CHAPTER 4
COLLECTING AND ANALYZING BACTERIOLOGICAL SAMPLES

Important note: As of July 1, 2008, all bacteriological data submitted to the TCEQ for decision making must come from a National Environmental Laboratory Accreditation Conference (NELAC) accredited laboratory. As a result of these accreditation requirements, some analytical elements formerly included in this chapter were removed to avoid redundancy or confusion with the NELAC Standard, test methods, laboratory SOPs, etc. However, program-specific requirements and some guidance pertaining to laboratory analysis are still included in this chapter. These should be incorporated into laboratory protocols when running bacteria samples for the TCEQ surface water quality monitoring programs.

Bacteriological Sample Collection

Sample Collection Bottles
In the majority of circumstances, the TCEQ staff acquires their bacteria sample containers from the Support Services Division at the TCEQ Headquarters in Austin, Texas. These containers are purchased from IDEXX and quality assurance documentation is maintained on each lot received. This allows for a controlled source of bacteriological sample bottles and ensures that quality control checks have been performed. The IDEXX containers have lot numbers etched on the bottles which are documented throughout the collection and analysis process. Others using this SOP must ensure that the source of bacteriological sample bottles is controlled and their quality is confirmed and documented. If laboratories provide the sample containers, then the NELAC requirements related to Chapter 5, Appendix D.3.1.a must be followed.

Sampling Considerations
The indicator organisms used for determining support of the contact-recreation use are *Escherichia coli* (*E. coli*) in freshwater and *Enterococcus* in marine waters.

Baseline bacteriological samples should be collected at all routine monitoring sites under all flow conditions. The contact-recreation use currently applies to all surface waters, not only swimming areas.

In streams and rivers, take care to find an undisturbed location if other work such as flow measurement or sediment collection, is being done at the site.

When collecting samples from a bucket of water (bridge site), collect the bacteriological sample before other samples. Pour water into the bacteriological-sample container. Never immerse water-sample containers in the bucket; doing so could introduce contamination.

Sample Collection
Before collecting bacteriological samples, care should be given to sanitizing hands and arms. Use a commercial hand sanitizer or at least 70% ethyl alcohol.
Never pre-rinse the sample container. When submerging the sample container, take care to avoid contamination by surface scums. The surface film is enriched with particles and bacteria not representative of the water mass.

Flowing streams. Dip the open sample container to a depth of 0.3 m, or roughly half the depth in very shallow streams. Avoid contact with the sediment. With the open end facing upstream, push the mouth of the container upstream at this depth until full. Always hold the mouth of the sample container upstream of the sampler, the sampling apparatus, and any disturbed sediments.

Reservoirs and coastal waters. Dip the sample container to a depth of 0.3 m. At that depth, push the mouth of the sample container away from the boat, the sampler, sampling apparatus, and any disturbed sediment.

Leaving sufficient headspace for air and shaking, fill the sample container to the top (not the 100 mL or 250 mL line). This is to allow the lab to process the sample according to their procedures.

Sample Labeling
Label each sample with the station number, parameter, date, and time collected.

Sample Collection Documentation
Complete either the Request for Analysis (RFA) Tag or Chain of Custody (COC). Indicate the type of bacteriological analysis that needs to be run. Record the lot number of the sample bottle on the RFA Tag or the COC. Provide specific field notes on the RFA Tag or COC to aid the laboratory staff in determining what or if sample dilutions should be prepared. Always include the results of field conductivity measurements.

Sample Preparation
If the sample contains residual chlorine, add 0.1 mL of 10 percent sodium thiosulfate (Na₂S₂O₃) to the sample, unless the sample container already contains sodium thiosulfate.

Safety note: Although sodium thiosulfate has a low toxicity, it can cause eye irritation. Wear safety glasses when preparing sodium thiosulfate solution and when adding it to a sample.

Sample Preservation
Place samples on ice immediately after collection. No more than one bacteria sample per gallon of cooler capacity may be placed inside the cooler; these should be evenly spaced inside the cooler and completely covered with wet ice. Lower the temperature of the samples as quickly as possible to <6 °C but do not allow the samples to freeze.

Sample Shipping
Sample shipping should be coordinated between sample collectors and laboratories. TCEQ staff—ship samples to the TCEQ Houston Laboratory between Monday and Wednesday, arriving no later than Thursday. Samples may be shipped to LCRA on Thursday or Friday, but only by prearrangement.

Quality Control Samples
Collect one large sample (>200 mL) at the same time you collect field splits for other parameters. This will serve as your sample and bacteriological laboratory duplicate. It is preferable to collect bacteriological laboratory duplicate samples where elevated bacteria concentrations have occurred in the
past. Further, it is preferable to vary the location where duplicate samples are collected over time. Field splits and field blanks are not required for bacteriological analysis.

**Sample Holding Time**

*Holding time* is defined as the amount of time between sample collection and the initiation of sample analysis. Plan field collection activities to ensure that samples can be prepared by the laboratory within the holding time. Do not report results from samples that are not prepared within the time limit or are qualified by the laboratory as having exceeded the holding time.

Laboratories are required to process bacteriological samples within 8 hours of sample collection whenever possible. This 8-hour holding time includes 6 hours for transport and 2 hours for processing, so field personnel should submit samples to the lab within 6 hours when it is possible. In cases when transport conditions necessitate delays in sample preparation longer than 8 hours, the holding time may be extended up to 48 hours for *E. coli*. However, if the sample holding time is extended beyond 8 hours, the extension should be minimized, whenever possible. This extended holding time applies only to *E. coli* analysis using IDEXX Colilert/Quantitray-2000. *Enterococcus* samples must be processed within the 8 hour time limit.

