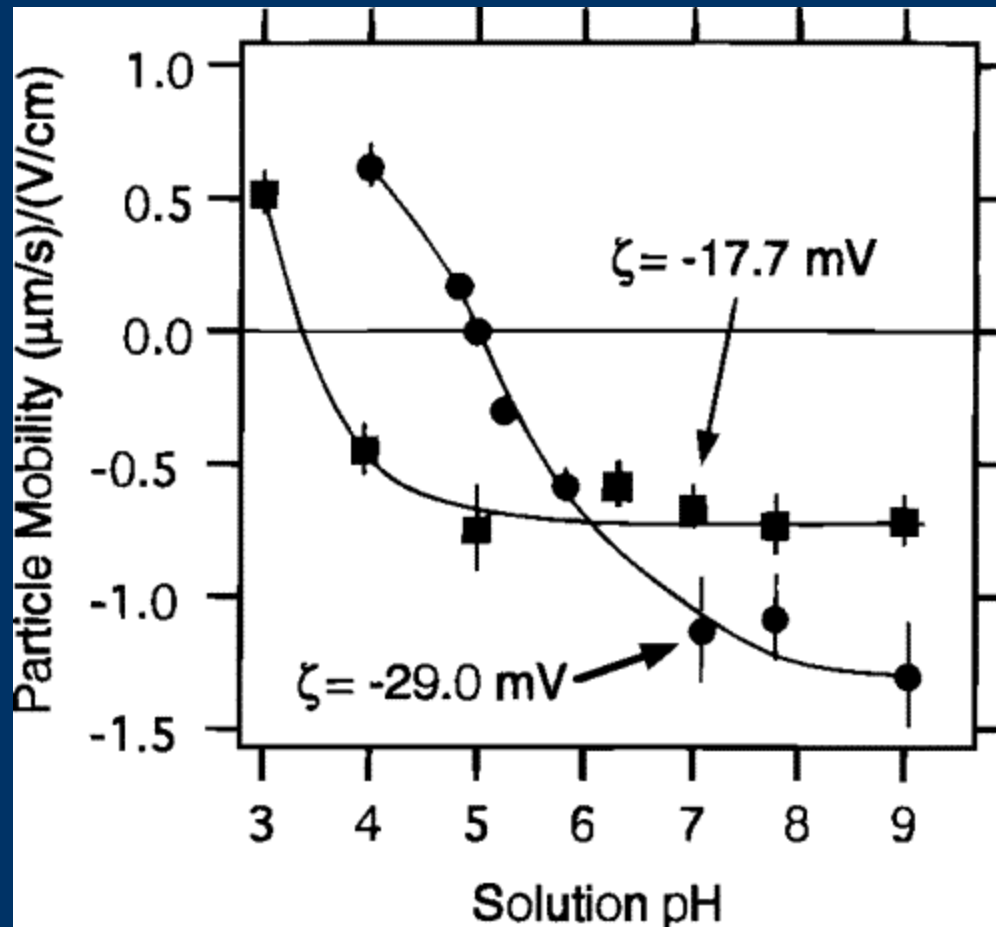


Table 4–2. Classification of the L- α -amino acids of proteins based on their relative hydrophilicity (tendency to associate with water) or hydrophobicity (tendency to avoid water in favor of a more nonpolar environment).

Hydrophobic	Hydrophilic	
Alanine	Arginine	Histidine
Isoleucine	Asparagine	Lysine
Leucine	Aspartic acid	Serine
Methionine	Cysteine	Threonine
Phenylalanine	Glutamic acid	
Proline	Glutamine	
Tryptophan	Glycine	
Tyrosine		
Valine		

Elution from Adsorbent Filters

- Choice of eluants
 - Beef extract
 - Amino acids
 - w/mild detergents
- Considerations
 - Efficiency of elution
 - Compatibility with downstream assays
 - Volume
 - Contact time

- Negatively charged treated w/cations
(Millipore HA, nitrocellulose)
 - 3-95% recovery pure water
 - 40-90% recovery from salt water
(Filterite, fiberglass)
 - 10-60% recovery
- Positively charged
(Cuno 1MDS, charge-modified cellulose/fiberglass)
 - 50-96% recovery from pure water
 - 5-20% recovery from salt water

Combined Sampling

- Hollow Fiber Ultrafilter
 - 25-50% virus recovery
 - 25-50% bacteria recovery
- Microporous Filters
 - Filterite ~40% recovery of Giardia and Cryptosporidium
 - Spun Polypropylene ~10-15% recovery of Giardia and Cryptosporidium
 - 1MDS $\approx$ Spun Polypropylene

Recovery from Water

- Factors that effect filter adsorption and elution efficiencies:
 - Cation speciation and concentration (Lukasik, et al. 2000; Katayama, et al. 2002)
 - pH (Lukasik, et al. 2000)
 - Presence of humic and fulvic acids (Sobsey and Hickey, 1985; Guttmann-Bass and Catalano-Sherman, 1985)
 - Volume of water filtered (Toranzos and Gerba, 1989)
 - Clay particles (Bentonite) (Sobsey and Cromeans, 1985)
 - Turbidity (Kuhn and Oshima, 2002; Simmons, et al. 2001)

Reconcentration and Purification (Viruses)

- Organic Flocculation
- Adsorption to minerals (e.g. aluminum hydroxide, ferric hydroxide)
- Hydroextraction (dialysis with Polyethylene Glycol (PEG))
- Spin Column Chromatography (antibodies covalently linked to gel particles)
- IMS (Immunomagnetic separation)
- Ligand capture

Virus Detection Techniques

Targets:

- Nucleic Acid
 - PCR methods
- Protein/Lipid
 - Immunological methods
- Whole Organism
 - Microscopy (EFM or EM)
 - Culture

Indicator Organisms

Pathogen Detection and Monitoring

- Pathogen detection
 - technically demanding,
 - often tedious,
 - slow to produce results,
 - Often unreliable
 - expensive.
- Done routinely in the health care field (clinical diagnostic microbiology):
 - often essential to patient treatment and care.
 - provides national surveillance of infectious disease epidemiology

Indicators: Background and Rationale

Besides nutrients and organic matter, human and animal fecal wastes contain large numbers of microbes (~100 billion/gram).

