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**Standard Operating Procedure #7: Collecting and
Processing Water Quality Samples for Fecal Indicator
Bacteria, Version SCPN_WATER_QUALITY_SOP07_20160901**

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Standard Operating Procedure #7: Collecting and Processing Water Quality Samples for Fecal Indicator Bacteria

Version SCPN_Water_Quality_SOP7_20160901				
Revision History Log				
Previous version number	Revision date	Section and paragraph	Change(s) made	Approved by

Only changes in this specific SOP will be logged here. Record the previous version number and date of revision; identify sections and paragraphs where changes were made, who approved the revision, and the new version number. More detailed descriptions of the changes made and the reason for the changes is provided at the end of this SOP.

This Standard Operating Procedure (SOP) describes procedures for collecting, handling, and processing total coliforms and *Escherichia coli*, a fecal indicator bacteria, in streams of SCPN parks. We are employing the IDEXX method, which is approved by the U.S. Environmental Protection Agency (EPA) for *E. coli* enumeration in ambient waters, and is used by water quality monitoring agencies in the four states where SCPN parks are located:

- Arizona Dept. of Environmental Quality—Surface Water Section
- Colorado Dept. of Public Health and Environment—Laboratory Services Division
- New Mexico Environment Dept.—Surface Water Quality Bureau
- Utah Dept. of Environmental Quality—Division of Water Quality.

Protocols and other reference documents of these organizations should be periodically checked for updates and saved to the SCPN server to ensure that SCPN is following similar procedures, and thus collecting comparable data.

This SOP supplements the American Public Health Association (APHA), EPA, and IDEXX methods with background information and approaches to sample collection, transport and processing that are specific to SCPN monitoring sites.

Equipment

Please see Appendix F for a list of the equipment needed for collecting and processing water quality samples for fecal indicator bacteria.

1 Detecting fecal coliform bacteria

Coliform bacteria are a group of microorganisms that are commonly found in water, soil, vegetation, and in the intestinal tract of animals. The majority of coliform bacteria are not pathogens. *E. coli* is a type of fecal coliform bacteria that only occurs in the intestinal tract of warm-blooded animals, and some strains can cause illness. Because coliform bacteria are easy to detect and are often associated with other disease-causing organisms, they are commonly used as an indicator of contamination in stream water.

Coliform bacteria, including *E. coli*, ferment lactose using specific enzymes. Detection of these enzymes in enzyme substrate tests indicates that coliform bacteria are present. For detecting total coliforms and *E. coli*, SCPN uses Colilert®, Colilert®-18, and Quanti-Tray®/2000 from IDEXX Laboratories, Inc., which are EPA-approved for *E. coli* enumeration in ambient waters (USEPA 2003). The Colilert® reagent reacts with the specific enzymes produced by total coliform and *E. coli* bacteria. After 18 to 24 hours of incubation at 35°C (depending on which test kit is used), a yellow color indicates the presence of total coliforms. If *E. coli* is present, the sample will fluoresce upon exposure to UV light. From these results, the Most Probable Number (MPN) of bacteria, an estimate of bacteria density in the sample (APHA1998), can be calculated in units of MPN/100mL.

2 Sample collection and handling

Bacteria are often associated with sediment particles, and USGS recommends that depth-integrated sampling be conducted for bacteria sampling in the same way that it is conducted for sediment (Wilde et al. 1999). However, most of the streams SCPN monitors are not deep enough, except during storm events, to obtain a meaningful depth-integrated sample. During storm events, stream conditions often preclude safe wading and collection of depth-integrated samples. Instead, grab samples will be collected at the center of flow.

Recommended sample collection sites in a stream and methods for collecting are presented in 7-1. The following supplies are needed to collect a bacteria sample:

- 100 mL sterile sample bottle in sealed plastic bag
- gloves
- Colilert® or Colilert-18® reagent
- Quanti-tray
- Tray sealer

Be sure to check expiration dates on all IDEXX supplies. Obtain ice for storage of the stream samples until they can be processed and incubated.

