Approved EPA Method 1603
Method ID/SOP for E. coli by Membrane Filtration
Method: EPA 1603
Modified m-TEC

Applicable Matrices: Scope and Application:

Modified m-TEC is a single step, EPA approved membrane filtration method used for the enumeration of E. coli in disinfected waste waters and ambient waters. The chromogen found in modified m-TEC, (5-bromo-6-chloro-3-indolyl-B-D-glucuronide), will catabolize to glucuronic acid and a red-magenta compound by E. coli that produce the enzyme, B-D-glucuronidase. Incubation is the same as for regular m-TEC (EPA1103.1).

Detection Limit:

Modified m-TEC can detect one (1) colony forming unit of Escherichia coli bacteria.

Method Summary: Procedures:

Sterilize filter funnels (via autoclave at 121 C. for 15 m. @ 15 psi.). Shake sample vigorously to distribute bacteria, then pour an aliquot of sample into a filter funnel that will most likely give rise to 20-60 colonies. Filter sample through 0.45 gridded membrane filter (Gelman type) while applying vacuum with vacuum pump (Gast type). Rinse inside of filter with three successive rinses of sterile buffered dilution water (approx. 30 mLs. per rinse). Record time of filtration (within 6 hours hold time for waste water E. coli) and milliliters of sample filtered. Place filter with sterile (alcohol flame) tweezers grid side up onto a prepared media plate containing 4-5 mLs. of modified m-TEC media, from Aquaplates, and cover. Be sure the filter is in contact with the media on all of the growing surface.

Also, filter a sterile sample (negative control) as well as a positive control (E. coli –ATCC culture). Be sure to re-sterilize filter funnels by autoclave, or disinfect by immersing funnels in boiling water for five minutes between filtrations. Alternately, a UV light may be used between filtrations as well.

Place plates with filters upside down in 35.0 +/- 0.5 degree incubator for 2, +/- 0.5 hours. This will help revive stressed organisms.

After two hours, place the filters in a water-tight container and put in 44.5 C. +/- 0.2 C. water bath incubator with gable cover for 22 +/- 2 hours. Remove plates, place right side up, and count red-magenta colonies which are E. coli. Plates with greater than 100 CFU’s may be inaccurate as the E. coli will compete at that level and growth inhibition is possible. Dilute highly turbid samples, or samples that may be high in colony count. Report E. coli results as colony forming units per one hundred milliliters of sample. (CFU/ 100 mLs.).

Safety:

There are no special safety requirements for Modified m-TEC method. Observe standard microbiological procedures for handling microbes, including disinfection of work area after filtration. Alcohol flames may not be visible when burning, use caution when lighting tweezers. Hold tweezers slightly down when lit to prevent flaming alcohol from running back into the analysts hand.
Definitions:

For Method EPA 1603, those colonies which are red-magenta in color after the incubation period are *E. coli* on Modified m-TEC agar.

Equipment and Supplies:

- Incubator capable of maintaining 35 degrees C +/- 0.5 degrees C continuous operation. Lab line type.
- Wet bath incubator with gable cover or solid block incubator capable of maintaining 44.5 C +/- 0.2 C.
- Prepared Media Plates – Modified m-TEC from Aquaplates
- Slant tubes and racks for holding and maintaining control organisms
- NIST Traceable thermometers
- Autoclave
- DI Water
- Filter funnels
- Filter manifold
- Vacuum pump
- Tweezers
- Alcohol / lighter
- Tubing for vacuum
- 3mm nichrome loop or equivalent
- Sterile buffered dilution water
- Autoclavable bottles with tops or foil for media prep.
- Sterile pipets and graduated cylinders
- 10X Stereoscope, optional with modified m-TEC.
- Bunsen burner or other type flame source.

