Total Coliform and *E.coli* Bacteria

WATER

W-6
Total
Coliform
and *E. coli*
Bacteria

Purpose

Total coliform refers to a broad class of bacteria that are readily cultured from water or environmental samples and refers to the shape of the bacteria when viewed under a microscope. Most are free-living organisms found naturally in soil and sediment. Coliform bacteria should not be present in well water; this indicates contamination in drinking water. These bacteria are commonly found in soil and open surface water samples, such as streams, rivers, lakes and ponds.

E. coli is a species of fecal coliform bacteria that is commonly found in the gut of warm-blooded animals. While most *E. coli* are harmless to humans, some strains such as *E. coli* O157:H7 have been shown to be pathogenic and sometimes even lethal. More importantly, *E. coli* can indicate the presence of a wide variety of disease-causing microorganisms in fecal matter and indicate that some sort of fecal matter is contaminating the water.

High levels of *E. coli* indicate a greater risk of illness from waterborne pathogens of all types, including viruses, intestinal parasites, and other bacteria that may be more harmful. *E. coli* should never be detected in drinking water, and only small concentrations are allowed in waters used for primary or secondary contact recreation, as described in the interpretation section.

The Petrifilm product recommended for this test can detect both the general category of coliform bacteria and also the *E. coli*. A special dye in the gelling agent reacts with the bacteria and will turn pink when there is a coliform colony forming unit. It will turn blue and may have a gas bubble when there is an *E. coli* colony forming unit. A colony forming unit is created from inoculation with a microscopic bacteria that will divide and grow into a colony, or dot on the plate, and will be visible to the naked eye after an incubation period. This is the basis of the test. This "quick test" can be performed at home, but you will have to wait for the colonies to form during the incubation period before you can count them and record your data.

Tools

3M Petrifilm EC Count Plate

Total Coliform and *E. coli* (short for *Escherichia coli*) are two types of bacteria that can be cultured in a quick test on 3M Petrifilm or in a lab on petriplates. Petrifilm EC plates were originally designed for counting *E. coli* and coliform bacteria in the food and dairy industries. This product is a ready-made culture medium system that contains nutrients, a cold-water-soluble gelling agent, and red and blue indicator dyes to help identify and count bacteria colonies. Sterile (one-use) 1-milliliter (ml) pipettes are also needed for this test, along with your water sample and an incubator, or relatively warm place to incubate the plates.

Storage and Disposal

Store unopened Petrifilm plate pouches at temperatures below 46º F. Allow pouches to come to room temperature before opening. Return unused plates to the pouch. Prevent exposure to moisture after opening pouches. Store resealed pouches in a cool dry place for no longer than one month. Exposure of plates to temperatures above 77º F and/or humidities above 50 percent can affect performance of the plates. Do not use plates that show orange or brown discoloration. Expiration date and lot number are noted on each package of Petrifilm plates. After use, Petrifilm EC plates will contain viable bacteria. Put all used Petrifilm in a plastic bag, inside another plastic bag, and take to sanitary landfill or transfer station. Alternatively, mix a mild chlorine bleach solution (10 percent) and put one dropper full on each exposed plate to kill the bacteria before disposal.

Procedure

1. Organize the water samples, and line them up in numerical order. Remove one plate for each sample from the pouch, including a plate for your blank, which should be distilled water or tap water that you know has been treated by a municipality or rural water district. Return unused plates to the pouch, put inside a resealable plastic bag, and return it to the refrigerator.

2. Label the plates with a permanent marker or dark ballpoint pen. At the top, write on the plastic outer layer, the date, the sample ID number, and a one or two-word description of the sample, for example, "north pond."

3. Remove sterile 1 ml pipettes from your kit. You will need one for each water sample. Remove the first pipette from its cover, being careful not to touch the tip, by opening the plastic wrapper at the bulb end.

