



**Subject: Presence/Absence Total Coliform and *E. coli* by Colilert®  
(SM 9223 B-2004)**

## Approval

Title	Name	Signature	Date
Laboratory Supervisor	Kelley E. Keenan		02-01-18
Quality Assurance Officer	Jim Sumner		02-01-18

## Document Revision History

Effective Date	Revision number	Review Type	Evaluators	Revisions
12-01-00	0	Internal	Jim Sumner (ETS)	Original document
10-11-11	1	Internal	Kelley Keenan (ETS)	<ul style="list-style-type: none"> <li>Changed bacteria culture name.</li> <li>Corrected typos.</li> </ul>
04-01-13	2	Internal	Jim Sumner (ETS)	<ul style="list-style-type: none"> <li>Updated procedure and references to the approved analytical method identified in USEPA Method Update Rule II (MUR II), May 18, 2012.</li> </ul>
09-14-15	3	Internal	Kelley Keenan (ETS)	<ul style="list-style-type: none"> <li>Updated section C for reporting using Public Water Supply Laboratory Data Submittal (LDS) system. Included Public Water Supply Laboratory Data Submittal (LDS) guidance document as Exhibit B6.3.</li> </ul>
02-01-18	4	Internal	Jim Sumner (ETS)	<ul style="list-style-type: none"> <li>Updated procedure to include NELAP requirements.</li> <li>Additional guidance included in SOP.</li> <li>Method number revised and additional QC requirements included based on 2017 MUR.</li> </ul>

## Scope and Application

Colilert® is an enzyme substrate test which utilizes hydrolysable substrates for the simultaneous detection and confirmation of total coliform and *E. coli*.

## Summary of Method

Colilert® reagent is used for the detection and confirmation of total coliform and *E. coli* in drinking water. This product utilizes nutrient indicators that produce color and/or fluorescence when metabolized by total coliform and *E. coli*. When the reagent is added to the sample and incubated, it can detect these bacteria at 1 CFU/100 mL within 24 hours with as many as 2 million heterotrophic bacteria/100 mL present.

Colilert® procedures are based on Standard Methods 9223 B-2004.

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## Sample Collection, Preservation, Shipment and Storage

Samples must be analyzed within **30-hours** of collection.

Samples must be collected in sterile 120-mL plastic containers containing **Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>** (sufficient to dechlorinate 5.0 mg/L residual chlorine).

## Quality Control

**Bottle Sterility:** Sterile bottles are purchased from an outside supplier. Sterility is verified on 2% of the purchased containers (SOP B3). The Certificate of Analysis supplied by the manufacturer is maintained in the laboratory's QC files.

**Consumable Testing:** Before new lots of consumables (Colilert® reagent, sample bottles) are used for testing and at 90 days for a given lot, those consumables must be tested (Exhibit B6.2). New consumables are tested to ensure that the consumable does not auto fluoresce and that the Colilert® reagent produces the expected results with known bacteria.

1. Fill a bottle to the 100 mL mark and pour into a graduated cylinder. Each new vendor of bottles must be checked to ensure that the 100 mL mark is 100 ± 2.5 mL. Note the result on the Quality Control - Sample Bottle Sterility benchsheet (SOP B2).
2. Fill 4 sterile Colilert bottles to the 100 mL mark with sterile water (SOP B11).
3. Using a sterile inoculating loop, inoculate the sterile water (100 mL) with the bacteria identified in Table B6.1. Follow the above test procedure and compare test results to the expected results identified in Table B6.1. Each bottle must be checked at 24 and at 48 hours. Immediately after Colilert® reagent has been added to the bottles, check the bottles for auto fluorescence under the UV lamp. If fluorescence occurs, repeat steps 2 through 3 above and check for fluorescence. If fluorescence is confirmed, a new lot of Colilert® reagent must be purchased and the supplier notified.

Table B6.1:

Organism	Expected Result
Blank	Clear, no fluorescence
<i>E. coli</i>	Yellow, Fluorescent
<i>Enterbacter aerogenes</i>	Yellow, no fluorescence
<i>Pseudomonas aeruginosa</i>	Clear, no fluorescence

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**PE:** Twice every calendar year (January and July), a single-blind QC check sample (QCS) or performance evaluation sample (PE) is analyzed. This sample is provided by an approved proficiency testing (PT) provider.