- ❖ About 1/3rd the mass of human fecal matter is microbes.
- ❖ Most are beneficial or essential in the gut; not pathogens.
- ❖ Some gut microbes are human pathogens; they cause disease.

What is Measured as Microbial Indicators and Why?

- Microbial indicators have been used for more than 100 years (since late 1800s) to detect and quantify **fecal contamination** in water, food and other samples
 - Concerns were for bacteria causing water- and foodborne illness, such as:
 - *Salmonella typhi*: the cause of typhoid or enteric fever
 - *Vibrio cholerae*: the cause of cholera
 - *Shigella dysenteriae* and other *Shigella* species: dysentery

What is Measured as Microbial Indicators and Why?

- Focus was and still is on detecting primarily human (or maybe animal) fecal contamination as the source of these and other enteric bacterial pathogens
- Detect fecal contamination by measuring:
 - common enteric bacteria residing in the gut and shed fecally
 - Chemicals associated with the gut or with anthropogenic fecal contamination
 - Something else associated with and predictive of fecal contamination

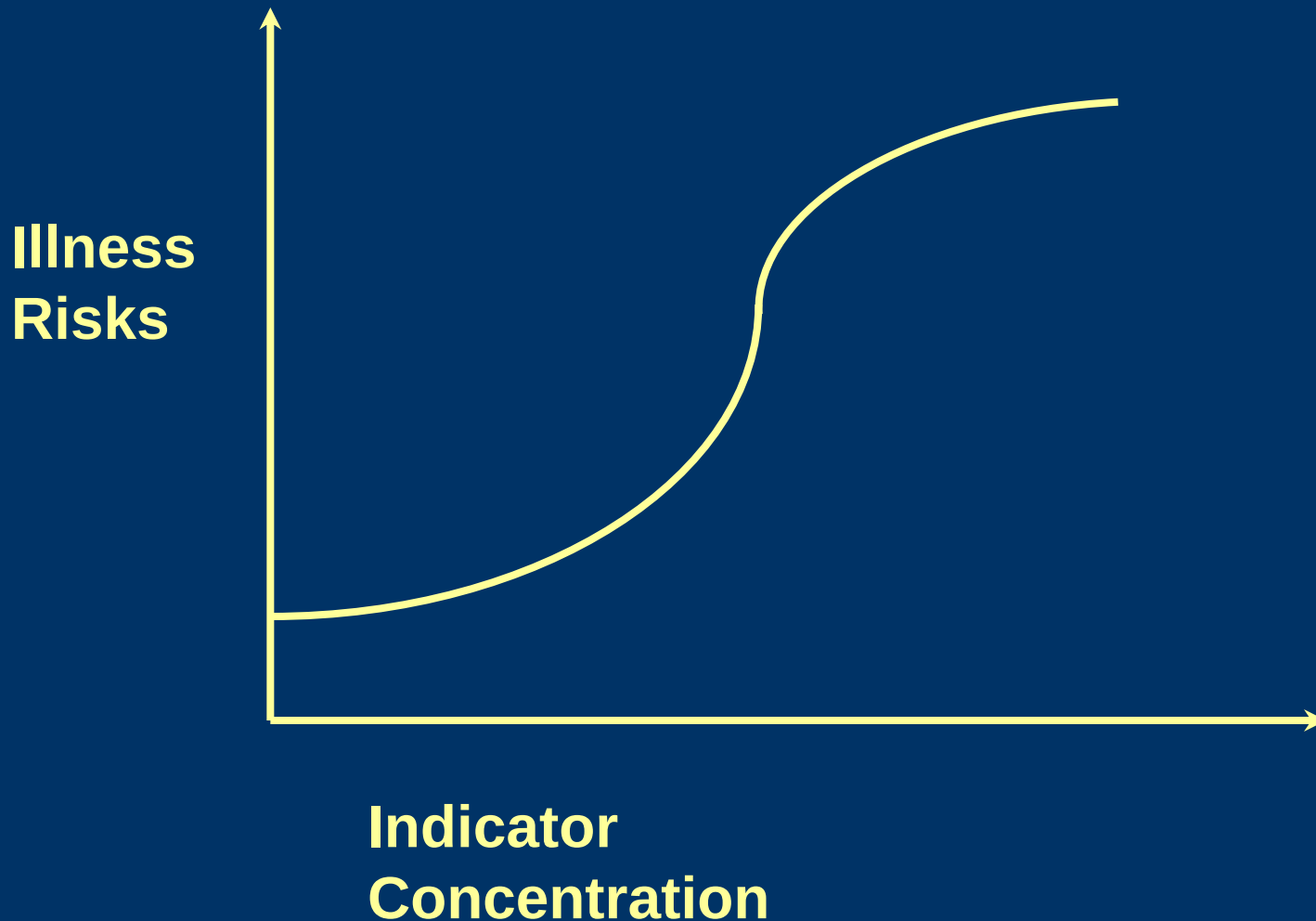
Some Purposes and Uses of Indicators

- Indicate presence of fecal contamination
- Indicate possible presence of pathogens
- Predict human health risks
- Indicate pathogen responses to treatment;
treatment efficacy

Criteria for an Ideal Indicator of Fecal Contamination

- Applicable to all types of water (and other relevant samples).
- Present in feces, sewage and fecally contaminated samples when pathogens are present; numbers correlate with amount of fecal contamination; outnumber pathogens.
- No "aftergrowth" or "regrowth" in the environment.
- Survive/persist $>$ than or $=$ to pathogens.
- Easily detected/quantified by simple lab tests in a short time.
- Constant characteristics.
- Harmless to humans and other animals.
- Numbers in water (food, etc.) are associated with risks of enteric illness in consumers (dose-response relationship).

