



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L305

IDEXX Quanti-Tray 2000 for *E. coli*/Total Coliform Enumeration

Standard Operating Procedure

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Table of contents

1 Purpose	2
2 Scope	2
3 Responsibilities	2
4 Definitions	2
5 Safety and Hazards	3
5.1 Required PPE	3
5.2 Emergency Procedures	3
6 Equipment and Materials	4
6.1 Equipment	4
6.2 Reagents and Consumables	4
7 Procedure	4
7.1 Definitions	4
7.2 Biases and Interferences	5
7.3 Sample Collection	5
7.4 Sample Preservation and Storage	5
7.5 Sample Processing	6
7.6 Incubation	6
7.7 Reading Results – Total Coliforms	6
7.8 Reading Results – <i>E. coli</i>	7
7.9 Determining MPN	7
7.10 Interpreting Results	7
7.11 Review and Sign-Off	7
7.12 Expected Results for Control Organisms	7
8 Quality Control and Acceptance Criteria	8
8.1 QA/QC for Each New Lot	8
8.2 Precision	8
8.3 Acceptance Criteria	8
9 Waste Disposal	8

10 References	9
11 Training Record	9
12 Appendix A: Troubleshooting	9
13 Appendix B: IDEXX MPN Tables	9
14 Appendix C: Estimated Time	9

1. Purpose

This procedure describes the collection and analysis of water samples for the enumeration of *Escherichia coli* (*E. coli*) and total coliform bacteria using the IDEXX Colilert-18 / Quanti-Tray 2000 method. Results are reported as Most Probable Number (MPN) per 100 mL (APHA et al., 2012).

This method is applicable for analyzing drinking water, surface water, groundwater, and wastewater to assess microbial contamination levels.

2. Scope

- **Applies to:** Enumeration of *E. coli* and total coliform bacteria in drinking water, surface water, groundwater, and wastewater using the IDEXX Colilert-18 defined substrate method with Quanti-Tray 2000
- **Does not apply to:** Non-coliform bacterial analysis, viral or parasitic pathogens, or samples requiring membrane filtration methods
- **Location:** Biogeochemistry Lab and field sampling sites
- **Detection limit:** 1 MPN per 100 mL

3. Responsibilities

Role	Responsibility
Lab PI / Lab Director	Approve SOP, verify and sign off on results
QA Manager	Review completed bench sheets, sign lab notebook
Trained Personnel	Collect samples, perform analysis, record results
New Students	Complete training before independent use

4. Definitions

- **Colilert:** A proprietary IDEXX defined-substrate technology that detects total coliforms and *E. coli* simultaneously by enzymatic hydrolysis of ONPG (for coliforms) and MUG (for *E. coli*).

- **Coliform bacteria (total coliforms):** A group of facultatively anaerobic, gram-negative, rod-shaped bacteria that ferment lactose; includes *E. coli* and many non-fecal genera; used as an indicator of sanitary water quality.
- **Defined substrate technology (DST):** A quantitative detection method based on the enzymatic hydrolysis of a chromogenic or fluorogenic substrate specific to the target organism.
- ***E. coli*:** *Escherichia coli*; a fecal indicator bacterium detected by Colilert via MUG hydrolysis, which produces a fluorescent product visible under UV light at 365 nm.
- **MPN (Most Probable Number):** A statistical estimate of the number of organisms per 100 mL, derived from the pattern of positive and negative wells in the Quanti-Tray/2000 using the IDEXX MPN table.
- **Quanti-Tray/2000:** A sealed 97-well tray into which the sample- reagent mixture is sealed and incubated; provides positive/negative results for 49 large wells and 48 small wells, allowing MPN calculation up to 2,419 per 100 mL.
- **Total holding time:** The maximum time from sample collection to the start of incubation; for Colilert, the sample must be incubated within 6 hours of collection or 30 hours if held at 4 °C.

5. Safety and Hazards

Hazards

- **Biological:** Water samples may contain live microorganisms and pose a threat of infection. Treat all samples as potentially infectious.
- **Physical:** UV lamp (365 nm) can cause eye damage with prolonged exposure.
- **Chemical:** Colilert-18 reagent is not classified as hazardous per the manufacturer's SDS, but gloves and safety glasses are recommended.

5.1. Required PPE

- ☐ Clean disposable nitrile gloves
- ☐ Safety glasses (especially during UV reading and reagent handling)
- ☐ Lab coat
- ☐ Closed-toed shoes

5.2. Emergency Procedures

Water quality can range from potable to highly contaminated with assorted pathogens. Take every precaution to protect yourself. Wash hands frequently. In case of exposure to potentially contaminated water, wash affected area with soap and water immediately. Refer to [SOP-L002 Laboratory Safety](#) and [SOP-L003 Hazardous Materials Handling](#).

