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40 CFR Part 136

Guidelines Establishing Test Procedures for the Analysis of Pollutants; Analytical Methods for Biological Pollutants in Wastewater and Sewage Sludge; Proposed Rule

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 136

[OW–2004–0014; FRL–7952–7]

RIN 2040–AE68

Guidelines Establishing Test Procedures for the Analysis of Pollutants; Analytical Methods for Biological Pollutants in Wastewater and Sewage Sludge; Proposed Rule

AGENCY: Environmental Protection Agency (EPA).

ACTION: Proposed rule.

SUMMARY: This proposed regulation would amend the ''Guidelines Establishing Test Procedures for the Analysis of Pollutants'' under section 304(h) of the Clean Water Act (CWA), by adding analytical test procedures for enumerating the bacteria, *Escherichia coli* (*E. coli*) and enterococci, in wastewater; and by adding analytical test procedures for enumerating fecal coliforms and *Salmonella* in sewage sludge to the list of Agency-approved methods. Specifically, EPA is proposing both membrane filter (MF) and multiple-tube fermentation (MTF, *i.e.*, multiple-tube, multiple-well) methods for *E. coli* and enterococci bacteria in wastewater, and MTF methods for fecal coliforms and *Salmonella* in sewage sludge. EPA's approval of these methods will help Regions, States, communities, and environmental laboratories better assess public health risks from microbiological pollutants.

DATES: Comments must be received on or before October 17, 2005.

ADDRESSES: Submit your comments, identified by Docket ID No. OW–2004–0014, by one of the following methods:

I. Federal eRulemaking Portal: *http://www.regulations.gov.* Follow the on-line instructions for submitting comments.

II. Agency Web site: *http://www.epa.gov/edocket.* EDOCKET, EPA's electronic public docket and comment system, is EPA's preferred method for receiving comments. Follow the on-line instructions for submitting comments.

III. E-mail: *OW-docket@epamail.epa.gov,* Attention Docket ID No. OW–2004–0014.

IV. Mail: Water Docket, Environmental Protection Agency, Mailcode: 4101T, 1200 Pennsylvania Ave., NW., Washington, DC 20460.

V. Hand Delivery: EPA Water Center, EPA West Building, Room B102, 1301 Constitution Avenue, NW., Washington, DC, Attention Docket ID No. OW–2004–0014. Such deliveries are only accepted during the Docket's normal hours of operation, and special arrangements should be made for deliveries of boxed information.

Instructions: Direct your comments to Docket ID No. OW–2004–0014. EPA's policy is that all comments received will be included in the public docket without change and may be made available on-line at *http://www.epa.gov/edocket,* including any personal information provided, unless the comment includes information claimed to be Confidential Business Information (CBI) or other information whose disclosure is restricted by statute. Do not submit information that you consider to be CBI or otherwise protected through EDOCKET, regulations.gov, or e-mail. The EPA EDOCKET and the Federal regulations.gov Web sites are ''anonymous access'' systems, which means EPA will not know your identity or contact information unless you provide it in the body of your comment. If you send an e-mail comment directly to EPA without going through EDOCKET or regulations.gov, your e-mail address will be automatically captured and included as part of the comment that is placed in the public docket and made available on the Internet. If you submit an electronic comment, EPA recommends that you include your name and other contact information in the body of your comment and with any disk or CD–ROM you submit. If EPA cannot read your comment due to technical difficulties and cannot contact you for clarification, EPA may not be able to consider your comment. Electronic files should avoid the use of special characters, any form of encryption, and be free of any defects or viruses. For additional information about EPA's public docket visit EDOCKET on-line or see the **Federal Register** of May 31, 2002 (67 FR 38102).

Docket: All documents in the docket are listed in the EDOCKET index at *http://www.epa.gov/edocket.* Although listed in the index, some information is not publicly available, *i.e.*, CBI or other information whose disclosure is restricted by statute. Certain other material, such as copyrighted material, is not placed on the Internet and will be publicly available only in hard copy form. Publicly available docket materials are available either electronically in EDOCKET or in hard copy at the Water Docket, EPA/DC, EPA West, Room B102, 1301 Constitution Ave., NW., Washington, DC. The Public Reading Room is open from 8:30 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The telephone number for the Public Reading Room is (202) 566–1744, and the telephone number for the Water Docket is (202) 566–2426.

FOR FURTHER INFORMATION CONTACT: Robin K. Oshiro, Office of Science and Technology (4303–T); Office of Water, U.S. Environmental Protection Agency, Ariel Rios Building, 1200 Pennsylvania Avenue, NW., Washington, DC 20460, (202) 566–1075 (e-mail: *Oshiro.Robin@epa.gov*).

SUPPLEMENTARY INFORMATION:

A. Does This Action Apply to Me?

EPA Regions, as well as States, Territories and Tribes authorized to implement the National Pollutant Discharge Elimination System (NPDES) program, issue permits that must comply with the technology-based and water quality-based requirements of the Clean Water Act (CWA). In doing so, NPDES permitting authorities, including States, Territories, and Tribes, make several discretionary choices when they write a permit. These choices include the selection of pollutants to be measured, monitoring requirements, permit conditions (*e.g.*, triggers), and, in many cases, limits in permits. EPA's NPDES regulations (applicable to all authorized State NPDES programs) require monitoring results to be reported at the intervals specified in the permit, but in no case less frequently than once per year. Monitoring results must be conducted according to test procedures approved under 40 CFR part 136 (see 40 CFR 122.41(j)(4), 122.44(i)(1)(iv) and 122.44(i)(2)). Therefore, entities with NPDES permits may potentially be regulated by actions proposed in this rulemaking. In addition, when an authorized State, Territory, or Tribe certifies Federal licenses under CWA section 401, they must use the standardized analysis and sampling procedures. Categories and entities that could potentially be regulated include:

Category	Examples of potentially regulated entities
Federal, State, Territorial, and Indian Tribal Governments.	Federal, State, Territorial, and Tribal entities authorized to administer the NPDES permitting program; Federal, State, Territorial, and Tribal entities providing certification under Clean Water Act section 401.
Industry	Facilities that must conduct monitoring to comply with NPDES permits.

Category	Examples of potentially regulated entities
Municipalities	POTWs that must conduct monitoring to comply with NPDES permits.

This table is not intended to be exhaustive, but rather provides a guide for readers regarding entities likely to be regulated by this action. This table lists types of entities that EPA is now aware could potentially be regulated by this action. Other types of entities not listed in the table could also be regulated. To determine whether your facility is regulated by this action, you should carefully examine the applicability language at 40 CFR 122.1, (NPDES purpose and scope), 40 CFR 136.1 (NPDES permits and CWA), 40 CFR 503.32 (Sewage sludge and pathogens). If you have questions regarding the applicability of this action to a particular entity, consult the appropriate person listed in the preceding **FOR FURTHER INFORMATION CONTACT** section.

B. What Should I Consider as I Prepare My Comments for EPA?

1. *Submitting CBI.* Do not submit this information to EPA through EDOCKET, regulations.gov or e-mail. Clearly mark the part or all of the information that you claim to be CBI. For CBI information in a disk or CD–ROM that you mail to EPA, mark the outside of the disk or CD–ROM as CBI and then identify electronically within the disk or CD–ROM the specific information that is claimed as CBI. In addition to one complete version of the comment that includes information claimed as CBI, a copy of the comment that does not contain the information claimed as CBI must be submitted for inclusion in the public docket. Information so marked will not be disclosed except in accordance with procedures set forth in 40 CFR part 2.

2. *Tips for Preparing Your Comments.* When submitting comments, remember to:

I. Identify the rulemaking by docket number and other identifying information (subject heading, **Federal Register** date and page number).

II. Follow directions—The agency may ask you to respond to specific questions or organize comments by referencing a Code of Federal Regulations (CFR) part or section number.

III. Explain why you agree or disagree; suggest alternatives and substitute language for your requested changes.

IV. Describe any assumptions and provide any technical information and/or data that you used.

V. If you estimate potential costs or burdens, explain how you arrived at your estimate in sufficient detail to allow for it to be reproduced.