Dose-Response Relationship Between Indicator Density in Vehicle (Water) and Risk of Illness in Exposed Individual or Population: Hypothetical Example

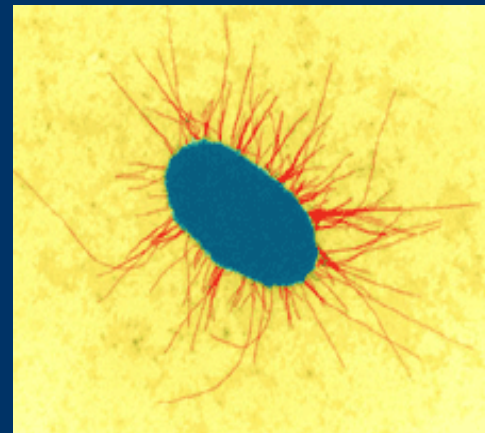


Current Bacterial indicators of Fecal Contamination

Coliform bacteria:

Members of the *Enterobacteriaceae*; Gram-negative, non-sporeforming rods, ~1-2 micrometer, facultative anaerobes, ferment lactose, producing gas; possess Beta-galactosidase activity, oxidase negative, some motile with peritrophic flagella

Coliforms: Operational definitions of bacterial groups; have changed over time

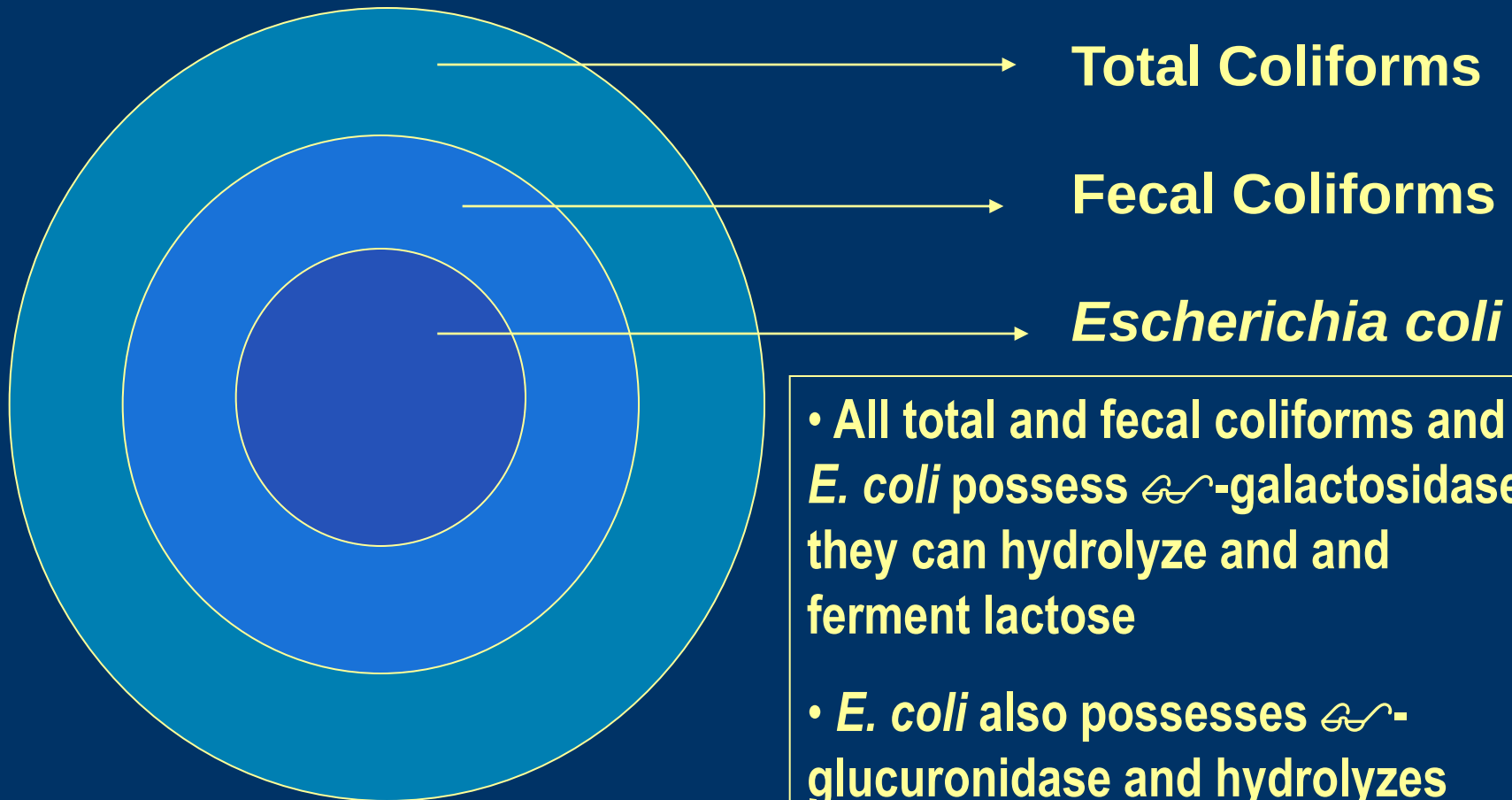


Coliforms

Coliform Groups:

- Total coliforms:
 - drinking, bathing and shellfish water standards
 - not feces-specific (some have environmental sources).
- Fecal ("thermotolerant") coliforms (FC):
 - detect by growing at elevated temperature of 44-45°C
 - ditto total coliforms in feces-specificity, but less so
 - Used in drinking, recreational and shellfishing waters
- *E. coli*: the "fecal" coliform; the predominant coliform in the gut and in feces
 - Detect & distinguish from other total & fecal coliforms by -glucuronidase activity
 - may occur naturally in tropical environments (and possibly elsewhere)
 - Used in drinking, recreational and shellfishing waters

Relationships among Total and Fecal Coliforms and *E. coli*



- All total and fecal coliforms and *E. coli* possess β -galactosidase; they can hydrolyze and ferment lactose
- *E. coli* also possesses β -glucuronidase and hydrolyzes glucuronide substrates

Current Bacterial indicators of Fecal Contamination

- Properties: Gram positive, cocci shape, nonmotile, occur in pairs or short chains, cells ~1 micrometer diameter, primarily in human and animal intestines, catalase-negative, facultative anaerobes (prefer anaerobic conditions), complex and variable nutritional requirements, perform simple fermentation, resistant to many Gram positive antibiotics,

Fecal streptococci (FS):

- Mostly Lancefield group D (and some group Q) streptococci and enterococci
- Similar levels as coliforms in feces and fecal waste
- Survive better than coliforms in environmental waters
- not feces-specific.

