GUAM ENVIRONMENTAL PROTECTION AGENCY
Environmental Monitoring and Analytical Services

STANDARD OPERATING PROCEDURE

Total Coliform and E. coli in Aggregates by the Enzyme Substrate Method (Colilert ®/Colilert-18 ®)

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GEPA Administrator — 10.14.2025 Date

Periodic Review:

Signature	Title	Date

Revision Page

Date	Section	Summary of Changes	Initials
		Added "Samples must be stored in a cooler at <10°C with ice packs (blue ice) during transit to the laboratory." "Once received by the laboratory, samples should be stored at 4°C up to the time of analysis." "Samples must be analyzed within 72 hours from the date of collection."	
7/25/25	6.3 - 6.5		AC
08/04/25	10.2	Added "Presence is indicated as DET (detected), and absence as ND (non-detected) in the LIMS."	AC
08/07/25	10.1	Added "Project Name/Contractor," and "g. Notes (including condition of sample: wet or dry, and scooped or chipped"	AC

CONTENTS

1 SCOPE AND APPLICATION

1.1 Colilert®/Colilert-18® is used for the simultaneous detection and confirmation of total coliforms and *E. coli*. It is an EPA approved method listed on SM 9223 in the Standard Methods.

1.2 Imported sand/rock aggregates are tested for the presence of total coliforms and *E. coli* using the fluorogenic/chromogenic enzyme substrate method Colilert®/Colilert-18® with results in 24 hours or less. Sand/rock aggregate samples, if not contaminated, should not test positive for the presence of total coliforms and *E. coli*.

2 METHOD PRINCIPLE

2.1 The Colilert®/Colilert-18® test is based on the ability of coliforms to produce the enzyme β-galactosidase which cleaves the media substrate o-nitrophenyl-β-d-galactopyranoside (ONPG) producing a color from the release of o-nitrophenyl. In addition, the enzyme β-glucoronidase produced by *E. coli*, a fecal coliform, forms a fluorescent substance when it hydrolyses 4-methylumbelliferyl-β-d-glucoronide (MUG). This combination of substrates allows detection of both coliforms and *E. coli* within 24 hours. The Method Detection Limit (MDL) is 1 CFU/100 mL with as many as 2 million heterotrophic bacteria/100 mL present.

2.2 Colilert®/Colilert-18® is a primary water test. The test method is modified for the analysis of coliforms in aggregates. In this modified method, an amount of sand/rock aggregate sample is first rinsed with an amount of sterilized water to capture any of the target organisms. The rinsate is then tested for the presence of total coliform/*E. coli* using the Colilert ®/Colilert-18 ® method.

3 INTERFERENCES

Residual chlorine in the water sample may cause a false negative. To avoid this, sample bottles with sodium thiosulfate are used. The amount of thiosulfate should be sufficient to dechlorinate the sample.

4 DEFINITIONS

4.1 Total Coliforms - coliforms are defined as rod-shaped Gram-negative organisms which ferment lactose with the production of acid and gas when incubated at 35 °C. Coliforms are abundant in the feces of warm-blooded animals, but can also be

found in the aquatic environment, in soil, and on vegetation. In most instances, coliforms themselves are not the cause of sickness, but they are easy to culture and their presence is used to indicate that other pathogenic organisms of fecal origin may be present. Because of this they are commonly used as a bacterial

4.2 *Escherichia coli* (*E. coli*) - is a Gram-negative, facultatively anaerobic, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded animals. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination. The harmless strains are part of the normal flora of the gut, and can benefit their host by producing Vitamin K and preventing colonization of the intestine with pathogenic bacteria. *E. coli* is expelled into the environment within fecal matter. Fecal-oral transmission is the major route through which pathogenic strains of the bacteria cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for fecal contamination. *E. coli*, a member of the coliform group, is the organism that is used as a positive control in the Colilert ®/Colilert-18 ® test method.

4.3 *Klebsiella pneumoniae* - will produce a positive total coliform result but does not produce the enzyme β-glucoronidase, thus will not fluoresce when expose to a 365 nm UV light in a dark environment. They are normally found in the soil but may also come from the mouth, skin, or intestines of animals. This is the control organism that is used in the Colilert®/Colilert-18® test as a positive for total coliform but negative for *E. coli*.