VI. Provide specific examples to illustrate your concerns, and suggest alternatives.

VII. Explain your views as clearly as possible, avoiding the use of profanity or personal threats.

VIII. Make sure to submit your comments by the comment period deadline identified.

3. *Docket Copying Costs.* Copies of analytical methods published by EPA are available for a nominal cost through the National Technical Information Service (NTIS); U.S. Department of Commerce; 5285 Port Royal Road; Springfield, VA 22161, or call (800) 553–6847. Copies of the EPA methods cited in this proposal may be obtained from Robin K. Oshiro; Office of Science and Technology (4303–T); Office of Water; U.S. Environmental Protection Agency; Ariel Rios Building; 1200 Pennsylvania Avenue, NW., Washington, DC 20460, or call (202) 566–1075. Copies of several of the EPA methods cited in this proposal may also be downloaded from the EPA Office of Water, Office of Science and Technology, home page at *http://www.epa.gov/waterscience/methods/.* Copies of all methods are also available in the public record for this proposal.

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I. Statutory Authority

EPA is proposing this action pursuant to the authority of sections 301(a), 304(h), 405(d) and (e), and 501(a) of the Clean Water Act (''CWA'' or the ''Act''), 33 U.S.C. 1311(a), 1314(h), 1361(a). Section 301(a) of the Act prohibits the discharge of any pollutant into navigable waters unless, among other things, the discharge complies with a National Pollutant Discharge Elimination System (NPDES) permit issued under section 402 of the Act. Section 304(h) of the Act requires the Administrator of the EPA to ''* * * promulgate guidelines establishing test procedures for the analysis of pollutants that shall include the factors which must be provided in any certification pursuant to [section 401 of this Act] or permit application pursuant to [section 402 of this Act].'' Section 501(a) of the Act authorizes the Administrator to '' * * * prescribe such regulations as are necessary to carry out this function under [the Act].'' EPA generally codifies its test procedures in the Code of Federal Regulations (including analysis and sampling requirements) for CWA programs at 40 CFR part 136, though some specific requirements are in other sections (*e.g.*, 40 CFR 503.8).

II. Explanation of Today's Action

A. Methods for NPDES Compliance Monitoring

This proposal would make available membrane filter (MF) methods and a suite of Multiple Tube Fermentation (MTF) methods (*i.e.*, multiple-tube, multiple-well) including culture and enzyme-substrate techniques available for enumerating (*i.e.*, determining organism density) *E. coli* and enteroccoci in wastewaters and fecal coliforms and *Salmonella* in sewage sludge as part of State, Territorial, Tribal, and local water quality and sewage sludge monitoring programs.

EPA selected the methods based on data generated by EPA laboratories, or submissions to the ATP program. Since multiple studies using different method versions and different statistical analyses generated the EPA laboratory data, the test procedures in today's rule must be evaluated against the end-users' needs based on data quality objectives. EPA recommends that all new proposed

alternative methods be compared to the appropriate EPA approved reference method before adopting it for that matrix to ensure that the proposed method generates data of comparable quality. For full details regarding alternative microbial methods, see the EPA Microbiological Alternate Test Procedure (ATP) Protocol for Drinking Water, Ambient Water, and Wastewater Monitoring Methods (EPA 821–B–03–004). Full citations for methods and validation data reports are provided in the References section and are included in the docket for today's proposed rulemaking.

1. Membrane Filtration (MF) and Multiple Tube Fermentation (MTF) Methods

Membrane filtration is a direct-plating method in which sample dilutions/volumes are filtered through 0.45 μm membrane filters that are subsequently transferred to petri plates containing selective primary isolation agar or an absorbent pad saturated with selective broth. The total sample volume to be analyzed may be distributed among multiple filters and diluted as needed, based on the anticipated water sample type, quality, and character (*e.g.*, organism density, turbidity). The goal is to obtain plates with counts within the acceptable counting range of the method. The acceptable counting range of membrane filter tests depends on the specific analytical technique and the target organism under study. Plates are incubated and target colonies are counted. A percentage of the target colonies may then be verified as specified by the method. Target colonies are detected by observing the presence of colonies that meet a specific morphology, color, or fluorescence under specified conditions. Colonies may be counted with the aid of a fluorescent light, magnifying lens or dissecting microscope. Results generally are reported as colony-forming units (CFU) per 100 mL. Organism density is determined by dividing the number of target CFU by the volume (mL) of undiluted sample that is filtered and multiplying by 100. If verification steps are performed, the initial target colony count is adjusted based upon the percentage of positively verified colonies and reported as a ''verified count per 100 mL'' (Standard Methods for the Examination of Water and Wastewater, 1998).

Membrane filtration is applicable to most tertiary treated wastewaters but has limitations where an underestimation of organism density is likely, such as water samples with high turbidity, toxic compounds, large numbers of non-coliform (background) bacteria. In addition, membrane filtration may have limitations where organisms are damaged by chlorine or toxic compounds, such as can be found in primary and some secondary treated wastewaters. To minimize these interferences, replicates of smaller sample dilutions/volumes may be filtered and the results combined. When the MF method has not been used previously on an individual water type, parallel tests should be conducted with a Multiple Tube Fermentation (MTF) to demonstrate applicability, lack of interferences, and at least comparable (*e.g.*, equivalent or better) recovery. For example, colonies from samples containing high-background levels or stressed organisms should be verified. If the MTF results are consistently higher than those obtained in MF tests, or there is an indication of suboptimal recovery, the user should use an appropriate recovery enhancement technique that the tester demonstrates is comparable to MTF. Further background information on MF tests is available in Standard Methods for the Examination of Water and Wastewater (1998).

In Multiple Tube Fermentation (MTF) tests, the number of tubes/wells producing a positive reaction provides an estimate of the original, undiluted density (*i.e.*, concentration) of target organisms in the sample. This estimate of target organisms, based on probability formulas, is termed the Multiple Tube Fermentation. MTF tests may be conducted in multiple-tube fermentation, multiple-tube enzyme substrate, or multiple-well enzyme substrate formats. In multiple-tube tests, serial dilutions may be used to obtain estimates over a range of concentrations, with replicate tubes analyzed at each ten-fold dilution/volume. The numbers of replicate tubes and sample dilutions/volumes are selected based on the expected quality of the water sample. Generally, for non-potable water samples, five replicate tubes at a minimum of three dilutions/volumes are used. Tubes are incubated, and positive results are reported and confirmed. Positive results are determined under specified conditions by the presence of acid and/or the production of gas using MTF tests, or by color change or fluorescence using enzyme substrate tests. Tests also may be conducted in a multiple-well format to determine MTF, using commercially prepared substrate media, multiple-well trays, and MPN tables provided by the manufacturer. Target organism density is estimated by comparing the number of positive tubes or wells with MPN tables. The MPN tables relate the number of positive tubes or wells to an estimate of the mean target organism density based on probability formulas. Results in both types of tests are generally reported as MPN per 100 mL.

The multiple-tube fermentation methodology is useful for detecting low concentrations of organisms (<100/100 mL), particularly in samples containing heavy particulate matter, toxic compounds (*e.g.* metals), injured or stressed organisms, or high levels of heterotrophic plate count bacteria (HPC). The membrane filtration technique may be more appropriate in instances where the toxins are water soluble; in such cases, the toxin may be eliminated while the organisms are retained on the filter. Multiple-tube tests are applicable to sewage sludge analysis. Since MPN tables assume a Poisson distribution, samples must be adequately shaken to break up any clumps and provide even distribution of bacteria. If the sample is not gently shaken, the MPN value may underestimate the actual bacterial density. The overall precision of each multiple-tube test depends on the number of tubes used and sample dilutions/volumes tested.

Unless a large number of tubes are used (five tubes per dilution/volume or more), the precision of multiple-tube tests can be very poor. Precision is improved when the results from several samples from the same sampling event are processed, estimated separately, and then mathematically combined using the geometric mean. Further background information on multiple-tube tests is available in the Standard Methods for the Examination of Water and Wastewater (1998).