Additional quality control guidance is provided in QAP-Q5.

## Interferences

Water samples containing humic or other material may be colored. If there is background color, compare the sample to the IDEXX® color comparator. If the sample color interferes with easily distinguishing a positive sample, the sample must be rejected. If the sample is to be reported to the North Carolina Public Water Supply (PWS), then the state form must reflect the reason why the sample was rejected and the client will be required to recollect the sample.

## Equipment and Materials

IDEXX® Colilert® reagent  
IDEXX® color comparator  
IDEXX® sterile, screw cap bottles graduated at 100 ml  
6 watt, 365 nm UV light  
Incubator (35.0 ± 0.5°C)  
Thermometer in 0.5°C increments  
Sterile dilution water  
Inoculating loop  
*Escherichia coli*  
*Pseudomonas aeruginosa*  
*Enterobacter aerogenes*  
Quality Control, Colilert® Reagent (SM 9223 B-2004) Benchsheet  
Total Coliform / *E. Coli* by Colilert® (SM 9223 B-2004) Benchsheet

## Procedure

### A. Presence/Absence Total Coliform and *E. coli* by Colilert

1. Each sample bottle must contain at least 100 mL of sample. If the volume is below the 100 mL mark, then the sample must be rejected. If the sample is to be reported to the North Carolina Public Water Supply (PWS), then the state form must reflect that there was insufficient volume to initiate the test.

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2. Vigorously shake each sample 25 times. If the sample volume is over the 100 mL mark on the bottle, carefully remove the cap and hold in your hand (being sure that the cap does not get contaminated). Carefully pour small aliquots out of the sample container, until the volume reaches the 100 mL mark on the bottle.
3. Carefully separate one Snap Pack containing Colilert reagent from the strip, taking care not to accidentally open the adjacent pack. Colilert reagent must be stored at room temperature where it is not exposed to light.
4. Tap the Snap Pack to ensure that the entire Colilert reagent is in the bottom part of the pack.
5. Open one pack by snapping back the top at the score line, being careful not to touch the opening of the pack.
6. Add the reagent to the water sample contained in a sterile, transparent, non-fluorescent vessel (100 mL).
7. Aseptically cap and seal the vessel.
8. Shake until dissolved.
9. Incubate for 24 hours at  $35.0 \pm 0.5^{\circ}\text{C}$ .
10. Record the sample number, sample identification, Private or State Compliance, initiation (date/time/analyst), termination (date/time/analyst) and results on the Total Coliform / *E. Coli* by Colilert® (SM 9223 B-2004) Benchsheet (Exhibit B6.2).
11. Read the results at 24 hours. Compare each result against the comparator dispensed into an identical vessel.
  - a. If no yellow color is observed, the test is absent for total coliform and *E. coli*.
  - b. If the sample has a yellow color equal to or greater than the comparator, the sample is present for total coliform. If color is not uniform, mix by inversion then recheck.

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- c. If the sample is yellow, but lighter than the comparator, it must be incubated an additional 4 hours (but not more than 28 hours total). If the sample has coliform present, the color will intensify. If it does not intensify, the sample is absent for total coliform.
  - d. If yellow is observed, check the vessel for fluorescence by placing a 6 watt 365 nm UV light within 2 inches of the sample in a dark environment. Be sure the light is facing away from your eyes and towards the vessel. If fluorescence is present, the sample contains *E. coli*.
12. If the sample contains total coliform and/or *E. coli*, alert the supervisor immediately, so that the individual and PWS can be notified.

**B. Reporting results to clients and the North Carolina Public Water Supply (PWS).**

- 1. If the results for state clients are positive for total coliform and or *E. coli*, the client must be notified that day. Note in the comment section on the state form that the client has been notified or a message has been left. Make sure that the result section and the REPEAT section are marked. All the appropriate dates, times and signatures must be completed. All data must be uploaded into the Public Water Supply Laboratory Data Submittal (LDS) system (Exhibit B6.3). If the sample is positive for *E. coli*, the data must be uploaded on the day the test is completed. If the sample is positive for total coliform, the results must be submitted within 24-hours.
- 2. If the results are for Repeat samples, follow C1 for reporting. Additionally, make sure that the Repeat box is completed for each sample.
- 3. All results must be uploaded into the LDS system before the 10<sup>th</sup> of the following month.

