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Ohio Water Microbiology Laboratory

Overview

The U.S. Geological Survey (USGS) Ohio Water Microbiology Laboratory (OWML) addresses water-related public-health concerns for Ohio and the rest of the Nation. The OWML works with government agencies, academic institutions, and other partners to study the quality of national, state, and local water resources. The OWML is involved in investigations of processes that affect microorganisms in the environment and testing of new methods to improve detection and interpretation of microbiological presence in water.

The OWML is located in the USGS Columbus, Ohio, office and consists of a 1,000-ft^2 main laboratory area and a 300-ft^2 limited-use area. The main area is used for sample login and preparation, media and reagent preparation, membrane filtration, incubation, culture maintenance, and reagent preparation for molecular methods. The limited-use area is the only area in the building where products from molecular methods are manipulated. The limited-use area is kept separate from the main area to prevent cross-contamination of incoming samples. The OWML is equipped to conduct a wide variety of cultivation- and molecular-based microbiology methods, including membrane filtration, direct microscopy, polymerase chain reaction, and hybridization with gene probes.

OWML laboratory technician analyzing a water sample for coliphage using a single-agar layer technique

Mission

The OWML provides microbiological data of public-health significance from surface waters, ground waters, and sediments for a variety of study objectives. The goals of the OWML are to

- provide quality microbiological analytical services to USGS projects for the analysis of environmental samples for bacterial indicators, coliphage, enteric viruses, and protozoa.
- work with other government agencies, academic institutions, and public utilities to develop and (or) test methods for detection or enumeration of microorganisms of public-health significance in the environment.
- provide assistance to other USGS employees for project planning and training related to microorganisms of public-health significance.
- continually develop projects and programs that enhance our understanding of the processes that affect microorganisms in the environment.

OWML Capabilities

The OWML provides water-quality data on three major groups of microorganisms of public-health significance in the United States–bacteria, viruses, and protozoa. Surface water, ground water, and sediments are analyzed for these microorganisms. Pathogenic (disease-causing) organisms are of primary concern in our Nation's waters. Methods to detect these organisms are often costly and time consuming, thus, it is impractical to monitor directly for pathogens by traditional methods. Indicator organisms are used to assess the microbiological quality of water and provide information on the possible presence of pathogens. Indicator organisms are microorganisms that are associated with the intestinal tract of warmblooded animals and are consistently present in fecal waste. They occur in greater numbers than the associated pathogens of interest, and their presence in water indicates fecal contamination of the water. Descriptions of common bacterial and viral indicator organisms are given in the following pages of this fact sheet, as well as the methods used by the OWML to detect them. Also described are viral and protozoan pathogens and the detection methods used by the OWML.

U.S. Department of the Interior
U.S. Geological Survey

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Fact Sheet FS 2005–3072
June 2005

Bacterial Indicators

Coliforms

- Total coliforms are found in animal intestines, 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 and are often, but not always, 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.

Streptococci

- Fecal streptococci are found in feces; however, some species live on plants and in soil.
- Enterococci are a subset of fecal streptococci and are commonly present in the feces of warmblooded animals.
- Enterococci are more persistent in water than coliforms. They provide a different assessment of the transport of fecal contamination in ground water than coliforms because of their different shape and survival rate.

Spore formers

- Certain bacteria produce an environmentally resistant form called a spore.
- Pathogens that produce spore-like structures include the protozoa, *Cryptosporidium* and *Giardia.*
- *Clostridium perfringens* (*C. perfringens*), a spore-forming bacterium, is present in human and animal feces and may be useful as a surrogate for stress-resistant organisms.
- *C. perfringens* is mainly an indicator of contamination from point sources, as well as a conservative tracer of past fecal contamination.

Bacterial Methods

Bacteriological indicators are routinely measured by means of membrane-filtration or most-probable-number techniques.

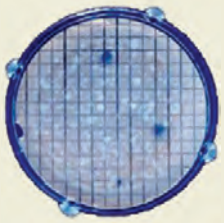
Membrane-filtration technique

Most-probable-number technique - Colilert quantitray

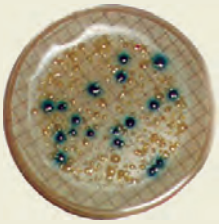
Total coliforms and *E. coli* – MI and Colilert methods

MI method

- Membrane-filtration method – allows simultaneous enumeration of total coliforms and *E. coli*.
- Medium contains a fluorogen that reacts with galactosidase in total coliforms, and a chromogen that reacts with glucoronidase in *E. coli*.
- Total coliforms fluoresce under ultraviolet light, and *E. coli* appear blue under ambient light, following a 24-hour incubation at 35 ºC.