A statistical comparison of results obtained by the MF and MTF methods showed that the MF method is more precise in enumerating target organisms than the MTF test, but differences in recovery were generally not statistically significant. However, based on susceptibility to interferences, MF tests may underestimate the number of viable bacteria, and the MTF method may overestimate the concentration because of the built-in positive bias of the method (Thomas, 1955). Because of susceptibility of some MF tests to interferences, verification of some MF results with confirmatory multiple-tube tests is critical. Additionally, some MTF tests require confirmation tests because of the false positive/false negative rates of the particular media. In general, although numerical results may not be identical, data from each method yield similar water quality information based on performance.

2. Methods for *E. coli* in Wastewater

EPA is proposing several methods for enumerating *E. coli* in wastewater. In Table 1, methods in the same row use the same technique, but are published by different entities. For example, ONPG–MUG is published in the ''Standard Methods'' manual and in the Association of Official Analytical Chemists (AOAC) manual, and is also available as a commercial product. Voluntary Consensus Standards (VCS) Methods are those developed or adopted by domestic and international voluntary consensus standard bodies. The American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Foundation (WEF) jointly publish methods approved by a methods approval program in Standard Methods for the Examination of Water and Wastewater (''Standard Methods''). The Association of Official Analytical Chemists (AOAC) also publishes methods that have met the requirements of the AOAC methods approval program. EPA methods are those that have been developed and validated by the US EPA.

TABLE 1.—PROPOSED METHODS FOR *E. coli* ENUMERATION IN WASTEWATER

Technique	Method [1]	EPA method	VCS methods		Commercial example
			Standard methods	AOAC	
Membrane Filter (MF)	Modified mTEC agar	1603	.....	.....	
Multiple Tube Fermentation (MTF)	ONPG–MUG	.....	9223B	991.15	Colilert® [2]
	ONPG–MUG	.....	9223B	.....	Colilert-18® [2]

[1] Tests must be conducted in a format that provides organism enumeration.
[2] Manufactured by IDEXX.

a. Membrane Filter (MF) Test for E. coli: Modified mTEC Agar (EPA Method 1603). The modified mTEC agar method is a single-step MF procedure that provides a direct count of *E. coli* in water based on the development of colonies on the surface of a filter when placed on selective modified mTEC media (USEPA, 2004a). This is a modification of the standard mTEC media that eliminates bromcresol purple and bromphenol red from the medium, adds the chromogen 5-bromo-6-chloro-3-indoyl-β-D-glucuronide (Magenta Gluc), and eliminates the transfer of the filter to a second substrate medium. In this method, a water sample is filtered through a 0.45 μm membrane filter, the filter is placed on modified mTEC agar, incubated at 35 ± 0.5 °C for 2 h to resuscitate injured or stressed bacteria, and then incubated for 23 ± 1 h in a 44.5 ± 0.2 °C water bath. Following incubation, all red or magenta colonies are counted as *E. coli.*

b. Multiple Tube Fermentation Tests for E. coli: ONPG–MUG (Standard Methods 9223B, AOAC 991.15, Colilert®, Colilert–18®). ONPG–MUG tests are chromogenic/fluorogenic enzyme substrate tests for the simultaneous determination of total coliforms and *E. coli* in water. These tests use commercially available media containing the chromogenic substrate ortho-nitrophenyl-β-D-galactopyranoside (ONPG), to detect total coliforms and the fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronide (MUG), to detect *E. coli.* All tests must be conducted in a format that provides quantitative results for ambient water. Colilert-18® should be used for testing marine waters with a minimum of a 10-fold dilution with sterile non-buffered, oxidant-free water. Media formulations are available in disposable tubes for the multiple-tube procedure or packets for the multiple-well procedure. Appropriate preweighed portions of media for mixing and dispensing into multiple-tubes and wells are also available. The use of commercially prepared media is required for quality assurance and uniformity.

For the multiple-tube procedure, a well-mixed sample and/or sample dilution/volume is added to tubes containing predispensed media. Tubes are then capped and mixed vigorously to dissolve the media. Alternatively, this procedure can be performed by adding appropriate amounts of substrate media to a bulk diluted sample (with appropriate dilutions for enumeration), then mixing and dispensing into multiple-tubes. The number of tubes, and number of dilutions/volumes are determined based on the type, quality, and character of the water sample. A multiple-well procedure may be performed with sterilized disposable packets. The commercially available Quanti-Tray® or Quanti-Tray®/2000 multiple-well tests uses Colilert® or Colilert-18® media to determine *E. coli* (IDEXX, 1999a,b,c). In these tests, the packet containing media is added to a 100-mL sample (with appropriate dilutions for enumeration). The sample is then mixed and poured into the tray. A tray sealer separates the sample into 51 wells (Quanti-Tray) or 96 wells (Quanti-tray/2000) and seals the package which is subsequently incubated at 35 ± 0.5 °C for 18 h when using Colilert-18® or 24 h when using Colilert®. If the response is questionable after the specified incubation period, the sample is incubated for up to an additional 4 h at 35 ± 0.5 °C for both Colilert® tests.

After the appropriate incubation period, each tube or well is compared to the reference color ''comparator'' provided with the media. If the sample has a yellow color greater or equal to the comparator, the presence of total coliforms is verified, and the tube or well is then checked for fluorescence under long-wavelength UV light (366-nm). The presence of fluorescence greater than or equal to the comparator is a positive test for *E. coli.* If water samples contain humic acid or colored substances, inoculated tubes or wells should also be compared to a sample water blank. The concentration in MPN/100 mL is then calculated from the number of positive tubes or wells using MPN tables provided by the manufacturer.

3. Methods for Enterococci for Wastewater

EPA is proposing several methods for enumerating enterococci in wastewater. Brief descriptions of the proposed MF and MTF methods are provided below. In Table 2, methods in the same horizontal row use the same technique, but are published by different entities.

TABLE 2.—PROPOSED METHODS FOR *Enterococci* IN WASTEWATER.

Methodology	Method [1]	EPA method	VCS methods		Commercial example
			ASTM	AOAC	
Membrane Filter (MF)	mEI agar	1600			
Multiple Tube Fermentation (MTF)	MUG media		D6503–99		Enterolert™ [2]

[1] Tests must be conducted in a format that provides organism enumeration.
[2] Manufactured by IDEXX.

a. Membrane Filter (MF) Test for Enterococci: mEI Agar (EPA Method 1600). The mE–EIA agar method is a two-step MF procedure that provides a direct count of bacteria in water, based on the development of colonies on the surface of a filter when placed on selective mE agar (USEPA, 2004b). This medium, a modification of the mE agar in EPA Method 1106.1, contains a reduced amount of 2–3–5-triphenyltetrazolium chloride, and an added chromogen, indoxyl-β-D-glucoside. The transfer of the filter to EIA is eliminated, thereby providing results within 24 h. In this method, a water sample is filtered, and the filter is placed on mEI agar and incubated at 41 ± 0.5 °C for 24 h. Following incubation, all colonies with a blue halo, regardless of colony color that are greater than 0.5 mm in diameter, are counted as enterococci. Results are reported as enterococci per 100 mL.

b. Multiple Tube Fermentation (MTF) Tests for Enterococci: 1. 4-methylumbelliferyl-β-D-glucoside (MUG) Medium (ASTM D6503–99, Enterolert™). This method utilizes a medium containing the fluorogenic substrate 4-methylumbelliferyl-β-D-glucoside (MUG) to determine enterococci concentrations. Enterolert™ is a commercially available test that utilizes this substrate test for the determination of enterococci in water (IDEXX, 1999a). Enterolert™ tests are incubated for 24 h at 41 ± 0.5 °C and may use the same quantitative formats available for the Colilert® tests, cited earlier in Section III–A. After incubation, the presence of blue/white fluorescence, as viewed using a 6-watt, 365 nm, UV light, is a positive result for enterococci. The concentration in MPN/100 mL is then calculated from the number of positive tubes or wells using MPN tables provided by the manufacturer. Enterolert™ is subject to the same interferences and cautions listed for the Colilert® tests. In addition, marine water samples must be diluted at least tenfold with sterile, non-buffered oxidant-free water (Enterolert™ is already buffered).

