

Water Quality Testing

Introduction: A group of bacteria, called coliform bacteria, that inhabit the intestinal tract of humans is used as an indicator of water quality. Large numbers of coliform bacteria in a water sample indicate that the water has become contaminated with sewage. Since that sewage may contain pathogens, the water is considered unsafe. The coliform bacteria themselves do not generally cause problems, but their presence is an indication that the water may be harmful.

Coliform bacteria are identified by their appearance on a particular type of medium, m-Endo broth. The green sheen of coliforms on this medium allows both their identification and quantitation.

Purpose: To determine the number of coliform bacteria in a sample of water.

Safety: The ethanol and Bunsen burner are a fire hazard. Students must be careful not to drip burning ethanol into the beaker of ethanol, which can cause the entire beaker to burn. Hair and sleeves must be kept well away from the flame. Students should know where fire extinguishers are and how to use them.

Materials (per group of students)

- bottle of water to be tested
- analytical filter funnels with sterile membrane and pad (disposable)
- side-arm flask and adapter to hold filter funnel
- aspirator or other vacuum source
- tubing to attach the side-arm flask to the aspirator
- 2.0 ml boiled and cooled m-Endo Broth in a sterile tube
- 1 ml sterile pipette or sterile dropper
- small petri plate
- forceps
- beaker of 95% ethanol
- bunsen burner
- incubator

Procedure

1. Place the analytical filter unit in the side-arm flask and make sure that it is tightly seated in the adapter.
2. Attach the side arm flask to the aspirator or vacuum source.
3. Remove the lid of the filter unit.
4. Carefully pour the 100-ml water sample into the top of the filter unit.
5. Turn on the vacuum source (the water, if it is an aspirator). The water in the top of the filter unit should begin to pass through the filter into the flask below. If nothing happens, check the seal between the filter unit and the flask and check to be sure that you have a good vacuum.
6. Wait until all the water has passed through the filter and the filter appears dry.
7. While you are waiting for the water to filter, prepare the petri dish of m-Endo broth.
 - a) Label the bottom of the empty sterile petri dish with your initials and the date.
 - b) Using a sterile pipette or dropper, remove 1.0 ml of sterile m-Endo broth from the tube provided.
 - c) Place the 1.0 ml of m-Endo broth into the small sterile petri dish provided.
8. When the water has passed through the filter and the filter appears dry, turn off the vacuum.
9. Remove the filter unit from the flask and carefully separate the holder from the filter. Do not touch the filter. Leave the filter in place while you sterilize the forceps (next step).
 - a) Dip the forceps into the beaker of ethanol. Allow most of the ethanol to drain back into the beaker.
 - b) Place the forceps in the flame of a Bunsen burner for one second and remove.
 - c) Allow the alcohol to burn off the forceps.
 - d) Allow the forceps to cool for about 30 seconds before using them.

- Using the cooled, sterile forceps carefully remove the filter and the pad from the filter unit and place directly onto the 1.0 ml of m-Endo broth in the small petri dish. The pad should soak up the broth.
- Incubate the plate, right side up, at 37° C. overnight.
- Observe the colonies and count the very dark colonies with a greenish sheen (there may not be any if the water source was quite clean).

Results

m-Endo broth is a differential medium. Colonies of coliform bacteria produce a characteristic green sheen. This medium is used only to identify coliforms; therefore colonies without the sheen should be ignored. Count the number of dark colonies with a green sheen and calculate the number of coliforms per ml of the water sample. If the original water sample was diluted by your teacher in preparing the sample for filtration, that dilution must also be considered in determining the coliform count in the original water sample.

Teacher Instructions

In advance

Order m-Endo broth, disposable membrane filter units, small petri plates

2 days before lab

- Prepare m-Endo broth**
Prepare m-Endo broth exactly as described on the package. Prepare only enough for 2 days. Boil the medium and cool. **DO NOT AUTOCLAVE**. Use the medium the same day or store in the refrigerator overnight. Since the medium is not sterile it will not keep long. Aliquot about 2 ml into sterile tubes for use in the class.
- Preliminary Test of Water Sample**
The most difficult part of this lab is estimating the extent of bacterial contamination of your water sample so that you will get a reasonable number of colonies on the filter. It is recommended that you collect several water samples a day or two before the class and spread 0.1 ml of the water on nutrient agar. Incubate overnight at 37° C. If you get no colonies or just a few, the water is quite clean and you will need to use 100 ml per experiment. If there are 10-50 colonies, use 10 ml of the sample water diluted in 90 ml water. If there are more than 50 colonies, use 1.0 ml of the sample water diluted in 99 ml water. In all cases the students will be provided with 100 ml samples to filter.

Day of lab

Assemble equipment, media and water samples for each group.

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