4. Pipette exactly 1 ml of liquid from your first sample to the appropriate plate. The 1 ml mark is at the top of the stem of the pipette, before the bulb. Lift up the cover sheet on the plate, and gently release the water sample into the center of the pink circle. Then slowly roll the top cover back down onto the sample, which will spread it somewhat. Gently pick up and tilt the plate from side to side to distribute the water evenly. Discard the first pipette. Keep the sample plate flat. You may stack as many as 20 of these without risk of damage or cross contamination. Pipette the remainder of your water samples. (*Figure 6-1*)

5. The ideal incubation temperature for this test is 35°C (93°F). At this temperature, test plates can be counted in 48 hours (two days). A warm spot on top of your refrigerator would be ideal. Do not place near a heating vent, wood stove, or any other place where plates would reach temperatures higher than 95°F, and do not expose them to high intensity light or sunlight. Normal room lighting is generally okay. Place them in a sealed plastic bag to keep them from drying out. If you are incubating the plates at 80°F, they may be ready to read in three days. At 70°F, wait at least four days for the bacteria to grow.

6. Count the plates in a well-lit area, on a slide sorter, or other area with good lighting. First, count the number of pink dots. These are primarily coliform colonies and represent several different types of bacteria that are normal and natural in nature. In fact, in good healthy soils, it is preferable to have more of these rather than less, along with other microbes such as fungi, nematodes, and others. But in drinking water you would like to see zero if possible, although these aren't necessarily pathogenic or harmful. In streams, rivers, lakes and ponds, the presence of coliform simply indicate the presence of soil, sediment, or other source of coliform.

   If there are more than 20 or 30 pink colonies, count one small square and multiply by 20, or two small squares and multiply by 10, because there are about 20 squares in the plate. This will give you an estimate. If the entire plate turns into pink and blue blotches, write "tmtc" (too many to count) on your data sheet. Next time you collect a sample from this location you will need to dilute it, using a ratio of 1:10, 1:100, or more.

7. Now count the blue dots or colonies. (See *E. coli* test interpretation on the back of the Citizen Science pocket portfolio, MF-2683.) These are the *E. coli* that indicate fecal contamination. Follow the same procedure as above. Where there are more than 20 or 30 dots, count one or two squares and multiply.

   Your units are colony forming units (cfu) per 1 ml. Multiply by 100 to compare to national and other standards, which are usually calculated on a 100 ml basis.
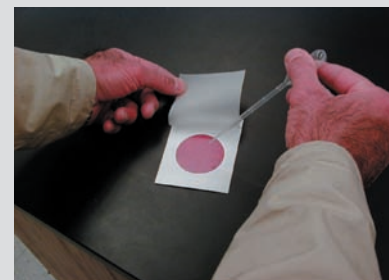

Figure 6-1. Use a 1-ml pipette to transfer the sample to the Petrifilm plate.

Warning: These plates are not designed for the detection of *E. coli* O157:H7, a highly pathogenic form of *E. coli* that can cause kidney complications and other problems. Like most *E. coli* or coliform media, this plate will not specifically indicate whether O157:H7 is present.

Caution: 3M has not documented Petrifilm EC plates for use in industries other than food and dairy, for example for the testing of water, pharmaceuticals or cosmetics. Do not use Petrifilm EC plates in the diagnosis of conditions in humans or animals.

See the back of the Citizen Science pocket portfolio, MF-2683, for E.coli interpretation, which illustrates best, good, fair and poor rating results. Or visit http://www.oznet.ksu. edu/kswater/images/Water_soil_sites.htm for examples.

Interpretation

The ratings below apply to surface water only. If you are using the Petrifilm to test your drinking water, the standard for both coliform and for *E. coli* is zero. For surface water, we are using the 200 colony forming units (cfu) per 100 ml criteria for contact recreation (two colony forming units per plate), and the 2,000 colony forming units per 100 ml (20 colony forming units per plate) level for noncontact recreation as our threshold levels for *E. coli* numbers or blue dots (colonies). There is no EPA standard for total coliform, but based on our testing of farms and streams in Kansas we have come up with suggested guidelines. On your data sheets you might also note if there are patterns with these data, as well as with *E. coli* at different times of year, at different sampling sites, or at high vs. base flow water levels.

What about livestock drinking water standards? Many surface water samples from farms are drinking water sources for cattle or other livestock. There are no published drinking water standards for farm livestock, and in general, *E. coli* will not cause health problems for cattle if it is in the water. However, the importance of *E. coli* is that it is an indicator organism and points to risk of other, more harmful organisms that could be in the water due to fecal contamination. For example, salmonella has been known to make cattle sick from contaminated pond water. Other communicable diseases could be spread through the fecal-oral route that might result in lower rates of gain, if not symptoms of a specific disease. In general, the higher the *E. coli* count, the higher the risk, especially for young animals, or those with compromised immune systems (due to stress or other factors).

