SOP-C-124a

Determination of Presence/Absence of Total Coliform and *Escherichia coli* by IDEXX Colilert®

Major Revision
Changes not indicated

Revision 19

Approval:

_____ _____ 8.23.23

Laboratory Manager Date

_____ _____ 8/23/2023

Concurrence Date

Effective date: 8-31-23

Renewal date: _____ Initials: _____

i. Identification of the method
a. IDEXX Colilert® (2022)
b. SM 9223 and 9223B (approved 2022)

ii. Applicable matrix or matrices
a. Drinking water

iii. Limits of detection and quantitation
a. Presence or absence

iv. Scope and application, including parameters to be analyzed
a. Total coliform, *Escherichia coli* (*E. coli*) in drinking water

v. Summary of the method
a. Bacteria are reacted with a prepared, specific reagent and incubated to allow bacteria to metabolize the chromogenic substrate reagent. Fluorescent and color changes indicate presence of the bacteria. Color change from clear to yellow indicate coliform bacteria, while fluorescence of the yellow under a UV light indicates presence of *E. coli*.

vi. Definitions
a. ATCC: American Type Culture Collection (1-800-638-6597) sets the industry standard identification for microorganisms.
b. Refer to QAM-Q-101, "Laboratory Quality Control," for standard QC definitions.
c. P/A= presence/absence coliform bacteria or *E. coli*

vii. Interferences
a. Interferences: more than 2 million heterotrophic bacteria per 100 mL
b. Sample color interference may be corrected for by comparison to an untreated sample
c. Noncoliform bacteria, such as *Aeromonas, Flavobacterium,* and *Pseudomonas* species, may produce small amounts of the enzyme, 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.
d. Some strains of *Shigella* and *Salmonella* spp. also may produce a positive fluorescence response.

viii. Safety

a. All aspects of this procedure comply with QAM-S-101 “Laboratory Safety”.
b. Coliform bacteria are indicators of the potential presence of pathogens; therefore, avoid all direct skin contact with sample.
c. All work areas are to be disinfected upon completion of work with a 10% bleach solution or 70% Isopropyl Alcohol.
d. Isopropyl and Ethyl alcohol are flammable. Analysts will take care to avoid igniting the alcohol when disinfecting the biosafety cabinet or other surfaces.
e. Personal protection equipment such as gloves and lab coats should be used when working with samples.

ix. Equipment and supplies

a. Autoclave: A sterilization chamber capable of 121˚C equipped with a pressure gauge and an emergency pressure relief valve. Used for sterilization of applicable equipment. The autoclave is only used for sterilization of waste prior to disposal.
b. Sample bottles: 100 or 250 mL HDPE bottles or equivalent that have been certified sterile by the manufacturer and contain sodium thiosulfate for chlorine removal. Bottles are labeled with an “S” to identify the TIAER container type. The lot number of each sample bottle is recorded on the COC when samples are received.
c. Incubator: A device with a chamber able to hold a constant temperature of 35˚C±0.5ºC.
d. IDEXX P/A comparator: Used to compare with incubated samples to determine presence or absence. The ID and expiration date of this comparator are recorded in each run.
e. UV lamp, long wave (365-366 nm)
f. 10% Bleach: Dilute a household bleach 1:10 in a spray bottle. Prepare fresh weekly.
g. Isopropyl alcohol (also Isopropanol)
h. Biosafety cabinet. See QAM-I-121, “Operation and Calibration of the Biological Safety Cabinet.”

x. Reagents and standards

a. Tryptic soy broth (TSB) or agar (TSA): a non-selective media, prepared in the lab or purchased pre-sterilized and ready to use
b. Sterile deionized water: Type II ASTM water purchased and certified sterile by the manufacturer. The vendor also supplies a certificate of analysis for chlorine residual, metals, ammonia/organic nitrogen, organic carbon and heterotrophic plate count. This water is stored away from incubators and sample prep area to avoid potential contamination.
c. Reagent snap-packs: Colilert® pre-formulated, buffer reagent pillows, or equivalent. Each batch is tested for positive and negative controls as described below. Store Colilert® in the dark.
d. Standards: Commercial standards are available for bacteria that represent positive and negative controls. Examples are "Quanti-Cult®" from IDEXX stable cultures of *Escherichia coli* (positive for coliform and *E. coli*- ATCC #25922 or 11775), *Pseudomonas aeruginosa* (negative for coliform and *E. coli*-ATCC #10145 or 27853) and *Klebsiella pneumoniae* (positive for coliform, but negative for *E. coli*-ATCC #31488).

xi. Sample collection, preservation, shipment and storage

a. The TIAER Lab does not collect samples.
b. Samples should be preserved on ice for shipment or delivery to the lab if more than 1 hour transpires from time of collection.
c. Samples can be held at room temperature between receipt and analysis.
d. Holding time is 30 hours total. Samples should be analyzed within 2 hours of receipt by lab.

xii. Quality control

a. In samples with excessive chlorine, a blue flash may be seen when adding Colilert. If this is seen, consider sample invalid and discontinue testing.
b. If a water sample has some background color, compare inoculated Colilert sample to a control blank of the same water sample.