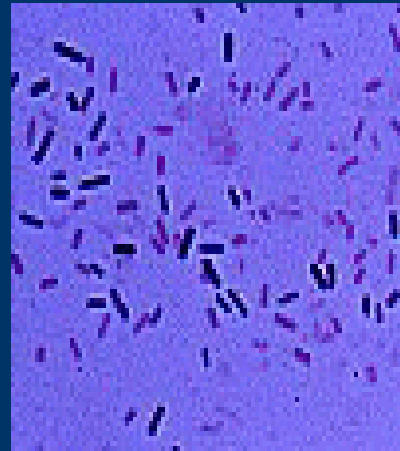
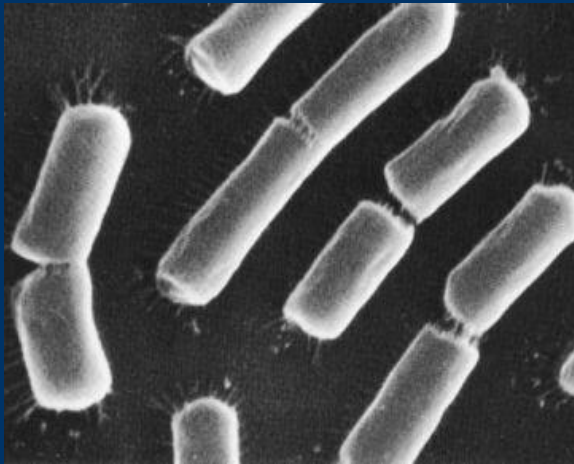
Enterococci:

- More feces-specific sub-set of FS
- Primarily *Enterococcus faecalis* & *E. faecium*
- Can grow in 6.5% NaCl
- Can grow at a pH range of 9.6 to 4.6
- Can grow at temperatures ranging from 10 to 45°C
- Optimum growth at 37°C
- EPA guideline for bathing water quality



Sulfite-reducing Clostridia and *Clostridium perfringens*:

- Anaerobic, Gram-positive, non-motile rods
- Form spores (terminal or sub-terminal)
- Reduce sulfite to hydrogen sulfide
- Can be pathogenic: foodborne disease (toxins), brain abscesses, pneumonia, wound infections, post-surgery infections.
- feces-specific?
- very (too?) resistant spores (can persist for decades of centuries!)
- may be an indicator for protozoan cysts and possibly viruses



Other Candidate Bacterial Indicators of Fecal Contamination

***Bacteroides* spp. and *Bifidobacteria* spp.:**

- most plentiful in feces (100X more than FC, FS and *E. coli*)
- strict anaerobes
- poor survival in the presence of air (oxygen)
- poor detection methods: requires strict anaerobic conditions
- Some *Bacteroides* species may be human-specific

***Rhodococcus coprophilus*:**

- plentiful in feces of some animals
- possible animal fecal contamination indicator

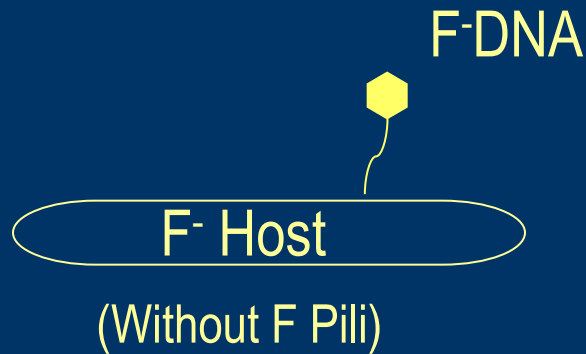
Microbial Indicators: No Ideal One

- Bacteria are not always reliable indicators of all pathogens
- Viruses and protozoa differ in size, response to environmental stressors and to treatment processes
- No single indicator fulfills the criteria of an ideal fecal indicator
 - There is no ideal indicator, really
- No single indicator is going to be suitable for all classes of pathogens
- No single indicator will reliably predict pathogen health risks in all media and under all conditions

Enteric Bacteriophages

- **Coliphages:** viruses infecting *E. coli* and maybe other coliforms
 - Somatic coliphages: attach directly to outer cell wall; several groups; some may not be feces-specific; host-dependent detection.
 - Male-specific (F+) coliphages: coliphages infecting "male" strains of *E. coli* (posses pili); may be feces-specific.
 - May distinguish human from animal fecal contamination by group classification (II & III human; I & IV animal); but, pigs may have, too.
- ***Bacteroides fragilis* phages:** may be human feces specific on certain host bacteria (USA studies do not show human-specificity); concentrations low but survive well in environment.
- ***Salmonella* phages:** in human and animal feces; may indicate presence of *Salmonella* bacteria; concentrations low but they survive well in environment.

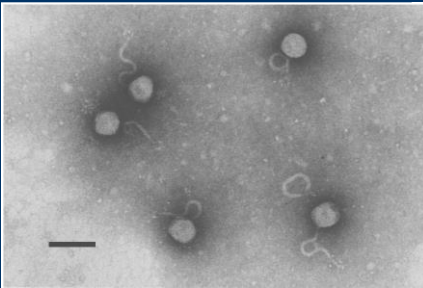
Types of Coliphages: Somatic (F⁻)



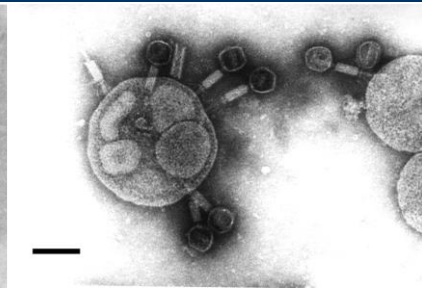
← Somatic
Infect host through receptors
on cell wall

