November 3, 2004

Office of Water Quality Water-Quality Information Note 2005.03

Subject: Field Methods—Buffered water for microbiology work

Purpose: The purpose of this Note is to announce that phosphate with magnesium chloride (PO_4-$MgCl_2$) buffered water is to be used with the following microbiology media: mTEC (*E. coli*), modified mTEC (*E. coli*), mEI (enterococci), MI (total coliform; *E. coli*), mFC (fecal coliform), KF (fecal streptococci), and mENDO (total coliform) and that the use of peptone buffer for these media is to be discontinued. The PO_4-$MgCl_2$ buffer may be purchased directly from Hardy Diagnostics in 99-mL (Catalog # D699; currently priced at $0.41 per bottle) and (or) 500-mL (Catalog # U193; currently priced at $3.89 per bottle) volumes. These prices are significantly less that what the NWQL must charge for buffer water made in-house (currently $4.48 per 99-mL bottle and $8.72 for 500 mL). The shelf life for the Hardy Diagnostics buffer is 330 days from the date of manufacture and they perform quality control for sterility, pH, and fill volume on each batch of bottles. For these reasons, the Office of Water Quality has decided that it would be best for Districts to order the buffer directly from Hardy Diagnostics rather than having it stocked by the National Field Supply Service at the NWQL.

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Background: It is current USGS practice to use phosphate buffer with magnesium chloride (PO_4-$MgCl_2$) with the following media: mTEC, modified mTEC, mEI, and MI (U.S. Environmental Protection Agency, 2000 and 2002); and to use peptone buffer with mFC, KF, and mENDO media (Britton and Greeson, 1987). According to the U.S. Environmental Protection Agency (USEPA) (1978), either PO_4-$MgCl_2$ or peptone buffer was acceptable for use with mFC, KF, and mENDO media. The purpose of both types of buffer is to increase the recovery of stressed organisms.

Research by individuals at the USEPA led to the conclusion that peptone buffer is not acceptable for use (Dr. Gerald Stelma, USEPS, oral communication, September 30, 2004; Dr. Kristen Brenner, USEPA, oral and written communications, October 6, 2004). The peptone serves as a nutrient source for bacteria, making it more prone to contamination than the phosphate with magnesium chloride (PO_4-$MgCl_2$) buffer. These studies showed that peptone buffer can become contaminated in as little as 30 minutes and that recoveries of initial bacterial concentrations increased 1,000 percent over a 24-hour period. The USEPA recommends that a 30-minute time limit be placed on processing serial dilutions in peptone buffer (U.S. Environmental Protection Agency, 1975).

As a result of those studies, the USEPA recommends the use of PO_4-$MgCl_2$ buffer with all of the following media: mTEC, modified mTEC, mEI, MI, mFC, KF, and mENDO (U.S. Environmental Protection Agency, 1978, 2000, and 2002; Gerald Stelma, oral communication, 2004; Kristen Brenner, oral communication, 2004).

References:

Britton, L.J., and Greeson, P.E., eds., 1987, Methods for collection and analysis of aquatic biological and microbiological samples: U.S. Geological Survey Techniques of Water-Resources Investigations, book 5, chap. A4, 363 p.

U.S. Environmental Protection Agency, 1975, Handbook for evaluating water bacteriological laboratories: U.S. Environmental Protection Agency, EPA-670/9-75-006.

U.S. Environmental Protection Agency, 1978, Microbiological methods for monitoring the environment—water and wastes: Cincinnati, Ohio, U.S. Environmental Protection Agency, EPA/600/8-78-017, 338 p.

U.S. Environmental Protection Agency, 2000, Improved enumeration methods for the recreational water quality indicators: enterococci and *Escherichia coli*: U.S. Environmental Protection Agency, Office of Science and Technology, EPA/821/R-97/004.

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

Questions should be directed to Kathy Fitzgerald (kkfitz@usgs.gov).

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