Bacteria Sampling Protocol

Pennsylvania Bacteriological Sampling Protocol

Applicability

This method applies to all surface waters of the Commonwealth of Pennsylvania, including streams, lakes, ponds, reservoirs, springs, and wetlands. The methods described below shall be used to assess recreational use attainment of surface waters. Use attainment decisions may be derived from other datasets on a case-by-case basis.

Sampling Frequency and Duration

Bacteriological sampling for determining water contact recreational use attainment should be conducted during the swimming season (May 1st through September 30th). At least two sampling groups must be collected per site to characterize a chronic impact. A sampling group consists of 5 bacteriological samples collected on different days during a 30-day period and spanning a minimum of 14 days. It is best to sample at varying times of the day to minimize the effect of timed releases from point source discharges.

Sampling Approach

Water Contact Recreational Use-
Waters supporting public beaches and aquatic life-use impaired waterbodies, with the highest potential to have water contact recreational use impairment, will be the focus of initial sampling efforts. Impairment sources include municipal point sources, combined sewer overflows, and agricultural sources relating to manure application, livestock grazing, and animal feeding. Other targeted sites may be placed in areas of important recreational activities or where land use practices may contribute to potential bacteriological problems. Additionally, during the swimming season Department of Health (DOH) and Department of Conservation and Natural Resources (DCNR) collect weekly samples for E.coli at public beaches for monitoring purposes (PA Code, Title 28. Chapter 18. §18.30). Closure notices when violations of criteria occur are also issued by DOH. In cooperation with DEP, DOH and DCNR provide a list of closures that DEP utilizes to focus future fecal coliform assessment sampling in areas where the closure lists indicates a possible recreational impairment.

Probabilistic Sampling

A random probabilistic design may be employed. The purpose of the probabilistic sampling design is to determine the magnitude of the bacteriological problem over a large scale rather than at the small waterbody size. Probabilistic sampling design will usually employ randomly selected sites at the watershed scale. The design will focus on random site selection and the number of sites is based on the desired target precisions and estiamted confidence levels.

The site selection process will be weighted based on Strahler stream order and land use. The actual site selection process is done using a grid system overlaid on a GIS map of all streams in the sample population, or all streams that could potentially be monitored for bacteriological analysis. During the grid process, the targeted number of sample sites are chosen at random.

Site Selection

Streams:
Two sampling sites at a minimum (each with two completed sampling groups of five samples) are normally required to delineate an impaired stream segment. Bacteriological data collection locations should be selected so that segments are properly bracketed by at least two sites, one upstream and one downstream from tributaries, point source discharges, and major changes in land use on a local level. In some cases more than two sites will be needed to assess a stream segment.

Lakes:
Samples from lakes with beaches or designated swimming areas are collected from the left perimeter, right perimeter, and center of the swimming area and each location sampled requires two completed sampling groups of five samples. If the beach or swimming area is less than 100 feet in length then only two samples are collected, each one a third of the distance from either end of the perimeter. If there is more than one beach or designated swimming area on a lake then each one must be treated individually and sampled accordingly. Lakes one square mile or less that have no designated beach or swimming area require one sampling site at the outfall with two completed sampling groups of five samples. Lakes having a surface arear greater than one square mile (640 acres) with no beaches require use of best professional judgment to determine the location and number of sampling sites. Number and distribution of docks, boating areas, residences, cabins, and access areas should be considered in locating sample sites. Large lakes with beaches and heavy water skiing and jet boating may require additional sampling in addition to the beaches.

Equipment

Bacteriological samples shall be collected using bottles that have been pre-sterilized and contain the proper amount of de-chlorinating agent (preservative). DEP supported sampling activities use 125-mL, screw-capped, polypropylene bottles with sodium thiosulfate added to neutralize the effects of residual chlorine.

Sample Collection Methodology

Ambient Surface Waters-
Streams: Sample in the main current, in the area of the greatest flow (mid-channel, mid-depth). Do not sample at the water's edge and avoid stagnant water.