Total coliforms on MI agar



E. coli on MI agar

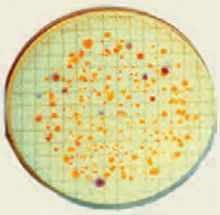
Colilert quantitray method

- Most-probable-number method – allows simultaneous enumeration of total coliforms and *E. coli.*
- Medium contains a chromogen that reacts with galactosidase in total coliforms, and a fluorogen that reacts with glucuronidase in *E. coli.*
- Total coliforms appear yellow under ambient light and *E. coli* fluoresce under ultraviolet light, following a 24-hour incubation at 35 ºC.

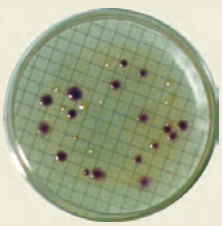
E. coli – mTEC and modified mTEC methods

mTEC method

- Two-step membrane-filtration method – allows detection of lactose fermentation and tests for the enzyme urease.
- *E. coli* colonies appear yellow, following a 2-hour incubation at 35 ºC and a 22-hour incubation at 44.5 ºC.
- Requires transfer of membrane to a urea-substrate medium – *E. coli* colonies are urease negative and will remain yellow.



E. coli on mTEC agar



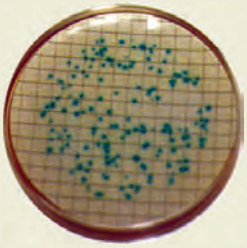
E. coli on modified mTEC agar

Modified mTEC method

- One-step membrane-filtration method – allows detection of *E. coli* without the secondary test for urease activity.
- Medium contains a chromogen that reacts with the enzyme glucuronidase in *E. coli.*
- *E. coli* colonies appear magenta, following a 2-hour incubation at 35 ºC and a 22-hour incubation at 44.5 ºC.

Enterococci – mEI method

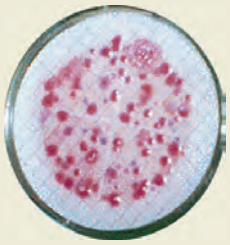
- Membrane-filtration method – allows detection of enterococci on a single medium, an improvement over the mE/EIA method.
- Medium contains a chromagen that reacts with the enzyme glucosidase in enterococci.
- Enterococci colonies have a blue halo, following a 24-hour incubation at 41 ºC; colony color is unimportant.



Enterococci on mEI agar

Clostridium perfringens - mCP method

- Membrane-filtration method – allows detection of the anaerobe *C. perfringens* by use of an oxygen-free chamber.
- Medium contains a chromagen that reacts with the enzyme acid phosphatase in *C. perfringens.*
- *C. perfringens* colonies turn magenta upon exposure to ammonium hydroxide vapors, following a 24-hour incubation under anaerobic conditions at 42 ºC.



Clostridium perfringens on mCP agar

Viral Indicators and Pathogens

Coliphage

- Coliphage are viral indicators that infect and replicate in coliform bacteria, primarily *E. coli*.
- Coliphage are found in high numbers in sewage and may be better than bacterial indicators to represent the survival and transport of viruses in the environment.
- Two main groups of coliphage are detected in separate tests:
  - Somatic coliphage infect the outer cell wall of its host and requires a laboratory strain, like *E. coli* CN-13, for detection.
  - F-specific (also called male-specific) coliphage infect the F-pili (produced at temperatures above 25 ºC) of its host and requires a laboratory strain, like *E. coli* F-amp, for detection.

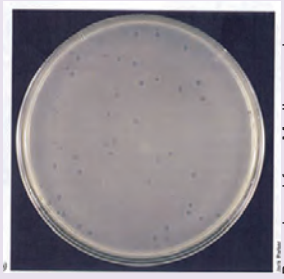
Enteric viruses

- There are more than 100 different types of human pathogenic viruses that may be present in fecal-contaminated waters; however, only a small number can be detected by available methods.
- Viruses are generally more persistant in the environment than bacteria and may not be completely removed by treatment processes.
- Because of their smaller size, viruses are often transported further in ground water than bacteria or protozoa.
- Some of the more common viruses included in monitoring programs are enterovirus, hepatitis A virus, rotavirus, reovirus, and calicivirus.