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Quanti-Tray Sealer	Lab bench	IDEXX Model B9-10894-04, Serial QT1757
UV light source (365 nm)	Lab bench	For <i>E. coli</i> fluorescence reading
Incubator	Lab	Set to 35 +/- 0.5 C; Catalog 150152632, Serial IMC40490982
Comparator	Lab refrigerator	Catalog 2100Q-01; stored in red zip-lock bag

6.2. Reagents and Consumables

Item	Catalog No.	Storage
IDEXX Colilert-18 reagent	98-27164-00	Room temperature
Sterile 100 mL IDEXX bottles (shrink-wrapped)	WV120SBT-200	Room temperature
Sterile 100 mL IDEXX bottles with sodium thiosulfate (for chlorinated samples)	WV120SBT-200	Room temperature
IDEXX Quanti-Tray 2000	–	Room temperature
Pipettes and sterile pipette tips	–	–
Disposable gloves and lab coat	–	–
Deionized water (for reagent preparation)	–	–

7. Procedure

7.1. Definitions

The following definitions apply throughout this SOP:

- **Analytical batch:** The set of samples processed at the same time.
- **Control cultures:** Known positive and negative cultures used to verify each lot of medium. *E. coli* serves as the positive control; *Staphylococcus aureus* serves as the negative control.
- **Field duplicate (FD):** Two samples taken at the same time and place under identical circumstances, treated identically through field and laboratory procedures. Analysis of FDs indicates the precision associated with sample collection, preservation, storage, and laboratory procedures.
- **Laboratory reagent blank (LRB):** An aliquot of sterilized water treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is to determine if analytes or interferences are present in the laboratory environment, reagents, or apparatus.

- **Laboratory duplicate (LD):** Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of LDs indicates precision associated with laboratory procedures but not with sample collection, preservation, or storage.

7.2. Biases and Interferences

- Water samples containing humic material or other colored substances may interfere with visual readings. If there is background color, compare inoculated trays to a control tray containing only water (SM, 9223 A) (APHA et al., 2012).
- Chlorinated water may inhibit the growth of coliform bacteria. If the water has been chlorinated, use the sodium thiosulfate bottles to neutralize the chlorine.

7.3. Sample Collection

1. Arrive at the site and record site number, date, and time
2. Label the bottle with: location (geographic area and stream or lake name), date, time, water temperature, and sampler's initials. Label the bottle before immersion using a black permanent marker or pre-printed labels.
3. Put on latex or nitrile gloves before handling bottles. Fingers contain contaminants such as nitrates; bug repellent and sunscreen are particularly troublesome. Once gloves are on, do not touch your face, the ground, or anything other than the bottles.
4. Collect the sample from flowing (not stagnant) water, facing upstream
5. Immerse the bottle completely, approximately 10 cm (4 inches) deep, with the mouth pointing upstream so no water flows over your hand into the bottle. Do not allow the bottle to contact the stream bottom where sediments can be disturbed. Collect water 6–12 inches below the surface.
6. Fill the bottle to the 100 mL line on the first immersion. Pour off any excess and cap immediately. Do not underfill, overfill, or re-dunk. If too much water is poured off, use a new 100 mL container.
7. Do not touch the bottle mouth or inside of the cap. Be careful not to contaminate the sample with surface film, contact with human skin, or breathing into the bottle or cap. If the stream is too shallow to fully immerse the bottle, collect as much as possible while avoiding the bottom, and note the depth in field notes.

Tip

Collect one field duplicate (FD) sample every two weeks of sampling. Duplicate sites are selected at random. The duplicate is the second sample when two samples are collected at the same location. Duplicates document repeatability of sample collection and reproducibility of laboratory results.

7.4. Sample Preservation and Storage

1. Keep samples cool during transport using a cooler with ice

2. Store at 4 C until analysis; do not freeze
3. Analyze within 6 hours of collection
4. Record the location number, bottle numbers, and collection time in the field sample log
5. Samples must remain in the possession of the researcher who both collects and analyzes them

7.5. Sample Processing

1. Turn on the Quanti-Tray sealer
2. Set the incubator to 35 C
3. Allow samples to reach room temperature before analysis
4. Aseptically add one packet of Colilert-18 reagent to the 100 mL water sample
5. Replace the lid and shake well until the reagent has fully dissolved
6. Label the Quanti-Tray with a permanent marker, recording information that identifies the sample
7. Open the Quanti-Tray by holding it at the top with the well side facing your palm. Push the top edge inward with your free hand while squeezing the tray into a circle. Gently pull the foil tab away from the plastic side. Be careful not to tear the tab. Do not touch the inside of the tray.
8. Pour the sample mixture into the Quanti-Tray, avoiding contact with the foil tab
9. Tap the small wells 2–3 times to release air bubbles. Allow foam to settle.
10. Place the filled Quanti-Tray on the rubber insert, making sure each well fits its corresponding hole
11. Push the insert with the tray into the sealer, non-foil-tab end first, until the sealer grabs the tray and pulls it through. If you need to reverse the motor, press and hold the reverse button. However, do not reverse the motor if the rubber insert is completely inside the sealer.
12. The sealer distributes the sample mixture into the wells, seals them, and partially ejects the sealed tray
13. Remove the rubber insert and tray from the sealer

Important

If the sealer jams or malfunctions, do not force the tray. Contact the lab manager for assistance.

