CHAPTER 4
COLLECTING AND ANALYZING BACTERIOLOGICAL SAMPLES

National Environmental Laboratory Accreditation Program (NELAP)

All bacteriological data submitted to the TCEQ for decision making must come from a lab accredited by the National Environmental Laboratory Accreditation Program (NELAP). As a result of these accreditation requirements (established in 2008), some analytical elements formerly included in this chapter were removed to avoid redundancy or confusion with the NELAP Standard, test methods, laboratory SOPs, etc. However, program-specific requirements and some guidance pertaining to laboratory analysis are still included. 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

IDEXX Containers

The preferred bacteriological sample containers are the 120 and 290 mL bottles from IDEXX. Containers purchased from IDEXX have quality-assurance documentation associated with each lot received, allowing for a controlled source of bacteriological sample bottles and ensuring that QC checks have been performed. The IDEXX containers have lot numbers etched on the bottom that are documented throughout collection and analysis. Record the lot number on the RFA or COC form. The lot number is a 5-digit number such as *KF007*.

IDEXX maintains "Certificates of Quality" for each lot of bacteriological sample bottles. To download IDEXX bottle-quality-control certificates go to the IDEXX "Water Microbiology" webpage (see Appendix A). Under "Quick Links", select "Quality Certificates" which opens the "Certificates of Quality Request Form" (see Figure 4.1). To download a certificate of quality for IDEXX sample bottles, select the product from the list provided, enter the lot number and click the "Request Certificate" button. The certificate can be saved electronically. The following product numbers are those most commonly used for SWQM purposes.

IDEXX Bottle	Product Number
120 mL	WV120ST-200, 120 mL Vessel
290 mL	WV290SBST-100, 290 mL Vessel

Other Containers

Others using this SOP must ensure that the source of bacteriological sample containers is controlled and their quality is confirmed and documented. If laboratories supply the sample containers, then the NELAC requirements related to Chapter 5, Appendix D.3.1.a must be followed.

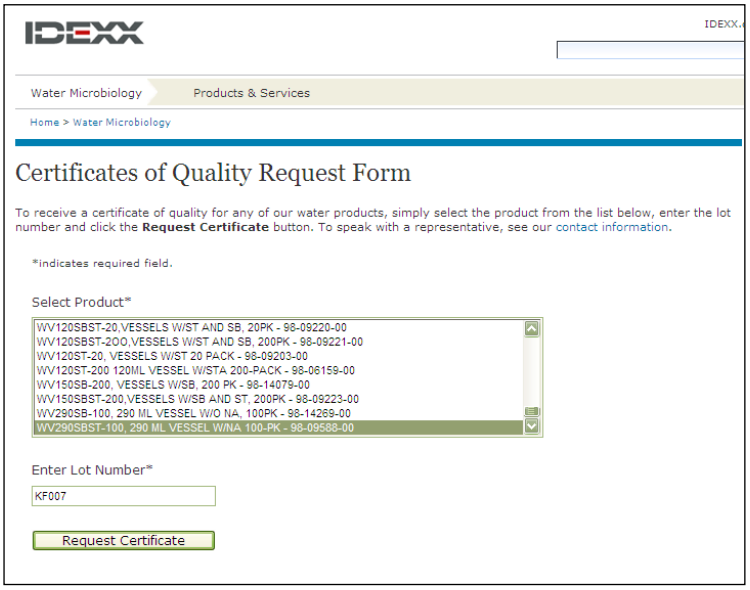
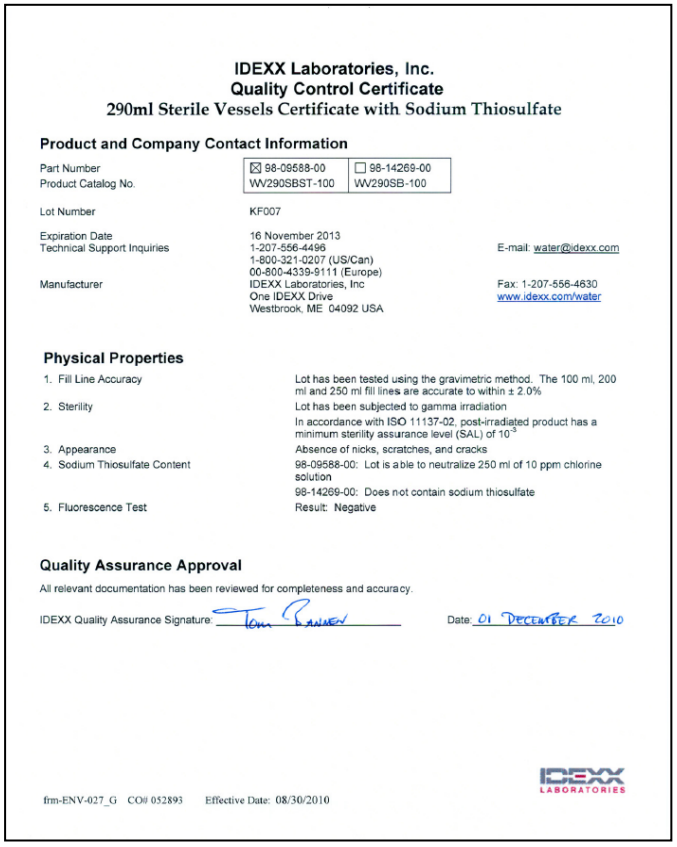


