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Office of Water Quality Water-Quality Information Note 2003.01

Subject: **Microbiology**—results of studies to compare analytical methods for indicator bacteria and coliphage sample analysis in ground water and surface water and recommendations for USGS-WRD programs and projects

The USGS Ohio District Microbiology Laboratory (ODML), in collaboration with the USGS National Water Quality Assessment Program (NAWQA), tested and compared analytical methods for indicator bacteria and coliphage. The purpose of this note is to describe results of the analytical method studies and how they were used to establish the standard procedures for microbiological monitoring in the USGS-WRD. Standard operating procedures for microbial sampling and analysis can be accessed at http://oh.water.usgs.gov/microbiol.html and in the updated version of the USGS National Field Manual for the Collection of Water-Quality Data (Chapters 7.1 and 7.2; in preparation) [http://water.usgs.gov/owq/FieldManual/Chapter7/index.html]. Specific guidance for the NAWQA Program's microbiological sampling can be accessed at http://oh.water.usgs.gov/micro/nawqa.html.

The objectives of the analytical method studies were to:

- test modified mTEC media, used for *Escherichia coli* (*E. coli*) determination, prepared by commercial suppliers and compare the quality and cost for use in surface-water studies,
- determine if the MI or mENDO/NA-MUG analytical method is most appropriate for use in enumerating *E. coli* and Total Coliform in ground-water studies, and
- define the holding times for processing of coliphage samples.

Tests, Test Results, and Recommendations

Surface-water studies.

Before initiating the analytical method studies, it was decided that the modified mTEC method (U.S. Environmental Protection Agency, 2000a) would be used to monitor NAWQA Program surface-water sites for concentrations of *E. coli*. The modified mTEC method is recommended by the U.S. EPA for use in monitoring fresh, estuarine, and marine surface waters. It was specifically developed to be used as a measure of recreational water quality. Although modified mTEC is more expensive than mTEC (U.S. Environmental Protection Agency, 1985), it offers several advantages over mTEC that outweigh the increased cost. Modified mTEC is a one-step procedure whereas mTEC is a two-step procedure. In addition, target colonies are easier to discern on modified mTEC than on mTEC.

The ODML conducted a study to test the quality and determine cost of commercial sources of modified mTEC. USGS personnel collected 40 samples from a variety of surface-water sites across the country. The samples were sent to the ODML for plating and method evaluation on three sources of modified mTEC:

1. Pre-poured plates that are not refrigerated during shipping and produced by Hach Company (Hach) (Loveland, CO)
2. Pre-poured plates that are not refrigerated during shipping and were from an experimental batch produced by Becton Dickinson (BD) (Cockeysville, MD)
3. Recently poured plates made from hydrated, refrigerated agar prepared by the ODML

RESULTS: Fewer *E. coli* were recovered using ODML medium than Hach or BD. This may be because the Hach and BD media have five times as much chromagen as the ODML medium. Among the three sources of modified mTEC, the highest recoveries were found using BD medium; however, the false-

positive rate was highest with the BD medium. Regardless, none of the differences between sources of media were greater than the analytical variability.

The cost (Sept. 2002) for 100 mL of medium or 20 plates, is as follows:
ODML—$64.00 plus 1/2 hour of preparation time in the study unit (hydrated medium)
Hach—$60.00 and no preparation time (hydrated medium as prepared plates)
BD—$30.00 plus 1-1/2 hours of preparation time in the study unit. This is for dehydrated medium that has not been tested by the USGS. BD has delayed production of the hydrated medium.

RECOMMENDATION: The use of dehydrated agar is usually preferred over prepared plates because the user has control over when the plates are poured and thus can more easily follow recommended holding times. When the BD dehydrated medium becomes available, the ODML will conduct a small study comparing recoveries and verification rates between the BD and the Hach medium. Until that is done, projects and programs that are monitoring for *E. coli* in surface water should use the Hach medium because the cost is lower than for the ODML medium.

