

Title: Bacteriological Sampling and Analysis	No: SOP 9.1	Page 1 of 6
Effective Date: 3/01/2013	Revision 1	
	Next Revision Date 3/01/2015	

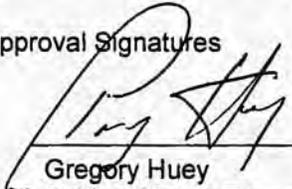
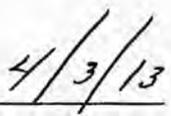
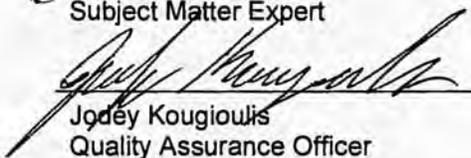
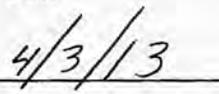
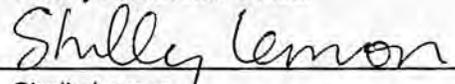
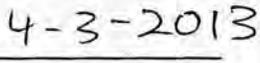
New Mexico Environment Department
Surface Water Quality Bureau

Standard Operating Procedure

for

Bacteriological Sampling and Analysis

Approval Signatures

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1.0 Purpose and Scope

This procedure describes the collection and analysis of ambient water and wastewater samples for total coliform and *Escherichia coli* (*E. coli*) bacteria using the IDEXX Laboratories, Inc. Colilert® method for both water quality standards assessment and permit compliance monitoring purposes.

2.0 Personnel Responsibilities

All personnel who collect or process samples for total coliform or *E. coli* analysis are responsible for implementing this procedure.

One individual within SWQB is designated as the "Bacteriological Equipment Manager." The manager is responsible for keeping the equipment used for bacteriological sampling in working order and ready for use. The manager is responsible for verifying that the dates on the lots have not exceeded their expiration dates.

3.0 Background and Precautions

The SWQB and the New Mexico State Laboratory Division (SLD) both use the IDEXX Laboratories, Inc. Colilert® procedures for enumeration of total coliform and *E. coli* by the most probable number (MPN) method. The procedure is explained in the Colilert® reagent snap packs and in *Standard Methods*, Part 9000 (APHA 2005). Background on the MPN method can be found in Oblinger and J. A. Koburger (1975).

Because *E. coli* can be an indicator of pathogens harmful to humans, precautions should be taken when sampling potentially contaminated water. Avoid accidental ingestion, contact with mucous membranes, eyes and skin to the extent possible, especially areas with cuts and abrasions. Wear splash protection and eye protection (i.e., goggles, gloves, and aprons) while working with bacteriological samples. Wash hands with soap and water or disinfecting hand cleaner as soon as possible after collecting samples and working with equipment. Hepatitis vaccinations are available for staff, if desired. Equipment exposed to potentially contaminated water should be cleaned using a dilute (1:10) bleach solution and rinsed in clean

Title: Bacteriological Sampling and Analysis	No: SOP 9.1	Page 2 of 6
	Revision 1	
Effective Date: 3/01/2013	Next Revision Date 3/01/2015	

water if possible. In the field, at a minimum, equipment should be thoroughly rinsed in clean water (e.g. the stream receiving the effluent above the point of discharge) immediately after use.

4.0 Definitions

None.

5.0 Equipment and Supplies

Equipment

There are two primary sets of IDEXX equipment available through the SWQB laboratory: a lab kit and a field kit.

Lab Kit:

- IDEXX 110V Incubator
- Fluorescent UV lamp, 6-watt, 365 nm
- IDEXX Quanti-Tray® sealer, “lab”
- IDEXX Quanti-Tray®/2000 rubber insert

Field Kit:

- Portable Incubator with DC power plug for vehicle operation
- IDEXX 6 watt fluorescent UV lamp, 365 nm
- IDEXX Quanti-Tray® sealer, “field”
- IDEXX Quanti-Tray®/2000 rubber insert
- 12 V Deep Cycle or Regular Automotive Starting Battery
- AC/DC inverter to power the portable incubator via a typical 110V outlet
- DC/AC 800 watt inverter and: +/- alligator clips to allow connection of sealer to vehicle alternator
- DC/DC adapter allowing vehicle power port conversion to +/- alligator clips for deep cycle battery power

Supplies

Materials for Samples Processed by SWQB

- IDEXX Colilert® Reagent “Snap Packs” for 24-28 hour incubation
- IDEXX Colilert®-18 Reagent “Snap Packs” for 18-22 hour incubation
- IDEXX Quanti-Tray®/2000 bacterial enumeration trays
- IDEXX Shrink Banded Sample Bottles