Viral Methods

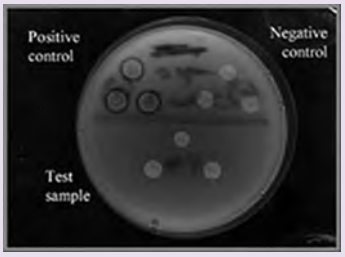
Coliphage – Single-agar layer (SAL) and two-step enrichment methods

- Documented and approved by U.S. Environmental Protection Agency (USEPA).
- Must be done by a trained analyst under sterile conditions.
- SAL method is a quantitative, 24-hour plaque assay method that is limited to sample volumes of 100 mL.
- 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.



Reproduced from Madigan and others, 2000

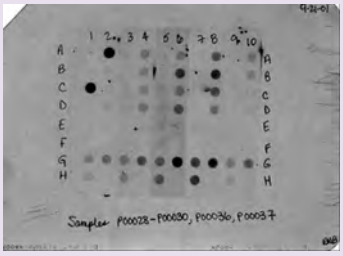
Single-agar layer method



Two-step enrichment method

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

- Must be done in a laboratory designed for molecular work by a trained analyst.
- Amplifies and detects the genetic material of specific viruses from large volumes of water.
- Detects the presence of actual pathogens; however, does not determine the infectious state of the viruses.

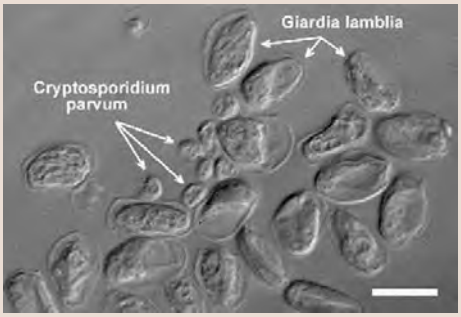


Detection of virus presence after RT-PCR

Protozoan Pathogens

Cryptosporidium and Giardia

- *Cryptosporidium* and *Giardia* are the principal protozoan pathogens that affect the public health acceptability of waters in the United States.
- Both protozoa are widely distributed in the aquatic environment and have been implicated in several waterborne-disease outbreaks.
- Both produce environmentally resistant forms (oocysts and cysts) that allow for their extended survival in water.



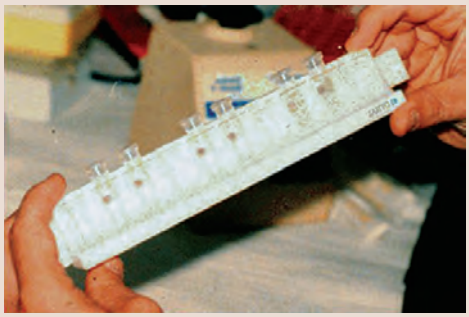
H.D.A. Lindquist, USEPA

Cryptosporidium and *Giardia*

Protozoan Method

Filtration/Immunomagnetic Separation (IMS)/ Immunofluorescence Assay (FA)

- Documented and approved by USEPA to detect *Cryptosporidium* and *Giardia* from large volumes of water by filtration, IMS, and FA microscopy.
- Does not identify to the species level, nor does it determine the viability or infectivity of the detected organisms.
- Must be done in the laboratory by a trained analyst.



Immunomagnetic separation

Quality Assurance/Quality Control

The OWML is committed to providing quality microbiological analytical services to the USGS. The quality assurance/quality control (QA/QC) program is designed to ensure the production of scientifically sound, legally defensible data of known and documented quality. The QA/QC manual identifies and documents practices and standard operating procedures for the activities in the OWML that affect quality of data. The manual is frequently updated as laboratory activities expand and change.

Web Site

Contact information for the OWML staff can be found at the OWML Web site:
http://oh.water.usgs.gov/microbiol.html

Other information that can be found on the OWML Web site includes

- information about microorganisms of public-health significance and the methods used to detect them
- field sampling methods and hold times
- current projects and publications
- guidance for microbiology within the National Water-Quality Assessment (NAWQA) program
- QA/QC manual
- price list for internal analytical services and instructions for sample submission
- references for methods described in this fact sheet

Data Management

A laboratory information management system (LIMS) is used by the OWML to store sample login information, sample results, and associated quality-control results. This information is backed up daily, and a copy of the information is stored at an offsite location. The LIMS is used to store QA/QC records: maintenance and calibration of laboratory equipment, maintenance of microbiological stock cultures and controls, and laboratory method QA/QC results.

The LIMS has been customized to produce reports of results that can be easily uploaded into the USGS National Water Information System (NWIS). Once the results have been loaded into NWIS, the information can be retrieved through NWIS Web: *http://water.usgs.gov/nwis.* Currently, water-quality data are updated annually on NWIS Web.

Additional Information

For additional information about the USGS and its programs, contact:

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