4. Methods for Fecal Coliforms in Sewage Sludge

EPA is proposing methods for enumerating fecal coliforms in sewage sludge (Table 3). Brief descriptions of the proposed MTF methods are provided below.

TABLE 3.—PROPOSED METHODS FOR FECAL COLIFORMS IN SEWAGE SLUDGE

Methodology	Method [1]	EPA method
Multiple Tube Fermentation (MTF).	LT–EC ..	1680
	A–1	1681

[1] Tests must be conducted in a format that provides organism enumeration.

a. Multiple Tube Fermentation (MTF) Tests for Fecal Coliforms:

1. LT–EC Medium (EPA Method 1680). The multiple-tube fermentation method for enumerating fecal coliforms in sewage sludge uses multiple-tubes and dilutions/volumes in a two-step procedure to determine fecal coliform concentrations (USEPA, 2004c). In the first step, or ''presumptive phase,'' a series of tubes containing lauryl tryptose broth (LTB) are inoculated with undiluted samples and/or dilutions/volumes of the samples and mixed. Inoculated tubes are incubated for 24 ± 2 h at 35 ± 0.5 °C. Each tube then is swirled gently and examined for growth (*i.e.*, turbidity) and production of gas in the inner Durham tube. If there is no growth or gas, tubes are re-incubated for 24 ± 2 h at 35 ± 0.5 °C and re-examined. Production of growth and gas within 48 ± 3 h constitutes a positive presumptive test for coliforms. Failure to produce gas is a negative reaction and indicates fecal coliform bacteria are not present. Turbidity without gas indicates an invalid test that requires repeat analysis.

Results of the MTF procedure using LTB/EC media are reported in terms of MPN/g dry weight calculated from the number of positive EC tubes and percent total solids (dry weight basis).

2. A–1 Medium (EPA Method 1681). The multiple-tube fermentation method for enumerating fecal coliforms in sewage sludge uses multiple-tubes and dilutions/volumes in a procedure to determine fecal coliform concentrations (USEPA 2004d). It should be noted that the Triton X–100 (polyethylene glycol p-isoloctylphenyl ether) is extremely volatile, and thus the medium must be used within one week (and preferably on the day of) preparation. In the first step, a series of tubes containing A–1 broth are inoculated with undiluted samples and/or dilutions/volumes of the samples and mixed. Inoculated tubes are incubated for 3 h at 35 ± 0.5 °C, then transferred to a water bath at 44.5 °C ± 0.2 °C. After 21 ± 2 h, tubes are examined for growth (*i.e.*, turbidity) and production of gas in the inner Durham tube. Production of growth and gas within 24 ± 4 h constitutes the presence of fecal coliforms. Failure to produce both turbidity and gas is a negative reaction and indicates fecal coliform bacteria are not present.

Results of the MTF procedure using A–1 media are reported in terms of MPN/g calculated from the number of positive A–1 tubes and percent total solids (dry weight basis).

5. Methods for *Salmonella* in Sewage Sludge

EPA is also proposing methods for enumerating *Salmonella* in sewage sludge (Table 4). Brief descriptions of the proposed MTF method are provided below.

TABLE 4.—PROPOSED METHODS FOR *Salmonella* IN SEWAGE SLUDGE

Methodology	Method [1]	EPA method
Multiple Tube Fermentation (MTF).	Modified MSRV.	1682

[1] Tests must be conducted in a format that provides organism enumeration.

a. Multiple Tube Fermentation (MTF) Tests for *Salmonella* in Sewage Sludge: Multiple Tube Fermentation (MTF) Test for *Salmonella* (EPA Method 1682). The multiple-tube fermentation method for enumerating *Salmonella* in sewage sludge uses multiple-tubes and dilutions/volumes in a multiple-step procedure to determine *Salmonella* concentrations (USEPA 2004e). In the selective phase, a series of tubes

containing tryptic soy broth (TSB) are inoculated with undiluted samples and/or dilutions/volumes of the samples and mixed. Inoculated tubes are incubated for 24 ± 2 h at 36 ± 1.5 °C. After incubation, six discrete, 30-µL drops from each TSB tube are spotted onto the selective Rappaport-Vassiliadis agar medium semisolid modification (MSRV). The drops are allowed to absorb into the agar for approximately 1 hour at room temperature, then incubated, inoculated side up, at 42 °C ± 0.5 °C for 16 to 18 hours in a humidity-controlled hot air incubator.

The plates are examined for the appearance of motility surrounding inoculations, as evidenced by a ''whitish halo'' of growth approximately 2 cm from the center of the spot. Growth from the outer edge of the halo is streaked onto labeled XLD plates for isolation with a sterile inoculating needle or loop. Two halos and chosen are stabbed using an inoculating loop into the halo's outer edge, which is then streaked onto individual XLD plates (one spot per XLD plate) that are then incubated for 18 to 24 hours at 36 °C ± 1.5 °C. After incubation, one of the plates is submitted to biochemical confirmation (the other is refrigerated for reference). Pink to red colonies with black centers on XLD plates are considered *Salmonella.*

In the confirmatory phase, isolated colonies exhibiting *Salmonella* morphology (pink to red colonies with black centers) are picked and inoculated into triple sugar iron agar (TSI) slants, lysine iron agar (LIA) slants, and urease broth, all of which are incubated for 24 ± 2 hours at 36 °C ± 1.5 °C. A positive TSI reaction is an acid butt (yellow in color) and an alkaline slant (red in color) with or without H_2S gas production. A positive LIA reaction is an alkaline butt (purple in color) and an alkaline slant (purple in color) with or without H_2S gas production. When H_2S gas production is present, the butts of both the LIA and TSI may be black, which would be considered a positive reaction for *Salmonella.* Urease is an orange medium and will change to pink or deep purplish-red if positive. A negative urease test is one that exhibits no color change after inoculation. *Salmonella* are negative for urease.

To confirm cultures via polyvalent O antiserum, growth on the slant portion of TSI (regardless of whether TSI is positive or negative) is emulsified using sterile physiological saline, and two discrete drops of emulsified growth are placed onto a slide. One drop of polyvalent O antiserum is to be added to the first drop of emulsified growth, and one drop of sterile saline is added to the second drop of emulsified growth as a visual comparison. The slide is observed under magnification for an agglutination reaction which indicates a positive result. In order for the original TSB tube to be considered positive for *Salmonella,* the associated inoculations should be MSRV positive, XLD positive, either TSI or LIA positive, urease negative, and polyvalent-O positive. Failure in any of these test constitutes a negative Salmonella reaction.

A total solids determination is performed on a representative sewage sludge sample and is used to calculate MPN/g dry weight. *Salmonella* density is reported as MPN / 4 g dry weight.

B. Request for Comment and Available Data

EPA is not proposing the use of EPA Method 1103.1 (mTEC) for *E. coli* or EPA Method 1106.1 (mE-EIA) for enterococci for use in wastewater because the validation test results for these methods showed that the false positive and false negative rates for these methods were unacceptably high. Specifically, the validation of Method 1103.1 had laboratory-specific rates combined over unspiked disinfected/secondary results ranging from 14.4% to 22.9% for false positives and from 8.9% to 16.9% for false negatives (USEPA 2004f). Additionally, the validation of Method 1106.1 had laboratory-specific rates combined over unspiked disinfected/secondary results ranging from 0.0% to 18.0% for false positives and from 55.4% to 60.5% for false negatives (USEPA 2004g).

EPA is not proposing to extend the holding time from 6 hours to 24 hours for fecal coliforms using Method 1680 (LTB/EC) from Class A aerobically digested sewage sludge or for *Salmonella* using Method 1682 (MSRV) from Class B thermophilically digested sewage sludge because the holding time studies for these methods showed significant differences in concentrations of these organisms using these methods after 24 hours holding time (USEPA 2004h).