Four Families

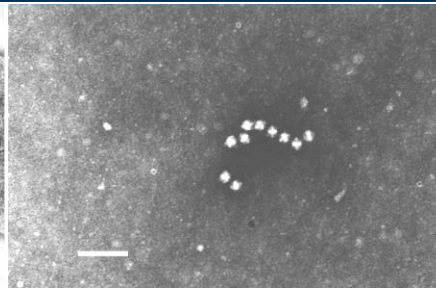
Siphoviridae



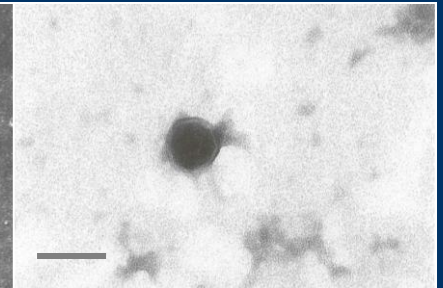
Myoviridae



Microviridae



Podoviridae

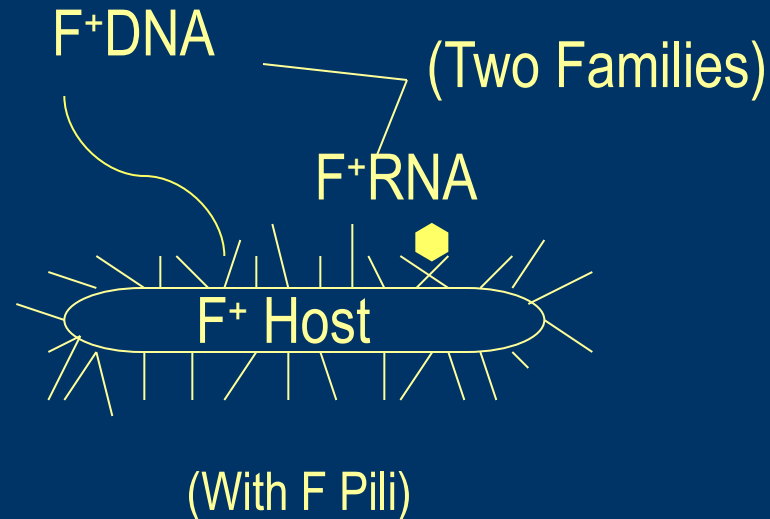
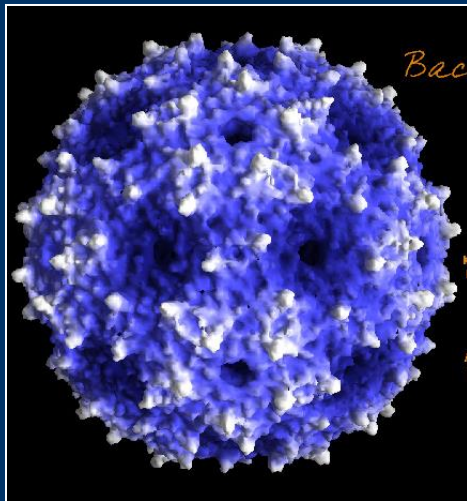


Bar = 100 nm; First three photos by Fred Williams, EPA

Types of Coliphages: Male-Specific (F^+)

Male-Specific

Infect host through receptors
on F pili



F^+ RNA = *Leviviridae*

Bacteriophage MS2. Valegard et al. Licensed for use, Inst. for Molecular Virology.
(linked to <http://www.bocklabs.wisc.edu/images/ms2.jpg>). 20 July 2001.

INDICATORS OF PROTOZOAN PARASITES

Currently, there is no universally reliable indicator of enteric protozoan parasites.

- Spores of *Clostridium perfringens* (a gut anaerobe) and thermostable aerobic bacteria (primarily *Bacillus* species) have been studied as indicators of water treatment efficacy for *Giardia*, *Cryptosporidium* and even enteric viruses (*C. perfringens* spores).
- No reliable indicator of enteric protozoan occurrence has been identified.

Chemical Indicators of Fecal Contamination

- Fecal sterols:
 - Coprostanol, Cholesterol and Cholestanol
 - Constituents of the fatty acids in cells
 - Chemical tracers of fecal contamination
 - Employs chemical methods: gas chromatography and HPL chromatography
 - Method sensitivity may be inadequate except where fecal contamination is high
 - Humans and animals have different dominant forms of fecal sterols
 - Use to possibly distinguish human from various animal sources

Other Chemical Indicators of Fecal or Anthropogenic Contamination

- Anthropogenic contamination indicators
 - Optical brighteners from detergents
 - Persistent in the environment.
 - Detected using low-tech black lights or mass spectroscopy.
 - May not reflect recent pollution; uncertain environmental persistence
 - Caffeine
 - Human source fecal contamination indicator
 - Chemical detection methods
 - Some other plants that have significant caffeine levels (e.g. watermelon)
 - Caffeine is easily degraded by soil microbes, so persistence is uncertain
 - Human pharmaceuticals and personal care chemicals
 - Antibiotics
 - Anti-inflammatory medications

Microbial Source Tracking

A large red 'X' is drawn over the text 'Microbial Source Tracking', indicating that the concept is being negated or is incorrect.

Microbial Source Typing

Background: MST

- What is MST?
 - The use of phenotypic or genotypic classification methods for determining the source of isolated microorganisms
 - Initially BST instead of MST
 - Based on several assumptions
 - Clonal population structure of bacteria
 - Within a given species of microorganism, some members (strains or types) have adapted to living under specific environmental conditions or within a specific host, thus display host specificity
 - Clonal composition of populations changes with locality or population
 - Clonal composition of populations is stable over time
 - Useful in management of fecal contamination sources
 - e.g. implementation of appropriate BMPS

Library-Based MST Approaches

- Phenotypic
 - ARA/MAR
 - Carbon Utilization Profiles
 - FAME
- Less stable
- Less specific

- Genotypic
 - rRNA methods
 - Restriction Analysis
 - 16s Sequencing
 - RFLP/PFGE
 - REP-PCR
 - Other Bacterial Genotyping/Sequencing
 - (Mitochondrial DNA)

Both require a sizable library for discriminate analysis

Library Independent MST Approaches

- FC/FS ratios
- Host-specific genetic markers (no growth required)
 - Bacteria (Bacteroides and Prevotella; Bifidobacteria; Rhodococcus)
 - 16s TRFLP or LH-PCR
 - DGGE
 - 16s sequencing
- Phage Analysis (B.fragilis phages, F+ and Somatic Coliphage, Salmonella phage)
 - Serotyping
 - Genotyping
- Direct detection of human or animal pathogens
 - qPCR detection of virulence factor/bacterial biomarker
 - qPCR detection of host-specific viruses
- Chemical targets (Fecal Sterols, Bile Acids, Caffeine, Fluorescent Whitening agents, Pharmaceuticals, other)

Choosing a Method

- Big concerns are cost and level of desired discrimination (inversely related)
- All methods still under development; none adequately standardized
- Most commonly used method is ribotyping
 - Also one of more expensive, but offers best discrimination
 - One of big problems is the library; temporal-spatial stability
- Some methods (e.g. phage analysis) can offer “quick and dirty” discrimination of human vs. animal, but currently lack adequate discrimination for good utility
- Best option probably to use multiple methods