c. Sample bottles are checked for sterility with non-selective growth media before first use. Record results in the Equipment Prep Log.
d. Sample bottles are checked for auto-fluorescence before first use. Record equipment used and results of test. Record results in the Equipment Prep Log.
e. Sample bottles are tested for volume before first use. Bottles are verified with Class A glassware. Record all raw data associated with volume checks. Record results in the Equipment Prep Log.
f. Media is checked for sterility, specificity and pH before first use. Record results in the Equipment Prep Log.
g. Media is stored separately from samples, as are other reagents and standards
h. Air quality is tested monthly with non-selective growth media (TSA). A settling plate is placed open on the workspace where sample analysis takes place for 15 minutes. The plate is incubated at 35˚C±0.5ºC for 48 hours. There can be no more than 15 CFUs (no more than 1 CFU/minute). Record all raw data associated with the Air Quality Test. Log air test in the Maintenance Logbook.
i. Replace the bulb in the UV lamp annually. Verify the intensity of the lamp at least semi-annually with a calibrated light meter. If intensity drops by more than 20% in a 6-month period, replace the lamp. Record results in logbook.
j. All microbiology incubators will have the temperature of at least the top and bottom shelves recorded at least twice a day at least 4 hours apart on each day that samples are analyzed.
k. Initial DOC must be 4 aliquots, prepared and analyzed according to the method, either concurrently or over a period of days.

xiii. Calibration and standardization

a. None

xiv. Procedure

a. Disinfect the work surface with 10% bleach for 15 minutes or 70% Isopropyl alcohol for 5 minutes immediately before beginning analysis.
b. Ensure that the incubator temperature is at the proper level for the test being performed (35˚C±0.5ºC) prior to use.
c. Ensure temperature distribution in incubator has been established. Record the logbook number and page of the distribution confirmation on the incubator, and transfer this information to each subsequent recording of data for this test.
d. If the sample has at least 1 inch (2.5 cm) of headspace, homogenize the sample by shaking 25 times each time the sample is opened. If the sample does not have adequate headspace, pour the entire sample volume into a sterile container large enough to ensure adequate mixing. Homogenize the sample in this container and aseptically transfer appropriate volumes for analysis. For drinking water testing, transfer exactly 100 mL to the original container for analysis.
e. Screen all water samples for chlorine presence in accordance with SOP-C-121, “Determination of Chlorine”. Sodium thiosulfate has normally been added prior to sample collection. If chlorine is still detectable, the sample will be discarded and the client notified that resampling should be done. For samples with only 100 mL available and with no dilutions, testing for chlorine may not be possible. Notify the client for such an occurrence as the client may want to resample.
f. A method blank of sterile water is used for a negative control for each batch of 20 samples or less. Transfer to a 100 mL sample bottle for analysis.
g. Duplicates are not required for the Presence/Absence procedure.
h. Aseptically remove water from the sample down to 100 mL.
i. Add the contents of the Colilert® snap-pack buffer to the sterile mixing bottle, and shake until dissolved. Ensure

that the lot of buffer been tested for positive and negative controls as described below. For each subsequent record of test data, record the logbook number and page where the passing controls can be found along with the lot number.

j. Incubate the filled sample bottle for 24-28 (maximum 28) hours at 35ºC±0.5ºC. Incubators may have preheated water in them to be used as heat sinks to prevent rapid temperature changes.
k. Repeat preparation steps for remaining samples.
l. Colilert®: After the incubation period observe the color of the sample. Samples that are as yellow or more yellow than the IDEXX P/A comparator are positive for Total Coliforms.
m. Samples that fluoresce blueish-white under a 6-watt, 365nm, UV light within 5 inches of the sample, in a dark environment are positive for *E. coli*.
n. If the chromogenic response is questionable, the incubation period may be extended for up to another 4 hours, but never exceed 28 hours maximum. The color comparator is used as needed.
o. For P/A testing, record as “Present” or “Absent” for total coliform or *E. coli*.
p. Enter values and quality control information into the QC module of ESDMS or LIMS.
q. Proceed as done with water samples.
r. Disinfect the work surface with 10% bleach for 15 minutes or 70% isopropyl alcohol for 5 minutes immediately after analysis.

xv. Data analysis and calculations

a. NA

xvi. Method performance

a. Method performance, data assessment and acceptance, corrective action: refer to QAM-Q-101, "Laboratory Quality Control"

xvii. Pollution prevention

a. Waste management and pollution prevention: refer to QAM-W-101, "Disposal of Laboratory Waste"

b. Negative samples are disposed down the drain. Positive samples are autoclaved before disposal. Lab Manager or designee initials for disposal on the reporting form.