Sampling procedure is as follows:

1. Carry a disposable bottle to the site in a sealed Ziploc bag. Label the disposable bottle with the site code using a permanent marker.
2. Put gloves on both hands.
3. Remove the shrink-band from the disposable bottle. Stand in the stream, facing upstream, at the center of stream flow and upstream of any streambed disturbance
4. Unscrew the lid and dip the bottle into the stream, being careful to avoid collecting streambed sediment.
5. If the collected water is above the “fill line,” carefully dispense the overflow back into the stream. This should be done immediately, before the water has settled. Close the bottle lid.
6. Place the vessel back in the Ziploc bag, seal it, and place it on ice immediately.
7. Process and incubate the sample within 8 hours of collection
8. Start a temperature logger to log incubation temperature at 30-second intervals

Record sampling information on the *Water Quality Datasheet*:

- Site code, site name and date of sample collection
- Type of reagent used (Colilert or

Table 7-1. Recommended location(s) and method for sample collection for bacterial analysis.

Site type	Potential location for sample collection (in order of preference)	Method of sample collection
Wadeable streams	- Center of flow - Near bank, if flooding precludes mid-channel sample	Water samples are collected in sterile polypropylene sample containers with leak-proof closures.

Coli-18)

- Time of incubation start
- Date and time of incubation end
- Was sample kept on ice from time of collection?
- Was incubator temperature logged during incubation (yes/no)? If so, record the temperature logger make/model and serial number, and the data file name.
- Lot numbers and expiration dates of IDEXX materials (disposable vessels, reagent, and Quanti-Tray 2000s®)

3 Coliform bacteria sample processing

Once collected, bacteria samples must be kept in a cooler with ice until ready to process. Samples must be processed and placed in the incubator within six hours of collection (EPA 2003). If for any reason the holding time must be exceeded, an additional two hours is acceptable, for a total of 8 hours (EPA 2007). Note this on the *Water Quality Datasheet* and flag it in the water quality database. After the incubation period there is a four-hour window in which to read the quantitrays. Therefore SCPN personnel must choose either a Colilert or Colilert-18 reagent based on their ability to read the trays at a reasonable time the next day.

3.1 The IDEXX Colilert® System

A step by step description of the IDEXX Quantitray-2000 coliform and *E. coli* method used in SCPN parks is presented in a video available on the IDEXX web site: <http://https://ca.idexx.com/water/trainingvideos.html>. The field methodology is entirely consistent with the video explanation but a few additional useful points are mentioned in the text below.

Prior to using the Quanti-Tray 2000 system, field crew members should familiarize themselves with the system. This includes using the Quanti-Tray 2000 Comparator (also by Idexx), which contains borderline yellow and fluorescing comparator wells for distinguishing threshold positive results from negative by comparison.

Generally, SCPN uses IDEXX Colilert® reagent, but may instead use IDEXX Colilert-18®, depending on the circumstances. These reagents are used in tandem with the Quanti-Tray 2000 enumeration method, and have identical counting ranges (from 1 to 2,419 MPN/100 mL without dilution). However, incubation times vary between reagents (Table 7-2), potentially introducing bias into sample processing and counting of positive vs. negative Quanti-Tray 2000 wells. Therefore, SCPN will ensure that results are qualified by recording the reagent type used for each sample and including this information in data interpretation. QA/QC procedures (blanks, replicates) will be conducted independently for each type of reagent (Colilert, Colilert-18).

3.2 Sample processing procedure is as follows:

1. Start up the Quanti-Tray Sealer. The sealer takes about five minutes to warm up. If using a vehicle battery, sealer must be connected via a 1000-watt or greater power inverter and vehicle must be running.
2. Put on gloves and add contents of one reagent package to a 100 mL water sample in a sterile bottle. Cap bottle and invert until dissolved, avoid creating foam.
3. If using Colilert-18, anti-foam drops must be added to eliminate air bubbles,

Table 7-2. A comparison of the features of two IDEXX® coliform and *E. coli* tests

Feature	Colilert	Colilert-18
Incubation & Definitive Results Time Window	24–28 hrs	18–22 hrs
Other valid results (coliform & <i>E. coli</i>)	Positives before 24 hrs and negatives after 28 hrs	Positives before 18 hrs and negatives after 22 hrs
Shelf life	Up to 12 months at 4–30°C	Up to 15 months at 2–25°C
Coliform positive signal	Yellow ≥ comparator	Yellow ≥ comparator
<i>E. coli</i> positive signal	Yellow and fluorescence ≥ comparator	Yellow and fluorescence ≥ comparator
Coliform & <i>E. coli</i> negative signal	Yellow < comparator	Yellow < comparator

Table information from: http://www.idexx.com/view/xhtml/en_us/water/comparison.jsf

which will get into the tray wells and displace the amount of water in each well

4. Pour sample/reagent mixture into a Quanti-Tray®/2000 and seal in the IDEXX Quanti-Tray® Sealer. Label with date, location, and start time of incubation (not collection time).
5. Place the sealed tray in a 35°C ± 0.5°C incubator for the time specified by the reagent type (Table 7-2).