4.4 *Enterobacter aerogenes* - is a gram-negative rod-shaped microorganism from the Enterobacteriaceae family, and forms part of the endogenous human gastrointestinal (GI) microflora. It also resides in soil, water, and dairy products. It is commonly responsible for infections in hospitals. This is also the control organism that is used in the Colilert®/Colilert-18®test as a positive for total coliform but negative for *E. coli*.

4.5 *Pseudomonas aeruginosa* - is a non-coliform organism. That is the organism used in the Colilert®/Colilert-18®test as a negative control for total coliform and *E. coli*.

4.6 Comparator - is a solution prepared by IDEXX used to show the lightest shade of yellow color that will confirm a positive for coliform. It will also show the minimum fluorescence for a positive *E. coli* result.

4.7 Colilert® /Colilert-18® - is a product of IDEXX laboratories, Inc. (800-321-0207). The Colilert®/Colilert-18®test is also referred to as an ONPG/MUG, or chromogenic/fluorogenic enzyme substrate test. The test is discussed in Standard

Methods under method number SM 9223. A positive for total coliform result will result in an about the same or darker yellow colored solution when compared with the comparator. A positive *E. coli* result will also be fluorescing when exposed to 6-watt, 365-nm UV light in a dark environment.

5 HEALTH AND SAFETY

5.1 Microbiological analyses involve the culturing of potentially pathogenic organisms. Gloves, lab coats and safety/UV glasses should be worn when handling samples, culturing media and equipment. All biologically contaminated materials in the laboratory, particularly media with growth, must be autoclaved prior to disposal. Contaminated media must never be discarded prior to autoclaving. Laboratory equipment and benches should be disinfected daily.

5.2 All laboratory acquired infections must be reported to the EMAS administrator, as must all accidents which may cause infection such as; accidental inoculation with syringes or needles, accidental oral aspiration of infectious material through a pipette, and spilling or spattering of pathogenic cultures on floors, table tops and other surfaces. A 6-watt ultraviolet light is used to detect fluorescence for *E. coli* or enterococci. Care should be taken not to look directly at the light. It should always be pointed away from the analyst during reading.

6 SAMPLE COLLECTION, HANDLING & PRESERVATION

6.1 Samples for microbiological analysis should be collected using aseptic sampling procedures. The sample collectors should be trained in performing this technique.

6.2 The amount of aggregate taken for testing should be more than what is required for analysis. Samplers should take more than ten grams of aggregate.

6.3 Samples must be stored in a cooler at <10°C with ice packs (blue ice) during transit to the laboratory.

6.4 Once received by the laboratory, samples should be stored at 4°C up to the time of analysis.

6.5 Samples must be analyzed within 72 hours from the date of collection.

7 APPARATUS AND MATERIALS

7.1 Colilert ® / Colilert -18 ® dry media in "Snap-Packs," stored in the dark at 4-30°C

7.2 Sterile sample containers (i.e., IDEXX 120 mL vessels with sodium thiosulfate; WHIRL-PAK)

7.3 Water bath incubator at 44.5 ± 0.2°C

7.4 Incubator at 35 ± 0.5°C

7.5 6 watt, 365 nm UV lamp

7.6 Colilert ® color comparator

7.7 Sterilized beakers

7.8 Sterilized spatulas

7.9 Weighing balance

7.10 Sterile water

8 QUALITY CONTROL

8.1 A sterile laboratory blank should be run with each day's samples

8.2 Positive and negative controls should be run on each new lot of Colilert®/Colilert-18®. The expected results for various types of bacteria are as follows:

Organisms	Expected Results
E. coli (fecal coliform)	Yellow, fluorescent
Coliform, fecal (e.g. *Klebsiella pneumoniae*)	Yellow, non fluorescent
Coliform, non-fecal (e.g. *Enterobacter aerogenes*)	Yellow, non fluorescent
Non-coliform (e.g. *Pseudomonas aeruginosa*)	Clear, non-fluorescent

8.3 Refer to GEPA Lab QA Manual (QA-01-01) for detailed QC checks for microbiology analytical methods.

9 ANALYTICAL PROCEDURES

9.1 Presence/Absence Test Procedure

9.1.1 Weigh about 10 grams of the sand/rock aggregate in a sterile container (i.e. IDEXX bottle or sterilized beaker).

9.1.2 Add 10 mL of sterilized water, as a rinse, into the container holding the sample and swirl/shake vigorously 25 times followed by the settling of the mixture for at least two minutes.