Lakes: Samples collected from the shoreline should be collected in water one meter or knee deep at a minimum. Samples from a boat are collected with the anchor up (so as not to disturb the sediment) , motor off, drifting toward or along shore, and water depth must be at least one meter.

a. After wading or boating to the desired sampling location, pause to allow disturbed sediment to settle. Do not collect water that is clouded by the sediment you disturb.
b. In flowing water, stand facing upstream.
c. Remove the cap from a 125-mL BacT bottle. Avoid touching the inside of the bottle, the threads at the top of the bottle, or the inside of the cap. If you accidentally touch any of these surfaces, a new bottle must be used.
d. DO NOT RINSE THE BOTTLE!
e. Sample water from a depth of 8 to 12 inches beneath the surface. If the water is less than 16 inches deep, collect the sample at mid-depth. If the water is less than 4 inches deep, find a new place to sample where the water is deeper.
f. Grasp the uncapped bottle near its base with the opening pointing downward and plunge it below the water surface. Turn the submerged bottle into the current and away from you. In slower current or lakes, push the bottle underneath the surface and away from you as if dipping water in an upstream direction.
g. DO NOT FILL THE BOTTLE COMPLETELY. Allow one-inch of air space. The sample is shaken at the laboratory prior to analysis and the air space makes this easier.
h. Recap the bottle carefully, making sure not to touch the inside of the bottle, its threaded top, or the inside of the lid and tighten.
i. Dry the outside of the bottle and either place an adhesive label on the bottle or write the sample information directly on the bottle in permanent marker.
j. Place each bottle inside a Ziploc plastic bag and seal it before putting them in the cooler with ice. Place all Bacteriological Analyses forms in an additional plastic bag and place them inside the cooler on top of the ice.
k. Deliver samples to the laboratory within 6 hours of collection for samples collected for assessment purposes or within 30 hours of collection for samples collected for monitoring purposes.

Quality Control

Each collector shall submit one blank and one duplicate sample for every 10 samples submitted for analysis. The blank sample will be used to test the laboratory's accuracy while the duplicate sample will test the laboratory's precision.

1. *To complete a blank sample*: Fill a sample bottle with sterile (autoclaved) water from DEP's laboratory. Label the bottle consistent with regular water samples, but be sure not to indicate on the bottle that the sample is a blank. Record the blank sample information on the same Bacteriological Analyses form as used for the regular water sample completed concurrently.

2. *To complete a duplicate sample*: Take a second sample bottle to the monitoring site. After filling the first bottle (the test sample), immediately fill a second bottle (the duplicate sample) from the same location using the same technique. Label the duplicate sample as you would the test sample, but do not indicate that the sample is a duplicate. Record the duplicate sample information on the same Bacteriological Analyses form used for the regular water sample completed concurrently.

Laboratory Results

Water Contact Recreation samples:
Fecal coliform bacteria are reported by the laboratory as the number of colony forming units per 100 milliliters (CFUs/100 mL). Analysis is ideally conducted by state-certified labs following Standard method 9222D (SIS code 31616), including recommended quality control analyses.

Coastal Beach Samples:
Coastal Beach samples are analyzed for E.Coli bacteria and reported as the number of colony forming units per 100 milliliters (CFUs/100 mL). Analysis is ideally conducted by state-certified labs following EPA Method 1603 (SIS Code MMTECMF).

Data Processing (Fecal Coliform)

Water Contact Recreation Samples:
A geometric mean is calculated for each sampling group (5 samples collected on different days in a 30-day period) and two complete sampling groups are required to characterize each site.

