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The current approved version of this procedure is located at **I:\COMONLAB\$ MethodSOPs (electronic)\WetChem_Micro**. *All ELS employees are responsible for ensuring they use only the current approved version of this procedure.*

1.0 Purpose

This document establishes the LCRA Environmental Laboratory Services Standard Operating Procedure for SM 9223B. The original method is presented here in its entirety, however, the sequence order and outline numbering system of the original method may differ from this ELS SOP. All ELS modifications, clarifications, additions, and detailed instructions are shown in gray shaded text boxes immediately after the pertinent original text.

2.0 Scope / Field of Application

Standard Methods for the Examination of Water and Wastewater
20th edition

9223 B. Enzyme Substrate Test

SM9223 A. Introduction

The enzyme substrate test utilizes hydrolyzable substrates for the simultaneous detection of total coliform bacteria and *Escherichia coli* enzymes. When the enzyme technique is used, the total coliform group is defined as all bacteria possessing the enzyme ß-D-galactosidase, which cleaves the chromogenic substrate, resulting in release of the chromogen. *Escherichia coli* are defined as bacteria giving a positive total coliform response and possessing the enzyme ß-glucuronidase, which cleaves a fluorogenic substrate, resulting in the release of the fluorogen. The test can be used in either a multiple-tube, multi-well, or a presence-absence (single 100-mL sample) format.

1. Principle

a. Total coliform bacteria: Chromogenic substrates, such as ortho-nitrophenyl-ß-D galactopyranoside (ONPG) or chlorophenol red-ß-D-galactopyranoside (CPRG), are used to detect the enzyme ß-D-galactosidase, which is produced by total coliform bacteria. The ß-D-galactosidase enzyme hydrolyzes the substrate and produces a color change, which indicates a positive test for total coliforms at 24 h (ONPG) or 28 h (CPRG) without additional procedures. Noncoliform bacteria, such as *Aeromonas* and *Pseudomonas* species, may produce small amounts of the enzyme ß-D-galactosidase, but are suppressed and generally will not produce a positive response within the incubation time unless more than 10^4 colony-forming units (CFU)/mL (10^6 CFU/100 mL) are present.

b. Escherichia coli: A fluorogenic substrate, such as 4-methylumbelliferyl-ß-D-glucuronide (MUG), is used to detect the enzyme ß-glucuronidase, which is produced by *E. coli*. The ß-glucuronidase enzyme hydrolyzes the substrate and produces a fluorescent product when viewed under long-wavelength (366-nm) ultraviolet (UV) light. The presence of fluorescence indicates a positive test for *E. coli*. Some strains of *Shigella* spp. also may produce a positive fluorescence response. Because *Shigella* spp. are overt human pathogens, this is not considered a detriment for testing the sanitary quality of water.

2. Applications

The enzyme substrate coliform test is recommended for the analysis of drinking and source water samples. Formulations also are available for the analysis of marine waters.

Water samples containing humic or other material may be colored. If there is background color, compare inoculated tubes to a control tube containing only water sample. In certain waters, high calcium salt content can cause precipitation but this should not affect the reaction.

Do not use the enzyme substrate test to verify presumptive coliform cultures or membrane filter colonies, because the substrate may be overloaded by the heavy inoculum of weak ß-D-galactosidase-producing noncoliforms, causing false-positive results.

COLILERT® from IDEXX (Catalog No. WP200 or equivalent)

Colilert Test Kit Introduction

Colilert simultaneously detects total coliforms and *E.coli* in water. It is based on IDEXX's patented Defined Substrate Technology® (DST®). When total coliforms metabolize Colilert's nutrient-indicator, ONPG, the sample turns yellow. When *E.coli* metabolize Colilert's nutrient-indicator, MUG, the sample fluoresces. Colilert can simultaneously detect these bacteria at 1CFU/100ml within 24 hours with as many as 2 million heterotrophic bacteria/100ml present.

Use COLILERT® for the analysis of drinking and fresh source water samples.

NOTE: Colilert® is not intended for marine water. For marine water use Colilert®-18, (IDEXX Catalog #WP200-18 or equivalent).

