

# STANDARD OPERATING PROCEDURE For SM 9223

## Enzyme Substrate Coliform Test Presence-Absence Procedure for the Analysis of Potable Water Samples

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SOP #: SM 9223

REVISION #: 2.2

DATE: December 2006

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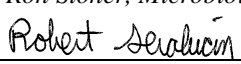
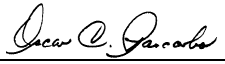
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# MassDEP

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## LIST OF REVISIONS

Rev. #	Date	Description of Revision	Page #
0	August 2000	None	
1.0	November 2001	Numerous edits	Throughout document
2.0	February 2003	Numerous edits	Throughout document
2.1	August 2005	Control Document statement added Table 1 added – QC elements, acceptance criteria, and corrective actions	Cover Page 8
2.2	December 2006	Replaced old DEP Logo with state seal + MassDEP	Title page & header



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## 1.0 SCOPE & APPLICATION

- 1.1 The enzyme substrate test is a presence/absence technique that utilizes hydrolyzable substrates in the growth medium for the simultaneous detection of total coliform bacteria and *E. coli* based on the detection of specific enzymes associated with these bacteria.
- 1.2 The Colilert® method may be used to test a 100-mL sample volume of potable water in compliance with the National Primary Drinking Water Standards – Total Coliform Rule.

## 2.0 SUMMARY OF METHOD

- 2.1 The commercially purchased medium is added to a 100-mL volume of sample, incubated for 24 hours at  $35 \pm 0.5^\circ\text{C}$ , and then checked for color and fluorescent reactions.

## 3.0 DEFINITIONS

- 3.1 When the enzyme substrate technique is used, the total coliform group is defined as all bacteria possessing the enzyme  $\beta$ -D-galactosidase, which cleaves the chromogenic substrate, ortho-nitrophenyl- $\beta$ -D-galactopyranoside (ONPG) resulting in the release of a chromogen which produces a yellow color in the growth medium.
- 3.2 Using this method, *E. coli* is defined as bacteria giving a positive total coliform response and possessing the enzyme  $\beta$ -glucuronidase which cleaves the substrate, 4-methyl-umbelliferyl- $\beta$ -D-glucuronide (MUG) which produces a fluorescent product in the growth medium when viewed under 366-nm ultraviolet (UV) light.

## 4.0 INTERFERENCES

- 4.1 Non-coliform bacteria, particularly *Aeromonas* and *Pseudomonas* species, may produce small amounts of the enzyme  $\beta$ -D-galactosidase, but are suppressed and generally will not produce a positive response within the incubation time unless more than 104 colony-forming units (CFU)/mL are present.
- 4.2 *Serratia* species may turn the medium yellow after 24 hours of incubation but the yellow color is typically brighter than that represented by the color comparator.
- 4.3 Some strains of *Shigella* species may produce a positive fluorescence response. This is not considered a detriment for testing the sanitary quality of water due to the pathogenic nature of *Shigella* spp.
- 4.4 Some water samples containing humic material may have an innate yellow color. If a water sample has some background color, compare inoculated Colilert® sample to a control of the same sample without the addition of the Colilert® medium.

## 5.0 SAFETY

- 5.1 Samples (and positive controls) may contain organisms that are pathogenic to humans. All precautions are to be taken to minimize exposure. All personnel must wear lab coats, safety glasses, and protective gloves while working in the laboratory. All personnel must be immunized against the hepatitis A and B viruses, and must receive on-the-job laboratory safety training.



## 6.0 EQUIPMENT AND SUPPLIES

- 6.1 Sterile Colilert® bottles (purchased from manufacturer) containing sodium thiosulfate.
- 6.2 UV lamp: 366-nm UV light (6 watt)
- 6.3 Color comparator (purchased from manufacturer)
- 6.4 Incubator capable of maintaining  $35 \pm 0.5^{\circ}\text{C}$  for 24-28 hours.

