Texas Stream Team Volunteer Water Quality Monitoring Program

E.coli Monitoring and Analysis Procedures

Training Requirements

Texas Stream Team supports certification programs in both the "core" field parameters covered in the Texas Stream Team Water Quality Monitoring Manual and bacteriological monitoring protocols for *Escherichia coli (E.coli)* bacteria. In addition to the "core" volunteer monitoring certification training, volunteer-certification in bacteriological monitoring includes:

1) bacteriological information background and sample collection procedures
2) media storage and preparation
3) proper dilution, plating, and incubation procedures
4) colony enumeration
5) proper documentation and safety procedures

In order to ensure the highest confidence levels among data users, Texas Stream Team recommends that bacteriological monitors attend an annual bacteriological QC session during which they are updated and evaluated on monitoring techniques and enumeration of bacterial colonies.

Scope and Application

Texas Stream Team monitors will perform bacteria tests on streams, lakes, swimming beaches, and springs. The primary reasons for bacteria testing are determination of baseline conditions. Tests results may also be used to identify potential contamination from broken or leaking septic tanks and sewer lines, wastewater treatment plants, animal holding operations and other point and nonpoint sources. Bacteriological monitors will develop sampling strategies to suit their objectives and budget, and sampling frequency will vary accordingly.

Results of the tests are evaluated against State of Texas contact recreation standards. If test results indicate contamination, Texas Stream Team advises the monitor to repeat and verify the initial results. If repeated high counts are found at a site over an extended period of time, the information will also be communicated to the appropriate local and state authorities.

Summary of Method

Coliscan Easygel is a method used to test for *E.coli* and general coliform bacteria. Dr. Jonathan Roth developed the technology for Micrology Laboratories, LLC. Easygel is not an agar, but is a pectin-gel. Easygel comes in a sterilized, two-piece unit, including a bottle of liquid medium and a petri dish treated with a special formulation.

With this method, a 1 mL to 5 mL of sample of water is collected using a sterile pipette and introduced into a 10 mL bottle of sterile liquid medium. The prepared medium is then plated on a treated petri dish, and incubated at a temperature of 33° C for 28 hours. Commercially available incubators, such as the Hovabator, are recommended. Incubator temperature is maintained and verified with the armored thermometer used in the Texas Stream Team Core kit. Monitors will conduct field blank quality control analysis for ten percent of sampling events.

Upon incubation, the general coliforms and *E.coli* produce enzymes that react with color reagents in the media to produce pink to red colonies (general coliforms) or dark blue colonies (*E.coli*). Two samples from each monitoring site are analyzed, and a mean value is reported. A blank sample will be analyzed during every fourth monitoring event to check for potential contamination issues.

Range and Accuracy
Monitors typically use a 1 mL sample, but sample volumes may range in size from 1 mL to 5 mL. The Coliscan Easygel test can detect as little as one bacterial colony per sample, and can be used to identify up to 200 colonies/sample. Concentrations exceeding 200 colonies/sample are recorded as too numerous to count (TNTC). A black and a white grid, which is the same size as the Petri dishes, is provided to assist monitors in counting *E.coli* colonies.

Accuracy of Coliscan Easygel is based on the reasonable performance of properly stored, pre-treated sterile plates, media, and pipettes. Extensive evaluation of the Coliscan Easygel method was conducted by Alabama Water Watch, Alabama Department of Fisheries, and Auburn University from February to September 1998 to confirm the accuracy of the Coliscan Easygel method. The results indicated this method is a reliable and valid tool for the detection of fecal contamination through a variety of concentrations.

In December 1999, Coliscan Easygel was approved by the U.S. EPA Region 4 for use in the bacteriological monitoring of surface waters as part of the program developed by the Alabama Water Watch under the direction of Dr. William G. Deutsch of the Dept. of Fisheries of Auburn University. As a result of this program and other studies, Coliscan Easygel has become the preferred method for bacteriological monitoring in water watch programs throughout the United States.