COLILERT®-18 from IDEXX (Catalog No. WP200-18 or equivalent)

Colilert-18 Test Kit Introduction

Colilert-18 simultaneously detects total coliforms and *E.coli* in water. It is based on IDEXX's patented Defined Substrate Technology® (DST®). When total coliforms metabolize Colilert-18's nutrient-indicator, ONPG, the sample turns yellow. When *E.coli* metabolize Colilert-18's nutrient-indicator, MUG, the sample fluoresces. Colilert-18 can simultaneously detect these bacteria at 1CFU/100ml within 18 hours with as many as 2 million heterotrophic bacteria/100ml present.

Use COLILERT®-18 for the analysis of drinking water, fresh source water and marine waters (*E.coli* only).

NOTE: Colilert®-18 can be used for *E.coli* (but not coliforms) in marine water.

COLISURE® from IDEXX (Catalog No. WCLS200 or equivalent)

Colisure Test Kit Introduction

Colisure simultaneously detects total coliforms and *E.coli* in water. It is based on IDEXX's patented Defined Substrate Technology® (DST®). When total coliforms metabolize Colisure's nutrient-indicator, CPRG, the sample turns from yellow to red/magenta. When *E.coli* metabolize Colisure's nutrient-indicator MUG, the sample fluoresces. Colisure can simultaneously detect these bacteria at 1CFU/100ml within 24 hours even with as many as 2 million heterotrophic bacteria per 100ml present.

Use COLISURE® for the analysis of drinking and fresh source water samples.

NOTE: Colisure® is not intended for marine water. For marine water use Colilert®-18, (IDEXX Catalog #WP200-18 or equivalent).

Definitions:

Laboratory Information Management System (LIMS) – Current version of Omega® by the Khemia Company.

3.0 Responsibilities

This method is restricted to use by or under the supervision of analyst trained and experienced with this method. Each analyst must be trained and able to read and understand the SOP.

3.1 Laboratory Analysts / Chemists - It is the responsibility of analyst/chemists to:

- 3.1.1 Read and understand this SOP and follow it as written.
- 3.1.2 Produce quality data that meets all laboratory and customer requirements.
- 3.1.3 Complete the required demonstration of proficiency before performing this procedure without supervision.
- 3.1.4 Enter laboratory sample and QC results into the LIMS data system for laboratory

supervisor review.

3.1.5 Repeat the required initial demonstration of laboratory capability each time a modification is made to the method.

3.2 Team Leader - It is the responsibility if the Team Leader to:

3.2.1 Ensure the all analyst/chemists have the technical ability and have received adequate training required to perform this procedure.

3.2.2 Ensure the all analyst/chemists have completed the required demonstration of proficiency before performing this procedure without supervision.

3.2.3 Review all data and QC for completeness, traceability and accuracy.

3.2.4 Produce quality data that meets all laboratory and customer requirements.

4.0 Sample Collection, Preservation and Storage

SM9060 B. Preservation and Storage

1. Holding Time and Temperature

a. General: Start microbiological analysis of water samples as soon as possible after collection to avoid unpredictable changes in the microbial population. For most accurate results, ice samples during transport to the laboratory, if they cannot be processed within 1 h after collection. If the results may be used in legal action, employ special means (rapid transport, express mail, courier service, etc.) to deliver the samples to the laboratory within the specified time limits and maintain chain of custody. Follow the guidelines and requirements given below for specific water types.

c. Nonpotable water for compliance purposes: Hold source water, stream pollution, recreational water, and wastewater samples below 10°C during a maximum transport time of 6 h. Refrigerate these samples upon receipt in the laboratory and process within 2 h. When transport conditions necessitate delays in delivery of samples longer than 6 h, consider using either field laboratory facilities located at the site of collection or delayed incubation procedures.

d. Other water types for noncompliance purposes: Hold samples below 10°C during transport and until time of analysis. Do not exceed 24 h holding time.

SDWA [EPA 40CFR141.21(f)(3)]: The time from sample collection to initiation of analysis may not exceed 30 hour. Systems are encouraged but not required to hold samples below 10 deg. C during transit.

5.0 Safety & Environmental

Review MSDS in LIMS for handling the following:

- **COLILERT® from IDEXX** (Catalog No. WP200 or equivalent)

- **COLILERT®-18 from IDEXX** (Catalog No. WP200-18 or equivalent)
- **COLISURE® from IDEXX** (Catalog No. WCLS200 or equivalent)
- **BD Bactrol™ Plus** *Escherichia Coli* ATCC (25922), Catalog #237912 or equivalent.
- **BD Bactrol™ Plus** *Klebsiella pneumoniae* ATCC (13883), Catalog #237905 or equivalent.
- **BD Bactrol™ Plus** *Pseudomonas Aeruginosa* ATCC (27853), Catalog # 237913 or equivalent.

