

*Implications and the Use of
Bacterial Indicators for the
Prediction of Microbial
Contamination of Water
(Continued)*

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Implications of Microbial Contamination of Water

- ▶ potential for transmission of disease causing microorganisms (pathogens) via water contact - oral fecal route
- ▶ contact includes ingestion, primary contact, and secondary contact
- ▶ Bacteria
- ▶ Viruses
- ▶ Protozoa



Difficulties With Routine Testing Of Pathogens in Water

- ▶ present in low numbers
- ▶ limited survival time
- ▶ numerous pathogens to analyze
- ▶ time and cost prohibitive



Testing of Indicator Organisms

- need an indicator of potential pathogen contamination of water which is easy, reliable, inexpensive, quick, etc.



Requirements of Indicator Organisms

- ▶ present when pathogens present in water
- ▶ absent in uncontaminated water
- ▶ present in higher numbers than pathogens in contaminated water
- ▶ better survival in water than pathogens
- ▶ easy to analyze



Coliform Bacteria - Indicators of Fecal Contamination

- ▶ bacteria in the family Enterobacteriaceae
- ▶ generally include the genera *Enterobacter*, *Klebsiella*, *Citrobacter*, and *Escherichia*
- ▶ present in the intestinal tract of numerous organisms
- ▶ gram negative, non-spore forming rods
- ▶ ferment lactose with acid and gas production in 48 hours at 35 °C



Total Coliform Bacteria

- ▶ inhabit the intestinal tract of animals
- ▶ sources: fecal material, soil, water, grain
- ▶ some capable of reproduction in the environment
- ▶ non-fecal examples include: *Klebsiella sp.*, *Citrobacter sp.*, *Enterobacter sp.*



Fecal Coliform Bacteria

- ▶ subset of the total coliform group
- ▶ separated from non-fecal coliforms by growth at 44.5 °C
- ▶ sources: fecal material
- ▶ capable of limited survival and growth in the environment
- ▶ primary example is *Escherichia coli* (*E. coli*)



Source Water vs. Drinking Water

- ▶ Drinking water and source water have different requirements for coliform analysis methods and holding times



Coliform Sample Holding Times

- drinking water - 30 hours
[40 CFR 141.21(f)]
- surface/source water - 6 hours
[40 CFR 141.74 (a)(1)]



Effects of Holding Time on Samples

- coliform numbers may decline
- coliform numbers may increase
- heterotrophic (non-coliform) bacteria may increase and inhibit detection of coliforms in the sample



Heterotrophic Interference

- ▶ Non-coliform bacteria which are better than coliforms at surviving in the environment may be present in the sample. If these organisms are given a chance to grow, they may out-compete coliforms during analysis and may limit the analysts ability to detect coliforms. The effect of HI is an underestimation of coliform numbers or a false negative result.



Holding Times Are Critical

- greater chance of heterotrophic interference in untreated source water - hence, shorter allowable holding time