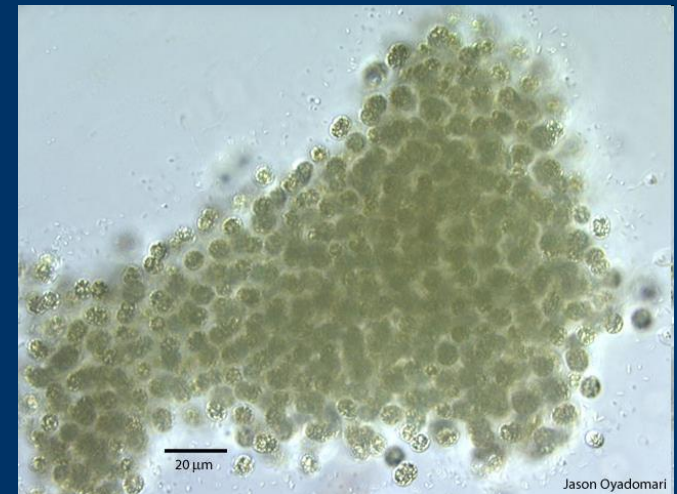
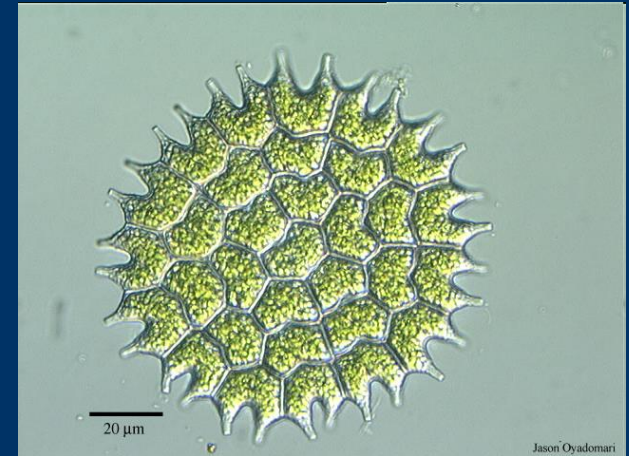
Buyer Beware!

- What MST methods can offer
 - Source typing
 - Rough cut between animal and human
- What MST methods cannot offer
 - Pin-point source
 - 100% solution

Algae

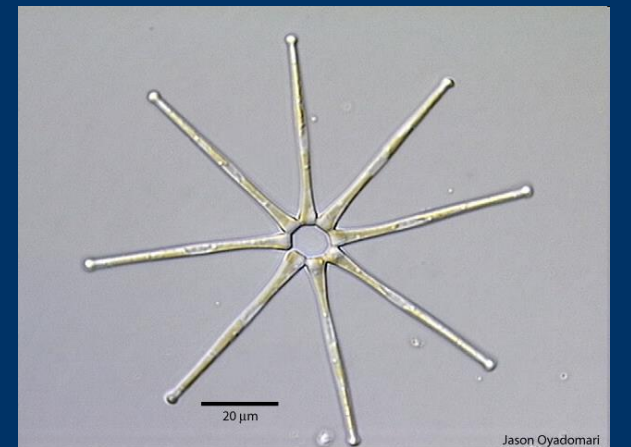
Algae Divisions

- **Chlorophyta** (green algae)
 - Least harmful, generally considered beneficial
 - Growth in reservoirs; mild taste and odor; some filamentous mat formers
- **Cyanophyta** (blue-green algae)
 - Prokaryotes
 - Most significant concerns for water quality
 - Taste and odor problems; filter cloggers; oxygen depletion; toxicity



Algae Divisions

- **Ochrophyta (Chromophyta)**
 - **Chrysophyceae** (Yellow-Green/Golden-Brown Algae)
 - Taste and odor problems; reservoir growth; filter cloggers
 - Frustules used for filtration
 - **Bacillariophyceae**
 - Diatoms
- **Dinophyta (Pyrrhophyta, Dinozoa)**
 - Dinoflagellates
 - Taste and odor problems
 - Red tide problems



Algae Divisions

- **Euglenophyta**
(protozoan-like algae)
 - Indicators of pollution
 - Filter cloggers
- **Cryptophyta**
(cryptomonads)
 - Taste and odor problems
- **Rhodophyta** (red algae)
 - Growth on reservoir walls and irrigation ditches



Taste and Odor

- **Dirty or Musty**
 - Geosmin and MIB (2-methylisoborneol)
 - blue-green algae, actinomycetes
- **Fishy, Cod liver Oil**
 - Chrysophyta, Pyrrophyta
- **Septic Odor**
 - Pryrophyta
- **Cucumber Odor**
 - Chrysophyta

Algal Toxins

- **Anatoxin** (e.g. *Anabaena*)
 - Staggering, paralysis, gasping, convulsions, death
 - 200 µg/kg LD₅₀
- **Microcystin** (e.g. *Anabaena*, *Microcystis*, *Oscillatoria*)
 - Jaundice, shock, abdominal pain/distention. Weakness, nausea, vomiting, severe thirst, rapid/weak pulse, death
 - 300-600 µg/kg LD₅₀
- **Saxitoxin/Neosaxitoxin** (e.g. *Anaphizomenon*)
 - Weakness, staggering, loss of muscle coordination, difficulty in swallowing, labored respiration, muscle paralysis, death, tingling around mouth or fingertips, slurred speech
 - 9 µg/kg LD₅₀
- **Hepatotoxin** (e.g. *Gleotrichia*)
 - Jaundice, abdominal pain/distention, weakness, nausea/vomiting
- **Cytotoxin** (e.g. *Gleotrichia*)
 - Skin irritation, gastrointestinal upset



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Isolated from Limonochia- an eutrophic lake in Ecuador



Isolated from the Ohio River

Anabaena sp. (blue-green/cyanobacteria)-A few species of this genus are planktonic; others are epiphytic, or form gelatinous masses. Among the planktonic forms several are coiled (Prescott 1982). Some of the planktonic species are capable of producing lethal microcystin toxins in large concentrations. *Anabaena* sp. will produce a grassy, musty, or nasturium odor at moderate concentrations. A rotten, septic, or medicinal odor is possible with large concentrations. Critical concentration for odor production is 530,000 cells/100 ml. (AWWARF 1987)