**Safety and Hazardous Waste Management**

Safety glasses, gloves and lab coats should be worn at all times while handling samples.

All samples (present or absent for coliform) must be disinfected with approximately 2 mL bleach per sample volume. Samples must be disinfected for at least 16 hours prior to disposal to the sanitary sewer (Exhibit B6.4).

Review Policy-P6: General Safety Policy and Policy-P9: Radiation Protection Policy for additional safety requirements.

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## Bacteria Procedures

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#### References

Standard Methods for the Examination of Water and Wastewater, 22<sup>nd</sup> Edition, 2012. American Public Health Association, 800 I Street, NW, Washington DC 20001-3710.

- Method: 9223 B-2004.

IDEXX Colilert®, 1 IDEXX Drive, Westbrook, ME, 04092.

TNI Standard. Management and Technical Requirements for Laboratories Performing Environmental Analysis. EL-V1-ISO-2016-Rev2.0. The NELAC Institute, PO Box 2439, Weatherford, TX 76086.

#### Exhibits

Exhibit B6.1: Total Coliform / E. Coli by Colilert® (SM 9223 B-2004) Benchsheet  
Exhibit B6.2: Quality Control, Colilert® Reagent (SM 9223 B-2004) Benchsheet  
Exhibit B6.3: Public Water Supply Laboratory Data Submittal (LDS) – ATTACHMENT  
Exhibit B6.4: Example Drinking Water Bacteria Sample Disposal Benchsheet

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**Exhibit B6.2: Quality Control, Colilert® Reagent (SM 9223 B-2004) Benchsheet**



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**Quality Control, Colilert® Reagent (SM 9223 B-2004)**

Colilert Source:	IDEXX Laboratories, Inc.
Lot Number:	
Expiration Date:	
Date Received:	
Received By (analyst's initials):	
IDEXX Un-buffered Sterile Water Lot #	

Organism	Initiation			24-hour Check					48-hour Check				
	Date	Time	Analyst	Date	Time	Analyst	Total Coliform Present or Absent (P or A)	Fecal Coliform Present or Absent (P or A)	Date	Time	Analyst	Total Coliform Present or Absent (P or A)	Fecal Coliform Present or Absent (P or A)
<i>Blank</i>													
<i>Escherichia coli</i>													
<i>Enterobacter aerogenes</i>													
<i>Pseudomonas aeruginosa</i>													

3 Month Date \_\_\_\_\_ Analyst \_\_\_\_\_ Auto-fluorescence Positive \_\_\_\_\_ Negative \_\_\_\_\_  
 3 Month Date \_\_\_\_\_ Analyst \_\_\_\_\_ Auto-fluorescence Positive \_\_\_\_\_ Negative \_\_\_\_\_  
 3 Month Date \_\_\_\_\_ Analyst \_\_\_\_\_ Auto-fluorescence Positive \_\_\_\_\_ Negative \_\_\_\_\_

SOP B6-Revision 4-Exhibit B6.2

**Exhibit B6.3: Public Water Supply Laboratory Data Submittal (LDS) – ATTACHMENT**

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**Exhibit B6.4: Example Drinking Water Bacteria Sample Disposal Benchsheet**

**2018 Drinking Water Bacteria Sample Disposal**

Samples received in week ending	Date samples disinfected	Analyst	Date samples disposed	Analyst
January 06				
January 13				
January 20				
January 27				
February 03				
February 10				
February 17				
February 24				
March 03				
March 10				
March 17				
March 24				
March 31				
April 07				
April 14				
April 21				
April 28				
May 05				
May 12				
May 19				
May 26				
June 02				
June 09				
June 16				
June 23				
June 30				

Note: All samples (present or absent for coliform) must be disinfected with approximately 2 mL of bleach per sample volume. Samples must be disinfected for at least 16-hours prior to disposal to the sanitary sewer.

SOP B7-Revision 4-Exhibit B7.4

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