9.1.3 Decant about 10 mL of the rinsate (in Section 9.1.2) into a new IDEXX bottle. Avoid adding aggregate into the new IDEXX bottle.

9.1.4 Add sterilized water up to the 100 mL mark in the new IDEXX bottle containing the rinsate (in section 9.1.3).

9.1.5 Carefully separate one Colilert ® or Colilert-18 ® snap pack from the strip taking care not to accidentally open adjacent packs.

9.1.6 Tap the snap pack to ensure all of the powder is in the bottom of the pack.

9.1.7 Open the pack being careful not to touch the opening of the pack.

9.1.8 Aseptically transfer the powder from the pack to the IDEXX bottle containing the rinsate and sterilized water (in Section 9.1.4).

9.1.9 Aseptically cap the bottle and sake until powder is dissolved.

9.1.10 When using the Colilert-18®, pre-warm the sample in 44.5 ± 0.2°C water bath for 7-10 minutes before incubating in the 35 ± 0.5°C air-type incubator.

9.1.11 For the regular Colilert®, incubate directly in the incubator.

9.1.12 Incubate in an incubator for the specified amount of time (Colilert ® - incubate for 24 hours and Colilert-18 ® - incubate for 18 hours). The temperature of the incubator should be 35 ± 0.5°C.

9.1.13 After incubation, read the results by comparing the results against the solution in a Colilert®/Colilert-18® comparator. The container of the comparator should be identical to the sample bottle utilized.

9.1.14 Interpret the results as follows:

Results	Interpretation
Clear solution or no yellow color	Test is negative for coliform
Yellow color is equal to or greater than comparator	Test is positive for coliform

Yellow color is less than comparator	Incubate sample for an additional 4 hours when color intensifies, test is positive. If not, the test is negative.

9.1.13 All total coliform-positive samples must be placed under an ultraviolet lamp (365-366 nm, 6-watt) within 5 inches in a darkened area. If *E. coli* is present, the medium will emit a blue fluorescence.

Use UV safety glasses for protection of your eyes.

9.2 **Procedural Notes**

9.2.1 If an inoculated Colilert® sample is inadvertently incubated more than 28 hours, and or Colilert-18® more than 22 hours, the following guidelines apply:
Lack of color is a valid negative test. A yellow color after 28 hours for Colilert® samples or a yellow color after 22 hours for Colilert-18® sample is not valid and should be repeated or verified.

9.2.2 Colilert®/Colilert-18® is already buffered and does not require the use of buffered water for dilutions.

9.2.3 Always add the Colilert®/Colilert-18® media to the proper volume of dilution water before adding the water sample aliquot.

10 DATA ACQUISITION, REDUCTION, AND DOCUMENTATION

10.1 When samples are received in the laboratory, the laboratory personnel verify that the Chain of Custody Form (Appendix A) is properly filled out. The Chain of Custody Form should contain the following information:

a. Project name/Contractor
b. Sampler's name and signature
c. Date and time of sample collection
d. Field sample ID
e. Source of sample (including name, location and sample type)
f. Analyses required
g. Notes (including condition of sample: wet or dry, and scooped or chipped)

The person who delivers the samples may relinquish custody and laboratory personnel may then receive and sign the Chain of Custody Form.

10.2 The results for presence/absence of total coliform and *E. coli* are determined according to the procedures above. A yellow color is confirmed positive for total coliform and both yellow and fluorescence is positive for *E. coli*. Results are

recorded in logbooks and in the GEPA Laboratory Information Management System (LIMS). Positive results are recorded with a plus sign (+)and negative results with a negative sign (-) in the Analytical Results Logbook (GEPA Log: MB-02-03), Appendix B; and presence as DET (detected) / absence as ND (non-detected) in the LIMS. An analytical results report is generated from the LIMS (Appendix C).

10.3 Laboratory data reports for Colilert®/Colilert-18® results will include presence/absence for total coliforms and *E. coli* for each sample. Results for all QC samples will also similarly be reported.

10.4 Daily incubator temperature readings are recorded in the Incubator # 2 Daily Temperature Log (Water Bath: 44.5 ± 0.2°C), GEPA Log: QC-02-02 (Appendix D) if using Colilert-18 ®, and in the Incubator # 3 Daily Temperature Log (Dry Incubator: 35.0 ± 0.5°C), GEPA Log: QC-02-03 (Appendix E) if using Colilert ®. Temperature readings are taken two times a day, four hours apart between readings.

