

STANDARD OPERATING PROCEDURE

For SM 9222B

Standard Total Coliform Membrane Filtration Procedure

SOP #: SM 9222B

SOP REVISION #: 3.0

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LIST OF REVISIONS

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0	August 2000	None	
1.0	November 2001	Numerous edits	Throughout document
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2.2	August 2005	Control Document statement added Section 11.8 added – coliform identification procedure using MICRO-ID Table 2 added – QC elements, acceptance criteria, and corrective actions	Cover Page 11 15
2.3	December 2006	Replaced old DEP Logo with state seal + MassDEP	Title page & header
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2.5	January 2010	Replaced EC-MUG method with SM9222G for <i>E. coli</i> confirmation Reference 16.2 added	5 & 11 13
3.0	September 2010	Revised SOP to limit application of method to testing finished drinking water and raw drinking water sources – no longer used to test non-potable water or wastewater Section 6.9 added – new UV light sterilizer. Section 11.9 – Referred coliform identification procedure using MICRO-ID to separate SOP. Section 12.5 – Revised duplicate range of logs calculation. Tables 1 and 2 revised – Removed non-potable water elements. Other minor edits	Throughout document 7 11 12 14-15 Throughout document



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1.0 SCOPE & APPLICATION

- 1.1 The total coliform membrane filtration technique is a highly reproducible method for the enumeration of total coliform bacteria in relatively large water sample volumes (i.e., assuming that the samples are not highly turbid and/or contain large amounts of non-coliform background bacteria).
- 1.2 The total coliform membrane filtration method may be used to test a 100-mL sample volume of potable water for the enumeration or detection (presence/absence) of total coliforms in compliance with the National Primary Drinking Water Standards - Total Coliform Rule (TCR). The method can also be used to enumerate total coliforms in raw drinking water sources. In our laboratory, this method is not used to enumerate total coliforms in non-potable water or wastewater.

2.0 SUMMARY OF METHOD

- 2.1 A well-mixed sample is drawn through a 0.45- μ m membrane filter which is then placed on a Petri dish containing m-Endo LES agar, incubated for 22 - 24 hours at $35 \pm 0.5^{\circ}\text{C}$, and observed for red colonies producing a golden metallic surface sheen, which are considered presumptive total coliform colonies.
- 2.2 Presumptive total coliform colonies are first tested using method SM9222G to determine if they are *E. coli* (i.e., must follow-up with this test). If colonies are confirmed as *E. coli*, no further testing is necessary – i.e., the sample is total coliform and *E. coli* positive. If colonies do not confirm as *E. coli*, they are subjected to the coliform confirmation procedure in Section 2.3.
- 2.3 Presumptive total coliform colonies are inoculated into lauryl tryptose broth (LTB) and brilliant green lactose bile broth (BGLBB) for coliform confirmation.

3.0 DEFINITIONS

- 3.1 When using the membrane filtration method, total coliforms are defined as Gram-negative, rod-shaped, facultative anaerobic, non-spore-forming bacteria that produce red colonies with a golden metallic surface sheen within 24 hours at 35°C on an Endo-type medium containing lactose.
- 3.2 The sheen may cover the entire colony or may appear only in a central area or on the periphery. The coliform group thus defined is based on the production of aldehydes from fermentation of lactose. While this biochemical characteristic is part of the metabolic pathway of gas production in the multiple-tube test, some variations in degree of metallic sheen development may be observed among coliform strains.

4.0 INTERFERENCES

- 4.1 The accuracy of the membrane filtration method is decreased when samples are highly turbid or contain high background (non-coliform) bacteria, both of which may interfere with detection of sheen colonies thereby masking the presence of coliforms.

5.0 SAFETY

- 5.1 m-Endo LES agar contains basic fuchsin that is a suspected carcinogen. Precautions must be taken to avoid respiratory and skin contact with this medium.



- 5.2 Samples (and positive controls) may contain microorganisms that are pathogenic to humans. All precautions are to be taken to minimize exposure. All laboratory personnel must wear laboratory coats, safety glasses, and protective gloves while working in the laboratory. All personnel must be immunized against the hepatitis A and B viruses, and must receive on-the-job laboratory safety training.

