Simultaneous Detection and Enumeration of Total Coliforms and Escherichia coli using m-ColiBlue24 Membrane Filtration Medium

Hach Company Method 10029
Rev. 2, 1999

1.0 Scope and Application

1.1 This test method describes a sensitive and differential membrane filter (MF) medium, using m-ColiBlue24 agar or broth, for the simultaneous detection and enumeration of both total coliforms (TC) and *Escherichia coli* (*E. coli*) in water samples in 24 hours or less on the basis of their specific enzyme activities and selective dye. m-ColiBlue24 is a nutritive, lactose-based medium, containing inhibitors to selectively eliminate growth of non-coliforms. It is analogous to an improved version of m-Endo. Total coliform colonies growing on the medium are highlighted by a non-selective dye, 2,3,5-Triphenoltetrazolium Chloride (TTC), which produces red colored colonies. Among the total coliform colonies, which grow up on the medium, any *E. coli* colonies are distinguishable by a selective blue color, resulting from the action of b-glucuronidase enzyme on 5-Bromo-4-Chloro-3-Indolyl-Beta-D-glucuronide (BCIG).

1.2 Total coliforms include species that may inhabit the intestines of warm-blooded animals or occur naturally in soil, vegetation, and water. They are usually found in fecal-polluted water and are often associated with disease outbreaks. Although they are not usually pathogenic themselves, their presence in drinking water indicates the possible presence of pathogens. *E. coli*, one species of the coliform group, is always found in feces and is, therefore, a more direct indicator of fecal contamination and the possible presence of enteric pathogens. In addition, some strains of *E. col*i are pathogenic (Reference 16.12).

1.3 This method, which has been validated for use with drinking water, source water, and wastewater in single-lab and multi-lab studies (References 16.8 - 16.10).

1.4 Since a wide range of sample volumes or dilutions can be analyzed by the MF technique, a wide range of *E. coli* and TC levels in water can be detected and enumerated.

2.0 Summary of Method

2.1 An appropriate volume of a water sample (100 mL for drinking water) is filtered through a 47-mm, 0.45-µm pore size cellulose ester that retains the bacteria present in the sample. The filter is then transferred to a 50-mm Petri plate containing an absorbent pad saturated with m-ColiBlue24 broth or m-ColiBlue24 agar plate and incubated at 35^{o} C for up to 24 hours. Both red and

blue colonies may appear; the blue colonies are specific to the presence of *E. coli* while the red colonies are specific to non-*E. coli* coliforms.

3.0 Definitions

3.1 Total Coliform Bacteria – Bacteria belonging to the genera *Klebsiella* sp., *Enterobacter* sp., *Cirobacter* sp., or *Escherichia* sp.

3.2 Coliform Positive Colony – A red or blue colony.

3.3 Coliform Negative Colony – A clear or white colony.

3.4 *Escherichia coli* or *E. coli* Bacteria – A genus within the total coliform group typified by possession of the enzyme *b*-Glucuronidase, ability to grow at 44.5^{o} C, and form indole from tryptophan.

3.5 *E. coli* Positive Colony – A blue colony.

3.6 *E. coli* Negative Colony - A non-blue colony.

4.0 Interferences

4.1 No interferences to the colony color development have been found in drinking water, source water, and wastewater samples. Similarly, particulates in water samples do not alter the efficacy of the medium, although excess particulates may cause colonies to grow together on crowed filters or slow the sample filtration process.

5.0 Safety

5.1 Standard safety practices appropriate to microbiology laboratories should be followed.

5.2 Solid and liquid waste materials containing or suspected to contain viable bacteria should be decontaminated using an autoclave or by using an appropriate disinfectant before discarding.

5.3 Refer to the appropriate Material Safety Data Sheets supplied for each reagent for comprehensive safety data essential to proper use.