Membrane Filter Method

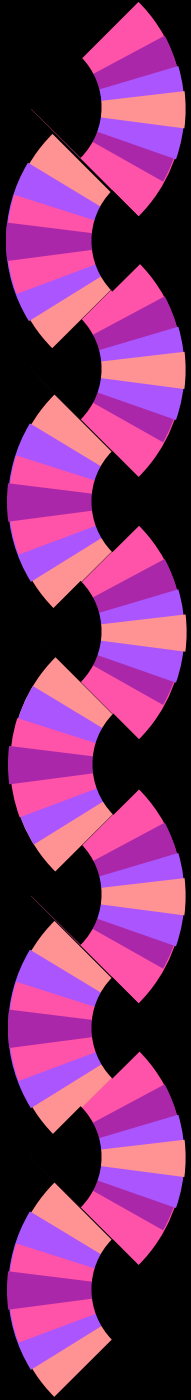
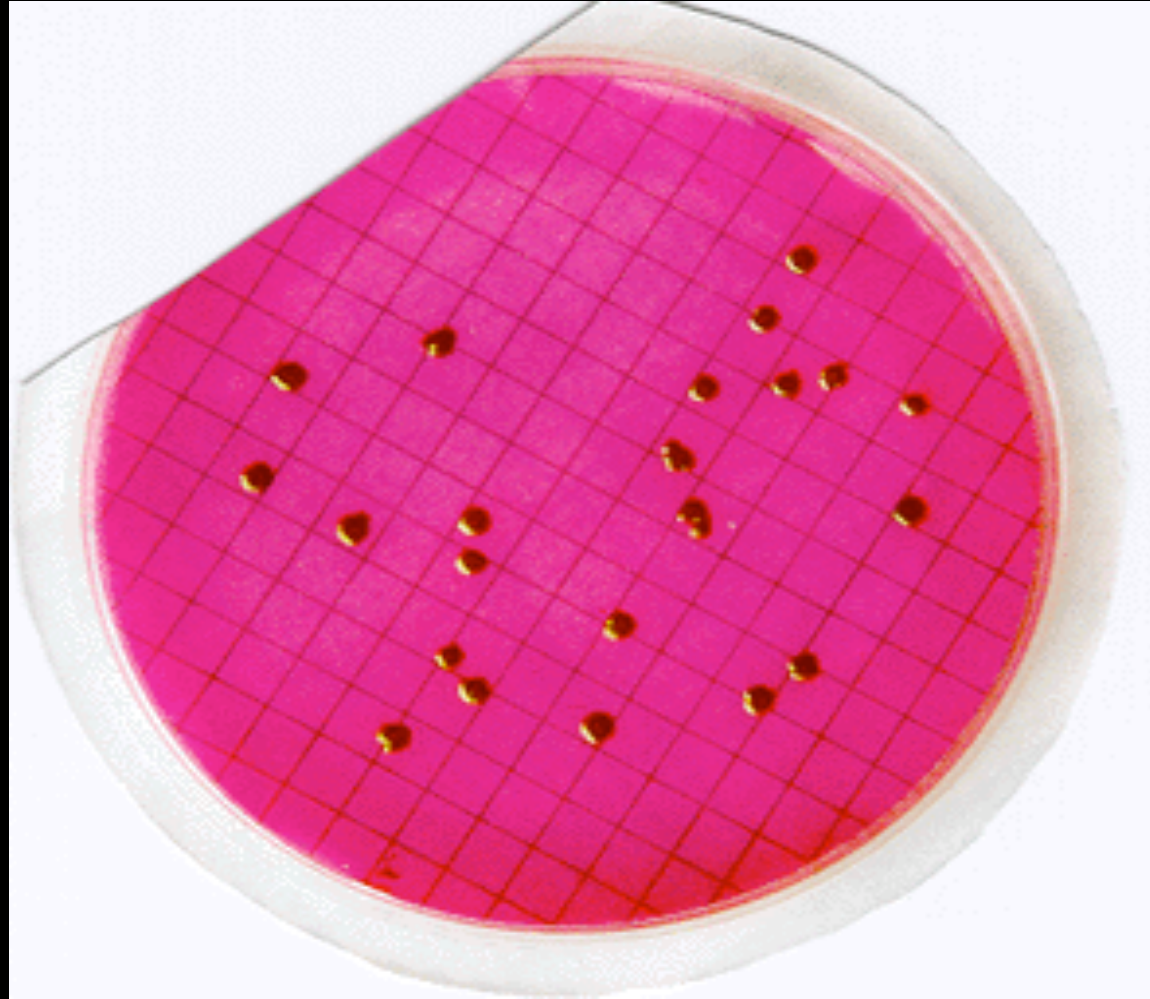
metallic green colonies on endo medium-
presumptive coliform positive

growth/gas in LTB and BGGBB - confirmed total
coliform positive

growth/gas in LTB and EC medium - confirmed
fecal coliform positive

growth and gas in LTB and growth and
fluorescence in EC + MUG - confirmed *E. coli*
positive

Membrane Filter Method (Endo agar with coliform growth)





Multiple Tube Method

5 day test

- growth/gas in LTB- presumptive coliform positive
- growth/gas in BGGBB - confirmed total coliform positive
- growth/gas in EC medium - confirmed fecal coliform positive
- growth and fluorescence in EC + MUG - confirmed *E. coli* positive



ADVANTAGES OF MF OVER MPN

Results in 24 hours as compared with 48-96

Much larger and more representative samples can be run

Numerical results from membrane filter have greater precision than MPN

The equipment and supplies are not bulky. More samples can be analyzed with less space and equipment