***Ground-water studies*.**

Before initiating the analytical method studies, it was decided that both the MI and the mENDO/NA-MUG membrane-filtration methods were suitable for testing ground-water samples for total coliforms and *E. coli*. Both methods have been validated for use with drinking water. These methods are especially suitable for enumerating bacteria in ground water because in ground-water studies, it is often desirable to enumerate both total coliforms and *E. coli*. Neither method is recommended for use with raw surface waters because non-target background growth may make plates difficult to read. The MI method (U.S. Environmental Protection Agency, 2000b) is a one-step method that takes 22-24 hours; the mENDO/NA-MUG method (U.S. Environmental Protection Agency, 1991) is a two-step method that requires a secondary 4-hour incubation in addition to the 22-24 hours primary incubation. MI medium requires the addition of an unstable antibiotic to the tempered agar before pouring the plates. The mENDO and NA-MUG media do not require the addition of any unstable components; however, two types of media need to be prepared.

The ODML compared method performance and ease-of-operation for MI and the mENDO/NA-MUG membrane-filtration methods:

- Personnel from two NAWQA study units analyzed ground-water samples using both methods and reported results.
- Students attending a two-day NAWQA training class on microbiological sampling and analysis methods further evaluated the two methods.

RESULTS: The comparison tests indicated that the MI method was the preferred method. The most important consideration to many participants was the convenience of the MI method, avoiding the 4-hour secondary incubation for the mENDO/NA-MUG method. Some individuals reported that it was hard to discern total-coliform positive colonies under ultraviolet light, as required by the MI method; however, others reported that it was also difficult to discern *E. coli* colonies under ultraviolet light, as required by the mENDO/NA-MUG method. *E. coli* is the most important bacterial indicator of fecal contamination and is very easy to identify and count on MI agar.

The MI medium is available in the agar format from a commercial supplier (S&S Microscience, West Palm Beach, FL) or from the ODML.

RECOMMENDATION: The commercial source of MI agar should be used as the standard medium. If the commercial supplier is unable to provide quality media in a timely manner, the ODML is an alternative supplier. The buffer required for the method, "sterile buffered water, $PO_4/MgCl_2$," is produced by the USGS Water Quality and Research Laboratory (OWQRL) in Ocala, Florida in 250 mL aliquots (Q453BACT) [http://owqrl.er.usgs.gov/owqrlcat3.asp]. This buffer may also be used with the mTEC and modified mTEC methods for *E. coli* and with the mEI and mE/EIA methods for enterococci (U.S. Environmental Protection Agency, 2000a).

***Coliphage holding-time study*.**

USEPA Method 1601, coliphage by the two-step enrichment procedure, is a presence/absence method and includes a 48-hour holding time for samples before analysis (U.S. Environmental Protection Agency, 2001a), which is a recommendation that is largely anecdotal. Because samples for coliphage will need to be transported to a remote microbiology laboratory for analysis, information on the stability of coliphage in samples of ground water is needed.

The ODML performed holding-time experiments by seeding ground-water samples with coliphage (isolated from sewage filtrate) and monitoring recoveries for 7 days. Experiments were done using *E. coli* CN-13 (somatic coliphage) and *E. coli* F-amp (F-specific coliphage) hosts and the single-agar-layer method; this method enumerates coliphage in water (U.S. Environmental Protection Agency, 2001b).

RESULTS: For F-specific coliphage, however, there was an initial drop in plaque counts after 24 hours. This drop may have been attributed to coliphage adapting to a new environment and may not apply to coliphage that are already present in ground water. After 24 hours, F-specific coliphage counts were stable from 48 to 96 hours and dropped slightly after 96 hours. For somatic coliphage, counts dropped slightly after 48 hours and remained stable for up to 7 days.

RECOMMENDATION: More work is needed to test a wide variety of ground-water samples. It would be ideal to test ground-water samples that are positive for coliphage (instead of spiking with sewage filtrate). Experiments can also be done using Method 1601, and determining for how long coliphage are detected using the presence/absence method. In the interim, we recommend the USGS retain the 48-hour holding time. If a sample exceeds the 48-hour holding time and the time expired is not more than 96 hours, the ODML laboratory will report results with a result qualifier.

References

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