

TABLE IA.—LIST OF APPROVED BIOLOGICAL METHODS—Continued

Parameter and units	Method ¹	EPA	Standard methods 18th, 19th, 20th Ed.	ASTM	AOAC	USGS	Other
	Sheepshead minnow, <i>Cyprinodon variegatus</i> , embryo-larval survival and teratogenicity.	1005.0 ³¹					
	Inland silverside, <i>Menidia beryllina</i> , larval survival and growth.	1006.0 ³¹					
	Mysid, <i>Mysidopsis bahia</i> , survival, growth, and fecundity.	1007.0 ³¹					
	Sea urchin, <i>Arbacia punctulata</i> , fertilization.	1008.0 ³¹					

Notes to Table IA:

¹ The method must be specified when results are reported.

² A 0.45 µm membrane filter (MF) or other pore size certified by the manufacturer to fully retain organisms to be cultivated and to be free of extractables which could interfere with their growth.

³ USEPA. 1978. Microbiological Methods for Monitoring the Environment, Water, and Wastes. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio. EPA/600/8-78/017.

⁴ APHA. 1998, 1995, 1992. Standard Methods for the Examination of Water and Wastewater. American Public Health Association. 20th, 19th, and 18th Editions. Amer. Publ. Hlth. Assoc., Washington, D.C.

⁵ USGS. 1989. U.S. Geological Survey Techniques of Water-Resource Investigations, Book 5, Laboratory Analysis, Chapter A4, Methods for Collection and Analysis of Aquatic Biological and Microbiological Samples, U.S. Geological Survey, U.S. Department of Interior, Reston, Virginia.

⁶ Because the MF technique usually yields low and variable recovery from chlorinated wastewaters, the Most Probable Number method will be required to resolve any controversies.

⁷ Tests must be conducted to provide organism enumeration (density). Select the appropriate configuration of tubes/filtrations and dilutions/volumes to account for the quality, character, consistency, and anticipated organism density of the water sample.

⁸ When the MF method has not been used previously to test ambient waters with high turbidity, large number of noncoliform bacteria, or samples that may contain organisms stressed by chlorine, a parallel test should be conducted with a multiple-tube technique to demonstrate applicability and comparability of results.

⁹ To assess the comparability of results obtained with individual methods, it is suggested that side-by-side tests be conducted across seasons of the year with the water samples routinely tested in accordance with the most current Standard Methods for the Examination of Water and Wastewater or EPA alternate test procedure (ATP) guidelines.

¹⁰ ASTM. 2000, 1999, 1996. Annual Book of ASTM Standards—Water and Environmental Technology. Section 11.02. American Society for Testing and Materials. 100 Barr Harbor Drive, West Conshohocken, PA 19428.

¹¹ AOAC. 1995. Official Methods of Analysis of AOAC International, 16th Edition, Volume I, Chapter 17. Association of Official Analytical Chemists International. 481 North Frederick Avenue, Suite 500, Gaithersburg, Maryland 20877-2417.

¹² The multiple-tube fermentation test is used in 9221B.1. Lactose broth may be used in lieu of lauryl tryptose broth (LTB), if at least 25 parallel tests are conducted between this broth and LTB using the water samples normally tested, and this comparison demonstrates that the false-positive rate and false-negative rate for total coliform using lactose broth is less than 10 percent. No requirement exists to run the completed phase on 10 percent of all total coliform-positive tubes on a seasonal basis.

¹³ These tests are collectively known as defined enzyme substrate tests, where, for example, a substrate is used to detect the enzyme β-glucuronidase produced by *E. coli*.

¹⁴ After prior enrichment in a presumptive medium for total coliform using 9221B.1, all presumptive tubes or bottles showing any amount of gas, growth or acidity within 48 h ± 3 h of incubation shall be submitted to 9221F. Commercially available EC-MUG media or EC media supplemented in the laboratory with 50 µg/mL of MUG may be used.

¹⁵ Samples shall be enumerated by the multiple-tube or multiple-well procedure. Using multiple-tube procedures, employ an appropriate tube and dilution configuration of the sample as needed and report the Most Probable Number (MPN). Samples tested with Colilert® may be enumerated with the multiple-well procedures, Quanti-Tray® or Quanti-Tray® 2000, and the MPN calculated from the table provided by the manufacturer.

¹⁶ Colilert-18® is an optimized formulation of the Colilert® for the determination of total coliforms and *E. coli* that provides results within 18 h of incubation at 35°C rather than the 24 h required for the Colilert® test and is recommended for marine water samples.

¹⁷ Descriptions of the Colilert®, Colilert-18®, Quanti-Tray®, and Quanti-Tray®/2000 may be obtained from IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092.

¹⁸ A description of the mColiBlue24™ test, Total Coliforms and *E. coli*, is available from Hach Company, 100 Dayton Ave., Ames, IA 50010.

¹⁹ Subject total coliform positive samples determined by 9222B or other membrane filter procedure to 9222G using NA-MUG media.

²⁰ USEPA. 2002. Method 1103.1: *Escherichia coli* (*E. coli*) In Water By Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC). U.S. Environmental Protection Agency, Office of Water, Washington D.C. EPA-821-R-02-020.

²¹ USEPA. 2002. Method 1603: *Escherichia coli* (*E. coli*) In Water By Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (modified mTEC). U.S. Environmental Protection Agency, Office of Water, Washington D.C. EPA-821-R-02-023.

²² Preparation and use of MI agar with a standard membrane filter procedure is set forth in the article, Brenner *et al.* 1993. "New Medium for the Simultaneous Detection of Total Coliform and *Escherichia coli* in Water." Appl. Environ. Microbiol. 59:3534-3544 and in USEPA. 2002. Method 1604: Total Coliforms and *Escherichia coli* (*E. coli*) in Water by Membrane Filtration by Using a Simultaneous Detection Technique (MI Medium). U.S. Environmental Protection Agency, Office of Water, Washington DC. EPA 821-R-02-024.

²³ A description of the Enterolert® test may be obtained from IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092.

²⁴ USEPA. 2002. Method 1106.1: Enterococci In Water By Membrane Filtration Using membrane-Enterococcus-Esculin Iron Agar (mE-EIA). U.S. Environmental Protection Agency, Office of Water, Washington DC. EPA-821-R-02-021.

²⁵ USEPA. 2002. Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI). U.S. Environmental Protection Agency, Office of Water, Washington, DC. EPA-821-R-02-022.