

Laboratory Support for Microbiological Monitoring Projects in the U.S. Geological Survey

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Rapid Method for Bacteria Concentrations

The USGS, in collaboration with the National Park Service and the University of Michigan, is conducting a 3-year study beginning in 2004 to investigate the use of a rapid method that will provide fecal-indicator bacteria concentrations in approximately 1 hour from sample collection. Standard plating methods to monitor the concentrations of fecal-indicator bacteria take 24 hours to obtain results. The goal of this project is to provide park managers with a tool that will give reliable results of the current day's bacteria concentrations.

To accomplish this goal, investigators will:

- Test the rapid method for E. coli and enterococci and determine how well its results correlate with results from standard plating methods. • Investigate the use of readily-measured variables (streamflow, rainfall, turbidity, rapid method results) as a predictive tool for E. coli and
- Test and compare the efficacy of the methods for estimating fecal-indicator bacteria concentrations.

Rapid method procedure:

Concentrate bacteria by filtration of water sample

- Recover bacteria from filter
- Add antibody/magnetic bead complex

Selectively capture bacteria by immunomagnetic separation

Rupture bacteria and measure the released bacterial ATP

• Results are in relative light units (RLUs)





Bacteria in Sediment

The USGS, in cooperation with the University of Toledo (UT), Cities of Oregon and Toledo, Toledo Metropolitan Area Council of Governments (TMACOG), and Ohio Water Development Authority (OWDA), is investigating the sources of fecal contamination to Maumee Bay so that future corrective measures can be taken and timely and accurate assessments of recreational water quality can be made. It has been hypothesized that the resuspension of accumulated E coli from bottom sediments may contribute to high E. coli concentrations in the water column.

The objectives of this 3-year project (2003 to 2005) are to:

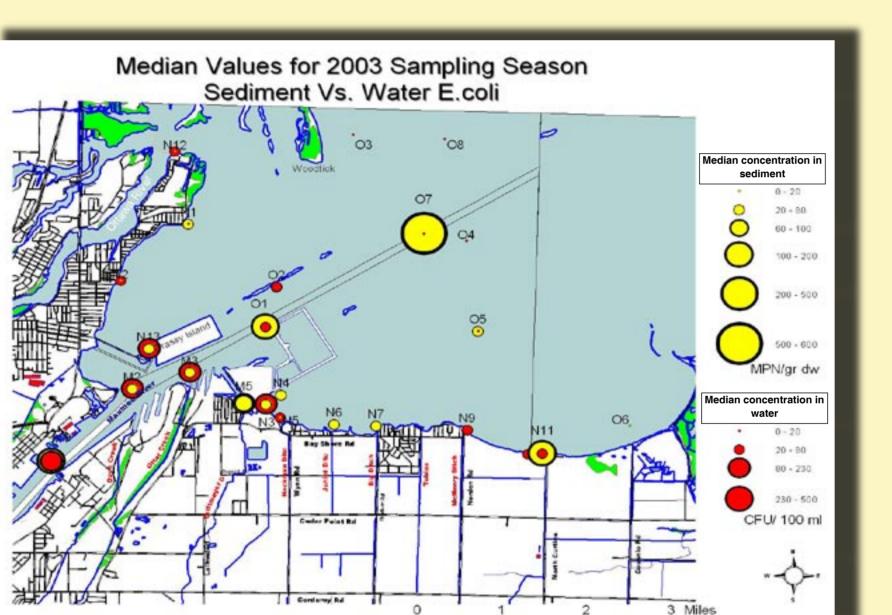
• Investigate the spatial and temporal distribution of *E. coli* in bed sediments at locations in the Maumee Bay area. • Examine the relations between environmental and water-quality variables and concentrations of E. coli in sediment and water in the nearshore area.