PPE includes lab coat, safety glasses, impermeable closed toe shoes and NeoPro NONLATEX gloves or equivalent.

All waste produced by microbiology methods must be collected in an autoclaveable biohazard bag specifically designed and manufactured to contain such materials. Refer to SOP 5-3F Microbiological Waste Management.

Refer to SOP 5-4-1A Pollution Prevention & Waste Minimization.

Refer to SOP 5-3A Emergency Preparedness & Spill Response.

All ELS personnel must comply with the LCRA Transmission Services Environmental and Safety Management System (ESMS) which is available on the LCRA intranet.

6.0 Apparatus & Materials

6.1 BLUE M STABIL-THERM® DRY TYPE BACTERIOLOGICAL INCUBATOR (model # 200A) or equivalent.

6.2 Lindberg/Blue Circulating water bath (model # WB1110A) or equivalent.

6.3 Precision Circulating water bath (model # 51221038) or equivalent.

6.4 IDEXX 120ml sterile disposable vessels with Sodium Thiosulfate and 100ml fill line (catalog # WV120ST-200) or equivalent for sample collection.

6.5 IDEXX Quanti-Tray® Sealer (Model 2X or equivalent).

6.6 IDEXX Quanti-Tray®/2000 (Catalog No. WQT-2K or equivalent).

6.7 IDEXX Presence/Absence Comparator for Colilert and Colilert-18 (Catalog No. WP104 or equivalent).

6.8 IDEXX Quanti-Tray®/2000 Comparator for Colilert and Colilert-18 (Catalog No. WQT2KC or equivalent).

6.9 Spectroline 365nm Longwave Ultraviolet light (Model EA-140 or equivalent).

6.10 Spectroline Fluorescence Analysis Cabinet (Model CM-10 or equivalent).

6.11 EM Science Bromothymol Blue 0.04% Aqueous (Catalog No. BX1562-1 or equivalent).

6.12 NIST (National Institute of Standards and Technology) calibrated thermometers. Working range above 30°C and graduated in increments of 0.5°C or less.

7.0 Reagents & Standards

1. Substrate Media

Formulations are available commercially*#(1) in disposable tubes for the multiple-tube procedure, in disposable multi-wells†#(2) for the multi-well procedure, or in containers that will hold 100-mL samples for the presence-absence approach.* Appropriate preweighed portions of the reagent for mixing and dispensing into multiple tubes for 10-mL test portions or other containers for 100-mL samples also are available. The need for good quality assurance and uniformity requires the use of a commercial substrate medium. Avoid prolonged exposure of the substrate to direct sunlight. Store media according to directions and use before expiration date.

Discard discolored media.

7.1.1 COLILERT® from IDEXX (Catalog No. WP200 or equivalent). Store at 2-30°C away from light.

7.1.2 COLILERT®-18 from IDEXX (Catalog No. WP200-18 or equivalent). Store at 2-25°C away from light.

7.1.3 COLISURE® from IDEXX (Catalog No. WCLS200 or equivalent). Store at 2-25°C away from light.

8.0 Procedure & Analyst's Notes

2. Procedure

NOTE: Check for presence of chlorine on all samples submitted without chlorine levels provided. If chlorine detected or inadequate sample volume provided to perform the check, notate in the Case Narrative and contact the Project Manager.

a. Multiple-tube procedure: Select the appropriate number of tubes per sample with predispensed media for the multiple-tube test and label. Follow manufacturer's instructions for preparing serial dilutions for various formulations. Aseptically add 10 mL sample to each tube, cap tightly, and mix

vigorously to dissolve. The mixture remains colorless with ONPG-based tests and turns yellow with the CPRG format. Some particles may remain undissolved throughout the test; this will not affect test performance. Incubate at 35 ± 0.5°C for period specified by substrate manufacturer.

The procedure also can be performed by adding appropriate amounts of the substrate media to the sample, mixing thoroughly, and dispensing into five or ten sterile tubes. Incubate as stated for multiple-tube procedure.

ELS does not use the Multiple-tube procedure.

b. Multi-well procedure: The multi-well procedure is performed with sterilized disposable packets. Add sample to 100-mL container with substrate, shake vigorously, and pour into tray. The tray sealer dispenses the sample into the wells and seals the package. Incubate at 35 ± 0.5°C for period specified by substrate manufacturer. The MPN value is obtained from the table provided by the manufacturer.

