OWQ WaQI Note

December 8, 2003

Office of Water Quality Water-Quality Information Note 2004.05

Subject: Microbiology—provides information on commercial sources of modified mTEC and MI media for use in NAWQA and other USGS programs.

The NAWQA Program began monitoring for microorganisms of public health significance in October 2002. For enumeration of *Escherichia coli* (*E. coli*) in surface water, NAWQA study units are using pre-poured modified mTEC plates produced by Hach Company (Loveland, CO). For enumeration of total coliforms and *E. coli* in ground water, NAWQA study units are using hydrated MI media produced by S&S Microscience (S&S) (West Palm Beach, FL).

The Becton-Dickinson (BD) Company (Cockeysville, MD) recently began production of dehydrated modified mTEC and MI media. The Ohio District compared the use, performance, and cost of the newly available BD dehydrated media with the Hach and S&S media currently used in the NAWQA program. The major advantage of using dehydrated media is that the user has control over when the media is prepared and plates are pored and can thus more easily follow the recommended holding times. The S&S hydrated MI media has cefsulodin, an unstable antibiotic, added to inhibit background growth prior to shipment from the manufacturer. There is some concern that the cefsulodin will break down during shipping or reheating the S&S media. With the preparation of dehydrated BD media, the cefsulodin is added fresh so that the breakdown of this compound is not a problem.

Tests and test results

Thirty-one surface-water samples and one sample of septic-tank effluent were collected by USGS employees. Samples were sent to the Ohio District Microbiology Laboratory (ODML) where they were analyzed for *E. coli* using the modified mTEC method (U.S. Environmental Protection Agency, 2002a) and for total coliforms and *E. coli* using the MI method (U.S. Environmental Protection Agency, 2002b). Each sample was evaluated on two sources of modified mTEC media (Hach and BD) and two sources of MI media (S&S and BD). Concentrations of *E. coli* ranged from 3 to 23,000 colonies per 100 milliliters (col/100 mL) and total coliforms ranged from 1,000 to 880,000 col/100 mL.

Differences in recoveries. Three different comparisons in recoveries were used to evaluate the substitution of dehydrated media (made by BD) for the currently used media (made by Hach or S&S): For *E. coli,* (1) modified mTEC media, Hach to BD and (2) MI media, S&S to BD. For total coliforms, (3) MI media, S&S to BD. The results of Wilcoxon signed-rank tests showed that the differences in recoveries for the three comparisons were all statistically significant at □=0.05. That leads to the following conclusions:

- For *E. coli*, BD modified mTEC recovered more bacteria than the Hach
- For *E. coli*, S&S MI media recovered more bacteria than the BD

- For total coliforms, S&S MI media recovered more bacteria than BD

Comparison to analytical variability. Six split replicate samples were analyzed to determine the analytical variability. To obtain a measurement of the analytical variability, the absolute-value differences between split samples were calculated. If the differences in recoveries between media sources were greater than the analytical variability, then these differences are expected to be true differences. If, on the other hand, the differences in recoveries between media sources were not greater than the analytical variability, then the differences may be due to analytical variability and not true differences between the sources of media.

In all three comparisons, the differences between sources of media were not significantly greater than the analytical variability as determined by the Wilcoxon rank-sum test. For total coliforms on S&S and BD media, although the difference was not significant, the p-value (p=0.17) was smaller than that found for the other two comparisons (p=0.41 and 0.63).

Verifications. A subset of colonies that were identified as *E*. coli was verified using biochemical tests to determine whether verification percentages were the same among sources of media. In these tests, colonies were verified as *E. coli* if they were EC gas-positive, indole positive, oxidase negative, and do not utilize citrate (U.S. Environmental Protection Agency, 2002a).

Verification percentages for the two sources of modified mTEC were the same. Of the 30 colonies isolated from each source of modified mTEC, 29 from BD and 29 from Hach were verified positive as *E. coli*; this was a verification percentage of 97 percent for both media.

Of the 30 colonies isolated from each source of MI, 14 from BD and 14 from S&S were verified positive as *E. coli* (verification percentages were 47 percent). This indicates a potential false-positive rate of 53 percent, which was considerably higher than the documented false positive rate of 4.3 percent for MI media (Brenner and others, 1993). The reason for the low verification percentage for MI was not identified in this study. Most colonies that failed the verifications tests on MI, however, did so because they were citrate positive; this test has been reported to be sensitive to false positives (Kristen Brenner, U.S. Environmental Protection Agency, oral commun., 2003).

Media performance and ease of use. For both sources of modified mTEC media, target colonies were easy to discern from non-target colonies. Preparing plates from dehydrated BD modified mTEC media was fairly quick and easy; a large batch could be prepared and autoclaved within an hour and stored in the refrigerator in dilution bottles for up to 6 months.

Although preparation procedures were not difficult for both sources of MI media, differences in quality were noted. On BD plates, fluorescent blue-white colonies (positive result) were easy to discern and identify as total coliforms. However, several technicians noted that the blue-white fluorescence was weak on some S&S plates. In addition, on several occasions on the S&S plates, green fluorescent colonies (negative result) were overgrown and their growth interfered with identifying and counting the target blue-white fluorescent colonies. The growth of green fluorescent colonies may have also caused an increase

in false positives for total coliforms (total coliforms were not verified in this study) and may explain why S&S media recovered more total coliforms than BD did.

