Understanding Coliform (Total Coliform and E. coli)

**Introduction** – The presence of certain bacteria are often a good indicator of water quality. Members of the coliform group serve as an indicator of pollutant vectors in the water system, specifically gaps in sanitary treatments. Coliform bacteria are quite common in the environment and most of them, by themselves, are not threats to the public health. The EPA recently revised the total coliform rule to eliminate public notification requirements based only on the presence of total coliforms. It is the detection of coliform bacteria associated with fecal material that is a true cause for concern.

**Approved Methods** – There are two different approved methodologies to test for bacteria. These are most probable number (MPN) and membrane filtration (MF). The MPN procedure can be further broken down into traditional multiple tube methods, which generally rely on physical indicators of gas production and chemical indicators of acid production by the growing bacteria and multiple well methods, which are also referred to as defined enzyme substrate tests. The approved methods are broken down as follows:

- MPN, 5 tube, 3 dilutions
- EPA Microbiological Manual p.114
- Standard Methods 9221 B-2006 followed by F-2006 for E. coli determination
- Multiple Well, Defined Enzyme Substrate – These methods can be used to determine total coliform and E. coli simultaneously
- Standard Methods 9223 B-2004
- Colilert/Colilert-18®
- MF
- EPA Microbiological Manual p.108
- EPA 1603
- EPA 1103.1
- mColiBlue-24®
Method Summary – All types of microbiology methods work on the same principle. A particular media is used to encourage the growth of the organisms you desire and suppress the growth of undesired organisms. Sometimes multiple stages are needed to selectively eliminate undesired organisms.

What You Should Know – While total coliform bacteria are common in the feces of warm-blooded animals they are also found in and on soil, vegetation, and aquatic environments. They are most useful as a negative indicator. The absence of total coliform bacteria shows that there is little chance of contamination from fecal sources. Fecal coliform bacteria have been traditionally used as the determining factor to identify fecal contamination. Since the mid 1990’s more studies have been done to show that the standard fecal coliform assays are not as precise as originally believed. It indicates the presence of bacteria that do not originate in mammalian feces often enough that a substitute has been sought. E. coli is a tighter group of bacteria that is almost exclusive to fecal contamination and is now being used as the indicator of choice.

The multiple tube methods use 5 replicates of a series of 3 decimal dilutions (i.e. a factor of 10 from one to the next) to determine the presence of the target organism. For coliforms that need verification of E. coli the test requires a minimum of 48 hours to perform and could extend up to 96 hours. The result from this test is a series of three numbers, i.e. 5-3-1, that correspond to the number of positive tubes at each level. This pattern is then read off of a MPN table to give the final result.

The substrate methods utilize the reaction of an enzyme produced by specific bacteria, coliforms in this case, to cause a visible change in the sample. The Colilert® methods turn yellow for the presence of any coliform and fluoresce under UV light for E. coli. These methods can have negative results reported as soon as 18 hours while positive results can be reported as soon as the indicators are available.

Membrane filtration methods involve passing a specific amount of sample through a sterile membrane filter. Bacteria are collected on the surface of the filter, which is then placed in a Petri dish. The dish contains either a pad saturated in the desired broth or an agar. The dish and membrane are incubated for a specific length of time at a certain temperature. After the incubation period any viable bacteria will have formed visible colonies on the surface of the membrane. Often two or three different types of bacteria can grow under the MF conditions but only one will be of a target color. A low power microscope is employed to discern smaller colonies and to verify the presence of a sheen on the surface of the colony.
Method Procedure

**Note** – This is not intended to be a standalone method and does not address all safety or quality control aspects that may be required. Quality control in a microbiological analysis can be particularly complex. Media checks and sterility checks are required for a large number of the material and processes involved in the test. Please consult your local regulations to comply with all requirements. The first procedure will address membrane filtration using modified mTec agar according to EPA 1603 while the second will follow SM9222 B-1997 with mEndo broth.

- Collect your sample in a sterile plastic or glass container. Standard Methods has an additional sampling requirement that there be a minimum of 2.5 cm headspace to facilitate mixing by shaking.

- Label the appropriate number of prepared modified m-Tec agar plates with the sample and QC ID’s.

- Place a sterile filter grid side up in a sterilized filter holder and insert in a 3 or 6 place vacuum manifold.

- Pour the appropriate amount of sample into each filter funnel. If less than 20 mL of sample is used an additional 20-30 mL of sterile phosphate buffered dilution water should be added to the funnel to ensure even distribution of the bacteria. Three different dilutions are recommended to ensure a countable number of colonies is obtained. The target number of colonies is 20-80.

- Turn on your vacuum and filter the samples until all liquid has been removed from the funnels. Continue vacuum and rinse the sides of the funnels with an additional 20-30 mL sterile phosphate buffered dilution water. Turn off vacuum.

- Use sterile forceps to aseptically transfer the filter into the labeled plate. Seat the filter with a rolling motion to avoid forming air bubbles under the filter. Replace the cover.

- Invert all plates and place in an incubator at 35 ± 0.5°C for 2 ± 0.5 hours.

- Transfer plates into a waterproof bag. Seal the bag and submerge in a water bath at 44.5 ± 0.2°C for 22 ± 2 hours.

- Remove the plates from the bath and use a 2-5x magnification to count the number of red or magenta colonies.

- There are multiple variations on the frequency and need for verification. Please consult the appropriate regulations for your needs.

- Calculate the number of E. coli per 100 mL according to the following formula. The plate should have 20-80 colonies for calculation purposes. Seek detailed guidance for too few colonies, too many colonies, or multiple plates with 20-80 colonies.
(E.coli)/100 mL = (number of colonies)/(volume of sample used (mL))×100

Collect your sample in a sterile plastic or glass container. Standard Methods has an additional sampling requirement that there be a minimum of 2.5 cm headspace to facilitate mixing by shaking.

Label the appropriate number of sterile Petri dishes with pads with the sample and QC ID’s.

Add the contents of one ampoule of mEndo broth onto the pad.

Place a sterile filter grid side up in a sterilized filter holder and insert in a 3 or 6 place vacuum manifold.

Pour the appropriate amount of sample into each filter funnel. If less than 20 mL of sample is used an additional 20-30 mL of sterile phosphate buffered dilution water should be added to the funnel to ensure even distribution of the bacteria. Three different dilutions are recommended to ensure a countable number of bacteria is obtained. The target number of colonies is 20-80.

Turn on your vacuum and filter the samples until all liquid has been removed from the funnels. Continue vacuum and rinse the sides of the funnels with an additional 20-30 mL sterile phosphate buffered dilution water. Turn off vacuum.

Use sterile forceps to aseptically transfer the filter into the labeled dish. Seat the filter with a rolling motion to avoid forming air bubbles under the filter. Replace the cover.

Invert all plates and place in an incubator at 35 ± 0.5°C for 22-24 hours.

Remove plates from the incubator and use 10-15x magnification to count the number of typical colonies per plate. The typical colony is pink to dark red with a metallic sheen. Consult Standard Methods for an in depth discussion of what is and is not a typical colony.

Calculate the number of total coliforms per 100 mL according to the following formula. The plate should have 20-80 colonies for calculation purposes. Seek detailed guidance for too few colonies, too many colonies, or multiple plates with 20-80 colonies.

(total)coliforms/100 mL = (number of colonies)/(volume of sample used (mL))×100

Standard Methods 9222 G-19997 can be used to determine the number of E. coli colonies present for each filter membrane.

We all like things that make life easier. Was this document helpful? Or do you disagree with something? Have something to add? Contact me at DavidS@envexp.com to let me know what you think.