Figure 4.1. IDEXX Certificates of Quality Request Form.



IDEXX Laboratories, Inc.
Quality Control Certificate
290ml Sterile Vessels Certificate with Sodium Thiosulfate

Product and Company Contact Information

Part Number Product Catalog No.	☒ 98-09588-00 WV290SBST-100	☐ 98-14269-00 WV290SB-100
Lot Number	KF007	
Expiration Date	16 November 2013	
Technical Support Inquiries	1-207-556-4496 1-800-321-0207 (US/Can) 00-800-4339-9111 (Europe)	E-mail: water@idexx.com
Manufacturer	IDEXX Laboratories, Inc One IDEXX Drive Westbrook, ME 04092 USA	Fax: 1-207-556-4630 www.idexx.com/water

Physical Properties

1. Fill Line Accuracy	Lot has been tested using the gravimetric method. The 100 ml, 200 ml and 250 ml fill lines are accurate to within ± 2.0%
2. Sterility	Lot has been subjected to gamma irradiation In accordance with ISO 11137-02, post-irradiated product has a minimum sterility assurance level (SAL) of 10^{-3}
3. Appearance	Absence of nicks, scratches, and cracks
4. Sodium Thiosulfate Content	98-09588-00: Lot is able to neutralize 250 ml of 10 ppm chlorine solution 98-14269-00: Does not contain sodium thiosulfate
5. Fluorescence Test	Result: Negative

Quality Assurance Approval

All relevant documentation has been reviewed for completeness and accuracy.

IDEXX Quality Assurance Signature: Tom Danner Date: 01 DECEMBER 2010

frm-ENV-027_G CO# 052893 Effective Date: 08/30/2010

IDEXX LABORATORIES

Figure 4.2. IDEXX Quality-Control Certificate.

Sampling Considerations

The indicator organisms used for determining support of the recreation use are *Escherichia coli* in freshwater and *Enterococcus* in marine waters and some saline inland waters.

Baseline bacteriological samples **should be collected at all routine monitoring sites under all flow conditions.** To maximize the processing time for the laboratory collect bacteriological samples last at a site. In streams and rivers, take care to find an undisturbed location if other work, like flow 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

Clean hands. Bacteria samples are the easiest to contaminate. Take steps to help eliminate possible contamination by using either an alcohol-based hand sanitizer that contains at least 60 percent alcohol prior to sample collection or wearing disposable latex gloves when collecting a sample.

Never prerinse the sample container. When submerging the sample container, take care to avoid contamination by surface scum. The surface film is enriched with particles and bacteria not representative of the water mass.

Leave sufficient headspace. The lab needs to mix the sample prior to processing to redistribute bacteria in the sample. Fill the sample container to the top (not the 100 mL or 250mL line.) This allows the lab to process the sample according to their procedures.

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 bag 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.

Sample Labeling

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

Documenting Sample Collection

Complete either a Request for Analysis (RFA) or Chain of Custody (COC) form. 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 Treatment in the Presence of Chlorine

Chlorine residual should be analyzed from samples collected downstream of chlorinated effluent discharges or in areas where the presence of chlorine is suspected. Test strips or a standard chlorine residual test may be used as a way to determine the presence of chlorine.

- If the sample bottle contains sodium thiosulfate, field testing for chlorine is not necessary—unless a high level of chlorine is suspected or requested by the lab analyzing your samples.
- If the sample bottle does not contain sodium thiosulfate, field testing for chlorine is necessary. If residual chlorine is present, add 0.1 mL of 10 percent sodium thiosulfate to the sample.