Bacteriological Monitoring Supplies & Equipment

Necessary Items - The items needed to conduct bacteriological monitoring using the Coliscan Easygel method include: sterile bacteriological bottles, Whirlpak™, Whirl-Pak Thio-Bags™; sterile Easygel medium and pre-treated Petri dishes; sterile pipettes; sterile diluent; an incubator; gloves; bleach; and seal able plastic bags. Easygel proprietary items like media and pretreated Petri dishes can be ordered directly from Micrology Laboratories at (888) EASYGEL or micrologylabs.com. Other equipment and supplies, including sterile diluent, can be purchased from a variety of sources like grocery and laboratory supplies stores. See the monitoring supplies section of the Texas Stream Team website for additional information.

Sample Media Storage and Disposal - When Coliscan Easygel reagents are received, the production date (if known) or arrival date, and the expiration date should be written on the box of media and petri dishes. Media bottles should be kept frozen until ready for use, allowing for a shelf life of up to one year. Thawed media is usable for up to two weeks when stored at room temperature. Medium can be refrozen but repeated freezing and thawing should be avoided. Pre-treated petri dishes should be stored at room temperature which also allows for a shelf life of one year. To dispose of expired media pour a teaspoon of bleach into the bottle, cap the bottle, shake well, place the bottle in a seal able plastic bag, and dispose in household trash.

Quality Control

Analyzing samples for *E.coli* can introduce challenges in ensuring contamination does not occur during sample collection and processing. It is important that all Texas Stream Team monitors use the same methods and procedures so that samples within and between streams can be compared to each other, and understanding the importance of quality assurance and quality control practices is crucial to generating credible environmental information. Quality assurance is the system used to make sure that all data collection activities are managed in a way that collected information meets the intended use of the project. Some examples of quality assurance measures include: the consistent Texas Stream Team training program, the use of consistent methods, written procedures, establishing data quality objectives, maintenance of records, and specifying the chain of custody procedures. Quality control procedures reassure that samples are being collected and documented in a consistent

and accurate manner at all sites by all monitors. Examples of quality control include: double rinsing of equipment prior to use, checking reagents for expiration dates, using data quality objectives to assess data validity, calibrating meters within 24 hours of use, and collecting field blanks on a routine basis. Together, quality assurance and quality control serve volunteer water quality monitors by bringing enhanced data credibility and use.

Cross Contamination – Efforts should be made to avoid contaminating sample containers, hands, tabletops, or any other surface or object. Do not touch bacterial colonies. The dishes should be taped shut and kept out of reach of children, pets, and curious wildlife. A disinfectant should be used to clean tabletops or other areas that colonized plates have touched. Monitors should wash hands before and after handling the plates.

Field Blank – Field blanks are used to assess potential contamination from sample handling, airborne materials, equipment, media, and other sources. A field blank usually consists of a sterile diluent sample of 2 mL that is taken to the site and poured into a properly labeled sample container during the first bacteria sampling event of that day. The blank sample is collected in the same type of container, labeled as a field blank, and handled and analyzed along with all the bacteria samples collected on that day. It is used to identify errors or contamination in sample collection and analysis. The frequency of a bacteria field blank is one with every 10 samples. If less than 10 samples are collected in a month, include at least one field blank for any month bacteria samples are collected. Report the results of the field blank on your data form. There should be no *E.coli* colony growth on the field blank samples. If *E.coli* growth occurs on the blank, discard all data collected on that day. Document the results on the data sheet and consult with your trainer.

Bacteriological Sample Collection Procedures

1.0 Sample Site Location - Establishing sampling site locations should follow procedures outlined in the Texas Stream Team Volunteer Environmental Monitoring Manual section *2.00 Choosing a Monitoring Location.* In streams, rivers, and lakes care should be taken to collect the bacteriological sample at an undisturbed location.