Media cost. An analysis was done to compare costs of media from the various sources. The time to prepare plates and the cost of plates were considered in computing the cost for BD modified mTEC and for S&S and BD MI. The cost per plate is as follows:

BD modified mTEC—$1.38

Hach modified mTEC—$3.00

BD MI—$1.15

S&S MI—$1.91

Implications

For modified mTEC, the BD media recovered more *E. coli* than Hach media in pre-pored plates, the differences in recoveries between BD and Hach were not significantly different from the analytical variability, and verification percentages were the same. The BD media was easily prepared and costs less than the Hach. Therefore, the BD dehydrated media will be substituted for Hach pre-poured plates in the NAWQA program and is recommended for other USGS programs.

For MI, the S&S media recovered more total coliforms and *E. coli* than the BD; however, the differences in recoveries were not significantly greater than the analytical variability and the recovery of more total coliforms may have been due to more false positives on the S&S plates. Most importantly, because problems were sometimes encountered in discerning target colonies on the S&S media, the BD dehydrated media will be substituted for S&S media in the NAWQA program and is recommended for other USGS programs.

Because of uncertainty about staffing at the Ocala Water-Quality and Research Lab (OWQRL), they will not be supplying kits for these methods at this time. Instructions for making the media from kits and a list of recommended sources of supplies are attached and can be modified for field use.

References

Brenner, K.P., Rankin, C.C., Roybal, Y.R., Stelma, Jr., G.N., Scarpino, P.V., and Dufour, A.P., 1993, New medium for the simultaneous detection of total coliforms and *Escherichia coli* in water: Applied and Environmental Microbiology, v. 59, p. 3534-3544.

Myers, D.N. and Sylvester, M.D., 2003, National field manual for the collection of water-quality data – Biological indicators: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A7, 44 p.

U.S. Environmental Protection Agency, 2002a, Method 1603—*Escherichia coli* in water by membrane filtration using modified membrane-thermotolerant *Escherichia coli* agar: Washington, D.C., EPA 821-R-02-23, 9 p.

U.S. Environmental Protection Agency, 2002b, Method 1604—Total coliforms and *Escherichia coli* in water by membrane filtration using a simultaneous detection technique (MI medium): Washington D.C., EPA 821-R-02-024, 14 p.

WaQI Notes are archived on the Office of Water Quality web site,
http://water.usgs.gov/usgs/owq/WaQI/index.html

Attachment

U.S. EPA Method 1603 (821-R-02-023)
Detection of *E. coli* in water by membrane filtration using modified mTEC media
http://www.epa.gov/nerlcwww/1603sp02.pdf

Kit:
- 4.56g dehydrated modified mTEC agar
- 100mL reagent-grade deionized water

Directions:
Combine agar and water in a 250mL flask with a stir bar. Heat and stir until dissolved completely. Autoclave (covered) for 15 minutes. Temper media before pouring plates. (You can easily hold the flask in your bare hand.)

U.S. EPA Method 1604 (821-R-02-024)
Detection of total coliforms and *E. coli* in water by membrane filtration using MI media
http://www.epa.gov/nerlcwww/1604sp02.pdf

Kit:
- 3.65g dehydrated MI agar
- 100mL reagent-grade deionized water
- 2mg cefsulodin (measured by OCALA into a test tube or vial)
- one syringe filter, sterile - 0.22µm pore size, 33mm dia.
- one 3cc syringe, sterile
- one sterile, test tube or vial
- 2mL of reagent-grade water

Directions:
Combine agar and water in a 250mL flask with a stir bar. Heat and stir until dissolved completely. Autoclave (covered) for 15 minutes.
Prepare the antibiotic (cefsulodin solution) as follows:
1. Add the 2mL of reagent-grade water to the test tube or vial containing the cefsulodin.
2. Filter sterilize the cefsulodin solution using the filter and syringe into the sterile test tube or vial.
3. If not used immediately, refrigerate until use. The cefsulodin solution is only good for four hours after preparation.
4. Temper media. (You can easily hold the flask in your bare hand. Note: if the media is too warm, the cefsulodin will break down, making the media unusable.)
5. Add 0.5mL of the sterile cefsulodin solution to the tempered media. Mix carefully, trying not to create bubbles, and pour plates.

	Qty.	Supplier[a]	Product/ Model Number	Description
BD modified mTEC dehydrated agar	bottle (100g)	Government Scientific	214884	media for determination/enumeration of *E. coli*
BD dehydrated MI agar	bottle (100g)	Government Scientific	214882	media for determination/enumeration of *E. coli* and total coliforms
Millex Syringe Filters, sterile - 0.22µm pore size, 33mm dia.	box (50)	Fisher	SLGP033RS	used to sterilize cefsulodin solution before addition to MI agar
3cc Syringe, sterile	pack (100)	Fisher	SCH1144	used to sterilize cefsulodin solution before addition to MI agar
Cefsulodin (250mg)	bottle	Sigma	C8145	antibiotic used in MI agar

[a]Fisher 800/766-7000 [http://www.fisherscientific.com/]
Government Scientific Source, Inc. 800/248-8030 [http://www.govsci.com/]
Sigma-Aldrich 800/325-3010
[http://www.sigmaaldrich.com/Area_of_Interest/The_Americas/United_States.html]