Standard Operating Procedure

Colichrome® MF EC

Membrane Filter Method for E. Coli

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1.0 Scope and Application

1.1 The Colichrome® MF EC method consists of a medium which detects the presence or absence of *E. coli* and enumeration.

1.2 The method allows the detection and enumeration of *E. coli* in 24 to 48 hours, or less and does not require a confirmation step.

1.3 The detection limit is one target CFU/sample.

2.0 Summary of the Method

2.1 The Colichrome® MF EC medium determines the presence or absence (and enumeration) of *E. coli* in any size water sample (100 mL is required for drinking water). The sample (diluted or not) is passed through a 0.45um pore size, 47 mm diameter membrane filter using standard equipment and methodology. The filter is then placed into a 50 mm plate containing a pad saturated with the medium (if in broth form) or a layer of the medium which has been solidified with an added agar gelling agent. Incubate for 24 to 48 hours at 35°C±0.5°C. *E. coli* will appear as green/teal CFUs. The presence of *E. coli*, depending on conditions, can start to appear in as little as 16 hours.

2.2 Colichrome® MF EC medium contains nutrients to assure the growth of the target organisms, buffers to maintain appropriate pH, and inhibitors to reduce the growth of non-target organisms.

3.0 Definitions

3.1 Escherichia coli - Those bacteria which grow as green/teal colonies on the Colichrome® MF EC medium are a result of the production of glucuronidase enzymes. These bacteria are of fecal origin.

3.2 Bacteria that form colonies which are not green/teal on Colichrome® MF EC medium are other than *E. coli*.

4.0 Interferences

4.1 No known chemical substances normally encountered in drinking water or source waters have been observed to affect the color of *E. coli* colonies on the Colichrome® MF EC medium. If particulate or colloidal materials are suspended in water samples, they may interfere with filtering efficiency by clogging filter pores and they may cause some spreading of bacterial colonies as they grow on the filter surface during incubation. However these materials would very rarely prevent the accurate determination of the bacterial population.

4.2 Colonies exhibiting the color of the target organisms should not be included in the *E. coli* count if they are less than 0.5 mm diameter (except when the entire colony population is very large due to excessive crowding on the plate. In such a case the sample should be rerun at a higher dilution.). Small colored colonies of this nature should not be counted unless they are isolated into pure culture and then verified by approved procedures.

4.3 It cannot be safely assumed that colonies can be picked directly from the surface of the filter and used to inoculate confirmatory media directly, particularly if the colonies are green/teal or teal green, as they may be contaminated by cells from adjoining colonies that have traveled on the filter surface. Therefore, questionable colonies should be picked and streaked onto the surface of a differential medium to ensure their purity before further testing.

5.0 Safety

5.1 Standard safety practices should be observed by persons using these materials in the

laboratory.

5.2 Any materials containing living or viable microbes should be disinfected or sterilized by accepted standard methods before being discarded.

5.3 Refer to the MSDS for specific product information.

6.0 Equipment and supplies

6.1 Incubator set at 35°C±0.5°C with provision for maintaining materials at above 80% humidity.

6.2 Filter funnel apparatus for 47 mm membrane filters, with a vacuum source.

6.3 Dissecting microscope (10-15X) with built-in light sources.

6.4 Sterile disposable or properly cleaned (by well-known standard methods) glass or plastic ware including 1 and 10 mL pipettes, sample collection containers, flasks, graduated cylinders, and diluent containers.

6.5 Sterile forceps

6.6 Sterile 50 mm diameter petri dishes with absorbent pads

6.7 Sterile 0.45µm pore size, 47 mm diameter micropore filters for sample filtering

6.8 Sterile Dilution and Rinse water prepared in accordance with standard methods.

6.9 Biohazard bag

7.0 Reagents and Standards

7.1 Colichrome® MF EC medium of Roth Bioscience LLC is provided in a broth for using 1.8-2 mL/dish. The medium should be kept frozen (0°C or below) and has an expiration time of 12 months.

7.2 Preparation of the Medium for use: The broth medium should be thawed before use. The thawed medium can be kept refrigerated for up to 2 weeks. The broth medium may be refrozen if not all used in one procedure.

7.3 Make a 10% solution of sodium thiosulfate using reagent-grade water.

8.0 Sample collection, De-chlorination, Preservation, Shipment and Storage

8.1 Collect samples in a sterile, clean wide mouth glass or heat resistant plastic bottle with a leak proof closure, all of which is non-toxic in use. A pre-sterilized, sealable, non-toxic plastic bag may also be used for sample collection.

8.2 For potable water, open the tap and allow the water to run for 2-3 minutes and then collect the sample using aseptic technique to avoid contamination. For other sample types, aseptically collect water that is representative of the source.

8.3 Samples with residual chlorine should be neutralized at the time of collection by adding 1 mL of a 10% solution of sodium thiosulfate (or the equivalent) per liter of sample.

8.4 Samples should be tested as soon as possible after collection. If processing is not done within 1 hour, the sample should be held on ice or refrigerated at 2-8°C. Potable water samples should be tested or processed within a maximum holding time of 30 hours of collection and non-potable samples should be tested or processed within a maximum

holding time of 8 hours of collection.