6.0 Equipment and Supplies

6.1 Equipment

6.1.1 Air Incubator – Capable or operating at 35^{o} C ± 0.5^{o} C.

6.1.2 Vacuum pump.

6.1.3 Membrane filtration-funnel unit and flask.

6.1.4 Dissecting microscope, capable of 10-15X magnification. The microscope should be equipped with a fluorescent illuminator.

6.2 Supplies/Glassware – Cleanse all glassware thoroughly with a suitable detergent and hot water, rinse with hot water to removes traces of detergent residual, and rinse again with laboratory-pure water. Sterilize all glassware by autoclaving 15 min. at 121^{o} C or by heating in an oven for at least 1 hour at 170^{o} C.

6.2.1 Pre-sterilized 50-mm MF Petri plates with pads

6.2.2 45-mm pre-sterilized membrane filters.

6.2.3 Sterile forceps.

6.2.4 Sterile glass or plastic sample collection containers.

6.2.5 Sterile graduated cylinders.

6.2.6 Sterile pipettes.

6.2.7 Sterile MF filtration unit.

6.2.8 Side-arm flask.

6.2.9 Biohazard bag.

7.0 Reagents and Standards

7.1 Growth Medium

7.1.1 m-ColiBlue24 broth (Hach Number 2608420, 2608442, or 2608450) or m-ColiBlue24 agar plates (Hach Number 2805215).

7.2 Dechlorinating Reagent

7.2.1 Hach dechlorinating reagent Powder Pillow (1436369) containing sodium thiosulfate and sodium sulfate.

7.2.2 Prepare a 3% sodium sulfate solution by adding 44.18 g of sodium thiosulfate pentahydrate to approximately 500 mL of deionized water, then dilute to 1 L with deionized water.

7.3 Buffered Dilution Water

7.3.1 Magnesium Chloride and Potassium Dihydrogen Phosphate Buffer, 99mL per dilution bottle (Hach Number 1430598).

8.0 Sample Collection, Dechlorination, Preservation, Shipment and Storage

8.1 Water Sample Collection

8.1.1 Sample Collection Containers – Samples should be collected in sterile, clean glass or heat-resistant bottles. Pre-sterilized Whirl-Pak® Bags may also be used.

8.1.2 Sample Procedure – Potable water samples are taken by first flushing the tap 2-3 minutes to clear the service line. Collect samples using aseptic techniques to avoid contamination. For other samples, aseptically collect water representative of the source.

8.2 Dechlorination – Water containing chlorine or other halogens must be treated with sodium thiosulfate to allow accurate evaluation of microbial content. Add one dechlorinating reagent Powder Pillow (1436369) by aseptically cutting of the tip of an alcohol-rinsed pillow and pouring the contents into 100 mL of the chlorinated water sample. Alternatively, pipette 0.1 mL of a 3% sodium thiosulfate solution into 100 mL of the chlorinated sample. Pre-sterilized Whirl-Pak Bags contain sufficient sodium thiosulfate powder to neutralize a 100 mL chlorinated water sample.

8.3 Preservation, Shipment, Storage – Samples should be tested as soon as possible. If analysis cannot be done within 1 hr of collection, water samples should be held on ice or refrigerated to 2-8^{o} C for a maximum holding time of 8 hours from sampling.

9.0 Quality Control

9.1 m-ColiBlue24 undergoes quality control (QC) testing at the time of manufacture. A Certificate of Analysis is include with every m-ColiBlue24 shipment stating the m-ColiBlue24, as received by the analysis, is ready for use in analyzing water samples by the membrane filtration procedure[1]. It is recommended that the

[1] **Note:** Hach Company has verified the performance of Method 10029 (m-ColiBlue24) in source water, finished drinking, and wastewater using ancillary supplies and source of test organisms listed below. Brand

laboratory perform a QC check for detection and enumeration with each ne w lot of membrane filters and pads using test organisms derived from pre-chlorinated primary treated effluent or from an ATCC strain of organisms known compatibility with the medium.