7.6. Incubation

1. Incubate the sealed tray at 35 +/- 0.5 C for 18 hours
2. Do not stack trays or block airflow around them in the incubator

7.7. Reading Results – Total Coliforms

1. After 18 hours of incubation, remove the tray from the incubator
2. Count the number of large wells and small wells that are yellow and equal to or greater than the comparator, including the large overflow well at the top
3. Mark positive wells with a permanent marker as you count
4. Record the number of positive small wells and positive large wells in the lab notebook

7.8. Reading Results – *E. coli*

1. In a darkened area, view the Quanti-Tray under the UV lamp (365 nm)

UV Safety

Be careful to avoid eye damage when using the UV lamp. Do not look directly at the UV light source. Wear UV-protective safety glasses if available.

2. Count the number of fluorescent wells that are equal to or greater than the comparator
3. Record the number of fluorescent small wells and fluorescent large wells in the lab notebook

7.9. Determining MPN

1. Use the IDEXX MPN table (see Appendix) to look up the MPN based on the number of positive large wells and positive small wells
2. Cross-check results using the IDEXX MPN Generator software: <https://www.idexx.com/en/water/resources/mpn-generator/> (Windows only)
3. Record results on the bench sheet for both total coliforms and *E. coli*

7.10. Interpreting Results

MPN/100 mL	Risk Level
1–10	Low risk
11–100	Medium risk
Greater than 100	High risk

7.11. Review and Sign-Off

1. The completed bench sheet should be reviewed by the researcher, the laboratory director, and the QA manager
2. The Lab Director or QA Manager must sign the lab notebook to verify that the results are accurate

7.12. Expected Results for Control Organisms

Organism	Expected Result
<i>Escherichia coli</i>	Yellow wells, fluorescence
<i>Klebsiella pneumoniae</i>	Yellow wells, no fluorescence
<i>Pseudomonas aeruginosa</i>	Clear wells, no fluorescence
Method blank	Clear wells, no fluorescence

8. Quality Control and Acceptance Criteria

8.1. QA/QC for Each New Lot

For initial analyst demonstration of capability and for each new lot of Quanti-Tray 2000, perform the following checks:

1. **Reagent lot check:** Shine the UV lamp on the Colilert media snap packs. If the unopened lot is fluorescent, discard it.
2. **Blank control:** Dissolve one packet of Colilert media in 100 mL of distilled water. Check for fluorescence. The blank must show no fluorescence.
3. **Positive control:** Dissolve one packet of Colilert media and 1–2 Control Culture vials (stored in freezer) in 100 mL of distilled water. Incubate for 18 hours. Confirm fluorescence.
4. **Reagent water blank:** Analyze a sterile reagent water blank with each batch of samples to verify a negative result after 18–22 hours.
5. **Sterile bottle check:** Gravimetrically check each new lot of sterile bottles to confirm they are transparent and non-fluorescing.

8.2. Precision

Calculate precision for duplicate analyses using relative percent difference (RPD):

$$\text{RPD} = \frac{(A - B) \times 100}{(A + B)/2}$$

where A = MPN from aliquot A and B = MPN from aliquot B.

8.3. Acceptance Criteria

- Desired precision: +/- 20% RPD
- Desired detection limit: 1 MPN/100 mL
- If a precision value exceeds 20%, record in the comments section of the bench sheet: “These data are associated with an out-of-control duplicate analysis. The UCL = 20%.”
- If a blank value exceeds 1 MPN/100 mL, record in the comments section: “These data are associated with a blank value that exceeds the detection limit of 1 MPN/100 mL.”
- Samples cannot be reanalyzed because the sample volume is depleted after the initial analysis
- If data are unacceptable for any reason, the analyst should review their technique before conducting this analysis again

9. Waste Disposal

Waste Type	Disposal Method
Used Quanti-Trays and media	Autoclave before disposal, then standard trash
Used sample bottles	Autoclave before disposal, then standard trash

Waste Type	Disposal Method
Workspace surfaces	Disinfect thoroughly after each session

! Important

All materials that have contacted water samples or culture media must be autoclaved prior to disposal. Workspaces must be thoroughly disinfected after each analytical session.

10. References

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

11. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP in full
2. Complete [SOP-L002 Laboratory Safety](#) and [SOP-F001 Field Safety](#) training
3. Review the Colilert-18 manufacturer's instructions
4. Review sampling and analysis procedures with the Lab Manager
5. Observe a trained operator perform the complete procedure (collection through enumeration)
6. Perform the procedure under supervision with acceptable QC results
7. Be signed off by the PI or lab manager

12. Appendix A: Troubleshooting

TBD – add troubleshooting guidance for common issues (e.g., trays not sealing properly, ambiguous well color, incubator temperature deviations).

13. Appendix B: IDEXX MPN Tables

The original LaTeX document included two pages of MPN lookup tables (scanned PDFs). These should be included here as linked PDF files or reproduced as tables. The tables can also be accessed via the IDEXX MPN Generator at <https://www.idexx.com/en/water/resources/mpn-generator/>.

14. Appendix C: Estimated Time

Task	Time Required
Sample setup	Approximately 12 minutes
Incubation	18 hours
Reading and enumeration	Approximately 10 minutes