EPA requests public comments on the proposed methods for the bacterial indicators of fecal contamination. EPA invites comments on the technical merit, applicability, and implementation of the proposed *E. coli* and enterococci methods for wastewater monitoring, and for fecal coliform and *Salmonella* methods for sewage sludge monitoring. Commenters should specify the method and bacteria/organisms to which the comment applies. EPA encourages commenters to provide copies of supporting data or references cited in comments. EPA also requests public comments on acceptable characteristics of these test methods for specific matrix applications, on comparability criteria to determine equivalency of alternative test methods, supporting data, and examples of any available alternative equivalency testing protocols. Additionally, EPA requests comments on any other applicable methods for analyzing *E. coli* and enterococci in wastewater and for fecal coliforms and *Salmonella* in sewage sludge and for holding times for the proposed methods in sewage sludge not included in today's proposal. Method descriptions and supporting data may be submitted for additional test procedures that are applicable to enumerating these bacteria in wastewater and sewage sludge, respectively.

C. Editorial Revision and Clarification to 40 CFR Part 136

40 CFR part 136, Table I currently includes microbial (bacterial, and protozoan) methods for use in both wastewater and ambient waters. For clarification purposes, EPA proposes to move those methods which are applicable to ambient waters to a new Table IG.

D. Sampling, Sample Preservation, and Holding Times for NPDES Compliance Monitoring: Revisions to 40 CFR Part 136, Table II

40 CFR part 136, Table II specifies sampling, preservation, and holding time requirements. This proposal would make additions to these tables for sewage sludge methods added to Table IA. In addition, clarification is provided for the holding time for bacterial tests.

III. Statutory and Executive Order Reviews

A. Executive Order 12866: Regulatory Planning and Review

Under Executive Order 12866 (58 FR 51735 (October 4, 1993)), the Agency must determine whether the regulatory action is ''significant'' and therefore subject to OMB review and the requirements of the Executive Order. The Executive Order defines ''significant regulatory action'' as one that is likely to result in a rule that may:

(1) Have an annual effect on the economy of $100 million or more, or adversely affect in a material way the economy, a sector of the economy, productivity, competition, jobs, the environment, public health or safety, or State, local, or Tribal governments or communities;

(2) Create a serious inconsistency or otherwise interfere with an action taken or planned by another agency;

(3) Materially alter the budgetary impact of entitlements, grants, user fees, or loan programs or the rights and obligations of recipients thereof; or

(4) Raise novel legal or policy issues arising out of legal mandates, the President's priorities, or the principles set forth in the Executive Order.

It has been determined that this proposed rule is not a ''significant regulatory action'' under the terms of Executive Order 12866 and is therefore not subject to Executive Order 12866 review.

B. Paperwork Reduction Act

This action does not impose an information collection burden under the provisions of the Paperwork Reduction Act, 44 U.S.C. 3501 *et. seq.* This rule proposes to make available new test methods for *E. coli* and enterococci for use in wastewater monitoring programs, and new test methods for fecal coliform and *Salmonella* for use in sewage sludge monitoring programs, but EPA would not require the use of these test methods. This rule does not impose any information collection, reporting, or record keeping requirements.

Burden means the total time, effort, or financial resources expended by persons to generate, maintain, retain, or disclose or provide information to or for a Federal agency. This includes the time needed to review instructions; develop, acquire, install, and utilize technology and systems for the purpose of collecting, validating, and verifying information, processing and maintaining information, and disclosing and providing information; adjust the existing ways to comply with any previously applicable instructions and requirements; train personnel to be able to respond to a collection of information; search data sources; complete and review the collection of information; and transmit or otherwise disclose the information.

An Agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a currently valid OMB control number. The OMB control numbers for EPA's regulations in 40 CFR are listed in 40 CFR part 9.

C. Regulatory Flexibility Act

The Regulatory Flexibility Act (RFA) generally requires an agency to prepare a regulatory flexibility analysis of any rule subject to notice and comment rulemaking requirements under the Administrative Procedure Act or any other statute unless the agency certifies that the rule will not have a significant economic impact on a substantial number of small entities. Small entities include small businesses, small organizations, and small governmental jurisdictions.

For purposes of assessing the impacts of this rule on small entities for methods under the Clean Water Act, small entity is defined as: (1) A small business that meets RFA default definitions (based on SBA size standards) found in 13 CFR 121.201; (2) a small governmental jurisdiction that is a government of a city, county, town, school district or special district with a population less than 50,000; and (3) a small organization that is any not-for-profit enterprise which is independently owned and operated and is not dominant in its field.

After considering the economic impacts of today's proposed rule on small entities, I certify that this action will not have a significant economic impact on a substantial number of small entities. In determining whether a rule has a significant economic impact on a substantial number of small entities, the impact of concern is any significant *adverse* economic impact on small entities, since the primary purpose of the regulatory flexibility analyses is to identify and address regulatory alternatives ''which minimize any significant economic impact of the rule on small entities.'' 5 U.S.C. 603 and 604. Thus, an agency may certify that a rule will not have a significant economic impact on a substantial number of small entities if the rule relieves regulatory burden, or otherwise has a positive economic effect on all of the small entities subject to the rule.

This proposed regulation would approve testing procedures for the measurement of *E. coli* and enterococci bacteria in wastewater, and fecal coliforms and *Salmonella* bacteria in sewage sludge. The inclusion of these test methods in 40 CFR 136.3 is intended to make these test methods available to States and others for use in wastewater and sewage sludge monitoring programs. EPA is not establishing any compliance monitoring requirements for these pollutants.

EPA analyzed the annualized cost estimates to regulated entities (small governmental jurisdictions that have publically-owned treatment works (POTWs) and small businesses with water quality-based discharge permits) for adoption of the newly proposed test methods for *Escherichia coli* (*E. coli*) and enterococci in wastewater and found that all incremental costs results are negative (a cost savings) to regulated firms. The cost savings for the adoption of wastewater testing procedures are as follows.

The savings for facilities to shift from fecal coliform testing to *E. coli* Method 1603 will range from $36 million to $226 million. The savings to shift to *E. coli* Method 1103.1 will range from $35 million to $220 million. The savings for facilities to shift from fecal coliform testing to enterococci Method 1600 will range from approximately $36 million to $225 million. The savings to those currently employing *E. coli* Method 1103.1 and shifting to *E. coli* Method 1603 will range from approximately $0.9 million to $5.8 million, and those currently shifting from enterococci Method 1106.1 to enterococci Method 1600 will range from $7,000 to $48,000.

We continue to be interested in the potential impacts of the proposed rule on small entities and welcome comments on issues related to such impacts.

D. Unfunded Mandates Reform Act

Title II of the Unfunded Mandates Reform Act of 1995 (UMRA), Public Law 104–4, establishes requirements for Federal agencies to assess the effects of their regulatory actions on State, tribal, and local governments and the private sector. Under section 202 of the UMRA, EPA generally must prepare a written statement, including a cost-benefit analysis, for proposed and final rules with ''Federal mandates'' that may result in expenditures to State, local, and tribal governments, in the aggregate, or to the private sector, of $100 million or more in any one year. Before promulgating an EPA rule for which a written statement is needed, section 205 of the UMRA generally requires EPA to identify and consider a reasonable number of regulatory alternatives and adopt the least costly, most cost-effective or least burdensome alternative that achieves the objectives of the rule. The provisions of section 205 do not apply when they are inconsistent with applicable law. Moreover, section 205 allows EPA to adopt an alternative other than the least costly, most cost-effective or least burdensome alternative if the Administrator publishes with the final rule an explanation of why that alternative was not adopted.

Before EPA establishes any regulatory requirements that may significantly or uniquely affect small governments, including tribal governments, it must have developed under section 203 of the UMRA a small government agency plan. The plan must provide for the notification of potentially affected small governments, enabling officials of affected small governments to have meaningful and timely input in the development of EPA regulatory proposals with significant Federal

intergovernmental mandates, and informing, educating, and advising small governments on compliance with the regulatory requirements.

This rule contains no Federal mandates (under the regulatory provisions of Title II of UMRA) for State, local, or tribal governments or the private sector. The rule imposes no enforceable duty on any State, local, or tribal governments or the private sector. In fact, this rule should (on the whole) save money for governments and the private sector by increasing method flexibility, and allowing these entities to reduce monitoring costs by taking advantage of innovations. Thus, today's rule is not subject to the requirements of sections 202 and 205 of the UMRA.