Materials for SWQB QC

- Colilert® and Colilert®-18 Quanti-Tray®/2000 Comparator

Materials for Samples processed by SLD

- 125 ml, sterile polypropylene sample bottles (available from SLD)
- **Note:** SLD does not accept samples in IDEXX bottles
- “Do not tamper” seals for lids (available from SLD)
- Submittal form (download from <http://www.sld.state.nm.us/Documents/waterform.pdf>)

The Environmental Microbiology section at SLD should be consulted (505-383-9129) at least 2-3 weeks in advance of the proposed sampling date to assure availability of media and incubator space.

6.0 Step-by-step Process Description

Title: Bacteriological Sampling and Analysis	No: SOP 9.1	Page 3 of 6
	Revision 1	
Effective Date: 3/01/2013	Next Revision Date 3/01/2015	

***E. coli* sample collection**

Before going in the field, fill out a “Bacteria Record Sheet” (attachment to this SOP) and use this form to record all requested information for each sample.

Collect both routine and quality control (blank) samples. In most cases, collect one set of quality control samples with each group of samples collected on consecutive days within one week. Generally, no less than 5% of the samples should be quality control samples. Prepare the field blank using distilled water and process quality control samples at the same as a routine sample.

Collect samples in sterile bottles. Do not rinse sample bottles before use and do not remove the caps or shrink wrap until immediately before sampling. For compliance sampling, the samples must be collected directly into the sample bottles. Otherwise, collect samples directly into the sample bottles whenever possible. If it is necessary to transfer to the sample bottle from another vessel, note this in the comment field when the sampling event is entered into NMEDAS.

Verify that the bottle is properly labeled and remove and dispose of the shrink band or sealing tape. In a stream, it is preferable to sample where the stream is flowing, well mixed and more than 6 inches deep. Stand downstream of the sampling bottle to avoid getting streambed sediment in the sample. In a lake or reservoir, collect the samples from the side of a boat at the sampling station or from the water’s edge.

Wear disposable gloves if you are sampling effluent water or are working downstream of a wastewater treatment plant. Uncap the sample bottle. Holding the cap in one hand and the bottle in the other, submerge the bottle in as upright a position as possible to retain the sodium thiosulfate chlorine neutralizer. Collect a sample from the water column, minimizing the amount of surface material entering the bottle. Attempt to fill the bottle just to the 100-mL line. If the bottle is filled above the 100-mL line, immediately decant excess water. Carefully replace the cap.

After collecting the sample, ice or refrigerate it at a temperature less than 10°C. Use insulated containers to assure proper maintenance of storage temperature. Ensure that sample vessels are not totally immersed in water during transit.

Samples for Submittal to SLD

Use 125 ml, sterile polypropylene sample bottles containing sodium thiosulfate (available from SLD).

Note: SLD will not accept samples in IDEXX bottles. Contact SLD in advance of delivery of the samples to verify that the samples can be processed within the required time.

Note the temperature of the samples when they are submitted to SLD receiving. Fill out one submittal form for each sample and apply “Do Not Tamper” seals to bottles before submitting. Maximum transport time is 6 hrs from time of collection until time of delivery at SLD, and 8 hours from collection to the start of incubation.

Samples for IDEXX processing

Use IDEXX sample bottles (120-mL shrink-banded containers with sodium thiosulfate, IDEXX Part Number WV120SBST).

Processing IDEXX Samples in the SWQB Laboratory



Figure 1. IDEXX Bottle

1. Remove the samples from the cooler approximately 30 minutes prior to arriving at the processing site and allow to warm to ambient temperature. Add the IDEXX Colilert® reagent packet. Although the reagent will dissolve in chilled samples, it dissolves better if the samples are at room temperature.