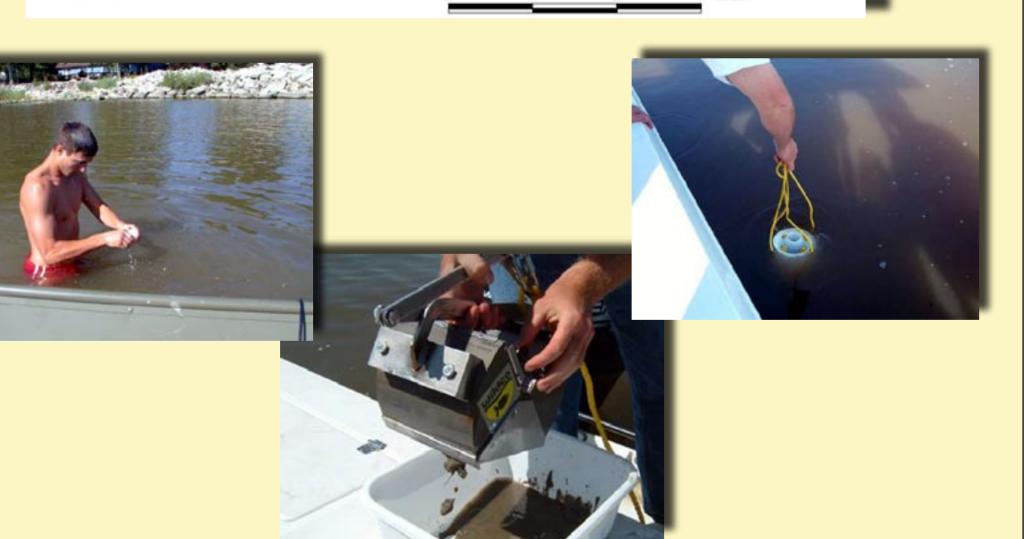
The work is being done in two data-collection phases:

- Phase 1 (2003) General patterns of E. coli concentrations in water and bed sediments collected throughout the Maumee Bay area were determined. • Phase 2 (2004) - Taking the results from phase 1, investigators will concentrate field efforts on a few "hot spots" of
- high *E. coli* concentrations.

Significant findings during phase 1

- Bed-sediment E. coli concentrations, determined by use of the Colilert method (Idexx Laboratories, Westbrook, Maine), ranged from <1 to 1,900 most probable number per gram dry-weight sediment. The highest concentrations were found at sites near or in the shipping channel (O1, O7), in the Maumee River (M1, M2), and at one nearshore site
- Water E. coli concentrations, determined by use of the modified mTEC method, ranged from <1 to 680 colonies per 100 milliliters. The highest concentrations were found in the Maumee River and some nearshore sites.
- Rainfall, wave height, and turbidity were related to E. coli concentrations at Maumee Bay State Park (N10). Water temperature, ultraviolet index, air temperature, and wind speed were not related to E. coli concentrations
- When antecedent wind directions were from the north, E. coli concentrations were generally higher at Maumee Bay State Park.





Predictive Modeling at Beaches

The USGS, in cooperation with Cuyahoga County Board of Health, Ohio Lake Erie Office, Cleveland Metroparks, and Lorain City Health Department, is working to establish an Internet-based nowcasting system for presenting beach advisories based on E. coli concentrations. To accomplish this goal, investigators will • Test the statistical models for Huntington (Bay Village, OH) during 2004 and 2005 and compare each model's performance with the use of the current method for assessing recreational

- Develop statistical models for predicting *E. coli* concentrations at Lakeview Beach (Lorain, OH) for future implementation of an Internet-based nowcasting system. • Implement an Internet-based nowcasting system in 2006 for
- presenting estimated bacterial water quality and posting beach advisories for Huntington.

Modeling at Lake Erie beaches

Building on past work, the USGS, in cooperation with Northeast Ohio Regional Sewer District and the Ohio Water Development Authority is working to identify predictive models that can be used to provide accurate and timely assessments of recreational water quality at Edgewater and Villa Angela (Cleveland, OH) and Lakeshore (Ashtabula, OH). In this 3-year study (2004 to 2006), investigators will:

- Improve the predictive ability of the current direction variable by incorporating more frequent (every 1 hour) • Obtain detailed information on local activity, such as number of swimmers, number of birds, number and
- types of debris, and boat activity at Edgewater to be used as possible explanatory variables in a model. • Obtain rainfall data that are more representative of the watershed affecting water-quality at Villa Angela. • Test the use of artificial neural networks (ANNs) for predicting recreational water quality at Lake Erie
- beaches. ANNs are a class of computational tools that operate analogously to the biological processes of the Collect data for predictive model development at Lakeshore.