6.0 EQUIPMENT AND SUPPLIES

- 6.1 Sample bottles: Sterile wide-mouthed borosilicate glass or plastic. For sampling chlorinated drinking water, a 120-mL sample bottle must contain 0.1 mL of a 3% solution of sodium thiosulfate. For potable water samples, the WES Microbiology Laboratory uses sterile 120-mL Corning® disposable wide-mouthed plastic ready-to-use bottles containing a sodium thiosulfate tablet (note: provides a final concentration of 100 mg/L $\text{Na}_2\text{S}_2\text{O}_3$).
- 6.2 Dilution bottles: Sterile bottles of the appropriate size to prepare positive control cultures to run with each sample batch.
- 6.3 Containers for culture media: Use sterile borosilicate glass flasks or beakers for preparation of media.
- 6.3.1 Culture dishes: Use pre-sterilized 50- x 9-mm plastic Petri dishes with tight-fitting lids. Reseal opened packages of disposable dishes for storage.
- 6.3.2 Culture tubes: Use screw-capped borosilicate culture tubes that are large enough to contain an inverted fermentation tube.
- 6.4 Filtration units: The WES Microbiology Laboratory has two stainless steel 6-funnel filtration manifolds and stainless steel as well as plastic filtration funnels. Discard plastic funnels with deep scratches on the inner surface.

Stainless steel funnel assemblies are placed on a stainless steel perforated rack for sterilization in the autoclave (15 minutes). The stainless steel funnels are placed in a UV light sterilizer (see Section 6.9) for storage. The plastic funnels are wrapped, typically in a clear plastic autoclavable instrument bag, and autoclaved prior to use.

Filtration funnels are sterilized by autoclaving for the initial filtration of a sample series. Following the filtration of a sample, the funnel(s) are placed in a UV light sterilizer and exposed to UV light for 3 minutes before re-using units between successive filtration series.

The filtration manifold is connected to two glass flasks in series with an electric vacuum pump securing a pressure differential of 138 to 207 kPa. The flask closest to the vacuum pump is for the protection of the pump from carryover water and must not be allowed to fill with water.

- 6.5 Membrane filters: Use 0.45- μm pore size, 47-mm diameter pre-sterilized, gridded membrane filters. Use only those filter membranes that have been found, through adequate quality control testing and certification by the manufacturer, to exhibit full retention of the organisms to be cultivated, stability in use, freedom from chemical extractables that may inhibit bacterial growth and development, a satisfactory speed of filtration (within 5 min), no significant influence on medium pH (beyond ± 0.2 units), and no increase in number of confluent colonies or spreaders compared to control membrane filters. Use membranes grid-marked in such a manner that bacterial growth is neither inhibited nor stimulated along the grid lines when the membranes with entrapped bacteria are incubated on a suitable medium. Preferably, use fresh stocks of membrane filters and, if necessary, store them in an environment without extremes of temperature and humidity.



- 6.6 Forceps: Smooth flat forceps, without corrugations on the inner sides of the tips. Sterilize before use by dipping in 95% ethyl or absolute methyl alcohol and flaming.
- 6.7 Incubator: Use incubator that provides a temperature of $35 \pm 0.5^{\circ}\text{C}$ and maintains a humid environment (60% relative humidity).
- 6.8 Microscope and light source: To determine colony counts on membrane filters, use a stereoscopic microscope at a magnification of 10-15x and a cool white fluorescent light source adjusted to give maximum sheen discernment. Do not use a microscope illuminator with optical system for light concentration from an incandescent light source for discerning coliform colonies on Endo-type media.
- 6.9 UV Light Sterilizer: Millipore Catalog # XX6370000.

7.0 MEDIA AND REAGENTS

- 7.1 Preparation of m-Endo LES Agar: Use commercial dehydrated media. Follow manufacturer's directions on the bottle for re-hydration. Store opened supplies of dehydrated media in a desiccator for up to one year.