Title: Bacteriological Sampling and Analysis	No: SOP 9.1	Page 4 of 6
	Revision 1	
Effective Date: 3/01/2013	Next Revision Date 3/01/2015	

2. Switch on the Quanti-Tray® Sealer and allow it to warm up until the green light on the cover comes on. Warm up times greater than 14 minutes improve sealing.
3. Fill out a “Bacteria Record Sheet” (attachment to this SOP) and use this form to record all requested information for each sample.
4. As appropriate based on read-time, select either Colilert®-18 (18-22 hr incubation) or Colilert®-24 (24 - 28 hr incubation) reagent.
5. Add one reagent packet directly to each sample bottle. Invert gently at 2-3 min intervals until completely dissolved.
6. Pour the solution into a Quanti-Tray®2000 incubation tray while holding it vertically. Gently tap the incubation tray to dislodge any bubbles that may be trapped in the cells.
7. Place the tray into the red rubber frame and insert the frame and tray into the bay in the front of the sealer.
8. Carefully ease the frame and tray forward until the sealer activates and feeds the tray automatically. The sealer may occasionally jam while processing the tray. If this occurs, press the reverse button on the cover and the track will reverse, depositing the frame on the bay. Wait until the green light activates again and repeat the loading process. The tray will be deposited on the counter behind the sealer.
9. Record initial incubator temperature on the Bacteria Record Sheet.
10. Start the incubation within 8 hours of sample collection. Place the sealed tray into the 35°C incubator and incubate 18-22 hrs for Colilert®-18 reagent or 24-28 hrs for Colilert® reagent.
11. After the appropriate incubation period, record the final incubator temperature on the Bacteria Record Sheet. Remove the sealed tray and record the incubation time. Do not read trays that have been incubated beyond the appropriate incubation time, as they may produce false positives.
12. Enumerate total coliform bacteria by counting the number of large and small cells that turn yellow after incubation. Enumerate *E. coli* by counting the number of large and small cells that fluoresce under UV illumination with the black light. For both total coliform and *E. coli*, the Colilert® and Colilert®-18 Quanti-Tray®/2000 Comparator should be used to verify positive or negative cells. The large window at the top of the tray should be counted as the 49th large cell.
13. Enter the sample date, method, sample ID (RID#), and the number of positive large and small cells counted into the IDEXX MPN generator (P:\SWQB PUBLIC\MAS Core Documents\IDEXX MPN Generator\IDEXXMPN.exe – be sure to enter the “Default Directory to Save Files” under “options” menu to record data). Press calculate and then log to record the mpn/100 ml and the upper and lower boundaries of the 95% confidence interval. *Record QA sample results (including zero) the same as routine results (MPN expressed as cfu/100 ml and 95% upper and lower confidence limits).*
14. Transfer IDEXX log file to the Bacteria Database Upload Form (attachment to this SOP) and enter additional data, as recorded on the Bacteria Record Sheet. Confirm that there are no data QA issues and then convert data file to csv for upload to NMEDAS database. Additional instructions and details are provided on the Bacteria Database Upload Form.

Title: Bacteriological Sampling and Analysis	No: SOP 9.1	Page 5 of 6
	Revision 1	
Effective Date: 3/01/2013	Next Revision Date 3/01/2015	

Processing IDEXX Samples in the Field

A field kit has been assembled for IDEXX sample processing in the field. Procedures are the same as for laboratory processing, however a 12 volt incubator and adaptors and voltage converters to operate the sealer off the electrical system of a vehicle are available. There is a checkout sheet for the field kit in the SWQB public folders: [SWQB PUBLIC\Sonde&Equipment Checkout](#). Use this sheet when taking the kit from the building.

Before leaving on a sampling run, it is advisable to test all field kit components to ensure that they are all working and present.

If it is necessary to seal Quanti-Trays in the field, the IDEXX sealer may be powered by the vehicle alternator.

To operate the 110V sealer,

power must be converted from the 12V supplied by the vehicle alternator, and the vehicle must be running to supply sufficient amperage. A DC/AC 800 watt converter is used for this power conversion. To use the 110V sealer:

1. Clamp the alligator clips on the leads to the converter to the respective terminals on the vehicle battery.
2. Plug the sealer into the converter.
3. Start the vehicle.
4. Turn the converter on.
5. Turn the sealer on.

Follow procedures 1-8 under SWQB laboratory processing.

Use the portable DC incubators for field incubation. These are supplied with a power cord that allows them to be plugged into a vehicle's cigarette lighter. However, they draw sufficient amperage to drain the vehicle battery if the vehicle is not running. There is also an AC/DC converter that converts 110V AC to 12V DC allowing the incubator to be powered by a standard home 3-prong electric outlet for use in a hotel room or field office. The power cord for the Thermotote[®] model can be inserted two ways: one orientation will heat the incubator (red lamp lights) and the other will cool it (green lamp lights).

To incubate the samples, follow steps 9-11 under SWQB laboratory processing.

It may be necessary to enumerate Quanti-Trays in the field. Follow steps 12 and 13 under SWQB laboratory processing. The biggest challenge to this task is finding a dark place to discern which cells fluoresce. Service station washrooms, WWTP laboratories and SWQB field offices are often used for this purpose.

