

Title: Bacteriological Sampling and Analysis	No: SOP 9.1	Page 1 of 7
	Revision 3	
Effective Date: 4/14/2016	Next Revision Date: 4/14/2018	

New Mexico Environment Department
Surface Water Quality Bureau

Standard Operating Procedure

for

Bacteriological Sampling and Analysis

Approval Signatures

	4/20/16
Charles Dentino Subject Matter Expert	Date
	4-20-16
for Vacant Quality Assurance Officer	Date
	4-20-16
Shelly Lemon Program Manager	Date

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 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).

E. coli can be an indicator of pathogens harmful to humans and precautions should be taken when sampling potentially contaminated water. Avoid accidental ingestion, contact with mucous

Title: Bacteriological Sampling and Analysis	No: SOP 9.1	Page 2 of 7
	Revision 3	
Effective Date: 4/14/2016	Next Revision Date: 4/14/2018	

membranes, eyes and skin to the extent possible, especially areas with cuts and abrasions. Hepatitis vaccinations are available for staff, goggles and disposable gloves may be desirable, and a disinfecting hand cleaner should be used as soon as possible after collecting samples. Equipment exposed to potentially contaminated water should be washed in soap and water and rinsed in clean water if possible.

4.0 DEFINITIONS

None.

5.0 EQUIPMENT AND SUPPLIES

5.1 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
- IDEXX 6 watt fluorescent UV lamp, 6-watt, 365 nm
- IDEXX Quanti-Tray® sealer, "lab"
- IDEXX Quanti-Tray®/2000 rubber insert
- IDEXX UV Viewing Cabinet

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
- AC/DC converter to power the portable incubator via a typical 110V outlet
- DC/AC 800 watt converter 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

Additionally, there is an extra power converter, fluorescent light and portable field incubator in the SWQB laboratory. If it becomes necessary for two teams to culture bacteria in the field, the lab sealer may be checked out. Prior to doing so, a notice must be sent to the SWQB ensuring that the lab sealer is not scheduled for use during that time. There is also a set of IDEXX equipment in the Las Cruces field office, and it may be possible to coordinate with staff to use this kit.

5.2 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 120 mL Bottles

Title: Bacteriological Sampling and Analysis	No: SOP 9.1	Page 3 of 7
	Revision 3	
Effective Date: 4/14/2016	Next Revision Date: 4/14/2018	

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://nmhealth.org/publication/view/form/1498/>)

The Environmental Microbiology section at SLD should be consulted (505-383-9122) at least 2 weeks in advance of the proposed sampling date to assure availability of media and incubator space.

6.0 PROCESS DESCRIPTION

6.1 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.

6.2 SAMPLES FOR IDEXX PROCESSING

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

6.3 *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 10% of the samples should be quality control samples. Prepare the field blank using distilled water and process quality control samples 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 SQUID.

Title: Bacteriological Sampling and Analysis	No: SOP 9.1	Page 4 of 7
	Revision 3	
Effective Date: 4/14/2016	Next Revision Date: 4/14/2018	

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 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.

6.4 PROCESSING IDEXX SAMPLES IN THE SWQB LABORATORY

1. Remove the samples from the cooler approximately 30 minutes prior to arriving at the processing site and allow them to warm to ambient temperature.
2. Switch on the Quanti-Tray® Sealer and allow it to warm up until the green light on the cover comes on.
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 hour incubation) or Colilert® (24 -28 hour incubation) reagent.
5. Add one reagent packet directly to each sample bottle. Invert gently at 2-3 min intervals until completely dissolved. This may take up to 30 minutes if samples are not completely warmed prior processing.
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 dock. 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.

Title: Bacteriological Sampling and Analysis	No: SOP 9.1	Page 5 of 7
	Revision 3	
Effective Date: 4/14/2016	Next Revision Date: 4/14/2018	

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 submit 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 Bacteria Database Upload Form: SWQB PUBLIC\MAS Core Documents\IDEXX MPN Generator\bacti_upload_from.. Press “Calculate MPN” 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. Confirm that there are no data QA issues and then press “Format Upload” to create the data file for upload to the SQUID database. Additional instructions and details are provided on the Bacteria Database Upload Form.

6.5 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, 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 present and working.

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.

Title: Bacteriological Sampling and Analysis	No: SOP 9.1	Page 6 of 7
	Revision 3	
Effective Date: 4/14/2016	Next Revision Date: 4/14/2018	

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 allow them to be plugged into a vehicle's power outlet. 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.

6.6 DISPOSAL OF QUANTI-TRAYS

Incubated Quanti-Trays are considered microbiological waste and must be disposed of accordingly. Trays collected in the field must be stored in a red "biohazard" bag and returned to the SWQB laboratory for storage. A storage container is kept in the SWQB laboratory walk-in cooler for staging of all enumerated Quanti-Trays, and is periodically transferred to SLD for disposal. Consult the SWQB Health and Safety Officer for more information on handling and storage of enumerated Quanti-Trays as necessary.

6.7 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 discarded. 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:

Title: Bacteriological Sampling and Analysis	No: SOP 9.1	Page 7 of 7
	Revision 3	
Effective Date: 4/14/2016	Next Revision Date: 4/14/2018	

Table1. Blank validation criteria.	Validation Criteria	Validation Codes and Action	
		Blank Sample	<1 cfu/100 mL

For each lot of Colilert 18200 or Colilert-2000 reagent, the following quality control procedure is recommended by the manufacturer:

Upon receipt of a new lot of IDEXX Colilert Colilert 18200 or 2000 reagent, fill out an SLD Isolate Request and Use Form (Appendix 1) and submit it according to the instructions. For a positive control, request E.coli strain ATCC 25922 or ATCC 11775 , or equivalent and for a negative control, request Psuedomonas Aeruginosa strain ATCC 10145 or strain ATCC 27853, or equivalent. Also, contact the head of the SLD Environmental Microbiology Section (currently Paul Torres paul.torres@state.nm.us; 383-9129) and let them know that you are submitting an Isolate Request Form. The Environmental Microbiology Section will also prepare sterile water or sterile 0.9% saline if needed. When the cultures are ready, pick them up from SLD and keep them sealed with parafilm or electrical tape at 4C in the dark. The cultures should remain viable for 4-6 weeks.

7.0 RELATED FORMS

Bacteria Record Sheet
Bacteria Database Upload Form

8.0 REVISION HISTORY

Original: 3/21/2011

Revision 1: 2/22/2013 – No significant changes.

Revision 2: 4/14/2015 – No significant changes. Made QA frequency consistent with SWQB QA policies.

Revision 3: 2/16/2016 – Updated data processing and upload instructions. Added QC instructions for positive and negative control.

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