## 7.0 REAGENTS AND STANDARDS

- 7.1 Colilert Presence-Absence Medium (purchased from manufacturer)
- 7.2 ASTM Type I reagent-grade water

## 8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

- 8.1 A 100-mL sample must be aseptically collected in a sterile disposable plastic bottle leaving at least 1" (2.5 cm) of headspace to allow for sufficient mixing of the sample prior to analysis. If the sample is chlorinated, make sure that the sample bottle contains the sodium thiosulfate tablet (Note: provides a final concentration of 100 mg/L  $\text{Na}_2\text{S}_2\text{O}_3$ ).
- 8.2 If tap cleanliness is in question, apply a solution of sodium hypochlorite (100 mg  $\text{NaOCl/L}$ ) to faucet before sampling.
- 8.3 Remove all attachments from the water tap (screens, etc.), open tap, and let run to waste for 2-3 minutes. Reduce the water flow to allow for filling of the bottle without splashing.
- 8.4 Keep sample bottle closed until it is to be filled, fill the bottle without rinsing, replace cap immediately, and secure the top with the attached plastic "lock".
- 8.5 Potable water samples must be analyzed as soon as possible but no longer than 30 hours after collection. Preferably, keep potable water samples at  $\leq 10^{\circ}\text{C}$  from the time of collection to the time of analysis.
- 8.6 A WES sample-tracking/chain-of-custody form must be filled out by the collector and sent with the samples.

## 9.0 QUALITY CONTROL

- 9.1 With each new lot of Colilert® medium purchased, test medium with un-inoculated sterile reagent water and sterile reagent water inoculated with: 1) Non-fluorescent *Pseudomonas* sp. (i.e., total coliform & *E. coli* negative); 2) *Klebsiella pneumoniae*, *Enterobacter aerogenes*, or *Enterobacter cloacae* (i.e., total coliform positive & *E. coli* negative); and 3) *E. coli* (i.e., total coliform & *E. coli* positive) control cultures.
- 9.2 With each batch of 20 or fewer samples, run a blank (sterile reagent water) and two positive control samples (i.e., sterile reagent water spiked with *Klebsiella pneumoniae*, *Enterobacter aerogenes* or *Enterobacter cloacae*, and sterile reagent water spiked with *E. coli*).



## **10.0 CALIBRATION AND STANDARDIZATION**

- 10.1 Each lot of sample containers used to measure sample volume must be checked to ensure that it meets a mass (grams) to measured volume (mL) ratio of one for reagent water at 4°C.
- 10.2 Refer to Laboratory Quality Assurance Plan for calibration and standardization procedures of laboratory equipment used for this analysis.

## **11.0 PROCEDURE**

- 11.1 Shake the sample well (25 times) and aseptically fill the Colilert® bottle to the 100-mL mark.
- 11.2 Without touching the perforated section of the Colilert® medium snap-pack, tap the medium down into the bottom of the snap-pack and open the pack by snapping along the perforations.
- 11.3 Empty the entire packet of medium into the bottle containing the sample. Cap the sample, shake, and incubate at  $35 \pm 0.5^{\circ}\text{C}$  for 24 hours.
- 11.4 After incubation, check the sample for a yellow color change that is at least as strong as the yellow color of the comparator. A yellow color that is equal to or deeper than that of the color comparator verifies that the sample is positive for total coliforms (See Figure 1).
- 11.5 If the sample is total coliform-positive as indicated in 11.4, expose the sample to 366-nm UV light using the hand-held UV lamp in the WES dark microscopy room. If the sample fluoresces (compare with comparator), then the sample is positive for *E. coli* (See Figure 1).
- 11.6 If the sample results are questionable after 24 hours of incubation, the sample may be incubated for an additional 4 hours (total of 28 hours), and rechecked for color and fluorescent reactions. If the color intensifies, the sample is total coliform positive; if it does not, the sample is negative. If an inoculated test is inadvertently incubated over 28 hours, the following guidelines apply:
- Lack of yellow color is a valid negative test
  - A yellow color after 28 hours is not valid and must be repeated.