Enzyme Substrate or Chromogenic Substrate Method

- Used with the Presence-Absence, the Multiple Tube Methods, or Quanti-Trays™
- Total coliform have the enzyme
 - β -D-galactosidase which hydrolyses
 - ortho-nitrophenyl- β -D-galactopyranoside (ONPG)
 - Yellow when hydrolyzed
- *E. coli* has the enzyme
 - β -glucuronidase which hydrolyses
 - 4-methylumbelliferyl- β -glucuronide (MUG)
 - Fluoresces when hydrolyzed



INDICATOR REQUIREMENTS

- ▶ Ubiquitous in wastewater
- ▶ Survives or is detectable at least as long as pathogens
- ▶ Easy to isolate and identify



Streptococcus and Enterococcus

• Fecal Strep

- *S. faecalis*
- *S. faecium*
- *S. avium*
- *S. bovis*
- *S. equinus*
- *S. gallinarum*

• Enterococcus

- Fecal Streps that survive in 6.5% sodium chloride
 - *S. faecalis*
 - *S. faecium*
 - *S. avium*
 - *S. gallinarum*



Florida Healthy Beaches Program

- Beginning with a 1998 pilot program, 11 Florida coastal counties began conducting beach water sampling every two weeks and reporting the results in local news media.
- August 2000, the beach water sampling program was expanded to include 34 Florida counties.
- August 2002, the coastal counties began collecting weekly samples. The coastal beach water samples collected by the county health departments are analyzed for enterococci and fecal coliform bacteria. High concentrations of these bacteria may indicate the presence of microorganisms that could cause disease, infections, or rashes. County health departments issue health advisories or warnings when these conditions are confirmed.



Enterococcus Results Description

- ▶ **GOOD** 0-35 *Enterococcus cfu/100 ml* of marine water
Moderate 36-104 *Enterococcus cfu/100 ml* of marine water
- ▶ **Poor** 105 or greater *Enterococcus cfu/100 ml* of marine water



Fecal Coliform Results Description

- ▶ **GOOD** 0-199 fecal coliform cfu/100 ml of marine water
- ▶ **Moderate** 200-399 fecal coliform cfu/100 ml of marine water
- ▶ **Poor** 400 or greater fecal coliform cfu/100 ml of marine water



Health Advisory or Warning

- A Poor rating may result in a resampling event to confirm poor conditions, otherwise a Health Advisory or Warning will be issued immediately. These indicate that contact with the water at this site may pose increased risk of infectious disease, particularly for susceptible individuals. A reading of **NR** means "No Result." This could indicate that no sample was taken at this point because of weather or other factors, or that an analysis result was not obtained from the laboratory.



Is the Origin of the Pollution Human Waste?

- ▶ There currently is no procedure for determining if microbial contamination is of human origin.
- ▶ The following methods are useful in estimating origins and risk assessment.



Heterotrophic Plate Count SM 9215

A direct quantitative measurement of the viable aerobic and facultative anaerobic bacteria in a water environment, capable of growth on a selective medium.

Drinking water can not exceed 500 cfu/1ml.

The higher the count the more suspect the water.



Total Coliform

- The total coliform group includes all the aerobic and facultative anaerobic, gram negative, nonspore forming, rod shaped bacteria that ferment lactose in 24 hrs. at 35 C. Includes genera : Escherichia, Citrobacter and Klebsiella
- Drinking water can have no coliform present.
- Recreational waters can exceed 1000 cfu/100ml
- Designed for drinking water not environmental waters particularly saline waters.



Fecal Coliform

Fecal coliform are part of the total coliform group. They are defined as gram negative nonspore forming rods that ferment lactose in 24 hrs at 44.5 C with production of gas and produce acidity with blue colonies in a membrane filter procedure. The major species is *Escherichia coli*.