xviii. Data assessment and acceptance criteria for quality control measures

a. Data assessment and acceptance comply with QAM-Q-101, "Laboratory Quality Control".
b. Positive and negative controls are analyzed with each new lot of snap-buffer pillows.
c. Prepare the bacteria cultures according to the manufacturer's instructions.
d. Analyze each of the three bacteria types in the same manner as a regular sample.
e. The positive and negative controls are counted as follows:
    i. Colilert®- *Escherichia coli* count positive for both yellow and fluoresced wells.
    ii. Colilert®-*Pseudomonas aeruginosa* count negative for both yellow and fluoresced wells.
    iii. Colilert®-*Klebsiella pneumoniae* count positive for yellow wells, but negative for fluoresced wells.
f. Each lot number of sterile pipettes, bottles, and dilution water are tested for sterility once per lot. Test sterile water with an equal volume of double-strength tryptic soy broth. Test supplies by pouring dilution water through each piece of equipment and into double-strength tryptic soy broth media of an equal volume. The broth with sample is incubated at 35ºC±0.5ºC for 48 hours. A change in color or clarity of the broth indicates presence of nonspecific bacteria that requires corrective action.
g. In the CAR 08-0233 file is a study done by an outside consultant lab showing that airborne contamination may be caused in the TSB by *Bacillis sphaericus.* This bacterium is not of interest in any of the methods tested for by TIAER Lab. However, all incidences of sterility check failures are documented by a CAR.
h. Record all reagents prepared in the Reagent Log. Record preparation of standards (controls) in the Standards Log.

i. No washable labware are used for this procedure. All supplies and sterile water for dilutions are purchased pre-sterilized.
j. Positives observed before 24 hours and negatives observed after 28 hours are valid readings.
k. For each lot number used, maintain copies of all manufacturers' certificates for sterility checks of containers and water in non-selective media, analysis of the water meeting ASTM Type II specifications for metals and heterotrophic bacteria. These records are maintained for a minimum of five years after the end of a project for which these data are collected.
l. Verify the volume mark of each lot of dilution water bottles, sample collection bottles and disposable pipettes by testing one bottle from each lot. Record the logbook and page number of verification on the box containing the lot confirmed for volume. On each subsequent recording of data, list the confirmation logbook number and page for that lot used.
m. Avoid prolonged exposure of the substrate media to direct sunlight. Discard colored media.

xix. Corrective actions for out-of-control data

a. All aspects of this procedure comply with QAM-Q-101, "Quality Control" and QAM-Q-105 "Corrective Action".

xx. Contingencies for handling out-of-control or unacceptable data

a. All aspects of this procedure comply with QAM-Q-101, "Quality Control".
b. If holding time has expired and data is not acceptable, resampling is the next option, if practical, as decided by the Program Manager.
c. See Table 1 for rejection codes.

xxi. Waste management

a. Waste management and pollution prevention: refer to QAM-W-101, "Disposal of Laboratory Waste".
b. Used supplies and other bacterial contaminated material are sterilized by autoclave prior to disposal and recorded in the Autoclave Log.

c. See QAM-I-110, “Operation and Calibration of the Autoclave.”

xxii. References

a. Colilert®, publication 06-12999-10, IDEXX Laboratories, Inc., 2022.
b. Quanti-Cult® Procedure, publication 06-01964-06, IDEXX Laboratories, Inc. (2013)
c. Standard Methods for the Examination of Water and Wastewater, (approved 2022), Methods 9223 & 9223B. QA/QC under SM 9020 (approved 2022).
d. National Environmental Laboratory Accreditation Conference (TNI, The NELAP Institute) standard, 2016.
e. CAR 08-0233, airborne contamination study
f. Appendix J, Uncertainty, FDA Screening and Testing Group, 2006.

xxiii. Any tables, diagrams, flowcharts and validation data

a. Drinking Water Rejection Codes, Table 1
b. Signage to inform clients of headspace requirements, Diagram 1
c. Example Drinking Water Worksheet, Attachment 1

Table 1. Drinking Water Rejection Codes

Code	Description
BR	Broken in transit
CL	Chlorine present (in sample)
EH	Exceeded hold time
EV	Excessive volume
FZ	Frozen Sample
HB	Heavy bacterial growth
ST	Heavy silt or turbidity present
IN	Insufficient sample information
BP	Invalid sampling point
IP	Invalid sampling protocol
LA	Lab accident
LR	Lab rejected
LT	Leaked in transit
NC	No field-measured chlorine residual (on form)
VO	Volume insufficient

Diagram 1: Signage to inform clients of headspace requirements

Attachment 1: Example Drinking Water Worksheet

Drinking water sample log			SOP-C-124			Reviewed and Approved 8/4/22 jrh								Total col.	E. coli	Total Coliform			E. coli		
Date	Analyst	Sample #	DPD ID	Cl2 check +or-	Reagent ID	Other/ comments	Incubator ID	Time in	Time out before 4 hrs	Date out	Time out	Analyst	Comparator ID	P/A Results + or -	P/A Results + or -	Large wells	Small Wells	MPN/ 100 mL	Large wells	Small Wells	MPN/ 100 mL
																na	na	na	na	na	na
																na	na	na	na	na	na
																na	na	na	na	na	na
																na	na	na	na	na	na
																na	na	na	na	na	na
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