

# STANDARD OPERATING PROCEDURE For SM 9213D

## Standard *E. coli* Membrane Filtration Procedure

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SOP #: SM 9213D

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# MassDEP

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## LIST OF REVISIONS

Rev. #	Date	Description of Revision	Page #
0	August 2000	None	
0	August 2000	Replaced old DEP Logo with state seal + MassDEP (December 2006)	Title page & header



## LIST OF TABLES

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<b>TABLE 1. RECOMMENDED FILTRATION VOLUMES FOR <i>E. COLI</i> MEMBRANE FILTRATION TEST .....</b>	<b>10</b>
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## 1.0 SCOPE & APPLICATION

- 1.1 This is a membrane filtration (MF) procedure used to enumerate *E. coli* in fresh, estuarine, and marine waters. Since a wide range of sample volumes or dilutions can be analyzed by the MF technique, a wide range of *E. coli* levels in water can be detected and enumerated. This method is highly reproducible for analyzing relatively large sample volumes assuming that the samples are not highly turbid.

## 2.0 SUMMARY OF METHOD

- 2.1 A well-mixed sample is drawn through a 0.45- $\mu$ m membrane filter which is then placed on a Petri dish containing m-TEC agar. The dish is incubated for 2 hours at  $35 \pm 0.5^\circ\text{C}$  to resuscitate injured or stressed bacteria and then incubated for 22 hours at  $44.5 \pm 0.2^\circ\text{C}$ . Following incubation, the filter is transferred to a filter pad saturated with urea substrate. After 15 min, yellow, yellow-green or yellow-brown colonies are counted using a stereoscopic microscope at a magnification of 10-15x and a cool white fluorescent light source.

## 3.0 DEFINITIONS

- 3.1 When using this membrane filtration method, *E. coli* is defined as bacteria that produce yellow, yellow-green or yellow-brown colonies when exposed to urea substrate for 15 minutes following incubation on m-TEC agar.

## 4.0 INTERFERENCES

- 4.1 The accuracy of the *E. coli* membrane filtration method is decreased when samples are highly turbid.

## 5.0 SAFETY

- 5.1 Samples (and positive controls) may contain microorganisms that are pathogenic to humans. All precautions are to be taken to minimize exposure. All personnel must wear lab coats, safety glasses, and protective latex gloves while working in the laboratory.

## 6.0 EQUIPMENT AND SUPPLIES

- 6.1 Sample bottles: Sterile wide-mouth borosilicate glass or plastic. For sampling chlorinated wastewater effluent, a 120-mL sample bottle must contain 0.1 mL of a 10% solution of sodium thiosulfate. The WES Microbiology Laboratory uses sterile 120-mL Corning® disposable wide-mouth 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 medium: Use clean 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.



- 6.4 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 inner surface.

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

Filtration funnels shall be sterilized for the initial filtration of a sample series. Following the filtration of a sample, the funnel(s) shall be placed in the UV light box and exposed to UV 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 shall not be allowed to fill with water.

- 6.5 Membrane filters: Use 0.45- $\mu$ m 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  $\pm 0.2$  pH 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 alcohol and flaming.
- 6.7 Incubators: A bacteriological incubator set at  $35 \pm 0.5^\circ\text{C}$  and a water bath with a temperature of  $44.5 \pm 0.2^\circ\text{C}$ .
- 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.

## 7.0 MEDIA

- 7.1 The need for uniformity dictates the use of commercial dehydrated media. Never prepare media from basic ingredients when suitable dehydrated media are available. Follow manufacturer's directions for rehydration. Store opened supplies of dehydrated media in a desiccator.

m-TEC Agar: (refer to the manufacturer's directions on the bottle for preparation)

Proteose peptone	5.0 g
Yeast extract	3.0 g
Lactose	10.0 g
Sodium chloride, NaCl	7.5 g
Dipotassium phosphate, $\text{K}_2\text{HPO}_4$	3.3 g
Monopotassium phosphate, $\text{KH}_2\text{PO}_4$	1.0 g
Sodium lauryl sulfate	0.2 g



Sodium desoxycholate	0.1 g
Bromcresol purple	0.08 g
Bromphenol red	0.08 g
Agar	15.0 g
Reagent-grade (Type I) water	1 L

Sterilize by autoclaving; Final pH  $7.3 \pm 0.2$ .