In cold weather, the incubator may take up to an hour to reach the desired temperature of 35 ± 0.5 °C, so plan accordingly (i.e., this may mean keeping the incubator running off the car battery during field work). In hot weather, the incubator may run the risk of overheating (beyond 35.5 °C) if it is kept in a vehicle or other unventilated space. Do not keep it in direct sunlight. Keep it in a cool, well ventilated place. The best option when working from a vehicle in remote locales may be to extend the power supply cord through a crack in the window and store the incubator on its side in the shade underneath the vehicle.

3.3 Dilutions

The above steps are appropriate for stream samples where *E. coli* colonies are expected to be within the range of the QuantiTrays (1 to 2,419 MPN/100 mL). If current conditions or previous sample results indicate *E. coli* colonies may exceed this range, the sample should be diluted. To do this, the 100 mL sample is mixed with sterile water in a sterilized bottle (sterile water is available from IDEXX in 100 mL quantities). Then, pour a 100 mL aliquot into the Quanti-Tray

and seal. Note the dilution factor in the notes section on the *Water Quality Datasheet*; multiply the tray result by this number to get the final result.

3.4 Counting bacteria in water samples

For IDEXX Colilert®, interpret Quanti-Tray 2000® results according to Table 7-2.

3.4.1 Total coliform

Count large and small wells that are equal to, or darker in color than corresponding wells on the Quanti-Tray 2000 comparator under natural light conditions, if possible. Enter the number of positive large wells and positive small wells on the field form. These numbers will later be entered in the water quality database, which will automatically calculate MPN/100 mL using a lookup table (provided by IDEXX).

3.4.2 *E. coli*

Count large and small wells that fluoresce equal to or greater than corresponding wells on the Colilert comparator tray under ultra violet light. Look for fluorescence with a UV light within 5 inches of the sample in a dark environment. Face light away from your eyes and towards the sample. Enter the number of positive large wells and positive small wells under “Bacteria Tray Readings” on page 4 of the *Water Quality Datasheet*. These numbers will later be entered in the water quality database, which will automatically calculate MPN/100 mL using a lookup table (provided by IDEXX).

3.5 Temperature monitoring and adjustments during bacteria sample incubation

As a quality control measure, SCPN will use temperature loggers to monitor the internal temperature of the incubator(s) being used for bacteria sample incubation. Presently, SCPN uses Hobo® Pendant temperature loggers, made by Onset Computer Corp. Follow guidelines in SOP #5 in setting up the loggers. (It is not necessary to log the “battery” channel data.) Use a 30-second logging interval, and download and visually inspect the data daily during a field trip in order to verify that the incubator is within the appropriate incubation temperature range (34.5 to 35.5 °C). If using the Thermotote 12® incubator, make very slight adjustments because the temperature dial is extremely sensitive. Turn the knob on the top of the unit with the small screwdriver housed inside the Thermotote—clockwise to increase temperature and counterclockwise to decrease temperature. If using the Micro Q incubator, the temperature is pre-set and no adjustments are necessary. Download a single data file at the end of each trip, and name it with the download date and the incubator type in name, e.g. “20101022_Thermotote.hobo.” These files are to be saved to the SCPN server, and will be used in bacterial data analysis and interpretation.

4 Safe handling and disposal of bacteria cultures

It is important to remember that by incubating and culturing the samples to enhance bacterial growth so that you may count them, you may also be encouraging the growth of colonies (or media) that may contain enteric pathogens, such as Salmonella, Shigella, and Legionella. As such, the waste culture media (such as would be used with IDEXX) should be treated as a biohazard and disinfected before it is discarded.

The following steps should be taken:

1. Wear gloves when handling bacteriological samples, and use hand sanitizer after.
2. Rinse the used IDEXX sample bottles well with tap water to remove all traces of stream water and Colilert reagent prior to being recycled.
3. While working in the field, store used quantitrays in autoclave bags, inside of Ziploc bags. In the lab, transfer autoclave bags into a tightly-sealed storage container. The storage container should have a biohazard label with a description of contents and SCPN contact information.
4. After each trip, arrange to have the Quanti-Trays autoclaved as soon as possible. They should be autoclaved at 121°C at 15 psi for at least 30 minutes.

5 Revision history

Provide the following information for each revision made to SOP #7.

New version:

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Date:

Author:

Sections affected:

Changes & reasons: