

COLIFORM IN DRINKING WATER ASSAY: PREP

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7/20/89, 16Aug93, 23July95, 22July97, 19July 98, 30June99, 9Aug00, 7Aug02, 24July05, 4Oct06, 23July09, 22July10
http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Drinking_Water/Drinking_Water.htm

Modified from: *Standard Methods for the Examination of Water and Wastewater*, 14th Ed, (1975).
pages 928-935.

The maximum number of coliform permitted in drinking water is 5/100 mL. It is impossible to plate out 100s of mLs of water, so the bacteria must be concentrated. This is accomplished by passing the sample (typically 250 mL) through a membrane filter which traps a sample's bacteria on its surface. This filter is then placed on top of an m-Endo MF medium-soaked pad which nourishes the bacteria. Coliform turns red on this medium, allowing enumeration of the number of coliform in the sample.

EQUIPMENT AND SUPPLIES:

Sterile 250 mL capped bottles (1/student)
sterile 47 mm petri dishes (1 per student)
sterile 47 mm memb. filters, 0.45 μ m pores
sterile 47 mm millipore pads
vacuum pump
3 vacuum hoses joined with "T" joint
2 strong hose clamps
1000 mL side arm filter flask
m-Endo Broth MF powder
sterilized repipet in 250 mL bottle

Filtering Station: millipore apparatus:

sintered glass platform in #8 stopper
glass cylinder, 300 mL capacity
800 mL beaker with 400 mL EtOH
150 mL beaker with 100 mL EtOH
triangle-tipped Tongs
blade-tipped (bent) tweezers in EtOH
paper towel (to touch off excess EtOH)
Bunsen burner
protective eye wear

MAKE THESE TWO ILLUSTRATIONS WITH LISTED FEATURES LABELED:

Vacuum Filtering Apparatus:

vacuum pump	filter flask
on/off power switch	#8 stopper
main vacuum line	screen platform
clamp 1: main vacuum line	membrane filter
T joint	glass cylinder, 300 mL
clamp 2: relief valve	cylinder clamp

Plate Ready for Incubation:

(exploded view):

50 mm petri dish top
47 mm membrane filter
47 mm pad with 2 mL m-Endo MF
50 mm petri dish bottom

I. COLLECTION OF WATER (Collect the same morning as performance of assay):

1. Determine and record the precise **name of your water district** in your notebook.
2. Run tap water at home until it is cold (to clear out pipes, at least a minute or so).
3. Label your sterile 250 mL capped bottle with your name. Fill with tap water, rinse several times, finally fill to neck, cap securely, maintaining sterility.

II. PREPARATION OF 50 mL of MEDIUM:

1. For up to 20 determinations, weight out **2.4 g m-Endo Broth MF** powder into 150 mL beaker.
2. Add **49 mL dH₂O** and **1 mL EtOH** to the powder.
3. Bring to boil, stirring with a thermometer. Remove from heat immediately.
4. Using sterile technique, pour into a sterilized repipet vessel which can deliver 2 mL aliquots. Securely screw down repipet to vessel. Clamp to ring stand for stability. (**NOTE**: Ensure that the volume is 2 mL by weighing a delivered aliquot. Adjust repipet setting if necessary.)

III. PREPARATION OF PETRI DISH WITH PAD AND MEDIA:

1. Label the *top* of a sterile 47 mm petri dish with initials, seat number, date collected & source.
2. Flame off EtOH from sterile bent blade-tipped **forceps**, pick up sterile **pad**, place in bottom of sterile **petri dish**, replace cover.
3. Repipet **2.0 mL m-Endo Broth** sterilely onto pad, replace lid, *keep bottom down*.

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IV. FILTER WATER THROUGH THE MILLIPORE FILTER:

Note: Wear safety goggles, tie back hair, *keep flammable materials (EtOH!) away from flame.*

1. **PRELIMINARY:** Check for adequate head space in the filter flask to receive the 250 mL water you are about to filter. If inadequate, empty out the flask into the sink, and replace the support platform. If necessary, re-sterilize surface of support platform with EtOH, turn on vacuum briefly to dry, then tightly clamp the main vacuum line to turn off the vacuum to the platform.
2. **SET UP MEMBRANE FILTER:** Flame off EtOH from blade-tipped tweezers, discard blue separating discs if necessary, pick up a sterile membrane filter, center it on screen platform. Turn on pump if not on yet, and open main vacuum line to hold membrane in place.
4. **STERILIZE THE CYLINDER (*Dangerous step*):** Ensure that all surfaces of the glass cylinder are immersed in 95% EtOH, touching the outside of the cylinder only, pick up with fingers, invert and allow excess EtOH to drip into 800 mL beaker, touching off the last drops on a paper towel. Grasp cylinder upside down with triangle-tipped tongs (Ekco, for instance), and, *away* from EtOH beakers and other students, announce “FLAMING” and *CAREFULLY* flame off the EtOH. It will flame up fairly high, but should burn off in a few seconds. Pass quickly through the flame once more to ensure that all of the EtOH has been removed.
5. **SET UP FILTER APPARATUS:** (The vacuum should still be on.) Grasp the outside of the cylinder with your fingers (it should not be too hot if you touched off the EtOH before flaming in step 3). Position it over the membrane filter, center it on the support platform, and clamp in place with spring clamp.
6. **FILTER WATER THROUGH:** With the vacuum on, pour your 250 mL water sample into the cylinder, monitoring that it is not leaking at the clamped joint. When all of the water has passed through, unclamp, remove the cylinder, and place it carefully upside down back in the EtOH.
7. **RELEASE VACUUM ON MEMBRANE FILTER:** Clamp the main vacuum line shut and open the relief valve to release the vacuum in the flask. With sterile, EtOH-free blade tweezers, gently lift the edge of the membrane filter and remove from the screen platform. (Caution: the membrane filter is brittle. Push it gently at the edge to separate it from the platform)
8. **TRANSFER MEMBRANE FILTER TO PREPARED PAD,** avoiding bubbles by lowering from one side first, centering it on the pad. (Hint: Rest it on far edge of petri dish, slowly pull it across the edge down toward you until it drops down onto the pad.)

V. INCUBATE TO DEVELOP COLONIES:

9. **INCUBATE** the plate without inverting (pad-side down) at 35°C for 24 hours.
10. **COUNT THE COLONIES:** Record the total number of colonies, and the number of coliform (red colonies). Divide by 2.5 to yield the number of bacteria per 100 mL. According to national health standards for drinking water, the number of coliform/100 mL should not exceed 5.
11. **RECORD RESULTS IN YOUR NOTEBOOK AND THE COMPUTER:** Enter your data in the following sequence and format into your notebook, and then into the class spreadsheet table:

Desk No. Initials Source in detail (including water district) Coliform/100 mL

Cut here.....

SET UP STATIONS:

Prep of m Endo MF media (demo desk):

- 150 mL beaker
- balance
- powdered m Endo MF
- 50 mL dH2O
- 95%EtOH
- 2 mL pipet
- ringstand with asbestos pad
- thermometer
- bunsen burner
- hot pad

Plate prep (demo desk):

- sterile repipet
- sterile 50 mm petri plates
- sterile 47 mm pads
- flat bladed tweezers
- 100 mL beaker with 50 mL EtOH

Four Filtering Stations (sides of room):

- four filtering stations
- (two stations per pump)