Brion, G.M., Neelakantan, T.R., and Lingireddy, S., 2001, Using neural networks to predict peak ryptosporidium concentrations: Journal of American Water Works Association, v. 93, no. 1, p. 99-105.

Ohio District Microbiology Laboratory Capabilities

The U.S. Geological Survey (USGS), Water Resources Discipline (WRD), Ohio District Microbiology Laboratory (ODML) supports several projects within the USGS. The ODML provides water-quality data on three major groups of microorganisms of public health significance in the United States—bacteria, viruses, and protozoans.

- Total coliforms are found in the human intestine, in soils, on vegetation, and in industrial wastes. They are used to assess drinking-water or ground-water quality.
- Fecal coliforms are total coliforms that are able to grow at elevated temperatures. They may be of fecal origin. • Escherichia coli (E. coli), a member of the fecal coliform group, is a
- natural inhabitant of the gastrointestinal tract of warmblooded animals and is direct evidence of fecal contamination. • E. coli is the USEPA-recommended indicator for monitoring fresh

Total coliforms and E. coli

recreational waters.

MI method – membrane filtration method

that allows the simultaneous enumeration

of total coliforms and E. coli. MI media

contains a fluorogen that reacts with an

enzyme (galactosidase) in total coliforms

(glucoronidase) in *E. coli*. Total coliform

E. coli colonies appear blue under ambient

Colilert quantitray method – most-probable

reacts with an enzyme (galactosidase) in total

light and a fluorogen that reacts with an enzyme

(glucuronidase) in E. coli creating fluorescence

under ultraviolet light.

coliforms causing a yellow color under ambient

enumeration of total coliforms and *E. coli*.

Colilert media contains a chromogen that

number method that allows for the simultaneou

and a chromogen that reacts with an enzyme

colonies fluoresce under ultraviolet light, and

Bacterial Indicators

in ground water.

- Fecal streptococci are found in feces; however, some strains are ubiquitous. • Enterococci are a subset of fecal streptococci and always present in the feces of warmblooded animals. They are more persistent in water than total coliforms and may provide a different assessment of the transport of fecal contamination
- Enterococci are the USEPA-recommended indicator for fresh and marine recreational waters.

- Certain bacteria produce an environmentally resistant form called a spore. • Clostridium perfringens is present in human and animal wastes and may
- be used as a surrogate for stress-resistant organisms. It is an indicator of present contamination, as well as a conservative tracer of past fecal

E. coli

Bacterial Methods

Enterococci mEI method

• 48-hour hold time before plating.

ammonium hydroxide vapors.

trained personnel.

42 degrees Celsius.

USEPA approved method for drinking, recreational, and

- shellfish-growing waters. • Requires a 24 hour incubation on one medium at 41
- degrees Celsius.

Clostridium perfringens

• Not field friendly – should be done in the laboratory

• mCP media contains a chromogen which causes

Viral Indicators and Pathogens

• C. perfringens are grown under anaerobic conditions at

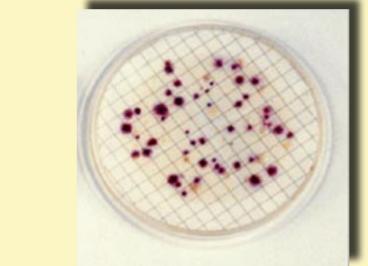
C. perfringens colonies to turn magenta upon exposure to

• A chromogen in the media causes enterococci colonies to have a blue halo for easy identification.



mTEC method – a two-step membrane filtration method that detects the breakdown of lactose and includes a test for the enzyme urease. E. coli colonies remain yellow on this

Modified mTEC method – a onestep membrane filtration method that contains a chromogen for the enzyme glucuronidase. E. coli colonies are