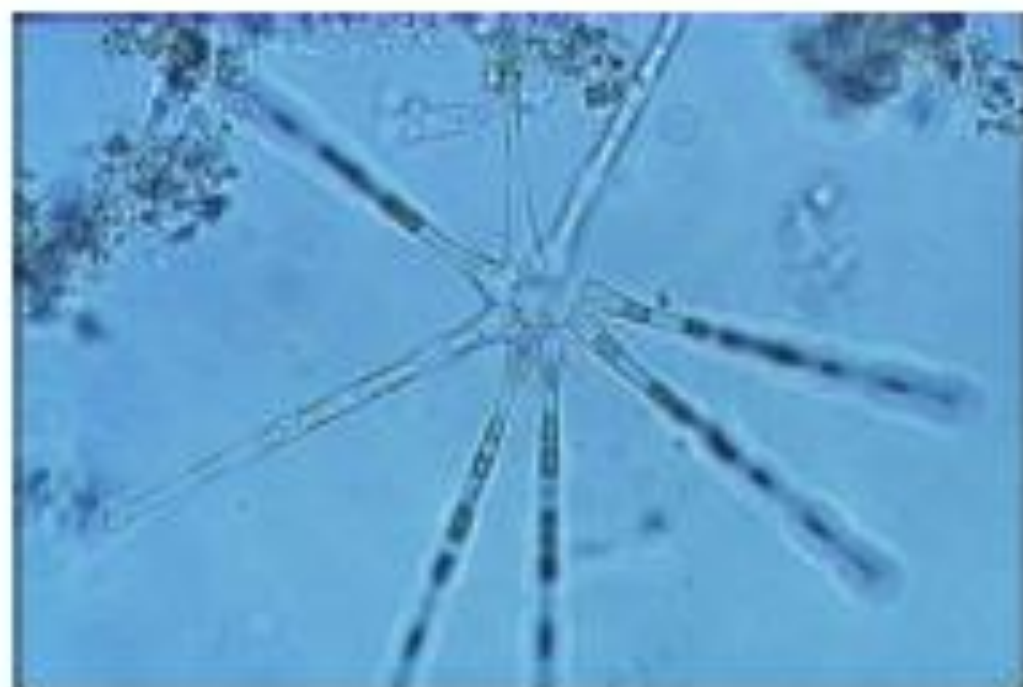
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Isolated from the Ohio River

Aphanizomenon sp. (blue-green/cyanobacteria)-Species of this genus have trichomes that often lie parallel in bundles. The cells are short cylindrical or barrel-shaped and are the same diameter throughout except at the apices. Each trichome contains 1 heterocyst and one akinete (Prescott 1982). This species is capable of producing taste and odor problems. Will produce a grassy, musty, or nasturium odor at moderate concentrations. A rotten, septic, or medicinal odor is possible with large concentrations. Critical concentration for odor production is 660,000 cells/100 ml or 20,000 colonies/100mls (AWWARF 1987). Microcystin toxins are also possible with this genera.



Asterionella sp. (diatom)—Species of this genus are generally planktonic, often very abundant, and are readily identified by the spoke-like arrangement of the rectangular frustules about a common center. Some species may form a bloom in favorable habitats and are often involved in water spoilage. The common species are usually found in hard water lakes (Prosser 1982). May produce a spicy garlicky odor in moderate concentrations and a fishy odor in large concentrations. Critical concentration for odor production is 300,000 cells/100 ml (AWWARF 1987).



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***Ceratium* sp. (Dinoflagellate).** This is one of the most common freshwater dinoflagellates and frequently—occurs as dominant phytoplankton in ponds, lakes and rivers. The horn-like extensions and transverse groove from which the 2 flagella arise are distinctive of the genus. It is a taste and odor producing algae.

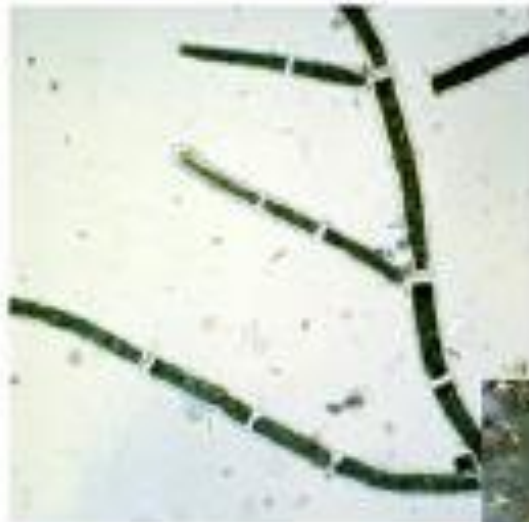


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Chlamydomonas

Chlamydomonas sp. (Chlorophyta, Green Algae)—Consist of spherical, ellipsoid, or ovoid cells, sometimes with one or two apical papillae, from which two flagella arise. Often it is found with a narrow or wide mucilaginous sheath. The chloroplast is a dense padded body that occupies the entire cell. The cell may contain 1 to many pyrenoids, which may be basal, or bilateral and scattered. Species of this genus have a habit of coming to rest, losing their flagella, and entering upon a quiescent phase. Vegetative cell division continues, ordinarily accompanied by the secretion of a mucilage, so that amorphous gelatinous masses are formed which contain many non-motile cells. This is known as the palmella stage. *Chlamydomonas* sp. Is capable of producing taste and odor in moderate concentrations. In large concentration, the odor may be fishy, medicinal, or septic. This alga is an indicator of fresh water pollution, and also sewage ponds.



Cladophora glomerata

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Cladophora sp. (Chlorophyta/Green Algae)—Is a very common algae found in polluted streams and rivers. It is an attached algae, but in some species the adults can become free-floating. It forms feathery tufts on substrates. The branches are smaller than the main axis, or at least tapering slightly toward the apices. The cells are cylindrical or swollen with thick cell walls are lamellate in most places. Occasionally the walls are thin and firm. The chloroplast is a parietal reticulum, which sometimes becomes fragmented and appears as numerous discs. Pyrenoids are present. Species in this genus are indicators of high pH and are generally found in hard or semi-hard water.