## **12.0 DATA ANALYSIS AND CALCULATIONS**

- 12.1 Record the presence-absence reaction for both total coliform and *E. coli* on the bench sheet generated by the LIMS.

## **13.0 METHOD PERFORMANCE**

- 13.1 The detection limit of this method is one colony-forming unit per sample volume or dilution tested.

## **14.0 POLLUTION PREVENTION**

- 14.1 Refer to the WES Environmental Management System (EMS) policy and SOPs regarding pollution prevention.
- 14.2 The quantity of media and reagents purchased should be based on expected usage during its shelf life. Actual media and reagent preparation volumes should reflect anticipated usage and stability.



## 15.0 WASTE MANAGEMENT

- 15.1 WES laboratories fully comply with all applicable federal, state, and local environmental regulations. WES is also committed to protecting the air, water, and land by minimizing and controlling all chemical releases from fume hoods, biological safety cabinets, and bench operations. Refer to the WES EMS policy and SOPs regarding waste management.
- 15.2 All positive sample bottles are placed in autoclave bags and autoclaved at 121°C for a minimum of 30 minutes. Decontaminated plastic bottles are rinsed for recycling.

## 16.0 REFERENCES

- 16.1 *Standard Methods for the Examination of Water and Wastewater*, 20th Edition, 1998. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC

## 17.0 TABLES AND FIGURES

**TABLE 1. Quality Control Elements, Acceptance Limits, and Corrective Actions for the Analysis of Total Coliform/*E. coli* in Potable Water Samples by the Enzyme Substrate Presence-Absence Coliform Test – SM 9223**

QC Elements	Frequency	Acceptance Criteria	Corrective Action
Sample storage	Every sample	Potable water samples are analyzed ASAP and no later than 30 hrs from collection. Although not required, potable water samples should be stored at $\leq 10^{\circ}\text{C}$ from time of collection to time of analysis.	Qualify data (H) as estimated value (exceeded holding time) and contact sample collector to obtain new sample
Positive control (equivalent to LCS and LFB; sterile reagent water spiked with <i>E. coli</i> )	With each batch of 20 or fewer samples ( $\geq 5\%$ )	Positive for total coliform (yellow) and <i>E. coli</i> (fluorescent)	Qualify data (J) as estimated value ( <i>E. coli</i> and/or TC negative for the positive control – media or other failure) and contact sample collector to obtain new sample
Positive control (equivalent to LCS and LFB; sterile reagent water spiked with <i>Klebsiella pneumoniae</i> , <i>Enterobacter aerogenes</i> , or <i>Enterobacter cloacae</i> )	With each batch of 20 or fewer samples ( $\geq 5\%$ )	Positive for total coliform (yellow); negative for <i>E. coli</i> (non-fluorescent)	Qualify data (B or J) as estimated value (if <i>E. coli</i> is positive or TC is negative in this control, respectively) and contact sample collector to obtain new sample
Negative control (equivalent to LRB; sterile reagent water)	With each batch of 20 or fewer samples ( $\geq 5\%$ )	Negative for total coliform (non-yellow) and <i>E. coli</i> (non-fluorescent)	Qualify data (B) as estimated value ( <i>E. coli</i> and/or TC positive for a sample, and for the negative control – laboratory contamination) and contact sample collector to obtain new sample