10.0 Procedure

10.1 Test Procedure

10.1.1 If using broth medium, aseptically open an ampoule containing m-ColiBlue24 and pour the broth onto the pad in a 50-mm MF Petri plate.

10.1.2 Place a sterile filter onto a sterile filter holder. Using a sterile graduated cylinders and pipettes, measure an appropriate sample volume. Pour water sample into the reservoir funnel and draw the water through the filter using a vacuum pump. Rinse the funnel with several 20-30 mL volume of sterile rinse water. With sterile forceps, transfer the filter to a Petri plate containing a pad saturated with m-ColiBlue24 or an agar plate of m-ColiBlue24. Invert the plate and incubate at 35^{o} C ± 0.5^{o} C for 24 hours.

10.2 Interpretation

10.2.1 If no blue or red colonies are present after 24 hours, the sample is free from total coliforms and *E. Coli*.

10.2.2 Examine the filters for colony growth. Colonies are typically readily visible, but a microscope may prove useful.

10.2.3 Presence /Absence detection for Drinking Water – A red colony is a total coliform positive result. A clear or white colony is a total coliform negative result. A blue colony is an *E. coli* positive result. A non-blue colony is an *E. coli* negative result.

10.2.4 Enumeration for Source Water and Wastewater – Refer to Standard Methods 9222B for appropriate dilutions of the sample to filter so that 20-

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names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent method performance may be achieved using materials and sources of organisms other than those specified here, but demonstration of equivalent performance that meets the requirements of this method and its use for regulatory compliance reporting purposes is the sole responsibility of the laboratory.

Membrane filters: Millipore 0.45µ membrane filters; Part Number XXXX
Pads and Petri Dishes: Pall/Gilman or Sartorius pre-sterilized, cellulose or glass pads with glass or plastic Petri dishes; Part Numbers XXXXX and XXXXXXX

80 coliform forming units (CFU) is present after incubation. Calculate the number of blue *E. coli* colonies and red non-E. coli coliform colonies according to Standard Methods 9222B. Total coliforms are counted as the sum of blue and red colonies.

11.0 Method Performance Characteristics

11.1 Drinking Water

11.1.1 The specificity of m-ColiBlue24 for recovery of total coliforms and *E. coli* following the EPA Protocol of June 30, 1992 is the following: *E. coli* false positive error – 2.5%; *E. coli* false negative error – 0%. Overall agreement between m-ColiBlue24 and the EPA reference method (m-Endo) – 98.8%. Total coliform false positive error – 26.8%; Total coliform false negative error – 1.6%.

11.2 Source Water[2]

11.2.1 *E. coli* false positive error – 2.3%; *E. coli* false negative error – 0%.

11.3 Wastewater[3]

11.3.1 The specificity of m-ColiBlue24 for recovery of total coliforms and *E. coli* following the EPA Protocol of March 2003 is the following: *E. coli* false positive error – 2.3%; *E. coli* false negative error – 4.9%.

12.0 Pollution Prevention

12.1 For information about pollution prevention that may be applicable to laboratories and research institutions, consult *Less is Better; Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington D.C., 20036.

13.0 Waste Management

13.1 It's the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and

[2] Grant, M.A. 1997. "A New membrane Filtration Medium for Simultaneous Detection and Enumeration of Escherichia coli and Total Coliforms." Applied and Environmental Microbiology, 63:3526-3530.

[3] "Guidelines Establishing Test Procedures for the Analysis of Pollutants; Analytical Methods for Biological Pollutants in Wastewater" (Federal Register / Vol. 66, No. 169 / Thursday, August 30, 2001 / Proposed Rules)

land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

13.2 See the MSDS for product composition information and further guidance on waste disposal.

13.3 For more information on laboratory waste management, consult Waste Management Manual for Laboratory Personnel, available from the American Chemical Society's department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington D.C., 20036.