Yeast extract	1.2 g
Casitone or trypticase	3.7 g
Thiopeptone or thiotone	3.7 g
Tryptose	7.5 g
Lactose	9.4 g
Dipotassium hydrogen phosphate, K_2HPO_4	3.3 g
Potassium dihydrogen phosphate, KH_2PO_4	1.0 g
Sodium chloride, NaCl	3.7 g
Sodium desoxycholate	0.1 g
Sodium lauryl sulfate	0.05 g
Sodium sulfite, Na_2SO_3	1.6 g
Basic fuchsin	0.8 g
Agar	15.0 g
Reagent-grade water	1 L
Ethanol, 95%	20 mL

- 7.1.1 In a sterile 2-L Erlenmeyer flask, re-hydrate 51 grams of commercial dehydrated product in 1 L of ASTM Type I reagent water containing 20 mL of 95% ethanol. Do not use denatured ethanol, which reduces background growth and coliform colony size. Bring to a near boil on a hot plate with constant stirring to dissolve agar, then promptly remove from heat and cool to 45 to 50°C . Do not sterilize by autoclaving. Final pH must be 7.2 ± 0.2 .
- 7.1.2 Using an auto-pipettor, dispense in 5- to 7-mL quantities into lower section of 50-mm plastic Petri dishes. Do not expose poured plates to direct sunlight; refrigerate in a lidded plastic container containing a card labeled with the name of the medium, stamped with the date of preparation, and labeled with the 14-day discard date. Discard unused medium after 2 weeks or sooner if there is evidence of moisture loss, medium contamination, or medium deterioration (darkening of the medium).



7.2 Lauryl Tryptose Broth

Tryptose	20 g
Lactose	5.0 g
Dipotassium hydrogen phosphate, K_2HPO_4	2.75 g
Potassium dihydrogen phosphate, KH_2PO_4	2.75 g
Sodium chloride, NaCl	5.0 g
Sodium lauryl sulfate	0.1 g
Reagent-grade water	1 L

- 7.2.1 In a 2-L Erlenmeyer flask, add 35.6 grams of commercial dehydrated product to 1 L of ASTM Type I reagent water, mix thoroughly and heat (using a hot plate with constant stirring) to dissolve. Dispense sufficient medium in fermentation tubes with an inverted vial to cover inverted vial at least $\frac{1}{2}$ to $\frac{2}{3}$ after sterilization. Close tubes loosely with heat-resistant plastic caps and autoclave broth at 121°C for 15 minutes. Remove tubes from the autoclave and allow them to cool. Discard any tubes with air bubbles in the fermentation tube. The pH of the medium after sterilization must be 6.8 ± 0.2 . Tighten screw caps and store medium at 4°C for no longer than three months. Tubes where evaporation exceeds 10% of the original volume are discarded.

7.3 Brilliant Green Lactose Bile Broth

Peptone	10 g
Lactose	10 g
Oxgall	20 g
Brilliant Green	0.0133 g
Reagent-grade water	1 L

- 7.3.1 In a 2-L Erlenmeyer flask, add 40 grams of commercial dehydrated product to 1 L of ASTM Type I reagent water, mix thoroughly and heat (using a hot plate with constant stirring) to dissolve medium. Dispense sufficient medium in fermentation tubes with an inverted vial to cover inverted vial at least $\frac{1}{2}$ to $\frac{2}{3}$ after sterilization. Close tubes loosely with heat-resistant plastic caps and autoclave broth at 121°C for 15 minutes. Remove tubes from the autoclave and allow them to cool. Discard any tubes with air bubbles in the fermentation tube. The pH of the medium after sterilization must be 7.2 ± 0.2 . Tighten screw caps and store medium at 4°C for no longer than three months. Tubes where evaporation exceeds 10% of the original volume are discarded.