EPA has determined that this rule contains no regulatory requirements that might significantly or uniquely affect small governments. This rule makes available testing procedures for *E. coli*, enterococci, fecal coliform, and *Salmonella* that may be used by a State, Territorial, Tribal or local authority for compliance with water quality standards (*E. coli*, enterococci) or sewage sludge (fecal coliforms, *Salmonella*) monitoring requirements when testing is otherwise required by these regulatory authorities. Thus, today's rule is not subject to the requirements of section 203 of UMRA.

E. Executive Order 13132: Federalism

Executive Order 13132, entitled ''Federalism'' (64 FR 43255, August 10, 1999), requires EPA to develop an accountable process to ensure ''meaningful and timely input by State and local officials in the development of regulatory policies that have federalism implications.'' ''Policies that have federalism implications'' is defined in the Executive Order to include regulations that have ''substantial direct effects on the States, on the relationship between the National Government and the States, or on the distribution of power and responsibilities among the various levels of government.''

This proposed rule does not have federalism implications. It will not have substantial direct effects on the States, on the relationship between the National Government and the States, or on the distribution of power and responsibilities among the various levels of government, as specified in Executive Order 13132. This rule makes available testing procedures for *E. coli* and enterococci in wastewater, and for fecal coliforms and *Salmonella* in sewage sludge. There is no cost to State and local governments and the rule does not preempt State law. Thus, Executive Order 13132 does not apply to this rule.

In the spirit of Executive Order 13132, and consistent with EPA policy to promote communications between EPA and State and local governments, EPA specifically solicits comment on this proposed rule from State and local officials.

F. Executive Order 13175: Consultation and Coordination With Indian Tribal Governments

Executive Order 13175, entitled ''Consultation and Coordination with Indian Tribal Governments'' (65 FR 67249, November 9, 2000), requires EPA to develop an accountable process to ensure ''meaningful and timely input by tribal officials in the development of regulatory policies that have tribal implications.''

''Policies that have tribal implications'' is defined in the Executive Order to include regulations that have ''substantial direct effects on one or more Indian tribes, on the relationship between the Federal Government and the Indian tribes, or on the distribution of power and responsibilities between the Federal Government and the Indian tribes.''

This proposed rule does not have tribal implications. It will not have substantial direct effects on Tribal governments, on the relationship between the Federal Government and Indian tribes, or on the distribution of power and responsibilities between the Federal Government and Indian tribes, as specified in Executive Order 13175. This rule makes available testing procedures for *E. coli* and enterococci in wastewater, and for fecal coliforms and *Salmonella* in sewage sludge. The costs to Tribal governments will be minimal (in fact, governments may see a cost savings), and the rule does not preempt State law. Thus, Executive Order 13175 does not apply to this rule.

In the spirit of Executive Order 13175, and consistent with EPA policy to promote communications between EPA and Tribal governments, EPA specifically solicits comment on this proposed rule from Tribal officials.

G. Executive Order 13045: Protection of Children From Environmental Health Risks and Safety Risks

Executive Order 13045: ''Protection of Children from Environmental Health Risks and Safety Risks'' (62 FR 19885, April 23, 1997) applies to any rule that: (1) Is determined to be ''economically significant'' as defined under Executive Order 12866, and (2) concerns an environmental health or safety risk that EPA has reason to believe may have a disproportionate effect on children. If the regulatory action meets both criteria, the Agency must evaluate the environmental health or safety effects of the planned rule on children, and explain why the planned regulation is preferable to other potentially effective and reasonably feasible alternatives considered by the Agency.

This proposed rule is not subject to the Executive Order because it is not economically significant as defined in Executive Order 12866, and because the Agency does not have reason to believe the environmental health or safety risks addressed by this action present a disproportionate risk to children. This action makes available testing procedures for *E. coli* and enterococci in wastewater, and for fecal coliforms and *Salmonella* in sewage sludge.

H. Executive Order 13211: Actions That Significantly Affect Energy Supply, Distribution, or Use

This rule is not subject to Executive Order 13211, ''Actions Concerning Regulations That Significantly Affect Energy Supply, Distribution, or Use'' (66 FR 28355 (May 22, 2001)) because it is not a significant regulatory action under Executive Order 12866.

I. National Technology Transfer and Advancement Act

Section 12(d) of the National Technology Transfer and Advancement Act of 1995, (''NTTAA''), Public Law 104–113, section 12(d) (15 U.S.C. 272 note), directs EPA to use voluntary consensus standards in its regulatory activities unless to do so would be inconsistent with applicable law or otherwise impractical. Voluntary consensus standards are technical standards (*e.g.*, material specifications, test methods, sampling procedures, and business practices) that are developed or adopted by voluntary consensus standard bodies. The NTTAA directs EPA to provide Congress, through the OMB, explanations when the Agency decides not to use available and applicable voluntary consensus standards.

This proposed rulemaking involves technical standards. Therefore, the Agency conducted a search to identify potentially applicable voluntary consensus standards. EPA's search of the technical literature revealed several consensus methods appropriate for enumerating *E. coli* and enterococci in wastewaters. Accordingly, methods for *E. coli* and enterococci published by Standard Methods for the Examination of Water and Wastewater, ASTM, and AOAC are included in this proposal and are listed in Table 1A at the end of this notice. No voluntary consensus standards were found for fecal coliforms

or *Salmonella* in sewage sludge. EPA welcomes comments on this aspect of the proposed rulemaking and, specifically, invites the public to identify potentially applicable voluntary consensus standards for enumerating *E. coli* or enterococci in wastewaters, and fecal coliforms and *Salmonella* in sewage sludge, and to explain why such standards should be used in this regulation.

IV. References

IDEXX. 1999a. Description of Colilert®, Colilert-18®, Quanti-Tray®, Quanti-Tray®/2000, Enterolert™ methods are available from IDEXX Laboratories, Inc., One Idexx Drive, Westbrook, Maine 04092.

IDEXX. 1999b. ''Quanti-Tray®: A Simple Method for Quantitation of Bacterial Density in Liquid Samples.''

IDEXX. 1999c. ''Quanti-Tray/2000®: Detection and Enumeration of Bacteria from High Bacterial Density Liquid Samples Without Dilution.''

USEPA. 2004a. Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC). December 2004. U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–04–025.

USEPA. 2004b. Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI). December 2004. U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–04–023.

USEPA. 2004c. Method 1680: Fecal Coliforms in Sewage Sludge by Multiple-Tube Fermentation Using Lauryl-Tryptose E. coli (LT–EC) Broth. December 2004. U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–04–026.

USEPA. 2004d. Method 1681: Fecal Coliforms in Sewage Sludge by Multiple-Tube Fermentation Using A–1 Broth. December 2004. U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–04–027.

USEPA. 2004e. Method 1682: *Salmonella* in Sewage Sludge by Multiple-Tube Fermentation Using Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium. December 2004. U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–04–028.

USEPA. 2004f. Results of the Interlaboratory Validation of EPA Method 1103.1 (mTEC) for *E. coli* in Wastewater Effluent. December 2004. U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–04–02.

USEPA 2004g. Results of the Interlaboratory Validation of EPA Method 1106.1 (mE–EIA) for *E. coli* in Wastewater Effluent. December 2004. U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–04–02.

USEPA. 2004h. Assessment of the Effects of Holding Time on Fecal Coliform and Salmonella Concentrations in Biosolids. December 2004. U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–04–029.

List of Subjects in 40 CFR Part 136

Environmental protection, Incorporation by reference, Reporting and recordkeeping requirements, Water pollution control.

Dated: August 10, 2005.

Stephen L. Johnson,

Administrator.

For the reasons set out in the preamble, title 40, chapter I of the Code of Federal Regulations, is proposed to be amended as follows:

PART 136—GUIDELINES ESTABLISHING TEST PROCEDURES FOR THE ANALYSIS OF POLLUTANTS

1. The authority citation for part 136 continues to read as follows:

Authority: Secs. 301, 304(h), 307, and 501(a) Pub. L. 95–217, 91 Stat. 1566, *et seq.* (33 U.S.C. 1251, *et seq.*) (The Federal Water Pollution Control Act Amendments of 1972 as amended by the Clean Water Act of 1977.)