Follow Quanti-Tray® Enumeration Procedure below.

c. Presence-absence procedure (P/A): Aseptically add preweighed enzymatic medium to 100-mL sample in a sterile, transparent, nonfluorescent borosilicate glass or equivalent bottle or container. Optionally, add 100-mL sample to the enzymatic substrate in a sterile container provided by the manufacturer. Aseptically cap and mix thoroughly to dissolve. Incubate as specified in manufacturer's instructions.

Colilert®, Colilert®-18 and Colisure® Presence/Absence (P/A) Procedure and Quanti-Tray® Enumeration Procedure

Note: Enumeration only valid for E.Coli

Record all required information in the following logbooks before continuing with the procedure:

COLILERT/COLILERT-18 TOTAL COLIFORM P/A Logbook

COLISURE TOTAL COLIFORM P/A Logbook

Colilert/Enterolert Quanti-Tray 2000 Logbook

1. Shake sample vigorously and decant to the 100ml fill line (±2.5 ml) if needed. **NOTE:** If the sample bottle does not contain 100ml ±2.5 ml, notify the Project Manager to request a replacement sample.
2. Carefully separate one Snap Pack from the strip taking care not to accidentally open adjacent pack.
3. Tap the Snap Pack to ensure that all of the Colilert, Colilert-18 or Colisure powder is in the bottom part of the pack.
4. Open one pack by snapping back the top at the scoreline. **Caution: Do not touch the opening of pack.**
5. Aseptically add contents of one pack to a 100 ml sample in a sterile, transparent, non-fluorescing vessel.
6. Cap vessel and shake until dissolved.

7. Sample is ready for the P/A Procedure. For the Enumertion Procedure, pour sample/reagent mixture into a Quanti-Tray or Quanti-Tray/2000 and seal in an IDEXX Quanti-Tray Sealer. **Caution: Do not touch the opening of the Trays.**
8. Place the vessel or sealed tray in a **35°± 0.5°C** incubator
9. For **Colilert®**, incubate at **35°± 0.5°C** for **24-28 hours**.
   For **Colilert®-18**, incubate at **35°± 0.5°C** for **18-22 hours**.
   For **Colisure®**, incubate at **35°± 0.5°C** for **24-48 hours**.
10. Read results according to the Result Interpretation table below. For the Enumeration Procedure, count the number of positive wells and refer to the IDEXX MPN table provided with the trays to obtain a Most Probable Number.
11. Document results in the required logbook.

Result Interpretation for Colilert and Colilert-18

Appearance	Result
Less yellow than the comparator$^{\Omega\dagger\dagger}$	Negative for total coliforms and *E.coli*
Yellow ≥ to the comparator$^{\Omega\dagger\dagger}$	Positive for total coliforms
Yellow and fluorescence ≥ to the comparator$^{\Omega\dagger\dagger}$	Positive for total coliforms and *E.coli*

$^{\Omega}$ IDEXX Presence/Absence Comparator for Colilert and Colilert-18 (Catalog No. WP104 or equivalent).

†† IDEXX Quanti-Tray®/2000 Comparator for Colilert and Colilert-18 (Catalog No. WQT2KC or equivalent).

- Look for fluorescence with a 6 watt, 365 nm, UV light within 5 inches of the sample, in a dark environment. Face light away from your eyes and towards the sample.
- **Colilert** results are definitive at **24-28 hours**. In addition, positives for both total coliforms and E. coli observed before **24 hours** and negatives observed after **28 hours** are also valid.
- **Colilert-18** results are definitive at **18-22 hours**. In addition, positives for both total coliforms and E. coli observed before **18 hours** and negatives observed after **22 hours** are also valid.
- **NOTE:** The Project Manager must be notified and a replacement sample requested for any samples that read positive after 28 hours for Colilert or 22 hours for Colilert 18.

Result Interpretation for Colisure

Appearance	Result
Yellow/gold	Negative for total coliforms and *E.coli*
Red or magenta	Positive for total coliforms
Red/magenta and fluorescence	Positive for total coliforms and *E.coli*

- Look for fluorescence with a 6 watt, 365 nm, UV light within 5 inches of the sample, in a dark environment. Face light away from your eyes and towards the sample.
- If the sample is pink or orange, incubate further (up to 48 hours maximum). A sample that remains pink or orange at 48 hours is negative for coliforms and E. coli.
- Samples are negative if at any time after 24 hours there is no red, magenta and/or fluorescence.
- Red/magenta or red/magenta with fluorescence observed before 24 hours is a valid positive.
- However, after 48 hours, heterotrophs may overwhelm Colisure's inhibition system. Therefore, red/magenta with fluorescence first observed after 48 hours is not a valid positive. **NOTE:** The Project Manager must be notified and a replacement sample requested for any samples that read positive after 48 hours.