Reagents and Standards:

- Use ACS Grade reagents and standards.
- Modified m-TEC prepared agar plates from Aquaplates
- Tryptic soy agar for keeping control microorganisms
- Sterile buffered dilution water (see below)
- Tryptic soy broth for culturing control microorganisms
- Stock Phosphate buffered dilution water – sterile. Prepare using commercially available buffers (Hach)
- Final pH should be 7.0 +/- 0.2
- Use phosphate buffered saline for initial and ongoing precision and recovery
- Individual chemicals for buffer solutions and/or medias if preparing from raw chemicals can be found in the July 2006 EPA Method for 1603

Interferences:

If testing a chlorinated system, record free residuals (by sampler) on collection form. Sodium Thiosulfate used in all containers for collecting samples from continuously chlorinated systems. If chlorinated, dechlorinate samples to prevent loss of bacteria in sample. Filter smaller volumes when samples are very turbid.

Sample Collection, Preservation, Shipment and Storage:
Aseptically collect at least 100 mLs of sample in sterile vessel. Keep samples at < 10 degrees C with ice or other effective measures during hold and transport times. Samples brought immediately to lab after being taken need only show evidence of cooling. Record temperature of sample upon receipt and record in Sample Log-In.

**Analysis of samples must begin within 6 hours from sample collection time.**

Samples collected from chlorinated systems must contain Sodium Thiosulfate in their collection vessel to reduce up to 10 mg/L chlorine residual.

**Quality Control:**

Also, filter a sterile sample (negative control) as well as a positive control (E. coli – ATCC type culture – Microbiologics). Be sure to re-sterilize filter funnels by autoclave, or disinfect by boiling water immersion for five minutes between filtrations. Use boil technique only after initial sterilization with autoclave.

All media preparations, slants, sub-plantings and other associated preparations or actions shall be listed in the Chemical, Media and Reagents Receipts and Preparation Manual. Slants shall be labeled with ATCC organism, date of planting or sub-planting, as well as media preparation date and type and reagent water and buffers used. All labels shall be able to be traced back to material receipts by referencing page number in media prep book from prep dates back through material receipts and ultimately NIST or ATCC or ACS grade verification via lot numbers, page numbers and/or date(s).

Do not sub plant more than 5 times from original stock culture. Use reference stock for preparation of working stock.

Samples may be periodically spiked with BioBalls (Sydney, Australia) to check for precision and recovery.

Keep records of Initial Performance as when culturing an unknown or new source. Compare counts between analysts periodically. Agreement should be within ten percent. Check for media growth for any stored media preparations. Discard any discolored media or media that shows mold or other unintended growth. Further confirmation and verification of atypical E. coli colonies may be done by transferring to trypticase soy broth and streaking to nutrient agar plates or slants. Incubate at 24 +/- 2 h. at 35.0 +/- 0.5 C. After growth, perform cytochrome test, where a deep purple color change within 15 seconds where the bacteria have been applied is indicative of (positive for) E. coli. Other tests may be performed, as E. coli are citrate-negative, cytochrome oxidase negative, EC growth with gas, and indole-positive. Commercially available applications are also available which combine general ID for the Enterobacteriaceae group.

**Calibrations:**

There is no calibration of required, except temperature checks of incubators with NIST traceable thermometer. Incubator temperatures on dry incubator shall be recorded at least twice daily for days in use, separated by at least four hours and once daily for wet bath incubator. Holding refrigerator temperatures shall be checked at least once daily and recorded.

**Calculations:**

Report final result in colony forming units E. coli per one hundred milliliters (CFU/100 mLs.).

**Pollution Prevention and Waste Management:**

After incubation and determination, sample bottles shall be disinfected with 10% sodium hypochlorite solution and then poured down sink. Rinse afterward to refill trap with fresh water to displace spent media and chlorine. Plastic
bottles and caps shall be recycled in local plastics recycling station

**Data Assessment of Acceptance Criteria for Out of Control Situations:**

Do not use media from lot of failed control. If blank fails on run, request re-sample of those sample that were run during the test that the blank failed on. Also, no growth on positive controls require investigation as to the cause, then corrective action employed, re-sampling and analysis.

Corrective action for out of control data such as failed blanks or media controls shall be as listed above. Client notified in writing if sample control failed and re-sample requested.

**Annual Method Detection Limit Study:**

N/A

**References:**

*USEPA: Method 1603 – Modified m-TEC.*