2. Section 136.3 is amended as follows:

a. In paragraph (a) by revising Table IA.

b. In paragraph (a) by adding Table IG after the footnotes of Table IF.

c. In paragraph (b) by revising references 54, 55, 56 and 59, and adding references 63 through 65.

d. In paragraph (e) by revising the entry for Table IA and adding an entry for Table IG in Table II.

§ 136.3 Identification of test procedures.

(a) * * *

TABLE IA.—LIST OF APPROVED BIOLOGICAL METHODS

Parameter and units	Method [1]	EPA	Standard methods 18th, 19th, 20th ed. [4]	Standard methods on-line [4]	AOAC, ASTM, USGS	Other
Bacteria: 1. Coliform (fecal), number per 100 mL.	Multiple Tube Fermentation (MTF), 5 tube 3 dilution, or.	p. 132 [3], 1680 [22 24], 1681 [23 24].	9221C E	9221C E–99	.....	
	Membrane filter (MF) [2], single step.	p. 124 [3]	9222D	9222D–97	B–0050–85 [5]	
2. Coliform (fecal) in presence of chlorine, number per 100 mL.	MTF, 5 tube, 3 dilution, or.	p. 132 [3]	9221C E	9221C E–99	.....	
	MF [12 16] single step [6]	p. 124 [3]	9222D	9222D–97	.....	
3. Coliform (total), number per 100 mL.	MTF, 5 tube, 3 dilution, or.	p. 114 [3]	9221B	9221B–99	.....	
	MF [2], single step or two step.	p. 108 [3]	9222B	9222B–97	B–0025–85 [5]	
4. Coliform (total), in presence of chlorine, number per 100 mL.	MTF, 5 tube, 3 dilution, or MF [2] with enrichment.	p. 114 [3], p. 111 [3]	9221B, 9222(B+B.5c).	9221B–99, 9222(B+B.5c)–97.	.....	
5. *E. coli,* number per 100 mL.	MTF, multiple tube/ multiple well,.	.....	9223B [12]	9223B–97 [12]	991.15 [11]	Colilert® [12 14], Colilert-18® [12 13 14]
	MF [2 6 7 8 9], single step	1603 [16 25]	.....	.....	.....	
6. Fecal streptococci, number per 100 mL.	MTF, 5 tube, 3 dilution,	p. 139 [3]	9230B	9230B–93	.....	
	MF [2], or	p. 136 [3]	9230C	9230C–93	B–0055–85 [5]	
	Plate count	p. 143 [3]	.....	.....	.....	
7. Enterococci, number per 100 mL.	MTF, multiple tube/ multiple well.	.....	.....	.....	D6503–99 [10]	Enterolert® [12 17]

TABLE IA.—LIST OF APPROVED BIOLOGICAL METHODS—Continued

Parameter and units	Method [1]	EPA	Standard methods 18th, 19th, 20th ed. [4]	Standard methods on-line [4]	AOAC, ASTM, USGS	Other
	MF [2 6 7 8 9] single step ..	1600 [18 25]	.....	.....	.....	
8. Salmonella, number per 100 mL.	MTF multiple tube	1682 [24 26]	.....	.....	.....	
Aquatic Toxicity:						
9. Toxicity, acute, fresh water organisms, LC50, percent effluent.	Ceriodaphnia dubia acute.	2002.0 [19]	.....	.....	.....	
	Daphnia puplex and Daphnia magna acute.	2021.0 [19]	.....	.....	.....	
	Fathead Minnow, Pimephales promelas, and Bannerfin shiner, Cyprinella leedsi, acute.	2000.0 [19]	.....	.....	.....	
	Rainbow Trout, Oncorhynchus mykiss, and brook trout, Salvelinus fontinalis, acute.	2019.0 [19]	.....	.....	.....	
10. Toxicity, acute, estuarine and marine organisms of the Atlantic Ocean and Gulf of Mexico, LC50, percent effluent.	Mysid, Mysidopsis bahia, acute.	2007.0 [19]	.....	.....	.....	
	Sheepshead Minnow, Cyprinodon variegatus, acute.	2004.0 [19]	.....	.....	.....	
	Silverside, Menidia beryllina, Menidia menidia, and Menidia peninsulae, acute.	2006.0 [19]	.....	.....	.....	
11. Toxicity, chronic, fresh water organisms, NOEC or IC25, percent effluent.	Fathead minnow, Pimephales promelas, larval survival and growth.	1000.0 [20]	.....	.....	.....	
	Fathead minnow, Pimephales promelas, embryo-larval survival and teratogenicity.	1001.0 [20]	.....	.....	.....	
	Daphnia, Ceriodaphnia dubia, survival and reproduction.	1002.0 [20]	.....	.....	.....	
	Green alga, Selenastrum capricornutum, growth.	1003.0 [20]	.....	.....	.....	
12. Toxicity, chronic, estuarine and marine organisms of the Atlantic Ocean and Gulf of Mexico, NOEC or IC25, percent effluent.	Sheepshead minnow, Cyprinodon variegatus, larval survival and growth.	1004.0 [21]	.....	.....	.....	
	Sheepshead minnow, Cyprinodon variegatus, embryo-larval survival and teratogenicity.	1005.0 [21]	.....	.....	.....	
	Inland silverside, Menidia beryllina, larval survival and growth.	1006.0 [21]	.....	.....	.....	

TABLE IA.—LIST OF APPROVED BIOLOGICAL METHODS—Continued

Parameter and units	Method [1]	EPA	Standard methods 18th, 19th, 20th ed.[4]	Standard methods on-line [4]	AOAC, ASTM, USGS	Other
	Mysid, Mysidopsis bahia, survival, growth, and fecundity.	1007.0 [21]	.....	.....	.....	
	Sea urchin, Arbacia punctulata, fertilization.	1008.0 [21]	.....	.....	.....	

[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, OH. 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, DC *http://www.standardmethods.org*.

[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, VA.

[6] Because the MF technique usually yields low and variable recovery from chlorinated wastewaters, the Multiple Tube Fermentation 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, MD 20877–2417.

[12] 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*.

[13] 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.

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

[15] Subject total coliform positive samples determined by 9222B or other membrane filter procedure to 9222G using NA–MUG media.

[16] USEPA. 2004. 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, DC EPA–821–R–04–025.

[17] A description of the Enterolert® test may be obtained from IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, ME 04092.

[18] USEPA. 2004. 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–04–023.

[19] USEPA. October 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fifth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA/821/R–02/012.

[20] USEPA. October 2002. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Fourth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA/821/R–02/013.

[21] USEPA. October 2002. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Third Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA/821/R–02/014.

[22] USEPA. December 2004. Method 1680: Fecal Coliforms in Sewage Sludge by Multiple-Tube Fermentation Using Lauryl-Tryptose *E. coli* (LTEC) Broth. December 2004. U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–R–04–026.

[23] USEPA. December 2004. Method 1681: Fecal Coliforms in Sewage Sludge by Multiple-Tube Fermentation Using A–1 Broth. December 2004. U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–R–04–027.

[24] Recommended for enumeration of target organism in sewage sludge.

[25] Recommended for enumeration of target organism in wastewater effluent.

[26] USEPA. December 2004. Method 1682: *Salmonella* in Sewage Sludge by Multiple-Tube Fermentation Using Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium December 2004. U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–R–04–028.

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TABLE IG.—LIST OF APPROVED MICROBIOLOGICAL METHODS FOR AMBIENT WATER

Parameter and units	Method [1]	EPA	Standard methods 18th, 19th, 20th ed.[4]	Standard methods on-line [4]	AOAC, ASTM, USGS	Other
Bacteria:	MTF [6 8 14] multiple tube	.....	9221B.1 / 9221F [11 13].	9221B.1 / 9221F–g599 [11 13].		
1. E. coli, number per 100 mL	multiple tube/multiple well.	.....	9223B [12]	9223B–97 [12]	991.15 [10]	Colilert® [12 16] Colilert-18® [12 15 16]
	MF [2 5 6 7 8], two step	1103.1 [19]	9222B / 9222G [18], 9213D.	9222B / 9222G–97 [18].	D5392–93 [9] ..	

