Standard Operating Procedure

Colichrome® MF ECC

(Dougen® Technology)

Membrane Filter Method for E. Coli and Total Coliforms

Roth Bioscience, LLC
1303 Eisenhower Drive S.
Goshen, IN 46526
(574) 533-3351
hello@rothbioscience.com
RothBioscience.com

1.0 Scope and Application

1.1 The Colichrome® Membrane Filter method consists of a medium which detects the presence or absence of *E. coli* and total coliforms simultaneously and also allows the enumeration of each.

1.2 The method allows the detection and enumeration of *E. coli* and other coliforms 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 ECC medium determines the presence or absence (and enumeration) of *E. coli* and other coliforms 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* CFUs will appear as blue/purple and other coliform CFUs will appear pink/magenta. The presence of *E. coli* and Coliforms, depending on conditions, can start to appear in as little as 16 hours**. (NOTE: If the sample contains Aeromonas spp., they may give a similar appearance to the true coliform).**

2.2 Colichrome® MF ECC 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. It is similar to the modification of m-TEC described by Duncanson and Cabelli (1986 paper presented at the National Meeting of AWWA). *E. coli* colonies growing on the medium appear blue to purple due to the combination of the enzymes glucuronidase and galactosidase affecting their respective substrates, 5-Bromo-4-Chloro-3-Indolyl-B-D-glucuronide (X-gluc) and 6-Chloro-3-Indolyl-B-D-galactoside (Salmon-gal). The teal green product of X-gluc hydrolysis combines with the pink/magenta product of the Salmon-gal hydrolysis to produce the blue to purple appearance of *E. coli* colonies. Coliform colonies (other than *E. coli*) are colored pink/magenta as a result of producing only galactosidase which acts on the Salmon-gal only.

3.0 Definitions

3.1 Escherichia coli - Those bacteria which grow as blue/purple colonies on the Colichrome® MF ECC medium as a result of the production of both glucuronidase and galactosidase enzymes. These bacteria are of fecal origin.

3.2 Total Coliforms - Those bacteria which make up the sum of the *E. coli* (blue/purple colonies) and other coliforms. The other coliforms will appear as pink/magenta colonies on the Colichrome® MF ECC medium because they produce galactosidase, but not glucuronidase and so cleave only the Salmon-gal substrate. Species of the genera *Citrobacter, Enterobacter, and Klebsiella* are main groups (other than *Escherichia*) of coliform bacteria.

3.3 Non Coliforms **-** Bacteria that form colonies which are not blue/purple or pink/magenta on Colichrome® MF ECC medium.

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 or other coliform colonies on the Colichrome® MF ECC 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(s) of the target organisms should not be included in the E. coli or other coliform counts 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 Colonies exhibiting a teal green color which is indicative of the production of glucuronidase without the production of galactosidase should not be counted as E. coli without isolation into pure culture and verification by approved procedures.

4.4 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 blue/purple 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.

4.5 Unlike media which utilize a fluorogen (such as MUG or MUGal) and a chromogen, where the fluorogen tends to diffuse rapidly into the surrounding medium, thus making the timing of reading the test results critical (before excessive diffusion occurs which may make neighboring colonies appear as false positives), the chromophores of the chromogens used in Colichrome® MF ECC are quite insoluble and little or no diffusion away from the target colonies occurs.

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 ECC 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 ECC 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 three plates of the medium, one to serve as a positive control, one to serve as a negative control, and one to serve as an uninoculated control.

Prepare 24 hour tryptone broth cultures of typical *E. coli, Enterobacter aerogenes or Klebsiellsa pneumonia, and Pseudomonas aeruginosa or Salmonella typhimurium.* Prepare serial dilutions of *E. coli and Enterobacter or Klebsiella combined* so that the combined inoculum will result in 20-80 CFU/100 mL and filter. Place the filter on the surface of one of the plates of Colichrome® MF ECC medium. Prepare serial dilutions of the *Pseudomonas or Salmonella* so that the inoculum will result in 20-80 CFU/100 mL and filter. Place the filter on the surface of the second plate of Colichrome® MF ECC medium. Filter 100 mL of sterile diluent and place the filter on the surface of the third plate of Colichrome® MF ECC medium. Use sterile filter apparatus for each prep.

Incubate the plates 24 to 48 hours at 35°C±0.5°C. The *E. coli/Enterobacter or Klebsiella* control should have both blue/purple (*E. coli*) and pink/magenta (*Enterobacter or Klebsiella*) colonies, the *Pseudomonas or Salmonella* control should have colorless colonies, and the diluent blank control should have no colonies.

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 ECC 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 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 1.8- 2 mL of the Colichrome® MF ECC broth, invert the dish and incubate at 35°C±0.5°C for 24 to 48 hours. (The pad should only be saturated and should not have pooled broth when tilted. Depending on the brand of dishes/pads, the proper amount of broth may vary. Too much broth results in possible spreaders on the membrane surface.) **If *E. coli* is the only target organism desired and the other coliforms and non-coliforms are undesired and may interfere with the growth of the *E. coli*, it is suggested that an initial "resuscitation period" of incubation for 2 hours at 35 C., followed by the remaining incubation at 44.5 C. will lessen the non-E.coli colony growth without negatively affecting the *E. coli* growth as *E. coli* is noted to grow at 44.5 C. Other coliforms and many other microbes will not grow well at 44.5 C.**

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 blue/purple and pink/magenta colonies is the Total Coliform Positive count. Blue/purple colonies are counted as *E. coli.* Pink/magenta colonies are counted as other than *E. coli* coliforms. Clear or white 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 blue/purple or pink/magenta colony at least 0.5 mm in diameter indicates the sample is total coliform positive. The presence of at least one blue/purple colony indicates the sample is positive for *E. coli.* The presence of at least one pink/magenta colony indicates the sample is positive for general coliforms.

12.2 General Coliform (excludes *E. coli*) - Quantitative Test

12.2.1 Count the number of pink/magenta CFUs present on the membrane filter and record as the number/amount of sample used for that test. For example, if the amount of sample was 10 mL and there were 20 pink CFUs, record as 20 per 10 mL. Then translate to the number of CFUs/100 mL. In this case, the 10 mL sample is 0.1 of 100, so the 20 CFUs should be multiplied X10, giving 200 CFU/100 mL sample. All pink/magenta CFUs should be counted as general coliforms. (Colonies should be at least 0.5 mm diameter to be counted.)

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

12.3.1 Count the number of blue/purple 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

blue/purple CFUs should be counted as *E. coli.* (Colonies should be at least 0.5 mm diameter to be counted.)

12.4 Total Coliforms - Quantitative Test

12.4.1 The sum of the number of general coliform CFUs and the number of *E. coli* CFUs from one sample equals the number of total coliforms in that sample. That is, the total number of pink/magenta CFUs and blue/purple CFUs = the total coliforms for that sample.

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* (blue/purple) 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 ECC 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 blue and purple colonies. Some operators may initially be confused in differentiating between purple and pink colonies as purple/blue colonies are a combination of the product of the color from both enzyme substrates (green-teal and pink-magenta). Normally, the purple/blue colonies appear first on the membrane because they contain color from both substrates, and the pink are very much lighter colored. However, for operators who are unsure of the colors, Roth Bioscience has available test media to differentiate whether the color is from only a single substrate (not E. coli) or a combination of both substrates (E. coli). This can be ordered as a separate item from Roth Bioscience.

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.