Colilert Procedural Notes

- Check each lot of Colilert before use with 365-nm ultraviolet light with a 6-watt bulb for autofluorescence. If the media exhibits faint fluorescence, use another lot that does not fluoresce. Record results in LIMS under chemical inventory in the "Notes" section.
- If a water sample has some background color, compare inoculated Colilert sample to the IDEXX Quanti-Tray®/2000 Comparator for Colilert and Colilert-18 (Catalog No. WQT2KC or equivalent) before incubation. If the sample color is equal to or greater than the comparator, invalidate sample and discontinue testing. Notify Project Manager to inform customer.
- If sample dilutions are made, multiply the MPN value by the dilution factor to obtain the proper quantitative result
- Use only sterile, non-buffered, oxidant-free water for dilutions. Check each batch of dilution/rinse water for sterility by adding 20 mL of water to 20 mL of Typticase™ Soy Broth (BBL™ catalog #297811 or equivalent). Incubate at 35±0.5°C for 24 hours and check for growth. Record results in the Microbiology Monthly QC logbook. Discard if growth is detected.
- Colilert is a primary water test. Colilert performance characteristics do not apply to samples altered by any pre-enrichment of concentration.
- In samples with excessive chlorine, a blue flash may be seen when adding Colilert. If this is observed, invalidate sample and discontinue testing. Notify Project Manager to inform customer.
- Always follow aseptic techniques when using Colilert.
- In the event of a 35.0°C dry incubator malfunction, set the temperature on the 41.0°C dry incubator to 35.0°C and transfer samples accordingly.

Colilert-18 Procedural Notes

- Check each lot of Colilert-18 before use with 365-nm ultraviolet light with a 6-watt bulb for autofluorescence. If the media exhibits faint fluorescence, another lot that does not fluoresce will be used. Record results in LIMS under chemical inventory in the "Notes" section.

- A slight tinge may be observed when Colilert-18 is added to the sample. If the sample color is equal to or greater than the comparator, invalidate sample and discontinue testing. Notify Project Manager to inform customer.
- If a water sample has some background color, compare inoculated Colilert-18 sample to the IDEXX Quanti-Tray®/2000 Comparator for Colilert and Colilert-18 (Catalog No. WQT2KC or equivalent) before incubation. If the sample color is equal to or greater than the comparator, invalidate sample and discontinue testing. Notify Project Manager to inform customer.
- Colilert-18 can be used for *E.coli* (but not coliforms) in marine water.
- If sample dilutions are made, multiply the MPN value by the dilution factor to obtain the proper quantitative result
- Use only sterile, non-buffered, oxidant-free water for dilutions. Check each batch of dilution/rinse water by adding 20 mL of water to 20 mL of Typticase™ Soy Broth (BBL™ catalog #297811 or equivalent). Incubate at 35±0.5°C for 24 hours and check for growth. Record results in the Microbiology Monthly QC logbook. Discard if growth is detected.[11.3]
- Colilert-18 is a primary water test. Colilert-18 performance characteristics do not apply to samples altered by any pre-enrichment of concentration.
- In samples with excessive chlorine, a blue flash may be seen when adding Colilert-18. If this is observed, invalidate sample and discontinue testing. Notify Project Manager to inform customer.
- Always follow aseptic techniques when using Colilert-18.
- In the event of a 35.0°C dry incubator malfunction, set the temperature on the 41.0°C dry incubator to 35.0°C and transfer samples accordingly.