Source Tracking

E. coli subtype matching in septic systems

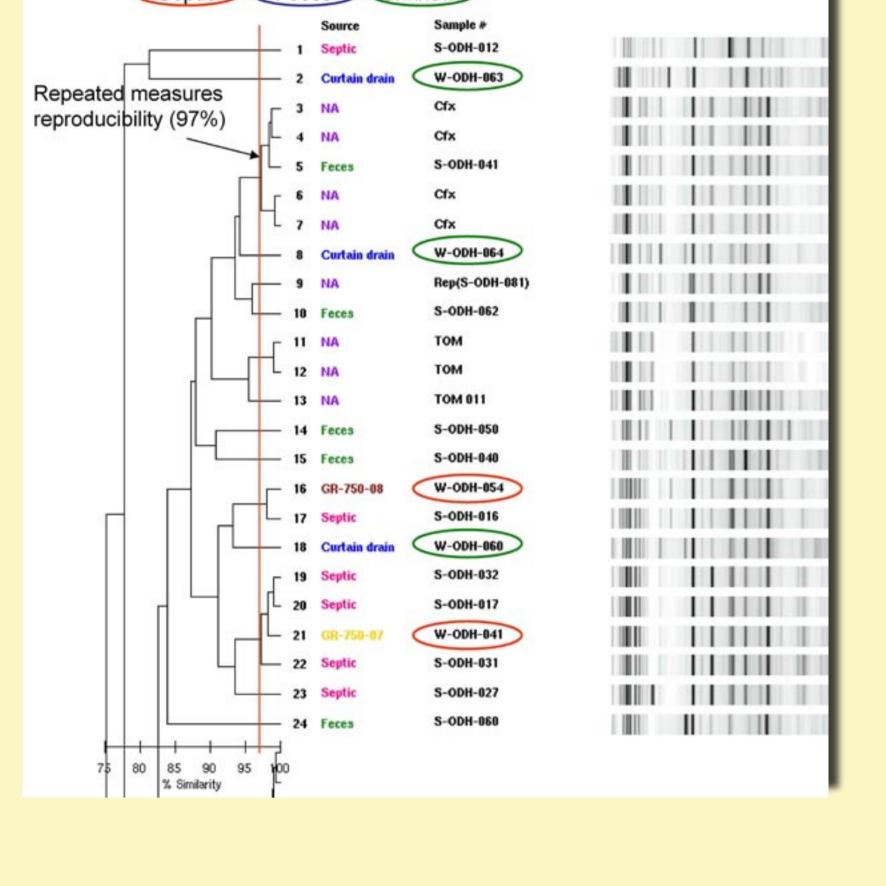
The USGS, in cooperation with the Ohio Department of Health, is testing the treatment effectiveness of septic systems sited on Ohio soils with shallow depth to a limiting layer. This 2-year effort began in spring 2003. Near-surface ground-water samples were analyzed for evidence of transport and incomplete treatment of sanitary waste in 20 sites within 7 counties in Ohio.

- E. coli and coliphage concentrations to indicate potential transport of fecal pathogens to ground water
- Genetic fingerprinting of E. coli (rep-PCR) from the septic system and the ground water to indicate a direct link between the source and the receiving water
- Elevated chloride to indicate presence of wastewater (hydrologic link)
- Elevated nitrate to indicate possible incomplete denitrification
- Nitrogen and oxygen isotope ratios to indicate whether nitrates were of organic or mineral origin

• Wastewater analytes to indicate transport of organic contaminants from the leach field into ground water

Results (collected and assembled but not vet reviewed):

- Of the 12 sites at which water was collected, 4 contained measurable concentrations of E. coli. • Of the 4 sites at which E. coli were collected, tests at 3 sites indicated passage of fecal-origin bacteria from the septic leach field
- to collection wells 4 feet from the leach field. At 1 site, passage from the septic leach field to the curtain drain was indicated. • Fecal-origin bacteria that could be traced to the septic leach field were not found beyond the curtain-drain perimeter (typically
- 10 feet) at 2 sites tested. • These data are provisional, and the sample size is too small to allow statewide interpretations to be drawn.