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***Closterium* sp.** (Chlorophyta/Green Algae)- Cells are crescent shaped, variously bowed, but in some species nearly straight, without apical spines. There is one axial chloroplast per semi cell, each with longitudinal ridges. Each cell may have few to many pyrenoids, which can be axial or scattered. The cells are either colorless or greenish brown. There is a terminal vacuole at the end of each cell (Prescott 1982). ***Closterium* sp** will produce a grassy odor in large quantities. The critical concentration for odor production is 20,000 cells/100 mls (AWWARF 1982).

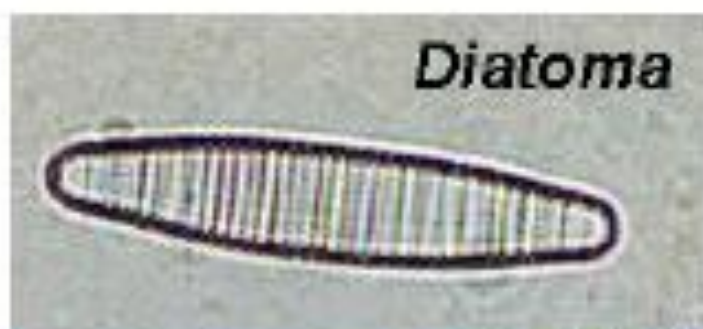
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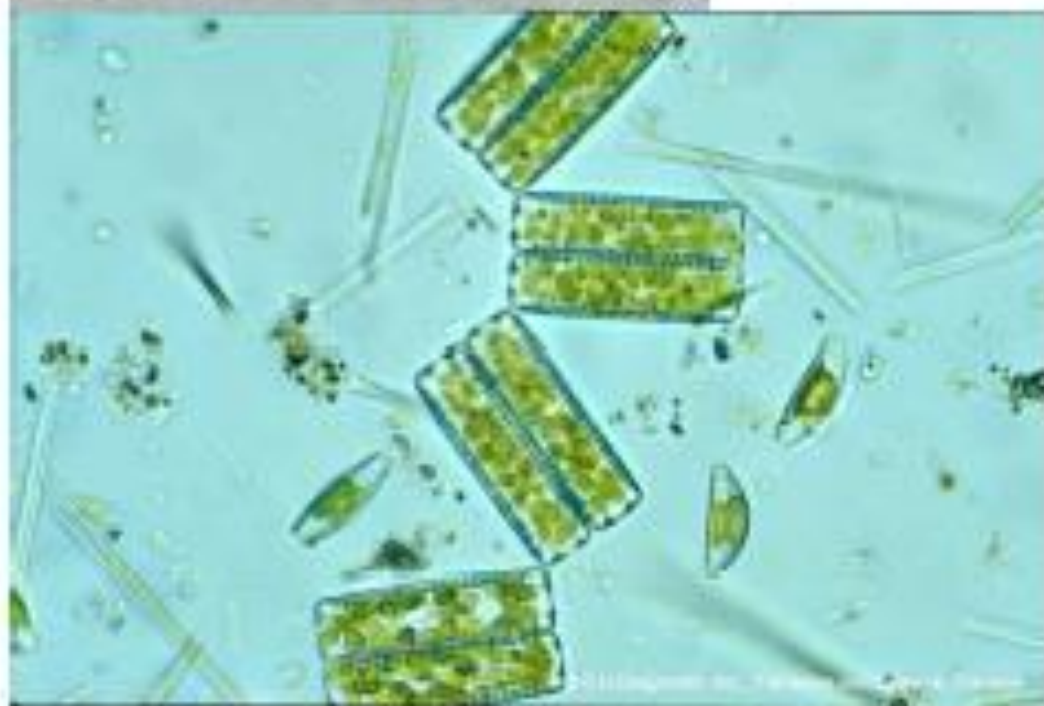
Isolated from the Coagulation Basin

***Cosmarium* sp.** (green)—There are thousands of species of this genera of algae, more than any other genera in the green algae (Prescott 1982). ***Cosmarium* sp.** will have two similar halves. Will produce a grassy odor in large concentration.



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Diatoma sp. (Diatom)—Frustules form zig-zag colonies that can be confused with *Tabellaria* when seen in girdle view. They are more common in running waters (rivers and streams) than in lakes or ponds.



*Euglena
gracilis*

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Euglena sp (Euglenophyta). Cells often changing shape when swimming. Numerous disc shaped chloroplasts are usually green but one species sometime is colored red because of a pigment (Haematochrome). The red pigment seems to be produced in response to intense light. Ponds may have a bright red film over the surface caused by *Euglena* blooms. This algae is found in eutrophic waters with high levels of organic material. It is a pollution indicator.

Isolated from Coagulation Basin

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Fragilaria
400x
45 microns

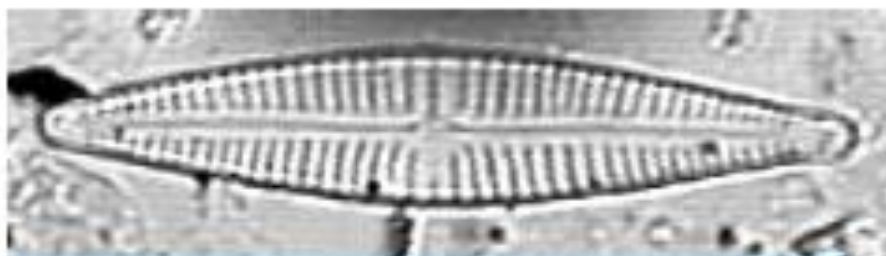
Fragilaria sp. (diatom)—Frustules quadrate or rectangular in girdle view, attached side by side to form ribbons (rarely in chains); valve view fusiform, the poles narrowed from enlarged central region (Prescott 1982). May produce a grassy, spicy, or geranium odor in moderate quantities and a musty odor in large concentrations.



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***Frustulia* sp.** Cells single, with raphe on both valves, usually rhomboidal or lanceolate, with straight to undulate margins. Raphe contained in a median rib extending most of the length of the valve. At the apices, the raphe rib has the appearance of a pencil tip. Striae are fine. ***Frustulia* sp.** seems to prefer oligotrophic to somewhat dystrophic waters with low to neutral pH.



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***Gomphonema* sp.** (diatom)—This epiphytic diatom is asymmetrical on the transverse axis. The striae are composed of puncta in a single series. The central striae usually ends with one isolated puncta. It is frequently attached on branched stalks (Prescott 1982).