To calculate a geometric mean of 5 samples, first compute the natural logarithm (ln) of each sample result and then calculate the average of the logarithm values. Finally, convert this product back to a normal value by computing the antilog of the product. The following examples illustrate how this is done:
Example 1

Sample 1:	130 CFU	ln (130) = 4.868
Sample 2:	380 CFU	ln (380) = 5.940
Sample 3:	240 CFU	ln (240) = 5.481
Sample 4:	100 CFU	ln (100) = 4.605
Sample 5:	180 CFU	ln (180) = 5.193

Average of logs = 5.217
Antilog of average = 184.4

Example 2

Sample 1:	120 CFU	ln (120) = 4.787
Sample 2:	390 CFU	ln (390) = 5.966
Sample 3:	220 CFU	ln (220) = 5.394
Sample 4:	130 CFU	ln (130) = 4.868
Sample 5:	180 CFU	ln (180) = 5.193

Average of logs = 5.242
Antilog of average = 189.0

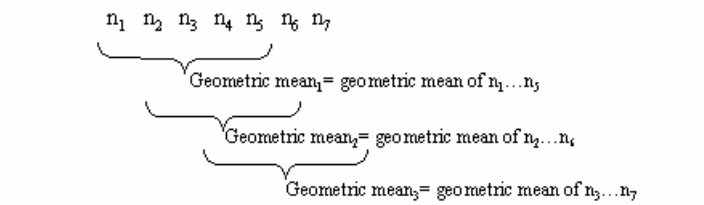
Now compare the values 184.4 and 189.0 to the swimming season criterion for fecal coliforms (200). No violation exists since neither value exceeds the criterion. We would conclude that this site is not impaired for recreational use.

Two violations of the 30-day geometric mean fecal coliform criterion for protection of water contact recreation as defined in Pennsylvania's Water Quality Standards occurring in the same or consecutive swimming seasons (May 1 – September 30) constitute use impairment. Generally, sampling efforts that generate less than two groups of five samples, for purposes of calculating geometric mean values for comparison to existing water contact recreational use criterion, will not be used to list waters as impaired. However, smaller or larger data sets may be considered depending on the frequency and/or duration of sampling. If a smaller incomplete data set supports the calculation of 1 complete sampling group (geometric mean of 5 samples collected on different days in a 30-day period and spanning at least 14-days) and that single mean greatly exceeds the geometric mean criterion value, the waterbody may be listed as impaired for water contact recreational uses. Incomplete data sets spanning multiple years that document good water quality, even in the absence of geometric mean values, are considered evidence that the waterbody is attaining the water contact recreational use if no likely sources of fecal coliform bacteria are present in the watershed.

Running Mean Method (Fecal Colifrom)

Where fecal coliform collection occurs frequently on a regular basis, an assessment can be conducted by calculating the running geometric mean of all fecal coliform results. This is accomplished by calculating the geometric mean of 5 samples as shown in examples 1 and 2 above for sequential groups of five samples as illustrated in the diagram below. All samples contributing to the running geometric mean must be taken over a period of no more than 30 days.

Calculating the running geometric mean:



When criteria is exceeded as in the previous Example 2, the temporal importance of the exceedance can be evalutated when there are enough samples to calculate running geometric means. No more than twice during a bathing season will sets of 3 consecutive grab samples, separated by 7 days, from waters attaining recreational use exceed the 400 CFU single sample maximum. No more than twice during a bathing season will two running geometric means of samples (as defined above), separated by 10 days, from waters attaining recreational use exceed 200 CFU.

Coastal Beach Samples:

Under, the Beaches Environmental Assessment and Coastal Health (BEACH) Act of 2000, Lake Erie is classified as a coastal beach. The BEACH Act indicator species for recreational use is E.coli. For E. coli data from coastal beach locations, where collection occurs frequently on a regular basis, assessment is best conducted using large datasets spanning multiple years utilizing the running geometric mean method. No more than twice during a bathing season will sets of 3 consecutive grab samples, separated by 7 days, from waters attaining recreational use exceed 235 CFU/mL. Second, no more than twice during a bathing season will two running geometric means of samples (as defined above), separated by 10 days, from waters attaining recreational use exceed 126 cfus/mL.