Source tracking at Ohio beaches

The USGS, in cooperation with Northeast Ohio Regional Sewer District, Ohio Water Development Authority, and Ashtabula Township Park Commission, is testing the use of microbiological source tracking techniques to help identify sources of fecal contamination at Lake Erie beaches. In this 3-year study (2004 to 2006), multiple lines of evidence will be used to identify sources:

• Spatial investigation. One approach is to physically locate potential sources and determine E. coli concentrations along a transient path from each

• F-specific coliphage genotyping. The four antigenically distinct groups of F-specific coliphage can be used to distinguish between human and

- animal sources. Groups II and II predominate in humans, and Groups I and IV predominate in animals (Hsu and others, 1995).
- Enterovirus and hepatitis A virus. The presence of human enteric viruses not only provides direct evidence that human sources contribute to elevated E. coli concentrations (Pina and others, 1998) but also provides information on the health risk for recreational users.
- Antibiotic resistance indexing of E. coli isolates is being tested to distinguish between human and wild-bird sources of fecal contamination. E. coli isolates from the guts of animals not previously exposed to antibiotics are less resistant to antibiotics than those E. coli from human or domestic
- animal sources (Guan and others, 2002). Guan, S., Xu, R., Chen, S., Odumeru, J., and Gyles, C., 2002, Development of a procedure for discriminating among *Escherichia coli* isolates from

animal and human sources: Applied and Environmental Microbiology, v. 68, no. 6, p. 2690-2698. Hsu, Fu-Chih; Shieh, Y.-S. Carol; van Duin, J.; Beekwilder, M.J.; and Sobsey, M.D., 1995, Genotyping male-specific RNA coliphages by

hybridization with oligonucleotide probes: Applied and Environmental Microbiology, v. 61, no. 11, p. 3960-3966.

Pina, S., Puig, M., Lucena, F., Jofre, J., and Girones, R., 1998, Viral pollution in the environment and in shellfish—human adenovirus detection by PCR as an index of human viruses: Applied and Environmental Microbiology, v. 64, no. 9, p. 3376-3382.

Monitoring for Pathogens

Enteric viruses in ground water

From 1999 through 2004, the USGS, in cooperation with the U.S. Environmental Protection Agency-Office of Research and Development, National Exposure Research Laboratory, studied the occurrence and factors related to the presence of enteric viruses and their indicators in aquifers serving small public water supplies.

• For enterovirus by RT-PCR, detections were in the same range as those found by other investigators sampling small and large public water supplies, but not for HAV, where a higher percentage of detection was found in this study. • In only 2 out of 9 wells were samples positive for a virus on more than one date.

• Of 11 samples that were positive for enteric viruses, bacterial indicators co-occurred in only 2 samples, and coliphage were not present in any. • The percentage of virus-positive wells that were also found to be positive by an indicator was 55.6 percent.

• Dissolved organic carbon and chloride concentrations, presence of nitrate and nitrite, and chloride to bromide ratios were related to the presence of one or more microbiological indicators or viruses.

38 0 0.0 169 0 0.0 32 0 0.0 106 0 0.0 34 1 2.9 121 1 0.8

[L, liter; mL, milliliter; P/A, presence/absence; SAL, single agar layer]

Pathogens in surface water

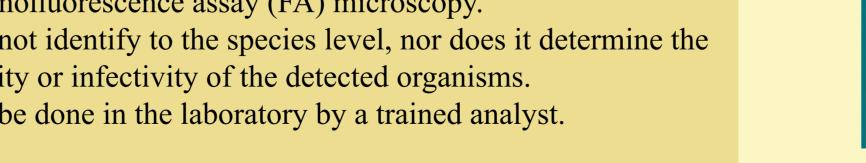
The USGS, in collaboration with the National Park Service, conducted a study from 2000 through 2004 to investigate the co-occurrence of waterborne pathogens and how they relate to concentrations of indicator organisms in the Cuyahoga River within the Cuyahoga Valley National Park. The indicator organisms and pathogens analyzed for in this study were E. coli, Salmonella, coliphage, enterovirus, hepatitis A virus, Clostridium perfringens, Cryptosporidium, and Giardia.