Colisure Procedural Notes

- Check each lot of Colisure before use with 365-nm ultraviolet light with a 6-watt bulb for autofluorescence. If the media exhibits faint fluorescence, another lot that does not fluoresce will be used. Record results in LIMS under chemical inventory in the “Notes” section.
- If sample dilutions are made, multiply the MPN value by the dilution factor to obtain the proper quantitative result
- Use only sterile, non-buffered, oxidant-free water for dilutions. Check each batch of dilution/rinse water for sterility by adding 20 mL of water to 20 mL of Typticase™ Soy Broth (BBL™ catalog #297811 or equivalent). Incubate at 35±0.5°C for 24 hours and check for growth. Record results in the Microbiology Monthly QC logbook. Discard if growth is detected.
- Colisure is a primary water test. Colilert performance characteristics do not apply to samples altered by any pre-enrichment of concentration.
- Always follow aseptic techniques when using Colisure.
- In the event of a 35.0°C dry incubator malfunction, set the temperature on the 41.0°C dry incubator to 35.0°C and transfer samples accordingly.

3. Interpretation

a. Total coliform bacteria: After the minimum proper incubation period, examine tubes or containers for the appropriate color change (Table 9223:I). ONPG is hydrolyzed by the bacterial enzyme to yield a yellow color. CPRG is hydrolyzed by the bacterial enzyme to yield a red or magenta color. If the color response is not uniform throughout the tube, mix by inversion before reading. Read manufacturer's instructions for interpretation guidelines. Some manufacturers suggest comparing sample tubes against a color comparator available through the manufacturer. Samples are negative for total coliforms if no color is observed in ONPG tests or if the tube is yellow when CPRG is used. If a chromogenic response is questionable after 18 or 24 h for ONPG, incubate up to an additional 4 h. If response is negative after 28 h for CPRG, incubate up to an additional 20 h. If the chromogen intensifies, the sample is total-coliform positive; if it does not, the sample is negative.

TABLE 9223:I. COLOR CHANGES FOR VARIOUS MEDIA

Substrate	Total Coliform Positive	*E. coli* Positive	Negative Result
ONPG-MUG	Yellow	Blue fluorescence	Colorless/no fluorescence
CPRG-MUG	Red or magenta	Blue fluorescence	Yellow/no fluorescence

See Colilert®, Colilert®-18 and Colisure® Presence/Absence (P/A) Procedure and Quanti-Tray® Enumeration Procedure above.

b. Escherichia coli: Examine positive total coliform tubes or containers for fluorescence using a long-wavelength (366-nm) ultraviolet lamp (preferably 6-W bulb). Compare each tube against the reference comparator available from a commercial source of the substrate. The presence of fluorescence is a positive test for *E. coli*. If fluorescence is questionable, incubate for an additional 4 h for ONPG tests and up to an additional 20 h for CPRG tests; intensified fluorescence is a positive test result.

See Colilert®, Colilert®-18 and Colisure® Presence/Absence (P/A) Procedure and Quanti-Tray® Enumeration Procedure above.

9.0 Quality Control

5. Quality Control

Test each lot of media purchased for performance by inoculation with three control bacteria: *Escherichia coli*, a total coliform other than *E. coli* (e.g., *Enterobacter cloacae*), and a noncoliform. Also add a sterile water control. If the sterile water control exhibits faint fluorescence or faint positive coliform result, discard and use a new batch of substrate. Avoid using a heavy inoculum. If

Pseudomonas is used as the representative noncoliform, select a nonfluorescent species. Incubate these controls at 35 ± 0.5°C as indicated above. Read and record results. Other quality-control guidelines are included in Section 9020.

Colilert, Colilert-18 and Colisure Quality Control Procedures

The following quality control procedure is recommended for each lot of Colilert, Colilert-18 and Colisure:

1. Inoculate 3 sterile vessels filled with 100 ml sterile water with the following and follow the Colilert®, Colilert®-18 and Colisure® Presence/Absence (P/A) Procedure above.

Use the following:

- **BD Bactrol™ Plus** *Escherichia Coli* ATCC (25922), Catalog #237912 or equivalent.
- **BD Bactrol™ Plus** *Klebsiella pneumoniae* ATCC (13883), Catalog #237905 or equivalent.
- **BD Bactrol™ Plus** *Pseudomonas Aeruginosa* ATCC (27853), Catalog # 237913 or equivalent.

2. Record in the Microbiology Monthly QC logbook if fluorescence is observed before incubation. Do not use the media lot if fluorescence is observed.
3. Results should match the Result Interpretation table above (see Colilert®, Colilert®-18 and Colisure® Presence/Absence (P/A) Procedure). If results do not match, invalidate the lot and do not use.

IDEXX Quanti-Tray® Sealer

- To determine adequate sealing, once a month, add a dye (e.g., Bromothymol Blue) to 100 mL of water and pour into a Quanti-Tray/2000.
- Seal as usual
- If dye is observed outside the wells, the sealer should not be used until the problem is resolved.
- Record results in the Microbiology Monthly QC logbook.

