



Adopt-a-Beach
***E. coli* Monitoring Instructions**

Methodology Summary

Using Petrifilm plates, you can easily screen lakes and streams for *E. coli* bacteria. After a one-milliliter water sample is placed on the Petrifilm plate, it is incubated. After the necessary amount of time, bacteria colonies are counted.

Equipment

Links on where to purchase materials can be found on the Adopt-a-Beach checklist

- Whirl-Pak bags
- Pipettes (1 ml)
- *E. coli* plates (Petrifilm)
- Gloves
- Bleach (for disposal)
- Sampling pole: A sampling pole can be made from a fishing pole, an extension rod, or a PVC pipe. Use a mop handle or alligator clips to attach the While-Pak bag to the end of the pole.

Storage

Do NOT refrigerate Petrifilm plates; store them at room temperature. Petrifilm will expire 30 days after it is opened. You will need new plates for each visit.

Incubation

Decide where you will incubate your sample when you return from the beach. Samples should be incubated at 35°C/95°F for 24 hours. If you do not have an incubator or other way to achieve this temperature, you can simply keep the sample in a sealed bag at room temperature for 48 hours as opposed to 24 hours. You should still see blue and red dots appear on the Petrifilm. However, without incubation, it is not as likely that you will see the gas bubbles that form, which confirm that the blue dots are *E. coli* colonies.

Sampling

1. Write the date and sample number in the corner of the Petrifilm plate. Observe consistent labeling and recording protocol for samples.
2. On the Whirl-Pak bag, include date, time, site, bacteria sampled for, and collector's initials.
3. Attach the While-Pak bag to your sampling pole. Wait to tear off the top until just before collecting the sample, making sure your fingers do not touch inside the bag.
4. Collect water sample using your sampling pole. You may need to wade into the water to

collect your sample. Fill the sampling bag at least half full with water by sweeping down through the water to elbow depth in a U-shaped motion.

- a. Collect sample at least 20 feet away from swimmers or other animals.
 - b. Try to avoid kicking up the bottom sediment of the sampling site.
 - c. Avoid sampling in the "swash" zone, the area of low wave/nearshore water.
 - d. Take samples from where water is at least one meter deep.
 - e. If you are collecting multiple samples, use a new sterile sample bag for each sample.
5. Carefully carry the sample back to shore and use a sterile pipette to withdraw one milliliter of water from the sample bag.
 6. Lift the top film of the Petrifilm plate and dispense the one-milliliter water sample onto the center of the pink circle.
 7. *Slowly* roll the top film down over the water sample to prevent air bubbles and distribute water evenly within the pink circle. Do not slide fingers or another object across the film.
 8. Leave plate undisturbed for one minute to permit the Petrifilm plate to "gel" with the water.
 9. Transport plates horizontally and gently to the incubator.
 10. Incubate the plates in horizontal position with clear side up for 24 hours.

If you are not using the water sample immediately (such as taking it back to school to perform testing), close the While-Pak bag making sure to include some air to avoid bacterial death. Place the bag on ice (4 degrees C) to slow bacterial growth. Sample should not be stored on ice for more than 6 hours before testing.

Interpretation

After the incubation period, red and blue dots will appear on the plate. Each dot is a bacteria colony. Blue dots (colonies) with gas (small bubbles next to the blue spot) are *E. coli* bacteria and red dots (colonies) are other coliform bacteria. Each colony is equivalent to 100 colonies per 100 ml of water. The Environmental Protection Agency's water quality standards for safe swimming require that no more than 235 *E. coli* colonies per 100 ml of water be found.

Disposal

After counting the bacteria, use a dropper to place one milliliter of bleach on the pink circle. Place used plates in a sealed plastic bag and dispose in garbage.

Health and Safety

- Always wear gloves when sampling and testing!
- Cleanse hands with alcohol wipes, or antiseptic lotion with alcohol between sites.
- Always bring a first aid kit with you to your site.
- Exercise extreme caution with students who are near the water.