Bacteriological Examination of Waters: Membrane Filtration Protocol

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Information History

pathogen, it is more practical to screen the water for the presence of fecal contamination by testing for the presence of an indicator microorganism. Indicator microorganisms are ones that have the following properties:

a) the microorganism is not found in water and will be present in the water only when a contamination event has occurred; and

b) the density of the microorganisms present should be proportional to the degree of contamination.

In the 1890's, it was suggested that Escherichia coli should be used as an indicator microorganism to detect the presence of pathogenic bacteria through the fecal-oral route (4). This bacterium was selected due to the work of Theodore Escherich in the 1880s (2). Escherich found that Bacillus coli , (now known today as E. coli) was distributed in the intestines (i.e., an enteric bacterium) and feces of animals and thus meets the properties of the indicator microorganism described above. Today, some water quality standards are still based on the detection of E. coli and/or related bacteria termed "coliforms" (1). Many different techniques can be used to detect the presence of these indicator microorganisms. Such techniques are ones that should have the following properties:

- The technique should be sensitive to detect the presence of the indicator, even at low concentrations.
- The technique needs to be able to process large amounts of water.
- The technique should be easy, cheap and can detect the presence of the indicator quickly.

In 1951, Goetz and

plate.

Table 1. Suggested sample volumes for membrane filtration to detect total coliforms (1). Note that filtering of 0.01 ml of sample is the same as filtering 1 ml of a 1/100 dilution of the original sample.

Table 2. Suggested sample volumes for membrane filtration to detect fecal

It is suggested that duplicate volumes are filtered for drinking water, and three different volumes (or dilutions) are filtered for all other sample sources. The membrane

filtered. However, the turbidity of the sample may limit the volume of sample that is practical to filter. High numbers of background bacteria or toxic substances may interfere with the test and result in underestimation of the density of coliforms.

Detection of total coliforms and fecal coliforms (1)

The characteristics that define the total coliform and fecal coliform groups are easily tested in the laboratory using culture media and incubation conditions that are selective for Gram-negative rods and/or differential for lactose fermenters. Growth media that are used include:

- Detection of total coliforms – mEndo agar LES
  This growth medium contains lactose and a pH indicator that changes color when acid is produced (from lactose fermentation). Coliforms typically will produce metallic (golden) sheen, which is due to the extensive production of aldehydes and acid from the fermentation of lactose. Some total coliforms may also be dark red, mucoid or have a dark center but without a metallic sheen; these are considered to be atypical total coliform colonies (7). E. coli will form colonies with a metallic sheen. It is important to note that some non-coliforms may exhibit red colonies. Filters used to detect the presence of total coliforms in a sample of water should be incubated at 35 ^{o}C for 22 - 24 hours.
- Detection of fecal coliforms - mFC agar.
  This growth medium contains bile salts. Enteric bacteria can grow in the presence of bile salts, while others cannot. mFC agar also contains rosolic acid, which inhibits bacteria other than fecal coliforms. Aniline blue, a pH indicator, turns dark blue upon acid production, helping in the identification of lactose- fermenting bacteria (7). Fecal coliforms form blue colonies on this medium; E. coli will form flat dark blue colonies. While rosolic acid is commonly used as part of mFC agar, it has been suggested that rosolic acid is not a necessary component of this medium (1, 6). Filters used to detect the presence of fecal coliforms in a sample of water should be incubated at 44.5 ^{o}C for 22 – 26 hours.
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Either liquid broth or a solid medium may be used for these assays. Solid media are commonly used and are specified in this protocol.

When reporting total and fecal coliforms in water samples, it is standard to use colony-forming units (CFU)/100 ml of sample.

RECIPE

Nitrocellulose membrane

47mm m

FIG 1 Nitrocellulose membrane. This membrane is used for the detection of total coliform and fecal coliform bacteria from samples of water. It has a 47 mm diameter and a pore size of 0.45 µm. The small pore size in the membrane filter will capture bacterial cells present in a sample of water during filtration.

rinse bacteria and sample remnants from the walls of the filter tower or funnel.

Positive Control Sample

100 ml of water with a 5 ml of a 1:100 dilution of an overnight culture of ***E. coli***

This sample is to be used as a positive control to ensure that you are able to detect E. coli from a water sample using the membrane filtration technique.

Negative Control Sample

Sterile Water

This sample is to be used as a negative control when using the membrane filtration technique.

Water Samples to be tested

Volumes of water needed for testing are indicated in Table 1.

Protocol

NOTES:

- Use aseptic technique throughout the procedure.
- Buchner Funnel or a Filtration column should be aut oclaved priou 0.349 0 Tci5on colu1

12. Repeat the procedure (steps 3 - 10) with the largest volume (or lowest dilution)
13. Incubate mENDO agar LES plates at 35 ± 0.5 ^{o}C for 22 – 24 hours and mFC plates at 44.5 ± 0.2 ^{o}C for 22 to 26 hours, all lid side down. In order to maintain the temperature within such a narrow range, a water bath is typically used for incubation of the mFC agar plates. These plates are placed in wate rtight plastic bags and then submerged in the water bath.

Day 2 (24 hours later):

14. After 22 – 24 hours, remove the mEndo agar LES plates from the 35 ^{o}C incubator and count the colonies that are dark red, mucoid, have a dark center or (more typically) produce a metallic sheen (Fig. 6). These are considered to be total coliform colonies.

FIG 6 Detection of total coliforms following 24 hour incubation at 35°C.
Metalli 0 Td ( )Tj /TT1 1 Tf -0.004 Tc 0.002 x T349 0 Td [(t)-20 (o)1 (t)-8 (a)-5 (l)-349 0 -0.001860 -0r66

FIG 8 Detection of fecal coliforms following 24 hour incubation at

SAFETY

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the ASM Curriculum Recommendations: Introductory Course in Microbiology and the Guidelines for Biosafety in Teaching Laboratories.
Care should be taken while working near a Bunsen burner. Ethanol and nitrocellulose membranes are flammable. Proper laboratory procedures should be followed when working with materials that may harbor microorganisms.

COMMENTS AND TIPS

It is recommend that the dilutions (step 1) be performed. Otherwise it may become difficult to determine the accurate cell density of coliforms present in water samples.
Demonstration that some bacteria in water can, indeed pass through a 0.45 µm pore-size filter can readily be demonstrated. Simply remove 5 – 10 mL of filtrate from the catchment vessel and inoculate a flask or test tube with a general purpose liquid growth medium, such as tryptic soy broth. Incubate the inoculated media at 35°C for 24 to 48 hours. The growth medium will be turbid, indicating that small but viable bacterial cells passed through the filter. A smaller pore-size filer (0.22 µm) is typically used to "filter-sterilize" water samples. A comparison between removals by these two types of filters could also be conducted.
It is recommended that you make your students aware of some of the EPA standards for testing that can be found at the following URLs:
http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/method_1603.pdf
http://water.epa.gov/type/oceb/beaches/upload/2006_06_19_beaches_rvsdman.pdf

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Laurent, P. 2002. Detection and enumeration of coliforms in drinking