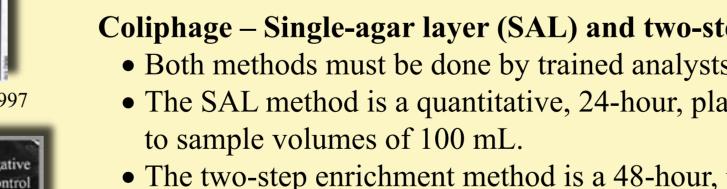
• More virus-positive samples were found at sites served by septic systems than those served by sewer lines.

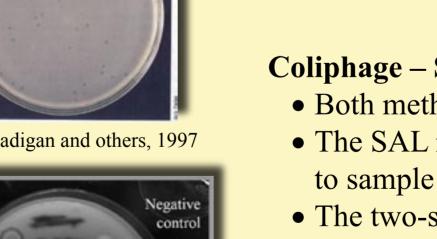
- All microorganisms analyzed for, except *Cryptosporidium*, were detected at least once at each of the four sampling sites. • Concentrations of E. coli exceeded the Ohio primary-contact recreational standard (298 colonies per 100 milliliters) in 87 percent of the river samples.
- Several samples that did not exceed the Ohio secondary-contact recreational standard (576 colonies per 100 milliliters) had detections of pathogens. • River samples in which the secondary-contact recreational standard was exceeded showed a higher percentage of the co-occurrence of pathogens that samples that did not exceed the standard
- Detections of hepatitis A virus were associated with higher median concentrations of coliphage and infectious enteroviruses.
- Geometric mean concentrations of *C. perfringens* tended to be higher in samples where hepatitis A virus was present than in samples in which the virus was

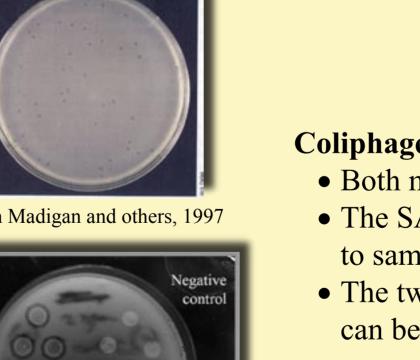
Cryptosporidium and Giardia

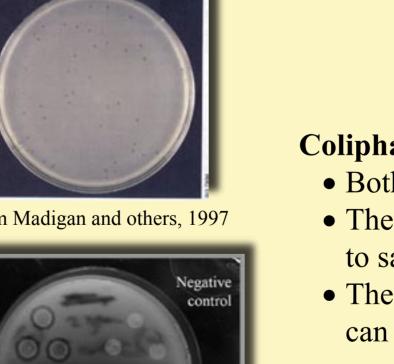
- Principal protozoan pathogens that affect the public health acceptability of waters in the United States.
- Widely distributed in the aquatic environment and have been implicated in several waterborne-disease outbreaks.
- Produce environmentally resistant forms (oocysts and cysts)
- viability or infectivity of the detected organisms.