1.1 Sample Containers - Collect bacteriological samples in sterile bacteriological bottles or Whirlpak bags. *Never pre-rinse the sample container.* For Whirlpack bags, squeeze out the top one inch of water from the bag and whirl the bag to seal. The sealed bag must retain at least 50 mL of sample but leave a small pocket of air. This airspace will help mix the sample when it is shaken just before making dilutions and membrane filtration. During every tenth sampling event (or a minimum of once per month), prepare one additional sample container and petri dish for a quality control field blank. 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 that monitors use the Whirl-Pak Thio-Bag™. These bags contain 10mg tablets of sodium thiosulphate to neutralize free chlorine in the sample.

1.2 Sample Labeling - Label each sample container with the station number, site name, date, and time collected.

If it is appropriate to process a field blank sample, this container will have the previously mentioned information plus a "field blank" label.

1.3 Collecting Samples - 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. Also be careful not to collect sediment from the bottom of stream or lake. The correct procedure for collecting samples is demonstrated during trainings. When it is appropriate, remember to collect the field blank from the 90 mL sterile diluent prior to conducting routine sampling at your site. This involves transferring the sterile diluent from its

original container to the routine sampling container while at your monitoring location.

In flowing streams, dip the open sample container to a depth of 0.3 m (1ft), 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, sampling apparatus, and any disturbed sediments.

In reservoirs and coastal waters dip the sample container to a depth of 0.3 m (1ft). At this depth, push the mouth of the sample container away from the boat, dock, shore, sampler, and any disturbed sediment.

When collecting samples from a bucket of water, collect the bacteria sample before other monitoring activities occur. Pour water into the bacteriological sample container. Never immerse water sample containers in the bucket. This could introduce contamination.

1.4 Sample Preservation & Hold Times - Place sample(s) on ice immediately after collection. Bacteriological samples must transported, processed (diluted and plated), and place in incubator within **6 hours** of sample collection. Do not report samples that are not processed within time limit. Record the hold time on the Texas Stream Team *E.coli* data sheet.


Figure A


Figure B


Figure C


Figure D

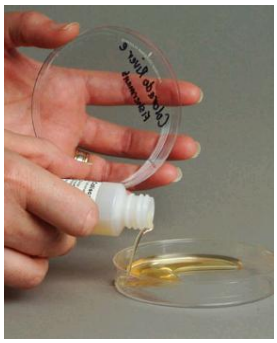


Figure E

Figure A depicts a monitor holding a Whirlpak bag
Figure B portrays a monitor labeling a petri dish with the appropriate monitoring information
Figure C shows a monitor using a 5mL pipette to remove a sample from the Whirlpak bag
Figure D shows the monitor placing the stream sample into the Coliscan Easygel bottle media
Figure E a monitor demonstrates pouring the media, which includes the stream sample, onto the petri dish

Analyzing *E.coli* Using Coliscan Easygel Pour Plate Method

2.0 Preparation – Prepare media for a minimum of two samples for each site. Bottles of media should be removed from the freezer in time to ensure they have reached room temperature (typically 2 - 3 hours) before use.

Prepare two Petri dishes per sample. During every tenth sampling event (or once per month minimally), prepare one additional Petri dish for a quality control field blank. Label the top of each Petri dish with: site name, date, volume of sample, and time the sample is poured into the Petri dish. The field blank consists of a sterile diluent sample of 9 mL, and the sample container and Petri dish will be labeled like the other samples and will also include "field blank" next to the site name.

2.1 Drawing Proper Sample Size - Shake the sample container vigorously, and then carefully open without touching the lip of the bag. Leave the pipette in the sterile wrapper until ready to draw the sample. Unwrap the

pipette from the bulb end and avoid contacting the tip with anything except the sample water. Submerge the bottom half of the pipette into the sample container and squeeze the bulb to expel the air. Draw the appropriate sample size (1 mL, 3 mL, or 5 mL) into the pipette by releasing the bulb slowly. Squeeze out any sample water in excess of the desired volume. Deposit the sample into the Easygel media bottle, cap, and swirl gently. Record the sample size on the Texas Stream Team *E.coli* data sheet. Draw a 1mL aliquot for field blanks.

