The Meadows Center for Water and the Environment
Texas State University

Texas Stream Team
E. coli Bacteria Field Guide

Sample Collection

ALWAYS wear gloves or use hand sanitizer prior to collecting bacteriological water sample to prevent contamination.

Note: If your sample site is downstream of a wastewater treatment plant outfall, the effluent might contain chlorine disinfectant that could debilitate bacteria. At these sites, Texas Stream Team recommends citizen scientists use the Whirl-Pak® bag that contains 10 mg tablets of sodium thiosulphate to neutralize free chlorine in the sample.

1. Before collecting your sample, label each Whirl-Pak® bag with the site ID, site description, date, and time collected. If it is appropriate to process a field blank sample, the Whirl-Pak® bag will have the previously mentioned information plus a "field blank" label. Refer to the Field Blank section on this field guide to see if a field blank is necessary.

2. If conducting a field blank, prepare your field blank Whirl-Pak® bag first before collecting your water sample. Transfer roughly 1 mL of the sterile diluent from its original container to the field blank Whirl-Pak® bag while at the monitoring site. This will serve as the "field blank."

3. Collect bacteriological samples in sterile Whirl-Pak® bags. *Never pre-rinse the bacteriological sample container because it is sterilized.*

> ***When collecting samples from water body***, dip the Whirl-Pak® bag to a depth of 0.3 m (1 ft), or roughly half the depth, in very shallow streams. Avoid contact with sediment to prevent contamination. With the open end facing upstream, push the opened mouth of the Whirl-Pak® bag upstream at this depth until full.
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> ***When collecting samples from a bucket of water***, collect the bacteria sample before other monitoring activities occur. Before grabbing your sample, rinse bucket twice, discard the water downstream of your sample site. Pour water from the bucket into the Whirl-Pak® bag. Never the Whirl-Pak® bag into the bucket. This could introduce contamination.

4. Squeeze out the top 1 inch of water from the Whirl-Pak® bag and whirl the bag away from you to seal. The sealed bag must retain at least 50 mL of sample but leave 1 inch of airspace to help mix the sample when it is inverted just before making the dilutions.

5. Place sample(s) on ice immediately after collection. Bacteriological samples must be transported, processed (diluted and plated), and placed in incubator within **8 hours** of sample collection. Do not report samples that are not processed within the time limit or holding time.

Field Blank

The frequency of a bacteria field blank is 1 with every 10 samples. If less than 10 bacteria samples are collected in a month, include at least 1 field blank per month. Follow routine handling, plating, and analysis procedures, and report the results on your Monitoring Form. There should be no *E. coli* bacteria colony growth on the field blank samples. If *E. coli* bacteria growth occurs, discard all data collected that day. Document the results on the Monitoring Form and consult your trainer.

Analyze *E. coli* Bacteria with Coliscan Easygel

Determining Sample Size

The ideal number of colonies resulting from a single prepared plate is 20-60, and not over 200. Since the number of resulting colonies is dependent on the sample size, it may be necessary to experiment with different sample volumes to determine the best sample size to achieve 20-60 colonies.

1. To establish a baseline for typical conditions, collect a 1 mL and a 5 mL sample volume during the first sampling event.

2. If the 1 mL sample volume results in *E. coli* bacteria colony counts of 0 or only a few colonies, the sample volume should be increased to 3 mL or 5 mL. Conversely, if the 5 mL sample results in >60 colonies, the sample size should be decreased to 1 mL or 3 mL for the next sampling event.

Note: Environmental and precipitation variables will influence bacteria levels. Waterbodies with low discharge often have more bacteria, and sampling should begin with 1 mL and 3 mL sample volumes. Pristine waters may require a 5 mL sample volume to achieve the preferred colony range.

Preparation

Note: Coliscan Easygel should be removed from the freezer in time to ensure they have reached room temperature (typically 2-3 hours) before use. You can also place them in water to help with thawing.

1. Prepare a minimum of 2 Petri dishes per sample, in addition to the field blank, if necessary.

2. Label the top of each Petri dish (the side without the treated film) with the station ID, site description, date, and the sample volume (1, 3, or 5 mL). If conducting a field blank, write field blank on the Petri dish as well.