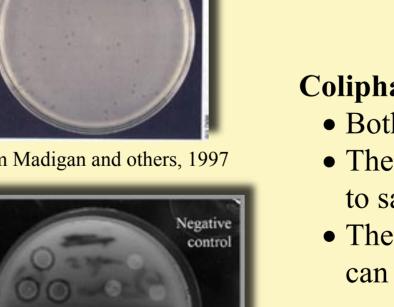


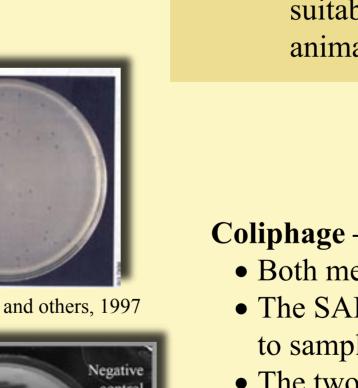


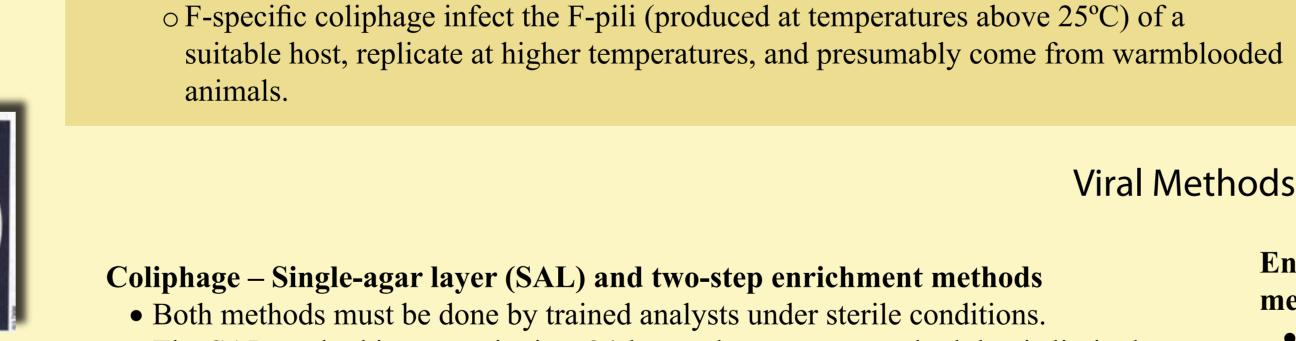




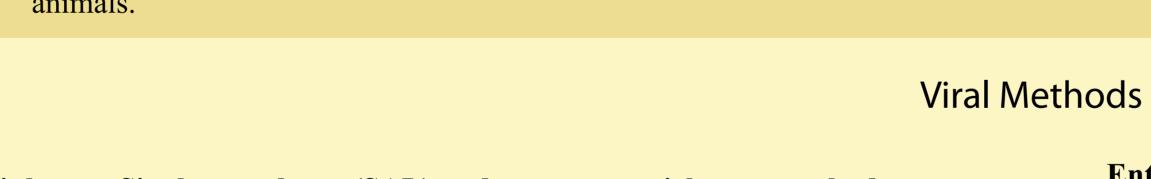








• Two types of coliphage:



• Coliphage are found in high numbers in sewage and are considered to be representative of

o Somatic coliphage infect the outer cell wall and are widely distributed in fecal-

• Coliphage are viruses that infect and replicate in coliform bacteria.

the survival and transport of viruses in the environment.

contaminated and uncontaminated waters.

Coliphage – Single-agar layer (SAL) and two-step enrichment methods • Both methods must be done by trained analysts under sterile conditions. • The SAL method is a quantitative, 24-hour, plaque-assay method that is limited

 Must be done in the laboratory by a trained analyst. • The two-step enrichment method is a 48-hour, presence/absence method that

can be used to analyze sample volumes of either 100 mL or 1 L.

water than bacteria or protozoans.

that allow for their extended survival in water and treated water. • Transmitted through the oral-fecal route. Photo Credit: H.D.A. Lindquist, USEPA

• RT-PCR amplifies and detects the genetic material of specific viruses in water. • This method does not determine the infectious state of the viruses. • Can be used for the detection of enterovirus, hepatitis A virus, rotavirus, reovirus, and Norwalk virus. Samples 100028-100030, 100036, 100037 Protozoan Pathogens

Enteric Viruses – reverse-transcriptase polymerase chain reaction (RT-PCR)

• There are more than 100 different types of human waterborne viruses.

• These human infectious agents are transmitted through the oral-fecal route.

• Viruses are generally more persistent in the environment and are not completely

• Because of their smaller size, viruses have different transport properties in ground

U.S. Environmental Protection Agency (USEPA) Method 1623 • Used to detect *Cryptosporidium* and *Giardia* in water by filtration, immunomagnetic separation (IMS), and immunofluorescence assay (FA) microscopy. • Does not identify to the species level, nor does it determine the • Must be done in the laboratory by a trained analyst.

More information about these microorganisms and references for the methods can be found at the Ohio District Microbiology Laboratory Web site: http://oh.water.usgs.gov/microbiol.html