NOTE: Once mixed with Easygel media, the prepared samples should be either be plated within 10 minutes, or kept on ice or in a refrigerator until plated.

2.2 Determining Sample Size – The ideal number of colonies resulting from a single prepared plate is 20 to 60, and not over 200. Since the number of resulting colonies is dependent on the sample size, it may be necessary to experiment with several sample volumes to determine the best probable sample size to achieve 20-60 colonies. Draw a 1mL aliquot for field blanks.

To establish a baseline for typical conditions, collect a 1mL and a 5mL sample volume during the first sampling event. If the 1mL *E.coli* colony reading results in zero or only a few colonies, the sample volume should be to increased to 3mL or 5mL. Conversely, if the 5mL sample results in more than 60 colonies, the sample size should be reduced to 1mL or 3mL during the next sampling event.

Environmental and precipitation variables will influence levels of bacteria. Urban creeks with low discharge often have abundant *E.coli* and other coliform growth, and the volunteer should begin sampling with 1mL and 3mL volumes. Pristine waters may require a 5 mL sample to achieve the preferred range of colonies.

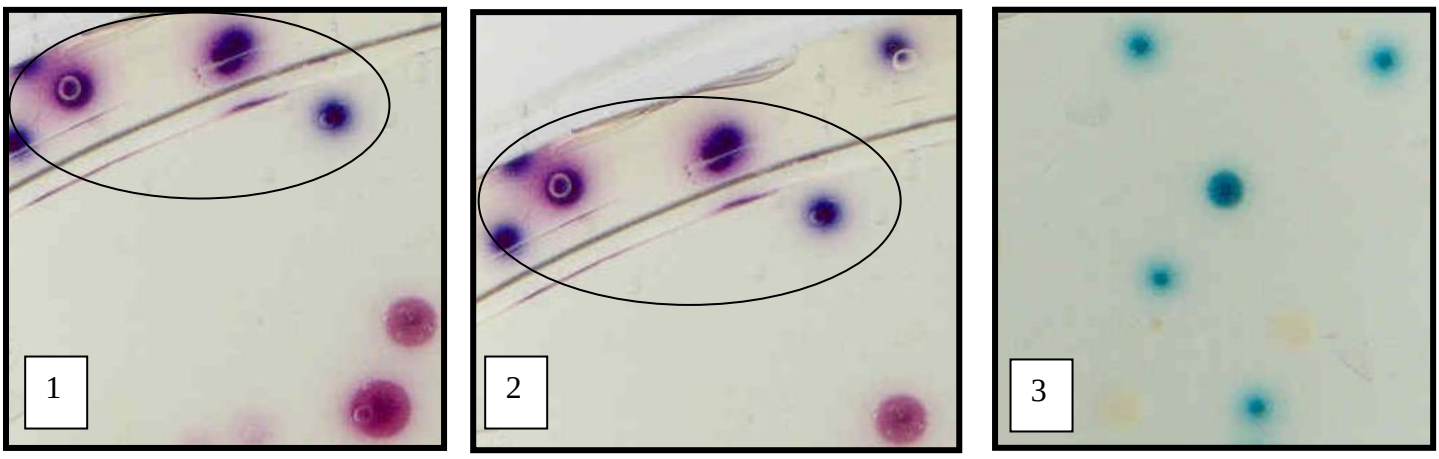
2.3 Plating the Sample – Pour the prepared sample (the Easygel media mixed with the water sample) slowly into the petri dish. Gently swirl until there is a smooth coating of prepared sample across the bottom of the petri dish (but be careful not to splash over the side or on the lid). Set on a level surface and allow five to forty-five 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.