Preparing the Sample

1. Invert the Whirl-Pak® bag with your sample water a few times, avoid touching the lip of the bag.

3. Unwrap the pipette from the bulb end when ready to draw the sample. Avoid contact of the tip with anything except the

sample water.

4. Submerge the bottom half of the pipette into the Whirl-Pak® bag and squeeze the bulb to expel the air. Draw the appropriate sample water volume (1, 3, or 5 mL) into the pipette by releasing the bulb slowly. Squeeze out any excess of the desired volume from the pipette.

6. Place the designated sample water volume from the pipette into the Easygel media bottle, cap, and swirl gently.

7. If a field blank is necessary, withdraw 1 mL of sample water from the Whirl-Pak® bag labeled field blank and place in the Easygel media bottle, cap, and swirl gently.

8. Your samples are now prepared. Record the sample sizes on the Monitoring Form.

Note: Once mixed with Easygel media, the prepared samples should either be plated within 10 minutes, kept on ice, or placed in a refrigerator and plated as soon as possible.

Plating the Sample

1. Pour the prepared sample slowly into the bottom of the Petri dish (the side with the treated film).

2. Gently swirl until there is a smooth coating of the prepared sample across the bottom of the Petri dish (be careful not to splash over the side or on the lid).

3. Set on a level surface and allow 5-45 minutes for the media to gel. This will help ensure that the sample will be spread uniformly across the Petri dish and help prevent shifting or pooling of the media after being placed in the incubator.

Incubation

1. Turn on the incubator far enough in advance to ensure a steady incubation temperature of 33° C +/- 3°C is reached before placing Petri dishes in incubator.

2. Place Petri dishes right-side up in the incubator. Colonies should be counted within **28-31 hours**.

3. Record the incubation start and end times and temperature on the Monitoring Form.

Counting *E. coli* Bacteria Colonies

1. Count the number of individual and distinct dark purple and dark blue colonies with and without pink diffused halos. **Do not** count the pink, white, light blue, or teal/turquoise-colored colonies.

2. Record the number of *E. coli* bacteria colonies on the Monitoring Form for each sample and/or field blank.

Data Reporting

Results of the analysis for the 2 samples per site plus the field blank are reported on the Monitoring Form as "colonies per 100 mL" of sample water. To arrive at that number, first determine the dilution factor.

> Dilution factor = 100 mL/ sample size
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> For example, if you collected a sample size of 1 mL in the pipette and added this to the Easygel media, your dilution factor is 100 mL/1 mL, or 100.

To determine the number of colonies per 100 mL, multiply the number of colonies counted x dilution factor.

> For example, if you counted 25 colonies and had a dilution factor of 100 (1 mL sample size), the result is 25 x 100 = 2500 *E. coli* colonies /100mL. If you counted 25 colonies and had a dilution factor of 33.3 (3 mL sample size), your result is 25 X 33.3 = 833 *E. coli* colonies/100mL. And, if you counted 25 colonies and had a dilution factor of 20 (5mL sample size) your result is 500 *E. coli* colonies/100mL.

This information should be entered on the Monitoring Form to document the results of each set of samples analyzed. Verify the dilution factor calculation is correct and marked accordingly on the Field Quality Control Checklist.

Note: Once the appropriate sample size is determined for a particular site (1, 3, or 5 ml), the sample size for both samples should remain the same for the next sampling event. For example, if after the first time you sample a site you determine that a 3 ml sample size yields the ideal number of E. coli colonies (between 20-60 and not over 200), then the sample size for both samples for that site should be 3 ml during the next sampling event.

Waste Disposal

1. To dispose of the used Petri dishes, lift the lid and pour 5 mL (about 1 teaspoon) of straight bleach or isopropyl alcohol into each dish.

2. Make sure the bleach has covered the entire dish and allow it to sit for a minimum of 15 minutes.

3. Place the dish in a sealed plastic bag and place in normal household trash.

4. Clean the inside of the incubator with diluted bleach solution and allowed to air dry before the next use.