Rapid Method For Determination Of Fecal-Indicator Bacteria Concentrations In Recreational Water

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Abstract

Standard plating methods to determine fecal-indicator bacteria concentrations take 24 hours to obtain results—too long to adequately assess water quality and take control measures. In a 3-year study (2004–2006), the U.S. Geological Survey, in collaboration with the National Park Service, is investigating the use of a rapid method (< 1 hour) for the determination of fecal-indicator bacteria concentrations in the Cuyahoga River within the Cuyahoga Valley National Park. The overall goal of this study is to determine whether the rapid method yields accurate enough results that it can be used by park managers in place of standard methods.

The immunomagnetic separation/adenosine triphosphate (IMS/ATP) rapid method requires less than 1 hour from sample collection to availability of results. In the original method, developed by researchers at the University of Michigan, bacteria are first concentrated from the water sample by serial filtration. Magnetic beads that are coated with antibodies for either *Escherichia coli* (*E. coli*) or enterococci are added to the concentrate. This mixture is then subjected to IMS, in which the bacteria-antibody bead complex is separated from extraneous materials in the sample by use of a magnet. The bacterial cells are then ruptured and ATP is released and measured with a microluminometer. Results are reported in relative light units (RLUs).

Daily samples, collected at three sites along the Cuyahoga River from May through August 2004, were analyzed by use of the rapid and standard methods for *E. coli* and enterococci. Standard plating methods included the use of modified mTEC media for the enumeration of *E. coli* and mEI media for enterococci. Throughout the sampling period, modifications were made to the original rapid method, including elimination of the filtration step and the addition of sonication of the water sample prior to IMS. The elimination of the filtration step increased the method's performance and strengthened the correlations to the standard methods. RLUs were significantly correlated to concentrations found by use of standard methods for *E. coli* (r=0.704) and enterococci (r=0.759). Further data analysis is needed before determining whether the addition of sonication significantly increases the correlations. Sampling is done bimonthly from October 2004–May 2005 and will be done daily during the recreational season of 2005 (June–September).

Introduction

The water quality of the Cuyahoga River within the Cuyahoga Valley National Park (CVNP) is a primary concern to park managers and to visitors of the park, who use the river for canoeing, swimming, or wading. With over 3 million visitors per year, the CVNP is a major destination and recreational resource. The 23-mile reach of the Cuyahoga River within the park receives discharges of stormwater, combined-sewer overflows, and incompletely disinfected wastewater from urban areas, resulting in frequent exceedences of Ohio bacteria standards for recreational water. These discharges result in a threat to the health of visitors who come into contact with the water during recreational use.

Park managers would like to promote the use of the river when it is of acceptable quality. Standard plating methods to monitor the concentrations of fecal-indicator bacteria take 24 hours to obtain results, too long to adequately assess water quality. The need for a more rapid method to determine the current day's concentrations of fecal-indicator bacteria is widely recognized and would be greatly beneficial to park managers and recreational users.

A 3-year study (2004-2006) is being done by the U.S. Geological Survey (USGS), in collaboration with the National Park Service. The study is investigating the use of a rapid method (< 1 hour) to determine concentrations of the fecal-indicator bacteria, *Escherichia coli* (*E. coli*) and enterococci, in the Cuyahoga River within the CVNP. Results from the rapid method will be compared to results obtained using standard plating methods to determine the rapid method's accuracy and the possibility of its use in place of the standard method.

Photo Credit: National Park Service

Approach

The 3-year study has been divided into two phases:

Phase 1 (2004-2005):

- Collaborate with University of Michigan to optimize the rapid method for the study area.
- Collect daily samples during the 2004 recreational season (May-September) at three sites (Old Portage, Botzum, and Jaite) – analyze each sample for *E. coli* and enterococci using the rapid and standard methods.
- Analyze initial data to determine how well the rapid method is performing in comparison to the standard method.

Phase 2 (2005-2006)

- Collect samples twice a month during the non-recreational season (October–April) at three sites (Old Portage, Botzum, and Jaite).
- Collect daily samples during 2005 recreational season (May-September) at four sites (Old Portage, Botzum, Jaite, and Independence) and possibly one or two tributaries.
- Analyze final data and write report.