2.4 Incubation - Turn on the incubator far enough in advance to ensure the appropriated temperature is reached before loading petri dishes. Place petri dishes right-side up in the incubator and maintain a steady incubation temperature of 33° C. At this temperature, colonies should not be counted for a minimum of 28 hours. For optimum results, count and record colonies at 28 hours of incubation. No counts should be made after 31 hours. Record the incubation time and temperature on the Texas Stream Team *E.coli* data sheet.

2.5 Counting *E.coli* Colonies - Count the number of individual and distinct dark purple and dark blue colonies. Teal or turquoise colored colonies with a clearly established dark center should also be counted as *E.coli.* Colonies which are pink and white should be ignored. Record the number of *E. coli* colonies on the Texas Stream Team *E. coli* data sheet.

The circled colonies in frame 1 and 2 are *E. coli*

Only count colonies in frame 3 as *E.*coli if the center of the colony is dark and well established.



Field Blanks The frequency of a bacteria field blank is one with every 10 samples. If less than 10 samples are collected in a month, include at least one field blank for any month bacteria samples are collected. Follow routine handling, plating and analysis procedures, and report the results on your data form. There should be no *E.coli* colony growth on the field blank samples. If *E.coli* growth occurs on the blank, discard all data collected on that day. Document the results on the data sheet and consult with your trainer. Trainers will work closely with monitors who have issues concerning field blank contamination to resolve the problem.

2.6 Data Reporting – Final results of the analysis for the two samples per site plus the blank are reported on the Texas Stream Team *E. coli* data sheet as "colonies per 100 mL" of sample water. To arrive at that number you must first determine dilution factor.

Dilution factor = 100 / sample size

For example, if you collected a sample size of 1 mL in the pipette and added this to the Easygel solution, your dilution factor is 100 / 1 or 100. Common dilution factors are: .5 mL sample = dilution factor of 200; 3 mL sample = 33.3; 5 mL sample = 20.

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

For example, if you counted 8 colonies and had a dilution factor of 33.3 (3mL sample size), your final result is 8 X 33.3 or 266 E. coli colonies/100mL. For a count of 11 colonies X dilution factor of 20 (5mL sample size) your result is 220 E. coli colonies/100mL.

This information should be entered on the Texas Stream Team *E. coli* data sheet to document the final results of each set of samples analyzed. Verify the dilution factor calculation is correct and marked accordingly on the Quality Data Review Checklist.

Data Sheet Example

Utilize sterile pipettes and two samples from one well-shaken container. The pipettes are discarded after use.

Sample # 1 Sample size 3 mL Dilution factor (100/sample size) 33.3

Colonies counted (dark blue/purple) 8 X dilution factor 33.3 =

Sample #2 Sample size 5 mL Dilution factor (100/sample size) 20

Colonies counted (dark blue/purple) 11 X dilution factor 20 = 220 colonies/100mL

FIELD BLANK E.coli Colony Growth (circle one) YES / (NO)

2.8 Waste Disposal - To dispose of the used Petri dishes, lift the lid and pour 5ml (about 1 teaspoon) of straight bleach or isopropyl alcohol into each dish. Make sure the bleach has covered the entire dish, and allow it to sit for a minimum of fifteen minutes. Place the dishes in a sealed plastic bag and place in normal household trash.

Periodically clean the inside of incubator with dilute bleach solution and allowed to air dry before the next use.

2.9 Data Review - The following Quality Data Review Checklist is used by the monitor, Texas Stream Team staff, and data user to verify that data are valid.

Quality Data Review Checklist	Yes / No	Comments
Monitor is certified		
Is the media expired		
Incubation temperature is 33 ° C (+/- 3 ° C)		
Incubation time is 28 – 31 hours		
Data form is complete		
Optimal colony number is achieved (<200)		
Dilution factor calculation is correct		
Colony Growth on Field Blanks		