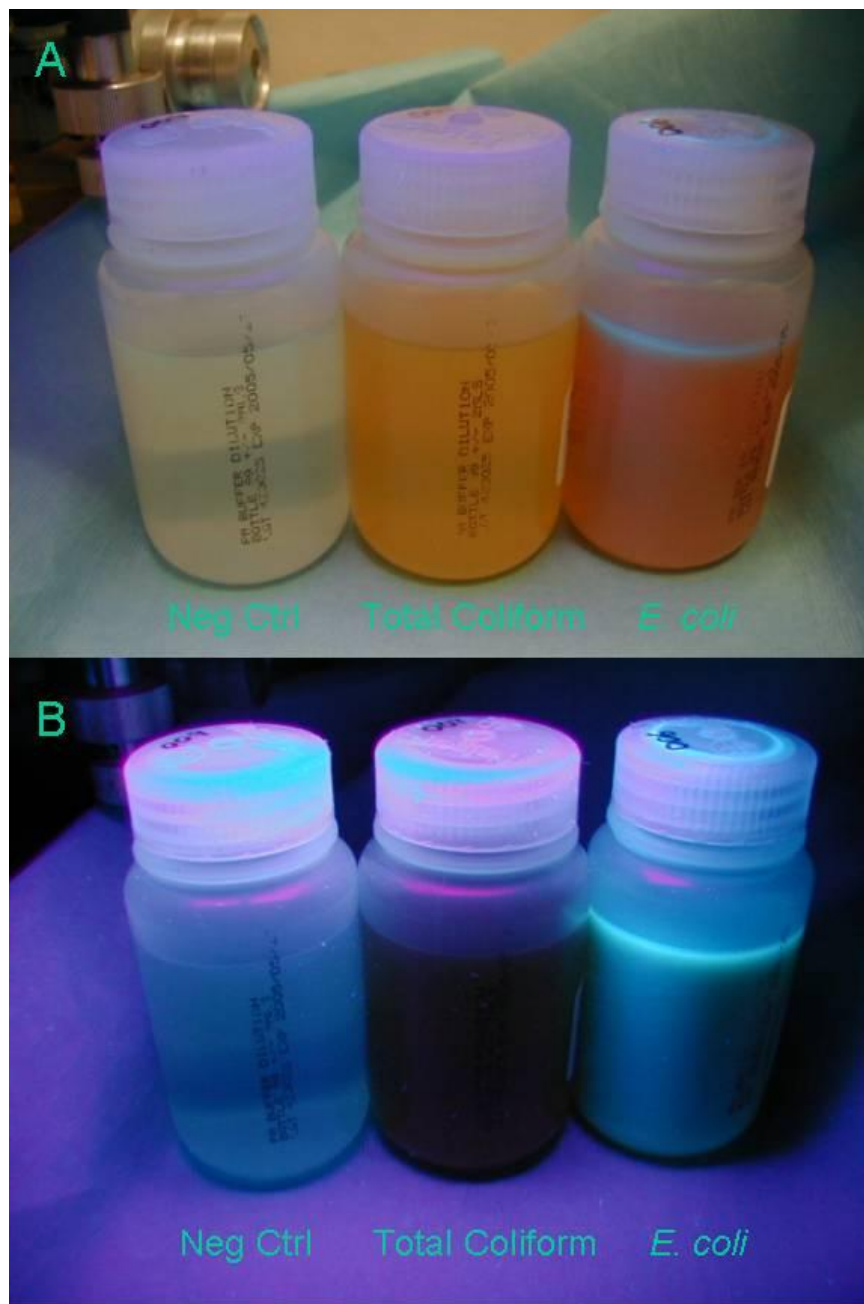


**TABLE 2. Interpretation of Colilert® Medium Quality Control Reactions**

Quality Control Organism	Yellow	Fluorescent
<i>Pseudomonas</i> sp. (Non-fluorescent strain)	No	No
<i>Klebsiella pneumoniae</i> , <i>Enterobacter aerogenes</i> or <i>Enterobacter cloacae</i>	Yes	No
<i>E. coli</i>	Yes	Yes

**TABLE 3. Interpretation of Colilert® Medium Reactions**

Reaction	Result
Yellow	Total Coliform Positive
Fluorescent	<i>E. coli</i> Positive



**FIGURE 1. Photographs of Colilert® Medium Reactions**

These photographs show the negative control (sterile reagent water), total coliform/*E. coli*, and total coliform/non-*E. coli* reactions on Colilert® medium, after 24 h incubation at  $35 \pm 0.5^\circ\text{C}$ . Under ambient light (Panel A), total coliform/non-*E. coli* and total coliform/*E. coli* bottles are yellow while the negative control is clear. Under UV light (Panel B), only the total coliform/*E. coli* bottle fluoresces.