E. coli Rating			
4 – Best	3 – Good	2 – Fair	1 – Poor
None detected. (For drinking water, this is the only acceptable level.)	*E. coli* detected, but less than 2 colony forming units per plate. (Safe for contact recreation, such as swimming.)	*E. coli* between 2 and 20 colony forming units per plate. (Not safe for contact recreation, but acceptable for non-contact recreation, such as boating.)	*E. coli* greater than 20 colony forming units per plate. (Not considered safe for non-contact recreation.)

Total Coliform Rating			
4 – Best	3 – Good	2 – Fair	1 – Poor
None detected. (For drinking water, this is the only acceptable level.)	Less than 20 colonies per plate.	20 to 200 colonies per plate.	More than 200 colonies or too many to count.

What to do if your test sample has *E. coli*?

If you obtain a test that indicates *E. coli* in a human drinking water source (farm well, or public water source), there are several steps to take. One is to re-check the sample, and make sure that your pipette or sample container was not the source of contamination. If the drinking water is from a public source, contact the officials in charge of water testing at that facility and let them know about your result. If it is from a home well, you can have it re-tested at a commercial lab to confirm the results. While waiting for confirmation, switch to bottled water. There are several alternatives if your well is contaminated. K-State Research and Extension fact sheets can help with water test interpretation and guide you through shock chlorination of a farm well as a treatment step. See *Private Wells - Safe Location and Construction*, MF-970; *Private Well Maintenance and Protection*, MF-2396; *Shock Chlorination for Private Water Systems*, MF-911; and *Understanding Your Water Test Report — Microbiological, Chemical and Nuisance Contaminants*, MF-912. For a list of labs, see the KDHE Web site: *http://public1. kdhe.state.ks.us/labaccredit/labaccredit.nsf/ frmfrontend?openform*.

If your surface water sample (river, stream, pond, or lake) has coliform, no action is required, as this is normal in the open environment. If *E. coli* is detected, interpret the test in light of the intended use. For example, if this is your favorite swimming spot, and there were more than two *E. coli* on the sample plates, you might not want to swim there any more. The next step is to determine if you would like to reduce the *E. coli* in that particular water body. For example, if cattle drink from that source, and there is an obvious activity or place on your farm contributing to the *E. coli*, then an action plan for remediation could be created. On some farm ponds, fencing the pond with inexpensive electric fence and limiting access to the pond or installing a concrete tank below the pond can reduce fecal contamination. Moving hay feeding sites, strategic location of mineral and salt, etc, also can make a difference. For more suggestions on grazing lands, see the Web site: *www. oznet.ksu.edu/glwqll.*

On other ag land, *E. coli* could be coming from recently applied manure or compost, or from livestock access to crop fields, feedlots or feeding areas. Household waste systems that are in need of repair are also possible sources of *E. coli*. Wildlife and pets can contribute *E. coli* to the environment, but unless they are in high concentration, they generally don't create a problem because sunlight (UV light), heat, and drying out in the environment kill *E. coli*.

Ordering Information

This fact sheet only describes the use of the 3M EC plates. A second product is available from 3M for assessing contamination on surfaces, such as kitchen work space, or garden vegetables that may have been contaminated from manure or compost. For these types of samples, order the 3M quick swabs, listed below.

3M Microbiology Products
3M Center, Building 275-5W-05
St. Paul, MN 55144-1000
1-800-328-1671
www.3M.com/microbiology
E-mail: *microbiology@3M.com*

These are the plates for the coliform and *E. coli* bacteria:
6484 50 EC plates
6414 500 EC plates
6432 50 quick swabs (for surfaces)
6433 250 quick swabs (for surfaces)

Ward's
P.O. Box 92912
Rochester, NY
14692-9012
1-800-962-2660
www.wardsci.com
18W 2972 sterile pipets (500)

Kansas State University Agricultural Experiment Station and Cooperative Extension Service