TABLE IG.—LIST OF APPROVED MICROBIOLOGICAL METHODS FOR AMBIENT WATER—Continued

Parameter and units	Method [1]	EPA	Standard methods 18th, 19th, 20th ed.[4]	Standard methods on-line [4]	AOAC, ASTM, USGS	Other
	single step	1603 [20], 1604 [21].				mColiBlue-24 [17]
7. Enterococci, number per 100 mL	MTF [6 8] multiple tube		9230B	9230B–93		Entero-lert® [12 22]
	multiple tube/multiple well.				D6503–99 [9].	
	MF [2 5 6 7 8] two step,	1106.1 [23]	9230C	9230C–93	D5259–92 [9]	
	single step, or Plate count.	1600 [24], p. 143 [3].				
Protozoa:						
8. Cryptosporidium	Filtration/IMS/FA	1622 [25], 1623 [26].				
9. Giardia	Filtration/IMS/FA	1623 [26]				

[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, OH. 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, DC *http://www.standardmethods.org*

[5] Because the MF technique usually yields low and variable recovery from chlorinated wastewaters, the Multiple Tube Fermentation method will be required to resolve any controversies.

[6] 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.

[7] 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.

[8] 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.

[9] 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.

[10] 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, MD 20877–2417.

[11] 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.

[12] 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.*

[13] 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.

[14] 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.

[15] 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.

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

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

[18] Subject total coliform positive samples determined by 9222B or other membrane filter procedure to 9222G using NA–MUG media.

[19] USEPA. 2004. 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, DC EPA–821–R–04–024.

[20] USEPA. 2004. 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, DC EPA–821–R–04–025.

[21] 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.

[22] A description of the Enterolert® test may be obtained from IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, ME 04092.

[23] USEPA. 2004. 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–04–022.

[24] USEPA. 2004. 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–04–023.

[25] Method 1622 uses filtration, concentration, immunomagnetic separation of oocysts from captured material, immunofluorescence assay to determine concentrations, and confirmation through vital dye staining and differential interference contrast microscopy for the detection of *Cryptosporidium.* USEPA. 2001. Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA. U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA–821–R–01–026.

[26] Method 1623 uses filtration, concentration, immunomagnetic separation of oocysts and cysts from captured material, immunofluorescence assay to determine concentrations, and confirmation through vital dye staining and differential interference contrast microscopy for the simultaneous detection of *Cryptosporidium* and *Giardia* oocysts and cysts. USEPA. 2001. Method 1623. *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA. U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA–821–R–01–025.

(b) * * *

REFERENCES, SOURCES, COSTS, AND TABLE CITATIONS:

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(54) USEPA. 2004. 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, DC December 2004, EPA–821–R–04–024. Table IG, Note 19.

(55) USEPA. 2004. Method 1106.1: Method 1600: Enterococci in Water by Membrane Filtration using membrane-Enterococcus-Esculin Iron Agar (mE-EIA). December 2004. U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–R–04–022. Table IG, Note 23.

(56) USEPA. 2004. 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, DC December 2004, EPA–821–R–04–025. Table IA, Note 16, and Table IG, Note 20.

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(59) USEPA. 2004. Method 1600: Enterococci in Water by Membrane Filtration using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI). December 2004. U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA–821–R–04–023. Table IA, Note 18, and Table IG, Note 24.

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(63) USEPA. 2004. Method 1680: Fecal Coliforms in Sewage Sludge by Multiple-Tube Fermentation Using Lauryl-Tryptose E. coli (LT–EC) Broth. December 2004. U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–R–04–026. Table IA, Note 22.

(64) USEPA. 2004. Method 1681: Fecal Coliforms in Sewage Sludge by Multiple-Tube Fermentation Using A–1 Broth. December 2004. U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–R–04–027. Table IA, Note 23.

(65) USEPA. 2004. Method 1682: *Salmonella* in Sewage Sludge by Multiple-Tube Fermentation Using Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium. December 2004. U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–R–04–028. Table IA, Note 26.

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(e) * * *

TABLE II.—REQUIRED CONTAINERS, PRESERVATION TECHNIQUES, AND HOLDING TIMES

Parameter No./name	Container [1]	Preservation [2 3 17]	Maximum holding time [4 17]
Tables IA, IG—Bacteria Tests:			
1–5 Coliform, total, fecal, and E. coli	PP,G	Cool, < 10 °C [18] 0.0008% $Na_2S_2O_3$ [5 18]	6 hours [19], 24 hours [20]
6 Fecal streptococci	PP,G	Cool, < 10 °C, 0.0008% $Na_2S_2O_3$ [5]	6 hours [19]
7 Enterococci	PP,G	Cool, < 10 °C, 0.0008% $Na_2S_2O_3$ [5]	6 hours [19]
8 Salmonella	PP,G	Cool, < 10 °C [18]	6[19] or 24 hours [21]
Table IG—Protozoa Tests:			
9 Cryptosporidium	LDPE	0–8 °C	96 hours [17]
10 Giardia	LDPE	0–8 °C	96 hours [17]

* * * * * * *

[1] Polyethylene (P) or glass (G). For microbiology, plastic sample containers must be made of sterilizable materials (polypropylene or other autoclavable plastic).

[2] Sample preservation should be performed immediately upon sample collection. For composite chemical samples each aliquot should be preserved at the time of collection. When use of an automated samples make it makes it impossible to preserve each aliquot, then chemical samples may be preserved by maintaining at 4°C until compositing and sample splitting is completed.

[3] When any sample is to be shipped by common carrier or sent through the United States Mails, it must comply with the Department of Transportation Hazardous Materials Regulations (49 CFR part 172). The person offering such material for transportation is responsible for ensuring such compliance. For the preservation requirements of Table II, the Office of Hazardous Materials, Transportation Bureau, Department of Transportation, has determined that the Hazardous Materials Regulations do not apply to the following materials: Hydrochloric acid (HCl) in water solutions at concentrations of 0.04% by weight or less (pH about 1.96 or greater); Nitric acid (HNO_3) in water solutions at concentrations of 0.15% by weight or less (pH about 1.62 or greater); Sulfuric acid (H_2SO_4) in water solutions at concentrations of 0.35% by weight or less (pH about 1.15 or greater); and Sodium hydroxide (NaOH) in water solutions at concentrations of 0.080% by weight or less (pH about 12.30 or less).

[4] Samples should be analyzed as soon as possible after collection. The times listed are the maximum times that samples may be held before analysis and still be considered valid. Samples may be held for longer periods only if the permittee, or monitoring laboratory, has data on file to show that for the specific types of samples under study, the analytes are stable for the longer time, and has received a variance from the Regional Administrator under § 136.3(e). Some samples may not be stable for the maximum time period given in the table. A permittee, or monitoring laboratory, is obligated to hold the sample for a shorter time if knowledge exists to show that this is necessary to maintain sample stability. See § 136.3(e) for details. The term ''analyze immediately'' usually means within 15 minutes or less of sample collection.

[5] Should only be used in presence of residual chlorine.

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[17] Holding time is calculated from time of sample collection to elution for samples shipped to the laboratory in bulk and calculated from the time of sample filtration to elution for samples filtered in the field.

[18] Sewage sludge samples collected for fecal coliform and *Salmonella* analysis do not require the addition of 0.0008% $Na_2S_2O_3$.

[19] Holding time for bacterial tests is 6 hours for transport of the sample to the laboratory, and an additional 2 hours to process the sample in the laboratory.

[20] An extended holding time of 24 hours is limited to sewage sludge Class A composted samples to be analyzed for fecal coliforms using either EPA Method 1680 (LTB/EC) or EPA Method 1681 (A–1) and Class B aerobically digested samples using EPA Method 1681 (A–1) only. Initial analysis of the sample in the laboratory must commence within 24 hours of sample collection.

[21] An extended holding time of 24 hours is limited to sewage sludge Class A composted samples to be analyzed for *Salmonella* using EPA Method 1682 (MSRV) only. Initial analysis of the sample in the laboratory must commence within 24 hours of sample collection.

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