Bacteria bottle dechlorination check.

- Test 2 bottles per lot of IDEXX 120ml sterile disposable vessels containing Sodium Thiosulfate or equivalent.
- Add 100 mL of 15 mg/L chlorine to each bottle and shake.
- Analyze for chlorine residual and record in LIMS.
- Results should be absent for the bacteria bottles. If chlorine is present, invalidate the lot and do not use.

Long-Wave Ultraviolet Lamp

- Lamp must produce wavelenth of 365 nm.
- Test UV lamp for proper fluorescence monthly using a know E. coli positive MMO-MUG comparator. Record results in the Microbiology Monthly QC logbook.
- If lamp fails to produce the required fluorescence, replace the bulbs.

Comparison Counting

- For routine evaluation, repeat counts on one or more positive samples monthly and compare the counts with those of other analysts who perform the same analysis.
- Replicate counts between analysts should agree within 10%.
- Record results in the Microbiology Monthly QC logbook.
- If results disagree more than the prescribed limit, notify the lab supervisor

Additional QC

- For the Quanti-Tray® Enumeration Procedure, an analytical QC batch consists of 10 samples and a sample duplicate.

- All positive and negative culture controls information can be found in LIMS under [Analytical: Standards/Reagents or Analytical: Chemical Inventory].

- Field samplers check for the presence of residual chlorine in non-potable water locations during field sampling if it suspected there is a chlorinated source and notate on COC.

10.0 Calculations and Data Reporting

4. Reporting

If performing an MPN procedure, calculate the MPN value for total coliforms and *E. coli* from the number of positive tubes as described in Section 9221C. If using the presence-absence procedure, report results as total coliform and *E. coli* present or absent in 100-mL sample.

Read results according to Colilert®, Colilert®-18 and Colisure® Presence/Absence (P/A) Procedure and Quanti-Tray® Enumeration Procedure above.

Count the number of positive wells and refer to the MPN table provided with the IDEXX Quanti-Tray®/2000 trays to obtain a Most Probable Number.

Follow the Quantitray reporting rules below:
1. Use a tray that has between 30 and 80 positive wells (small + large) if available.

2. If 2 trays fall in the 30-80 range, use the average of the extended counts.
3. If no trays fall in the 30-80 range, use the average of the extended counts.
4. If both trays are >2420, use the higher dilution.
5. If both trays are < 1 use the lower dilution.
6. If one tray is <1 or >2420, use the other tray.
7. Round result to two significant figures.
8. Report results as MPN/100mL.

11.0 References

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11.2 IDEXX Colilert®-18 insert # 06-02027-11 © 2002 IDEXX Laboratories, Inc.

11.3 IDEXX Colisure® insert # 06-03553-03 © 2002 IDEXX Laboratories, Inc.

11.4 EPA Manual for the Certification of Laboratories Analyzing Drinking Water (Criteria and Procedures Quality Assurance, Fourth Edition, March 1997)

11.5 Laboratory Quality Assurance Guidance for Colilert®/Enterolert® Analysis Under the Clean Rivers Program[1] – Revised 10/4/01 (from Section 9020 B of Standard Methods for the Examination of Water and Wastewater, 20th ed.)*

11.6 SOP 5-3F Microbiological Waste Management.

11.7 SOP 5-4-1A Pollution Prevention & Waste Minimization.

11.8 SOP 5-3A Emergency Preparedness & Spill Response.

Endnotes

*#(1) Colilert® and Colilert 18® for multi-tube, P/A, and tray formats, Colilert MW® for multi-tube
format, and ColisureTM for multi-tube and P/A formats, available from IDEXX Laboratories, Inc., Westbrook, ME.

†#2 Quanti-Tray® or Quanti-Tray®/2000, available from IDEXX Laboratories, Inc., Westbrook, ME.

*Colilert and Colilert-MW, Idexx Laboratories, Inc., Westbrook, Maine, or demonstrably equivalent product.

12.0 Appendices

None

13.0 Revision History

Revision 1 – Update to Standard Methods 20th Edition. Adds Colisure procedure.

Revision 2 - Updates SOP references. Section 8.0 – Adds residual chlorine check and documentation with qualification of data not meeting specifications. Section 9.0 - Adds bacteria sample bottle dechlorination verification and documentation.