Photo Credit: National Park Service

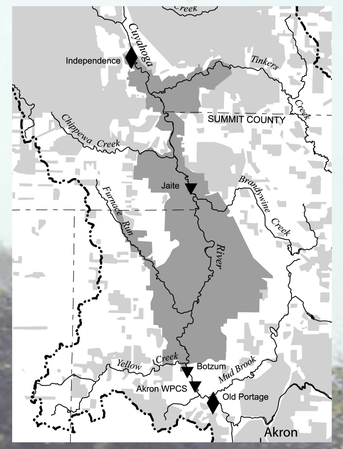


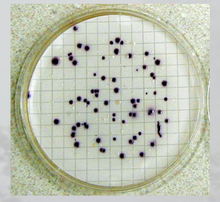
Figure 1. Location of the study area in northeastern Ohio.

Methods

Standard methods

E. coli concentrations are determined by use of the modified mTEC method (U.S. Environmental Protection Agency, 2002). Briefly, water samples are filtered through 0.45-µm membrane filters and placed on modified mTEC agar plates. The plates are incubated for 2 hours at 35°C and then 22 hours at 44.5°C. After incubation, colonies that are magenta in color are counted as *E. coli*. Results are reported as colonies per 100 milliliters (col/100 mL).

Enterococci concentrations are determined by use of the mEI method (U.S. Environmental Protection Agency, 1997). Water samples are filtered through 0.45-µm membrane filters and placed on mEI agar plates. The plates are incubated for 24 hours at 41°C. After incubation, colonies that have a blue halo are counted as enterococci. Results are reported as col/100 mL.



***E. coli* on modified mTEC agar**

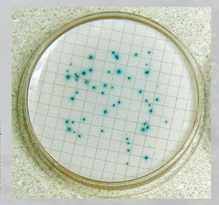


Photo Credit: Susanna Dziechciuk

Enterococci on mEI agar

IMS/ATP rapid method

The IMS/ATP rapid method was developed by researchers at the University of Michigan (Lee and Deininger, 2004). This method requires less than 1 hour from sample collection to availability of results. In the original method, bacteria are concentrated from the water sample by double filtration—a 20-µm prefilter followed by a 0.45-µm membrane filter. The 20-µm prefilter is used to remove large particles and sediment from the sample. The 0.45-µm filter is used to concentrate bacteria onto the filter. Bacteria are then removed from the 0.45-µm filter by washing with phosphate buffered saline (PBS) with Tween 20. Magnetic beads that are coated with antibodies for either *E. coli* or enterococci are added to the concentrate. This mixture is then subjected to IMS, in which the bacteria-antibody bead complex is separated from extraneous materials in the sample by use of a magnet. The bacterial cells are ruptured and ATP is released. An enzyme/substrate solution (luciferin-luciferase) is added and reacts to the ATP to produce light, which is measured with a microluminometer. Results are reported in relative light units (RLUs). Based on the amount of water filtered, results are converted to RLU/100 mL.

Photo Credit: Susanna Dziechciuk

Photo Credit: New Horizons Diagnostics Corporation

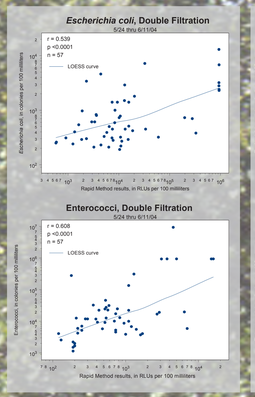
Results

Results from samples analyzed using the original rapid method provided by the University of Michigan were significantly correlated to results found by use of standard methods; however, the relations were influenced by a few high values and there were several outliers (figure 2). We suspected that sometimes the bacteria were trapped on the prefilter during filtration. To test this theory, the 20-µm prefilter and the 0.45-µm filter were placed on modified mTEC and mEI agar plates and incubated at the specified times and temperatures. Results showed that the bacteria were within a countable range on the 0.45-µm filter but were too numerous to count on the 20-µm prefilter. This indicates that the bacteria were being trapped on the prefilter and were not being analyzed during subsequent steps (data not shown).