7.4 Preparation of Buffered Rinse Water

- 7.4.1 To prepare stock phosphate buffer solution, dissolve 34.0 grams of potassium dihydrogen phosphate (KH_2PO_4), in 500 mL ASTM Type I reagent water, adjust to pH 7.2 ± 0.5 with 1 N sodium hydroxide (NaOH) and dilute to 1 L with ASTM Type I reagent water. Autoclave for 15 min @ 121°C or filter sterilize through a $0.22\ \mu\text{m}$ pore-size filter. Store solution at 4°C in a one-liter screw cap bottle. Discard solution if it becomes turbid.
- 7.4.2 To prepare magnesium chloride solution, dissolve 81.1 grams of magnesium chloride ($MgCl_2 \cdot 6\ H_2O$) in one liter of ASTM Type I reagent water. Autoclave for 15 min @ 121°C or filter sterilize through a $0.22\ \mu\text{m}$ pore-size filter. Store solution at 4°C in a one-liter screw cap bottle.
- 7.4.3 In a large carboy, add 95 mL of magnesium chloride solution and 24 mL of phosphate buffer solution to 5 gallons of ASTM Type I reagent water. Dispense the buffer from the



carboy into one-liter bottles. Place nine bottles of the buffer solution into the autoclave and autoclave at 121°C for 45 minutes.

7.4.4 Remove bottles of buffered water from the autoclave and store at room temperature.

7.4.5 A sterility check must be performed for each batch of buffered water prepared. Add 50 mL of buffered water and 50 mL of tryptic soy broth (TSB) (2x) to a sterile glass jar. Incubate for 48 hours at $35^{\circ} \pm 0.5^{\circ}\text{C}$, checking sterility at both 24 and 48 hours. Discard any batch of water showing turbidity or growth after incubation. Record the results of the sterility check in the autoclave log.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples must be aseptically collected in a sterile disposable plastic bottle leaving at least 1" (2.5 cm) of headspace to allow for sufficient mixing of the sample prior to analysis. If the sample is chlorinated, make sure that the appropriate concentration of sodium thiosulfate is added (see Section 6.1). If tap cleanliness is in question, apply a solution of sodium hypochlorite (100 mg NaOCl/L) to faucet before sampling.

8.2 Remove all attachments from the water tap (screens, etc.). Open tap and let run to waste for 2-3 minutes. Reduce the water flow to allow for filling of the bottle without splashing.

8.3 Keep sample bottle closed until it is to be filled, fill the bottle without rinsing, replace cap immediately, and secure the top with the attached plastic "lock".

8.4 Raw potable water source samples must be kept at 1 - 8°C from the time of collection to the time of analysis. Although not required, it is a good practice to also keep finished drinking water at 1 - 8°C from the time of collection to the time of analysis.

8.5 Potable water samples must be analyzed as soon as possible but no longer than 30 hours after collection. Raw potable water source samples must be analyzed within 8 hours of collection (i.e., maximum of 6 hours in the field and two hours in the laboratory).

8.6 All samples must be accompanied by a WES *Sample Tracking /Chain-of-Custody Form* filled out by the collector.

9.0 QUALITY CONTROL

9.1 Test each new lot of medium upon receipt against a previously acceptable lot for satisfactory performance using known total coliform positive (i.e., *E. coli* and/or *Enterobacter aerogenes*) and negative (i.e., *Staphylococcus aureus* or *Pseudomonas* sp.) control cultures.

9.2 Test each new batch of medium against known total coliform positive (*E. coli* and/or *Enterobacter aerogenes*) and negative (*Staphylococcus aureus* or *Pseudomonas* sp.) control cultures. Also test medium sterility with a blank control.

9.3 Filter a sterile buffered rinse water sample at the beginning and end of each filtration series. Incubate the rinse water control membrane culture under the same conditions as the sample.

9.4 If enough potable water sample volume is collected, run one sample in duplicate for each batch of 10 or fewer samples.

9.5 Run a positive control culture organism (*E. coli*) for each filtration series.



- 9.6 A colony count comparison between at least 2 analysts must be performed on an m-Endo LES agar plate each month that samples are received by the laboratory for SM 9222B testing. The colony counts must be within 10% and results documented in the *WES Microbiology Laboratory's Monthly QC Form*.

10.0 CALIBRATION AND STANDARDIZATION

- 10.1 Each lot of graduated cylinders or sample containers used to measure sample volume must be checked to ensure that it meets a mass (grams) to measured volume (mL) ratio of one for reagent water at 4°C.
- 10.2 Refer to Laboratory Quality Assurance Plan for calibration and standardization procedures of laboratory equipment used for this analysis.