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. Cool the samples as quickly as possible to < 6.0ºC 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 the 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 sample provides enough volume for the lab to analyze the ambient sample and a 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 collection and the initiation of analysis. Plan sample collection so that samples are set up within the required holding time. Do not report samples that are not prepared within the time limit or are reported from the laboratory as exceeding the holding time.

Laboratories are required to process bacteriological samples within **eight hours** of sample collection whenever possible. The 8-hour holding time includes 6 for transporting and 2 for processing. Field personnel should submit samples to the lab within 6 hours when possible. When transport conditions cause delays in sample preparation longer than 8 hours, the holding time may be extended up to 48 hours for *E. coli*. However,

any extension should be minimized. This extended holding time applies **only** to *E. coli* analysis using the IDEXX Colilert Quanti-Tray/2000.

Bacteria Sample	Holding Time
E. coli	up to 48 hours
Enterococci	up to 8 hours
Fecal coliform	up to 8 hours

Note: There is no extension of holding times for *Enterococcus*. Regions needing this analysis must contact the SWQM quality assurance office to arrange for the use of a local NELAP-accredited laboratory.

Selecting an Indicator for Classified Water Bodies

In monitoring for attainment of the recreational use the appropriate indicator must be determined before sample collection. The indicators for classified water bodies, which appear in Appendix A of the TSWQS, must be used at all times regardless of the specific conductance at the time of sampling.

Selecting an Indicator for Unclassified Water Bodies

- Unclassified water bodies within the watershed of a classified segment designated as **freshwater** should be sampled for *E. coli*.
- Unclassified water bodies within the watershed of a classified segment designated **high saline** should be sampled for enterococci.
    - However, if it can be demonstrated that the unclassified water body is not high saline through a historical review of the specific conductance data—the maximum of all measurements must be less than 10,000 µS/cm—then *E. coli* may be chosen as the indicator. See the following information on selecting the appropriate method for *E. coli*.
- Unclassified water bodies within the watershed of a classified segment designated **tidal** should be sampled for enterococci.
    - However, if it can be demonstrated that the unclassified water body is not tidal through a historical review of the specific conductance data, then *E. coli* may be chosen as the indicator. Due to variable conditions along the Texas coast these are addressed case by case.

Note: The chosen indicator must be used during all sampling events. Use that indicator regardless of the specific conductance value measured during sampling. This ensures that all data collected can be reasonably compared to the single most appropriate criterion.

Program-Specific Requirements and Guidance

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 enterococci in marine waters or high saline inland 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 (for example, membrane filtration) may also be used. In such cases, information 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.

Selecting a Method

E. coli

In monitoring for attainment of the recreational use, *E. coli* is the indicator for all non-tidal water bodies classified as freshwater in the TSWQS.

However, many inland water bodies with high specific conductance remain classified as freshwater in the TSWQS. There have been false positive results for *E. coli* when using the Colilert methods in these water bodies. Because of this issue, the TCEQ has revised the guidelines regarding the use of Colilert media and sample dilutions.

The following guidelines apply to freshwater bodies with *E. coli* as the designated indicator:

- Choose an *E. coli* analysis procedure after evaluating long-term specific-conductance results.
- Use Colilert-18 or Colilert-24 for freshwater samples with specific-conductance values less than 3,000 µS/cm.
- Use Colilert-18 for freshwater samples with specific conductance values greater than 3,000 µS/cm.

Enterococci

Enterococci are the indicator for water bodies classified as tidal, marine, or high saline inland listed in Appendix A of the 2010 TSWQS.

Fecal Coliform

Fecal coliform is used only in monitoring for attainment of the oyster-water use and not for the recreational use. Oyster waters are monitored by the Texas Department of State Health Services Seafood and Aquatic Life Group. The DSHS Seafood Safety Program is governed by the guidelines of the U.S. Food and Drug Administration's National Shellfish Sanitation Program. Information about the Seafood Safety Program is available at the DSHS website (see Appendix A).

Selecting Sample Dilutions

Dilutions, if 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.

For the detection of *E. coli* in freshwater and enterococci in marine and inland saline 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 suspended solids. If the bacterial level is unknown, other dilutions should be performed in addition to using the standard test volume. This applies to Colilert-18 or Colilert-24.

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, or turbidity) that might mask fluorescence. ***Note: When using Colilert-18 on samples with specific conductance values greater than 3,000 µS/cm the minimum dilution should be 1 to 10.

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 two significant figures.
- Report results as *E. coli* MPN/100 mL or CFU/100 mL, as appropriate.

Quality-Control Requirements

Laboratories should refer to the NELAP 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 NELAP 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 > 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.