Storage and Disposal of Used Quanti-Trays

Following the sample incubation period, Quanti-Trays are considered a bacteriological biohazard and must be handled and disposed of accordingly. Trays collected in the field must be stored in a red or

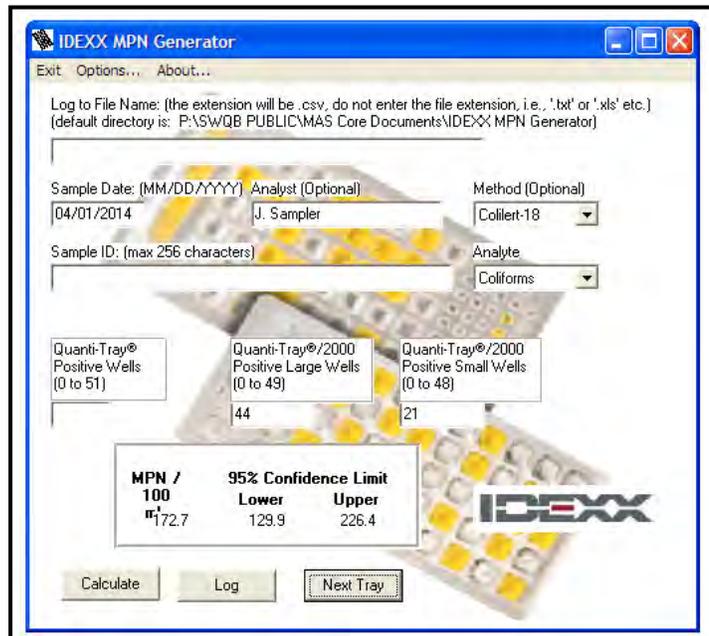


Figure 2. IDEXX MPN Generator Screenshot

Title: Bacteriological Sampling and Analysis	No: SOP 9.1	Page 6 of 6
	Revision 1	
Effective Date: 3/01/2013	Next Revision Date 3/01/2015	

orange “biohazard” bag inside a rigid container (e.g., small cooler, cardboard box, etc) and returned to the Runnels Building laboratory for storage. Used Quanti-Trays that are generated in the field or in the lab must be stored in an appropriate containment area until they can be properly disposed. A red bagged storage container is kept in the Runnels laboratory walk-in cooler and has been designated for staging of all used Quanti-Trays. This container is periodically emptied by Stericycle for proper disposal. Consult the *SWQB Chemical Hygiene Plan* for more information on handling and storage of used Quanti-Trays, as necessary.

Quality Assurance

If the samples are analyzed by SLD, the Laboratory should provide information to verify that the incubation temperature was maintained within the method-specified range, that the incubation began within 2 hours after sample receipt, and that the incubation time was within that required by the method.

If the samples were processed using SWQB equipment, the following sample rejection rules apply:

If both the temperature at the initiation and conclusion of the incubation were within $35 \pm 0.5^\circ\text{C}$, the data is not flagged (i.e. no qualifier code) . If either temperature is less than 34.5°C , the data is rejected (qualifier code = Er). If either temperature is between 35.5 and 38°C , the data is flagged and may only be used as supporting evidence for assessments (qualifier code = Ea). If either temperature is greater than 38°C , the data is rejected (qualifier code = Er).

The sealed trays should be read within the period indicated by the reagent. If trays are read before the nominal incubation time or more than 4 hours after, the data is rejected.

If the blank shows a value greater than the blank validation criteria value, then all of the results since the last in-range sample are rejected. This is summarized below:

	Validation Criteria	Validation Codes and Action	
Blank Sample	<1 cfu/100 mL	<1 cfu/100 mL, no code	≥ 1 cfu/100mL RB1, reject results

7.0 Related Forms

Bacteria Record Sheet
Bacteria Database Upload Form

8.0 Revision History

Revision 1 – 3/01/2013 – added information on the precautions one should take when working with potentially contaminated water and on the proper storage and disposal of used Quanti-Trays to be consistent with *SWQB’s Chemical Hygiene Plan*.

Original – 3/21/2011.

9.0 References

Oblinger, J.L. and J.A.Koburger (1975) Understanding and Teaching the Most Probable Number Technique. *J. Milk Food Technol.* 38(9): 540-545.

American Public Health Association (2005) Standard methods for the examination of water and wastewater. 21st edition. American Public Health Association