11.0 PROCEDURE

- 11.1 Sample Volume: For potable water samples, a volume of 100 mL must be used. If the sample is relatively turbid or is suspected of having high levels of background bacteria, the 100-mL volume may be divided among several filters (e.g., 5 filters with 20 mL filtered on each) as long as the total volume analyzed is 100 mL. For volumes of 20-100 mL, use a sterile graduated cylinder to measure sample volume. For sample volumes less than 20 mL, use a sterile wide-tip pipette.
- 11.2 For raw potable water source samples, the sample volume filtered will be based on the expected bacterial density (see Table 1).
- 11.2.1 Ideal sample volume will yield 20 to 80 coliform colonies and not more than 200 colonies of all types on a membrane filter. Filter three different volumes depending on the expected bacterial density. When less than 20 mL is to be filtered, add approximately 10 mL of sterile dilution water to the funnel before filtration or pipette the sample volume into a sterile dilution bottle and then filter the entire dilution. This increase in water volume aids in uniform dispersion of the bacterial suspension over the entire effective filtering surface.
- Note: Bacteria must not be suspended in any dilution water for more than 30 minutes at room temperature as death or multiplication may occur.**
- 11.3 Sterilize filtration units: Use sterile filtration units at the beginning of each filtration series as a minimum precaution to avoid accidental contamination. A filtration series is considered to be interrupted when an interval of 30 minutes or longer elapses between sample filtrations. After such interruption, treat any further sample filtration as a new filtration series and sterilize all funnels and bases in use.
- 11.3.1 Filtration units are to be sterilized in a UV light sterilizer for three minutes before use for a new sample and/or if a higher dilution of the same sample is to be filtered. **Note:** It is unnecessary to expose the funnel(s) to UV light between filtrations of dilutions of the same sample as long as the smallest dilution (volume) of the sample is filtered first and the funnel is rinsed thoroughly with sterile buffered water before proceeding to the next dilution.
- 11.4 Label Petri dishes: Label the bottom (side containing the agar) of each Petri dish with the: 1) LIMS sample number, and 2) dilution/volume filtered.



- 11.5 Filtration of sample: Using sterile forceps, place a sterile membrane filter (grid side up) over porous plate of filter base. Attach funnel to base and lock it in place. Shake sample vigorously at least 25 times. Pour appropriate volume of sample into the funnel. Turn on the vacuum and filter the sample completely. With filter still in place, rinse the interior surface of the funnel by filtering three 20- to 30-mL portions of sterile buffered rinse water. Rinsing between samples prevents carryover contamination. Turn off the vacuum, remove the funnel, and remove the membrane filter with sterile forceps (touching only within the outer 1/8" of the filter), and place it on the m-Endo agar surface with a rolling motion to avoid entrapment of air. Invert the Petri dish and incubate for 22 to 24 h at $35 \pm 0.5^{\circ}\text{C}$.

Note: Differentiation of some colonies may be lost if cultures are incubated beyond 24 h.

- 11.6 Counting: Using the low-power (10x to 15x) binocular dissecting microscope, with the cool white fluorescent light source directed to provide optimal viewing of sheen, count the typical coliform colonies and atypical colonies on the filter. Typical coliform colonies are pink to dark-red with a metallic surface sheen. The sheen area may vary in size from a small pinhead to complete coverage of the colony surface. Atypical coliform colonies can be dark red, mucoid, or nucleated without sheen. Generally, pink, blue, white, or colorless colonies lacking sheen are considered non-coliforms. The total count of colonies (coliform and non-coliform) on m-Endo agar has no consistent relationship to the total number of bacteria present in the original sample. A high count of non-coliform colonies may interfere with the maximum development of coliforms. Refrigerating cultures (after 22 h incubation) with high densities of non-coliform colonies for 0.5 to 1 h before counting may deter spread of confluence while aiding sheen discernment.

Samples of disinfected water may include stressed organisms that grow relatively slowly and produce maximum sheen in 22 to 24 h. Organisms from non-disinfected sources may produce sheen at 16 to 18 h, and the sheen subsequently may fade after 24 to 30 h.

- 11.7 *E. coli* confirmation: Test for *E. coli* all presumptive total coliform colonies using SM 9222G (Refer to SOP # SM 9222G). Colonies that confirm as *E. coli* are also confirmed as total coliforms, so no further confirmation is needed. If no colonies confirm positive for *E. coli*, then follow the coliform verification procedure in Section 11.8.
- 11.8 Coliform verification: Occasionally, typical sheen colonies may be produced by non-coliform organisms, and atypical colonies (dark red or nucleated colonies without sheen) may be coliforms. Verify all suspect colonies (i.e., all typical and atypical colony types) as described below.
- 11.8.1 Lactose fermentation: If *E. coli* is not confirmed by SM 9222G, swab the entire membrane with a sterile cotton swab and inoculate one test tube containing lauryl tryptose broth (LTB) and one test tube containing brilliant green lactose bile broth (BGLBB); incubate the LTB and BGLBB at $35 \pm 0.5^{\circ}\text{C}$ for 48 h. Gas formed in LTB and confirmed in BGLBB within 48 h verifies the colony as a coliform. If only the LTB tube from a particular colony produces gas, transfer growth from the positive tube to another BGLBB tube and retest.

Note: A single swab may be used to simultaneously inoculate more than one medium with a presumptive total coliform-positive culture. The inoculation order must be LTB and then BGLBB.

- 11.9 In special cases or if requested, biochemical tests can be performed to identify to genus and species those organisms that are confirmed as coliforms. The current assay used at WES is the Microbiological Identification System (MICRO-ID) produced by REMEL. This is a self-contained



testing system that employs qualitative biochemical procedures for the rapid differentiation of the Enterobacteriaceae (See SOP # Micro-ID).

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 For potable water samples, results are to be reported as Present/Absent unless quantification has been requested.

12.2 Quantification: Compute the count, using membrane filters with 20 to 80 coliform colonies and not more than 200 colonies of all types per membrane, by the following equation:

$$\text{Total coliforms} / 100 \text{ mL} = \frac{\text{coliform colonies counted} \times 100}{\text{mL sample filtered}}$$

12.3 Out-of-Range Plates: If no filter has a coliform count falling in the ideal range, total the coliform counts on all filters and report as number per 100 mL. For example, if duplicate 50-mL portions were examined and the two membranes had five and three coliform colonies, respectively, report the count as eight coliform colonies per 100 mL.

12.4 Confluent growth: If confluent growth occurs, covering either the entire filtration area of the membrane or a portion thereof, and colonies are not discrete, report results as "confluent growth with (or without) coliforms." If the total number of bacterial colonies (i.e., coliforms plus non-coliforms) exceeds 200 per membrane or if the colonies are not distinct enough for accurate counting, report the results as "too numerous to count" (TNTC) or "confluent," respectively. For drinking water, the presence of coliforms in such cultures showing no sheen may be confirmed by swabbing the entire surface of the filter and inoculating a tube of BGLBB. If gas is produced from the BGLBB tube within 48 h at $35 \pm 0.5^\circ\text{C}$, coliforms are present. For compliance with the EPA Total Coliform Rule, report confluent growth or TNTC with at least one detectable coliform colony (which is verified) as a total coliform positive sample. Report confluent growth or TNTC without detectable coliforms as invalid. For invalid samples, request a new sample from the same location within 24 h and select more appropriate volumes to be filtered per membrane, observing the requirement that the standard drinking water portion is 100 mL, or choose another coliform method that is less subject to heterotrophic bacterial interferences. Thus, to reduce interference from overcrowding, instead of filtering 100 mL per membrane, filter 50-mL portions through two separate membranes, 25-mL portions through each of four membranes, etc. Total the coliform counts observed on all membranes and report as number per 100 mL.

12.5 Calculation of Precision QC Criterion: For raw potable water source samples or potable water samples where enough volume is collected and enumeration is requested, determine the range of logs for the duplicate colony counts as follows (*Standard Methods for the Examination of Water and Wastewater*, 1998, Page 9-10):

Range of Logs for a Duplicate Set = Log (Count 1) - Log (Count 2)

If either result of a duplicate set is < 1, add 1 to both values before calculating the logarithms as follows:

Range of Logs for a Duplicate Set = Log [(Count 1) + 1] - Log [(Count 2) + 1]

Precision QC Criterion = 3.27 (Mean Range of Logs for 15 Most Recent Duplicate Sets)



13.0 METHOD PERFORMANCE

- 13.1 The detection limit of this method is one colony-forming unit per sample volume or dilution tested.

14.0 POLLUTION PREVENTION

- 14.1 Refer to the WES Environmental Management System (EMS) policy and SOPs regarding pollution prevention.
- 14.2 The quantity of media and reagents purchased should be based on expected usage during its shelf life. Actual media and reagent preparation volumes should reflect anticipated usage and stability.

15.0 WASTE MANAGEMENT

- 15.1 WES laboratories fully comply with all applicable federal, state, and local environmental regulations. WES is also committed to protecting the air, water, and land by minimizing and controlling all chemical releases from fume hoods, biological safety cabinets, and bench operations. Refer to the WES EMS policy and SOPs regarding waste management.
- 15.2 All positive sample plates, including filter positive control plates, are placed in autoclave bags and autoclaved at 121°C for a minimum of 30 minutes. Decontaminated plastic Petri plates are separated from agar for recycling; agar waste is disposed to the WES dumpster.

16.0 REFERENCES

- 16.1 *Standard Methods for the Examination of Water and Wastewater*, 20th Edition, 1998. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.
- 16.2 *Standard Methods for the Examination of Water and Wastewater*, 21st Edition, 2004. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.



17.0 TABLES

TABLE 1. Recommended Filtration Volumes by Sample Type

Sample Type	Recommended Filtration Volume (mL)							
	100	50	10	1	0.1	0.01	0.001	0.0001
Finished Drinking Water	X							
Wells, springs	X	X	X					
Lakes, reservoirs	X	X	X					
Water supply intake			X	X	X			
River water				X	X	X	X	X



TABLE 2. Quality Control Elements, Acceptance Limits, and Corrective Actions for the Analysis of Total Coliforms by the Membrane Filtration Procedure – SM 9222B

QC Elements	Frequency	Acceptance Criteria	Corrective Action
Sample storage	Every sample	Potable water samples analyzed ASAP and no later than 30 hrs from collection. Raw potable water source samples analyzed within a max of 8 hrs from collection (i.e., max of 6 hr in the field + 2 hr in the laboratory) and stored at 1-8°C from the time of collection to the time of analysis.	Qualify data (H or J) as estimated value (exceeded holding time or holding temperature, respectively) and contact sample collector to obtain new sample
Filter blank or negative control (equivalent to LRB; sterile buffered rinse water)	Beginning and end of batch	No colonies present	Qualify data (B) as estimated value (if TC colonies present in sample, and in one or more of the filter blanks – laboratory contamination); determine and eliminate the contamination source, and contact sample collector to obtain new sample
Filter positive control (equivalent to LCS and LFB; sterile buffered rinse water spiked with <i>E. coli</i>)	One per batch	Total coliform (TC) colonies present	Qualify data (J) as estimated value (TC colonies absent in the filter positive control – media or other failure); determine cause of and correct the problem, and contact sample collector to obtain new sample
Laboratory duplicate (for raw potable water source samples or potable water samples where enough volume is collected and enumeration is requested)	One per batch of 10 or fewer samples ($\geq 10\%$)	Range of Logs for Duplicate Counts = 0 - 0.40 (current laboratory calculated control limits for the ideal counting range of 20 - 80 colonies per plate)	Qualify data (J) as estimated value (precision criterion not met)
Field blanks	Not routinely included – only for special projects or if submitted by sample collector	TC colonies absent	If the sample is identified to the laboratory as a field blank, qualify data (J) as suspect due to possible sample contamination in the field (i.e., for all field samples collected in the same sampling event that have TC colonies)



Massachusetts Department of Environmental Protection
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QC Elements	Frequency	Acceptance Criteria	Corrective Action
Field duplicates	Not routinely included – only for special projects or if submitted by sample collector	Range of Logs for Duplicate Counts = 0 - 0.40 (current lab calculated control limits for the ideal counting range of 20 - 80 colonies per plate)	If the sample is identified to the laboratory as a field duplicate, qualify data (J) as not meeting the field precision criterion