Standard Operating Procedure
For Bacteriological Examination of Seawater by Membrane Filtration using mTEC Agar

Updated 4/22/2014 by Linda McFarland

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Scope and Application:

Fecal Coliform by mTEC method detects the presence of fecal coliform in seawater.

1.0 Summary of Method:

Fecal coliform analysis is performed by filtering a known volume of sample through a sterile girded membrane using a sterile funnel. The filter is then placed on mTEC agar and incubated for 2 hours at 35±0.5°C, then placed in a waterbath at 44.5±0.2°C for 22 hours. Any yellow, yellow-brown, or yellow-green colonies are used to calculate the result.

2.0 Interferences:

None noted

3.0 Sample, Collection, Preservation, and Storage:

All samples must be collected in sterile bottles and transported back to laboratory. The samples are then removed from coolers and stored at 1-10°C until time of analysis. A temperature blank is checked to assure no abuse has occurred.

4.0 Equipment and Supplies:

4.1 Sterile 250mL wide-mouth bottles (nalgene or glass preferred)
4.2 Sterile plastic Petri dish, with tight fitting lids (50 x 9mm)
4.3 Sterile girded membrane filters (47mm @ 0.45um)
4.4 Sterile funnel pre-calibrated at 100mL
4.5 Sterile forceps with blunt tip
4.6 Ziploc bags (quart and gallon size)
4.7 Sterile pipettes, graduated and various sizes
4.8 Sterile graduated cylinders (100mL)
4.9 Vacuum pump with manifold and filtration flask
4.10 1L Nalgene dilution/rinse water bottles (autoclavable)
4.11 Lighted Magnification source
4.12 Hot air Incubator set at 35±0.5°C
4.13 Circulating waterbath at 44±0.2°C
4.14 Autoclave, routinely service and checked
4.15 Autoclave sterility tape
4.16 Refrigerator (1-5°C)
4.17 Oven set at 170-180°C for sterilizing pipettes
4.18 Balance, top loading to 0.01g

5.0 Reagents and Standards:

5.1 mTEC agar: using a 2L flask dissolve 45.3g of dehydrated mTEC in 1000mL of DI water. Heat while stirring until agar is completely dissolved and boils for 1min. Autoclave at 121°C for 15min. cool to 44.5-50°C and pour 4-5mL into sterile Petri dishes. Allow agar to solidify, then place in large Ziploc bags and store in refrigerator for no longer than 2 weeks.
5.2 1N NaOH: brought pre-prepared.

5.3 Sterile Phosphate Buffered Saline (PBS):

Ingredients: sodium dihydrogen phosphate – monobasic 0.58g
sodium monohydrogen phosphate – dibasic 2.5g
sodium chloride 8.5g

Dissolve all ingredients in 1000mL of reagent grade lab water and dispense into rinse water bottles. Autoclave at 121°C for 15min. Final pH should be 7.4±0.2. Perform a Sterility check before using. Record in logbook.

A PBS 10X solution may also be purchased and used.

5.4 70% alcohol bench wipe: brought pre-prepared.

6.0 Quality Control:

6.1 Analyze a blank on each funnel before and after all samples. Run mid-blanks every 10 samples or less to ensure continued sterility. Also run blanks in-between sampling areas.

6.2 A fecal positive (*E. coli*), a fecal negative (*E. aerogenes*), and a gram-negative (*S. aureus*) are analyzed with each new batch of media.

6.3 A fecal positive (*E. coli*) and a fecal negative (*E. aerogenes*) are analyzed with each run.

6.4 Sterility checks are proformed:

6.4.1 On each lot of sterile Petri dishes.

6.4.2 On each lot of membrane filters.

6.4.3 On each batch of sample bottles, funnels, and forceps.

6.4.4 On each batch of dilution water.

6.4.5 On each batch of mTec agar.

6.5 Comparisons are to be made whenever a new lot of membrane filters is put into use.

6.6 Temperature checks: Check and log incubator, waterbath, and refrigerators temperatures and time twice a day at least four hours apart.

6.7 Thermometers are calibrated annually against a NIST certified thermometer, and labeled with corrections.

6.8 Balance is serviced annually and checked monthly using 4 "S" class weights.

6.9 Autoclave:

6.9.1 Temperature and pressure are recorded each run on a recording tape.

6.9.2 Maximum temperature is recorded each run with a maximum registering thermometer.

6.9.3 Cycle times are checked quarterly.

6.10 All water used in laboratory in DI water. Each year the DI system is checked for trace metals and total metals. Each month the DI system is checked for conductivity, residual chlorine, and heterotrophic plate count.