<table>
<thead>
<tr>
<th>Bacteria Sample</th>
<th>Holding Time</th>
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<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>up to 48 hours</td>
</tr>
<tr>
<td><em>Enterococcus</em></td>
<td>up to 8 hours</td>
</tr>
<tr>
<td>Fecal coliform</td>
<td>Up to 8 hours</td>
</tr>
</tbody>
</table>

*Note:* There is no extension of holding times for *Enterococcus*. Regions needing this analysis must contract with local labs that are NELAC- accredited.

**Program-Specific Requirements and Guidance Related to the Analysis of *E. Coli* and *Enterococcus* Samples Using IDEXX Methods**

This section discusses program-specific requirements and other program-specific guidance pertaining to IDEXX Colilert and Enterolert methods for detecting *E. coli* in freshwater and *Enterococcus* in marine waters (APHA, et al 2005; ASTM 2004).

Note: Information specific to IDEXX methods are discussed in this chapter. While the IDEXX methods are encouraged for laboratories supporting the state’s surface water quality monitoring programs, other appropriate methods (e.g., membrane filtration) may also be used. In such cases, information below that is specifically related to IDEXX methods may be disregarded. However, program-specific quality control requirements and some program-specific reporting rules are applicable no matter what method is chosen.

**Method Selection in High-Conductivity Freshwater**

In monitoring for TSWQS attainment, *E. coli* must be used as the indicator for all water bodies not classified as tidal or marine. However, many inland bodies in Texas with high concentrations of total dissolved solids are classified as freshwater in the TSWQS. There have been some false positive results for *E. coli* when using the Colilert-24 method in high-conductivity freshwater. Because of this problem, the TCEQ SWQM Program and the CRP have revised the procedures regarding use of Colilert-18 rather than Colilert-24, sample dilutions, and the discontinuation of both tests under certain circumstances.

The following guidelines apply to water bodies with conductivities greater than 3,000 µS/cm:
Choose an analysis procedure after evaluation of conductivity results over the long term.

- Use Colilert-24 to test for *E. coli* in water bodies with conductivity values less than 3,000 µS/cm.
- Use Colilert-18 in water bodies where conductivity values range from 3,000 through 10,000 µS/cm.
- Do not use either Colilert-24 or Colilert-18 in water bodies where conductivity values exceed 10,000 µS/cm. If the IDEXX method is discontinued at certain sites, go back to analyzing fecal coliform by membrane filtration, and report those results. The fecal coliform indicator can still be used to indicate support of contact recreation.
- If you are using Colilert-18 on samples with high conductivity, you can use it for other samples with conductivities less than 3,000 µS/cm.

**Sample Dilutions**

Dilutions, if they are necessary, should be determined based on the historical concentrations at the sampling site, field notes, and/or the examination of samples themselves. The goal of sample dilutions is to achieve quantifiable results and remove interferences. Ideally, at least one dilution should yield a result between 40 – 900 MPN/IDEXX tray (i.e., 40 – 900 MPN before result multiplied by dilution factor). Values measured outside this range are less precise than those within the range.

For the detection of *E. coli* in freshwater and *Enterococcus* in marine waters, the following dilutions are recommended as starting points for most common situations.

- **E. coli, standard test volume.** Use a single standard test volume of 100 mL for water that is determined through experience to be relatively free of bacterial contamination (< 2400 MPN/100 mL), as well as turbidity, color, and solids (interfering substances). If the bacterial level is unknown, other dilutions should be performed in addition to using the standard test volume.

- **E. coli, interference and/or high (>2400 MPN/100 mL) bacteria count.** Run multiple dilutions to achieve quantifiable results and remove interfering factors (suspended solids, color, turbidity) that might mask fluorescence.

- **Enterococci, standard test volume, all waters.** Use the standard test volume of 10 mL on all waters.

**Reporting Rules**

**IDEXX Methods:**
- Report the result from the tray with the smallest dilution (largest sample volume) which does not have all positive wells.
- If all trays have all positive wells, report the largest dilution (smallest sample volume).
- If all trays have no positive wells, report the smallest dilution (largest sample volume).

**All Bacteriological Methods:**
- Report final results as 2 significant figures.
- Report results as E. coli MPN/100 mL or CFU/100 mL, as appropriate.

**Quality Control Requirements**

Laboratories should refer to the NELAC Standard and test methods for a full listing of quality control
requirements related to bacterial analysis. The requirements discussed below are program-specific requirements which are not found in the NELAC Standard.

**Bacteriological Laboratory Duplicate**
Analyze a laboratory duplicate with every tenth sample. If fewer than 10 samples are collected in a month, analyze one duplicate per month.

When analyzing a laboratory duplicate for a microbiology sample, use another aliquot from the parent sample. To ensure that enough sample volume is available, collect “greater than” 200 mL of sample at stations for which samples are analyzed at full strength (100 mL). Remember to mix the sample well before dividing. For other samples that normally require dilution, take the appropriate dilution from the 100 mL sample. Add the reagent to each dilution and mix until the reagent is completely dissolved. Pour each dilution into a separate tray, seal it, and incubate it with the other samples.

**Comparison Counting**
For routine evaluation, repeat counts on one or more positive samples at least monthly. If possible, compare counts with an analyst who also performs the analysis. Replicate counts by the same analyst should agree within 5 percent, and those between analysts should agree within 10 percent. Record the results of comparison counts in an appropriate location.