Bacteroides

Culture methods are available to test for sewage contamination in buildings (M117 and M013) as well as new *Bacteroides* PCR based methods. The *Bacteroides* test has many advantages over the traditional culture tests for total coliforms, fecal coliforms, fecal streptococci, enterococci and *E.coli* tests, including:

- *Bacteroides* is specific for fecal contamination from all sources–animals, birds, and human.
- Coliforms, *E.coli*, fecal streptococci and enterococci can grow in water, soil, sediments and on vegetation in uncontaminated environments. (Note: *E.coli* can be found in pristine tropical environments).
- Because they are anaerobic, *Bacteroides* will not multiply in the environment.
- *Bacteroides* out number coliforms by 1.000:1 and outnumber *E.coli* by 10,000:1; therefore, the chance of finding *Bacteroides* is greatly increased.
- Traditional culture based tests rely on the presence of live bacteria. Unfortunately these bacteria often will not be viable in indoor environments. THIS MEANS THAT A NEGATIVE COLIFORM or *E.coli* RESULT FROM AN INDOOR SAMPLE MAY NOT PROVE THE ABSENCE OF FECAL CONTAMINATION. The *Bacteroides* test overcomes this limitation. Since PCR is used, the laboratory can detect live, nonviable or viable-but-not-culturable bacteria.
- Using Total *Bacteroides* (M095), it is now possible to determine the amount of fecal contamination from non-human and human sources.
- Using Human *Bacteroides* (M199) determines the amount of fecal contamination from human sources only.

Sampling Procedure

For sampling *Bacteroides* it is important to wear sterile nitrile gloves. Change the gloves frequently between samples if you are collecting many samples to prevent accidental cross-contamination.

Soil Samples

Use a 120 mL sterile bottle. (Preserved bottles are not required for this test but if it is present, that is OK). Do not use tools for digging the soil since they may have been previously contaminated with fecal bacteria unintentionally. Instead use the rim of the opened bottle to loosen the soil. Drag the bottle along the soil to be sampled to scoop the soil into the bottle. Repeat if necessary to collect get at least half a bottle of sample. Use a separate bottle for each sample.

Stormwater, Groundwater or Surface Water:

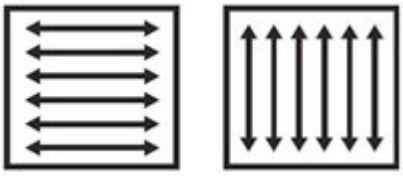
Use a 1000 mL sterile bottle as a minimum. A larger sample size is better in the event the *Bacteroides* concentration in these samples is very dilute. EMSL also supplies 10 L sterile containers for a fee. Use a separate bottle for each sample and each different bacterial test.

Well Water

Use a 1000 mL or 10 L sterile container.
REMEMBER that *Bacteroides* testing should not be done for an NPDES permit. EPA is still evaluating the test for this purpose. Use the culture fecal bacterial indicator tests specified in the permit instead.

Surface (wipe) Sampling

1. Obtain a sterile 1mL Butterfield's solution swab (EMSL Product ID 8708935) or a pre-moistened sterile transport swab (EMSL Product ID 878301 or 878301B to collect and transport samples (provided at your request by EMSL).
2. With sterile nitrile gloves on, prepare separate 4"x4" sampling grids for each sample to be taken. Do not re-use sampling grids since you will be cross-contaminating your samples. Alternatively purchase pre-packaged electrical, masking or paint tape to mark out the 4"x4" areas to be sampled and then swab inside that area. Do not re-use the tape since you will be cross-contaminating your samples.
3. Change nitrile gloves. With sterile nitrile gloves on, remove swab from sterile packaging.
4. Carefully remove the swab from the container being sure not to accidentally touch swabs to anything other than the site to be sampled.
5. Hold swab at an approximate 30° angle from the sampling surface, taking care not to contaminate any part of the swab.
6. Using firm, even pressure, move the swab slowly and thoroughly over an entire 4" x 4" sampling area using the picture below as a guide. Rotate the swab 180 degrees between the latitudinal and the longitudinal passes.
7. To minimize sampling variability between samples, get in the habit of doing 6 latitudinal and 6 longitudinal passes only. Use the picture below as a guide. If you are unfamiliar taking samples this way, it is a good idea to practice your sampling technique before going to the site.



8. After sampling is complete, carefully put swab back into its transport container and seal.
9. Label the samples using a permanent ink marker.
10. ALWAYS take background samples in non-fecally contaminated areas to determine if there is background contamination from an unidentified event that may bias your results.

Bulk Sampling

2"x2" sample size of carpet, wall board, flooring, clothing. Purchase sterile rubbing or isopropanol alcohol. Use disinfected scissors or cutting knives only. Do not re-use these without disinfecting between samples or you will be cross-contaminating your samples.

1. Don a pair of sterile, nitrile gloves. Pour the alcohol over the cutting blade and allow to air dry before each sample to be taken.
2. Use a clean, unused ziplock bag for each sample taken. Label each bag with a permanent marker. Use a different sample identifier for each sample taken.

Sample Shipping

- Be sure to label each sample with a unique identifier using a permanent marker.
- There are no special shipping requirements since *Bacteroides* will not multiply outside of the intestine.
- There is no hold time, however, ship the samples back to the lab as quickly as possible (overnight is ideal). For shipping, use an insulated container. No freezer packs are required.