Dispense in 5- to 7-mL quantities into lower section of 50-mm plastic Petri dishes and let solidify. 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).

Urea Substrate:

Urea	2.0 g
Phenol red	10 mg
Reagent-grade water	100 mL

Adjust pH to between 3 and 4. Store at 2 to 8°C. Use within 1 week.

## 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 head space 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 6.1)
- 8.2 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".
- 8.3 Samples shall be kept at  $< 4^{\circ}\text{C}$  from the time of collection to the time of analysis.
- 8.4 Non-potable water samples shall be analyzed within 8 hours of collection.
- 8.5 All samples shall be accompanied by a sample tracking sheet/chain-of-custody form filled out by the collector.

## 9.0 QUALITY CONTROL

- 9.1 Test each new lot of medium against a previously acceptable lot for satisfactory performance by making dilutions of a thermotolerant *E. coli* culture and filtering the appropriate volume to yield plates with counts in the range of 20-60 colonies per filter.
- 9.2 Filter a sterile buffered water sample at the beginning and end of each filtration series and insert a sterile rinse water sample after filtration of a series of 10 samples to check for possible cross-contamination or contaminated rinse water. Incubate the rinse water control membrane culture under the same conditions as the sample.
- 9.3 Run one sample in duplicate for each batch of 10 or fewer samples.



- 9.4 For each filtration series, run a positive control culture organism.

## 10.0 CALIBRATION AND STANDARDIZATION

- 10.1 The stainless steel funnels have been etched at the 100-mL point as determined by measuring liquid volume with a graduated cylinder. The 100-mL graduation of the plastic funnels has also been checked for accuracy with a graduated cylinder. If the 100-mL graduation is inaccurate on the funnel, the correct volume has been labeled on the funnel.

## 11.0 PROCEDURE

- 11.1 The sample size will be governed by the expected bacterial density (see Table 1).
- 11.2.1 An ideal sample volume will yield 20 to 60 *E. coli* colonies 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 sterile dilution water to the funnel before filtration or pipet the sample volume into a sterile dilution bottle, then filter the entire dilution. This increase in water volume aids in uniform dispersion of the bacterial suspension over the entire effective filtering surface.
- 11.2 Sterilize/sanitize 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 sanitized in the UV light box 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 between filtrations of dilutions of the same sample (for non-potable samples) 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. 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-TEC agar surface with a rolling motion to avoid entrapment of air. Invert the Petri dish, place the dish into a WhirlPak bag, seal the bag, and incubate for 2 hours at  $35 \pm 0.5^{\circ}\text{C}$ . Then place the dish, *completely submerged*, in a water bath at  $44.5 \pm 0.2^{\circ}\text{C}$  for 22 hours.
- 11.6 Counting: Place a filter pad in a Petri dish and saturate the pad with urea substrate. Transfer the membrane from the m-TEC plate to the urea substrate pad for 15 minutes. Using a low-power (10 to 15x) binocular dissecting microscope, count all yellow, yellow-green or yellow-brown colonies as *E. coli*.





## 12.0 DATA ANALYSIS AND CALCULATIONS

- 12.1 Quantification: Compute the count, using membrane filters with 20 to 60 *E. coli* colonies using the following equation:

$$E. coli/100 mL = \frac{E. coli colonies counted \times 100}{mL sample filtered}$$

- 12.3 Out of range plates: If no filter has an *E. coli* count falling in the ideal range, total the *E. coli* 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 *E. coli* colonies, respectively, report the count as eight *E. coli* 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".

## 13.0 METHOD PERFORMANCE

Reserved

## 14.0 POLLUTION PREVENTION

Reserved

## 15.0 WASTE MANAGEMENT

- 15.1 Dispose of all positive samples by discarding in autoclave bags and autoclaving for a minimum of 30 minutes.

## 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



## 17.0 TABLES

**TABLE 1. Recommended filtration volumes for *E. coli* membrane filtration test**

Sample Type	Recommended Filtration Volume (mL)							
	100	50	10	1	0.1	0.01	0.001	0.0001
Lakes, reservoirs	X	X						
Wells, springs	X	X						
Water supply intake		X	X	X				
Natural bathing waters		X	X	X				
Sewage treatment plant			X	X	X			
Farm ponds, rivers				X	X	X		
Storm water runoff				X	X	X		
Raw municipal sewage					X	X	X	
Feedlot runoff					X	X	X	
Sewage sludge						X	X	X