9.0 Quality Control

9.1 Colichrome® MF EC is tested for quality control at the time of manufacture and is certified to meet specifications. Each lot should be tested by the using laboratory by preparing a plate of the medium to serve as a positive control, negative control, and uninoculated control by spotting the filter with pure broth cultures.

Prepare pure broth cultures of typical *E. coli* and a non-*E. coli* coliform (ie. *Enterobacter*). Spot the filter in three locations, one with E. coli for the positive control, a second one with a non-E. coliform for the negative control, and a third with sterile diluent for the uninoculated control . Place the filter on the pad of one of the plates containing Colichrome® MF EC medium.

Incubate the plate 24 to 48 hours at 35°C±0.5°C. The *E. coli (positive)* control should have green/teal color, the non-*E. coli* coliform (negative) control growth should be colorless , and the diluent control should have no colony growth.

Colonies from the controls may be picked and tested further with various diagnostic media if desired.

10.0 Calibration and Standardization

10.1 Colichrome® MF EC calibration or standardization is not required.

10.2 Incubators should be tested daily to ensure maintenance of proper temperature. Thermometers used should be tested at least annually against an NIST certified thermometer.

11.0 Procedure

11.1 Test Procedure

11.1.1 Prepare Petri dishes and absorbent pads by adding 1.8 mL of medium onto the pad. This should wet the entire pad surface without any excessive overflow of the liquid medium. Dishes may be checked by tilting the dish to determine if excessive medium collects on one side. If so, that excessive medium can be removed (with a sterile fine tip dropper or pipette) so that the pad has no excessive medium running over the pad top. (This is a precaution so that when the membrane filter holding the filtered microbes will not be too wet, so that individual CFUs will grow as smooth edged circular colonies instead of irregular spreading colonies.)

11.1.2 Using proper technique, filter the sample through a 47 mm, 0.45µm pore size membrane filter. Rinse the filter funnel twice with at least 20 mL of sterile diluent/rinse to complete the filtration. Transfer the filter to a petri dish containing a pad saturated with Colichrome® MF EC broth, invert the dish and incubate at 35°C±0.5°C for 24 to 48 hours.

11.2 Interpretation

11.2.1 Check the filter surface for colony forming units. Generally, colonies are obvious and can be observed with the unaided eye in a normal room or daylight. However, the use of a 10-15X magnifying device is recommended for critical analysis.

11.2.2 The sum of green/teal colonies is the *E. coli* positive count. Clear or colorless colonies are counted as non-coliforms.

12.0 Data Analysis, Calculation, Interpretation and Reporting Results

12.1 Presence/Absence Test

12.1.1 The presence of at least one green/teal colony at least 0.5 mm in diameter indicates the sample is *E. coli* positive.

12.2 *E. coli* (Fecal) - Quantitative Test

12.2.1 Count the number of green/teal CFUs present on the membrane filter and record as the number of *E. coli* / amount of sample used for that test. Then translate to the number of *E. coli* CFUs/100 mL of sample (see 12.1.1). All green/teal CFUs should be counted as *E. coli.* (Colonies should be at least 0.5 mm diameter to be counted.)

13.0 Method Performance Characteristics

13.1 Specificity - In a study done to compare Colichrome® MF to the m-TEC Method for the detection and enumeration of *E. coli* from disinfected wastewater effluent, the false positive error was 3.8% and the false negative error was less than 1.0%. That is, of 105 CFUs judged to be *E. coli* (green/teal) which were picked and subjected to Enterotube® analysis, only 4 were identified as other than *E. coli*. And of 131 CFUs judged to be coliforms other than *E. coli* (pink/magenta) which were picked and subjected to Enterotube analysis, only 1 was identified as other than a true coliform.

13.2 Comparability - The Pearson Coefficient for the parallel analyses on the Colichrome® MF and the m-TEC methods within the same laboratory was 0.928. T- Test analyses indicated no significant differences between the methods at the 95% confidence level.

14.0 Pollution Prevention

14.1 Laboratory personnel should use pollution control techniques to minimize waste generation wherever possible. Where this is not possible at the source, recycling should be practiced.

15.0 Waste Management

15.1 Each laboratory is responsible to comply with all federal, state and local regulations governing waste management. Special emphasis should be placed on hazardous waste identification rules and land disposal restrictions and on protecting the air, water, and land by minimizing and controlling all release from fume hoods and bench operations. Compliance is also required with any sewerage discharge permits and regulations. Federal, state or local authorities should be contacted for further specific information.

16.0 Troubleshooting

16.1 Count only the green/teal colonies. For operators who are unsure of the colors, questionable colonies should be picked and streaked onto the surface of a differential medium to ensure their purity.

17.0 References

17.1 APHA (1995) Standard Methods for the Examination of Water and Wastewater. Edition 19.

17.2 Roth, J.N., W.J. Ferguson. (1993) Method Test Media and Chromogenic Compounds for Identifying and Differentiating General Coliforms and *Escherichia coli* Bacteria. United States Patent #5,210,022.

17.3 Umble, A.K., et al. (1999) Elkhart, Ind., Tests an Improved, Simplified Membrane

Filtration Method for *Escherichia coli* Detection and Enumeration. Water Environment Tech. Vol.11, No.4, 57-59.