6.11 All dry media is discarded at the expiration date/ or sooner if it discolors or becomes caked and loses the characteristic of a free-flowing powder.

6.12 All Liquid media and/or reagents are discarded at expiration date.

7.0 Sample Preparation and Procedure:

7.1 Disinfect work area with 70% alcohol – spray counter and wipe with paper towel or rag.

7.2 Label each Petri dish with sample ID and volume (if needed) on the bottom side of the dish. (metallic silver sharpie works best)

7.3 Place sterile funnels on vacuum manifold using proper aseptic technique.

7.4 Place sterile membrane filters on the funnels using sterile forceps.

7.5 Shake sample vigorously 25 times in an arcing motion.

7.6 Seat filter with a small portion of sterile PBS.

7.7 Depending on the sample volume needed you will use a sterile pipette (for volumes <10mL), a sterile graduated cylinder (for volumes <100mL), or pour directly into pre-calibrated funnel for 100mL volume.

7.8 Allow sample to flow through filter using a vacuum pump.

7.9 Wash down the sides of the funnel at least 3 times with 10-25mL of sterile PBS.

7.10 Remove filter with sterile forceps and place into Petri dish grid side up, making sure there are no air bubbles trapped under the membrane.

7.11 Invert dish and put aside till all samples are complete.

7.12 Place Petri dishes into quart-sized Ziploc bags, 12 dishes per bag. Then place this Ziploc into a 2nd Ziploc, and finally place 2nd Ziploc into 3rd Ziploc. Making sure all bags are sealed tightly and excess air has been squeezed out.

7.13 Place Ziploc bags containing dished in the incubator set at 35.0±0.5°C for 2hours.

7.14 Remove bags from incubator after 2hours and place in waterbath (44.5±0.2°C) for an additional 22±2hours. Use rust-resistant test tube racks with weights on top to hold plates under the surface of the water.

7.15 After full incubation remove plates and count colonies using a lighted magnification source. Only yellow, yellow-brown, and yellow-green colonies are to be counted. Record all results on seawater sheets.

8.0 Calculations:

8.1 For samples where only a 100mL volume was run count all appropriate colonies up to 80 and report that number as fecal coliform/100mL

8.2 For samples where more than one volume or <100mL was run use the dish with a count between 20-80 colonies. If more than one dish falls in this range or if all dishes are below 20 use calculation below:

8.2.1 Fecal coliform/100mL = $\frac{\text{number of colonies}}{\text{Volume of sample filtered (mL)}} \times 100$

e.g. if you had 7 colonies on a dish with 30mL volume and 19 colonies on the 100mL dish then the calculation would go as follows

$$FC/100mL = \frac{7 + 19}{30 + 100} \times 100$$

$$= 20$$

8.3 Data Entry: when entering data into computer program follow these guidelines.

8.3.1 Calculated results: results from volume other than 100mL frequently return fractional values. These should be rounded to one decimal place for data entry.

8.3.2 Greater than or less than Values: The acceptable range of colonies on a membrane using the mTEC method is 20-80. If no membrane contains colony counts in this range the lab result must be interpreted in order to be entered into the database. Use the following rules to determine the values to enter.

8.3.2.1 Case 1: No colonies on the membrane for any volume filtered. Enter the result as 1.

8.3.2.2 Case 2: There is colony growth on the membrane (or membranes), but none contains 20 or more colonies. Calculate the results using the formula in 9.2.1.

8.3.2.3 Case 3: The colony growth for all volumes filtered exceeds 80. Use the table below for data entry value.

Smallest Volume Filtered	Lab Result	Data Entry Value
100mL	>80	81
50mL	>160	162
30mL	>266.67	270
10mL	>800	810
3mL	>2666.67	2700

9.0 References:

9.1 EPA method 1103.1: *E. coli* in Water by Membrane Filtration Using mTEC.

9.2 FDA Checklist for Bacteriological Examination of Seawater by Membrane Filtration using mTEC agar.

10.0 Waste Management:

All waste generated from this analysis is to be put into the autoclave waste bags and sterilized before disposal.