Recreational waters can not exceed 400 cfu/
100ml



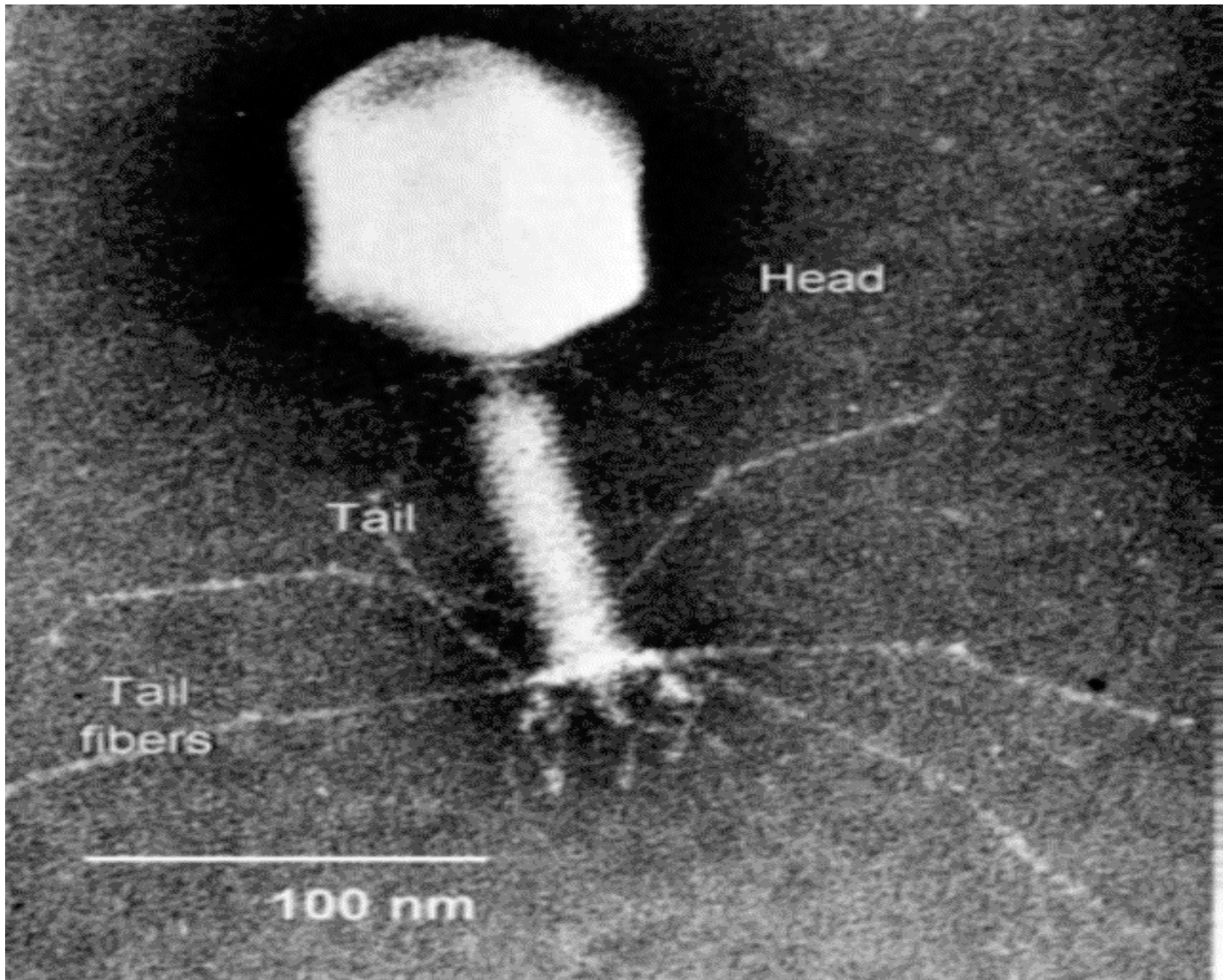
Enterococci EPA 1600

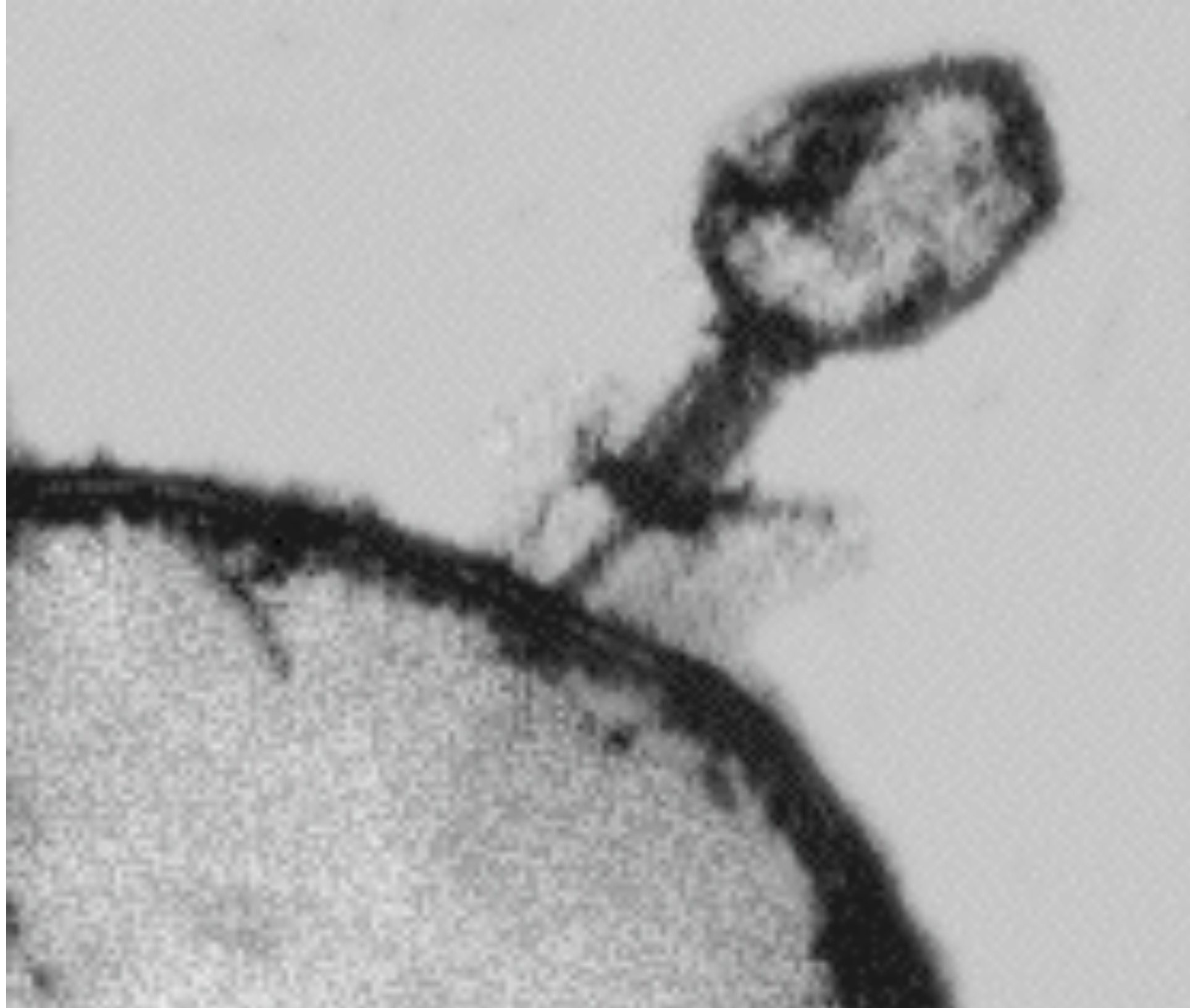
Enterococci are found in the feces of humans and other warm-blooded animals. Some strains are ubiquitous and not related to fecal pollution, the presence of enterococci in water is an indication of fecal pollution and the possible presence of enteric pathogens. A direct relationship can be constructed between the density of enterococci in water and swimming-associated gastroenteritis, the membrane filtration enterococci test can be used to determine the bacteriological quality of recreational marine and fresh waters.



Coliphage SM 9211D

- Coliphage are bacteriophages that infect and replicate in coliform bacteria and are present whenever total and fecal coliforms are found.
- Coliphage are more resistant to chlorine and saltwater than the bacteria.
- In sewage contaminated freshwater the coliphage are found as 10% of the fecal coliforms.









Useful References

- *Standard Methods for the Examination of Water and Wastewater*, 18th Edition, 1992, American Public Health Association, 1015 Fifteenth Street NW, Washington, D.C., 20005
- Total Coliform Rule (40 CFR 141.21)
- Surface Water Treatment Rule (40 CFR 141.7)
- <http://esetappsdo.h.doh.state.fl.us/irm00beachwater/default.aspx>