The prefiltration step was eliminated from the method on June 12, 2004, and subsequent analyses have been done with single filtration through a 0.45-um filter. The results from these analyses showed a weaker correlation than results from the double filtration, especially for enterococci (figure 3).

From July 22 through August 29, 2004, experiments were run to compare the results of the single filtration method to a direct method (no filtration). In the direct method, a small volume of sample (10-30 mL) was analyzed starting with the step that involved the addition of the antibody-coated magnetic beads. Results from these analyses showed stronger correlations to the standard methods than using the double or single filtration methods (figure 4).

Because the river samples are high in sediment and bacteria are known to attach to sediment particles, experiments were run to determine if sonication of the samples led to more consistent results and higher correlations. In this modification of the method, samples were not filtered. Instead, small volumes of the sample (10-30 mL) were subjected to sonication using a sonic dismembrator (Fisher Scientific, Pittsburgh, Pa.) for 30 seconds at the highest setting prior to the addition of the beads. Results from these analyses were similar to those for the direct method without sonication (figure 5).



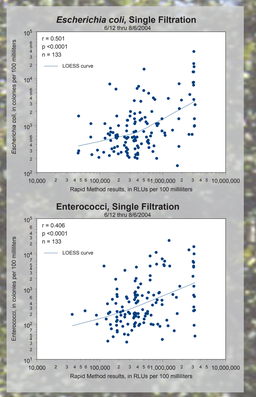


Figure 2. Relation between results from the double filtration rapid method and the standard method for *Escherichia coli* and enterococci. [r = Pearson's correlation coefficient; correlation is significant when p value is less than 0.05; n = number of samples]

Figure 3. Relation between results from the single filtration rapid method and the standard method for *Escherichia coli* and enterococci. [r = Pearson's correlation coefficient; correlation is significant when p value is less than 0.05; n = number of samples]

Conclusions

Based on the results from Phase 1 of the study, results from the rapid method were significantly correlated to standard methods for both *E. coli* and enterococci. In the original method, a double filtration was used. The prefiltration step was eliminated from the original rapid method because it was determined that significant amounts of bacteria were lost during this step. The single filtration step through a 0.45-µm filter was also eliminated because it showed weaker correlations to the standard method than the double filtration method. Using the direct method (no filtration), the correlations between the rapid and standard methods were much stronger than with the double or single filtration methods. Analytical time using the direct method is even less than the double and single filtration methods. Sonication of the samples prior to IMS did not seem to improve correlations over the direct method without sonication; therefore, it is not being used in subsequent analyses. Samples will be analyzed during the nonrecreational season (October 2004–April 2005) bimonthly, using the direct rapid method. Intensive daily sampling will resume in May 2005 through September 2005 at the same three sites plus two or three additional sites. All samples will also be analyzed using the standard method. In 2005, we will determined whether the relations between the rapid method and standard method are the same at all sites.

Acknowledgments

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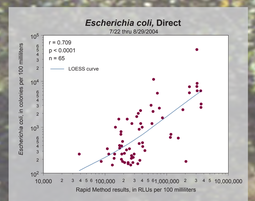

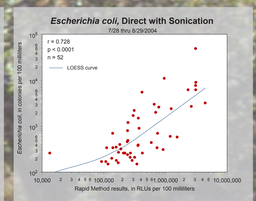

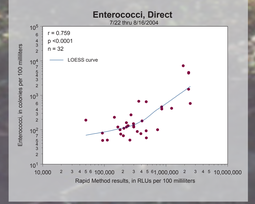

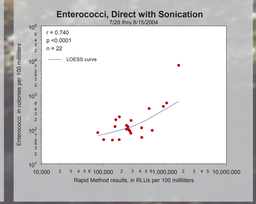

Figure 4. Relation between results from the direct rapid method and the standard method for *Escherichia coli* and enterococci. [r = Pearson's correlation coefficient; correlation is significant when p value is less than 0.05; n = number of samples]

Figure 5. Relation between results from the direct rapid method with sonication and the standard method for *Escherichia coli* and enterococci. [r = Pearson's correlation coefficient; correlation is significant when p value is less than 0.05; n = number of samples]