11 REFERENCES

11.1 American Public Health Association, *Standard Methods for the Examination of Water and Wastewater*, 20th edition, 1998.

11.2 IDEXX, *"Colilert® " Product Instructions*, Number 06-12999-08, 2017.

11.3 IDEXX, *"Colilert -18® " Product Instructions*, Number 06-02027-24, 2017.

11.4 U.S. Environmental Protection Agency, *Microbial Methods for Monitoring the Environment*, EPA-600/8-78-017, December 1978.

11.5 U.S. Environmental Protection Agency, *National Primary Drinking Water Regulations*, 40 CFR Part 141, "Analytical Methods for Drinking Water Contaminants", 1994.

11.6 U.S. Environmental Protection Agency, *Manual for the Certification of Laboratories Analyzing Drinking Water*, 5th , EPA 815-R-05-004, January 2005.

Appendix A

Chain of Custody Form

GUAM ENVIRONMENTAL PROTECTION AGENCY

CHAIN OF CUSTODY RECORD

PROJECT NAME

REQUESTED

ASSIGNED LAB ID #

SDG #

SAMPLER PRINT/SIGN

SAMPLER PRINT/SIGN

FIELD SAMPLE ID	DATE	TIME	COMPOSITE	GRAB	SAMPLE LOCATION	CONTAINER QUANTITY				NOTES

RELINQUISHED BY: PRINT	SIGNATURE	DATE	TIME	RECEIVED BY: PRINT	SIGNATURE	DATE	TIME
RELINQUISHED BY: PRINT	SIGNATURE	DATE	TIME	RECEIVED BY: PRINT	SIGNATURE	DATE	TIME
RELINQUISHED BY: PRINT	SIGNATURE	DATE	TIME	RECEIVED FOR LAB BY: PRINT	SIGNATURE	DATE	TIME

REMARKS

Appendix B

Analytical Results Logbook

(GEPA Log: MB-02-01)

Guam EPA Laboratory, Analytical Results Logbook

GEPA Log: MB-02-03

Total Coliform and E. coli in Aggregate by Chromogenic Substrate (Colilert ___), SM 9223

SDG: _____ Analyst: _____

Lab ID (Work Order No.)	Field ID	Sample Aliquote (g) in 100 ml sterile water	After adding Colilert to Sample and Shaking		Incubation (35.0 ± 0.5°C)				Coliform Result*		Notes
					Start		End (____ hrs.)		Presence (+) / Absence (-)		
			Is sample lighter in color than comparator (Y/N)*	Is Colilert completely dissolved? (Y/N)*	Date	Time	Date	Time	Total Coliform (Yellow Color)	E. Coli (Fluorescent)	
Positive/Positive Control		100 ml	QC Analyzed on:_____ QC Analyzed by:_____								(E. Coli)*
Positive/Negative Control		100 ml									(E. aerogenes)*
Negative/Negative Control		100 ml									(P. Aeruginosa)*

*Criteria: If sample is darker than comparator prior to incubation, samples must be re-analyzed using a different Lot# of Colilert or an alternative procedure. Colilert must be completely dissolved prior to incubation. For sample results to be acceptable, QC results must be as follows: Positive/Positive Control Results = (+/+); Positive/Negative Control Results = (+/-); Negative/Negative Control Results = (-/-)

Sample bottles checked to ensure they are not autofluorescent (Y/N): _____

Colilert Media Lab ID:_____, Lot # _____ Expiration Date:_____

Reviewed by:_____

Date Reviewed:_____

Appendix C

LIMS Analytical Results Report

Guam Environmental Protection Agency
Analytical Services Program
EMAS Laboratory

GUAM ENVIRONMENTAL PROTECTION AGENCY 1973

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ALL LIVING THINGS OF THE EARTH ARE ONE

Analysis Method:	**SM 9223 B**	Report No:	**03850**
Analyte:	**Total Coliforms and E. Coli**	Report Date:	**07/28/2025**
Matrix:	**Sand**	Date Analyzed:	**07/26/2025**
Date Collected:	**7/25/2025**	Analysts:	**JF/AC**

Lab Sample ID	Field ID	Location	Analytes	Instanteous Results
2507005-01	TLLU #1	Port Authority	Total Coliforms	ND
			E. coli	ND
2507005-02	TLLU #2	Port Authority	Total Coliforms	ND
			E. coli	ND
2507005-03	GCXU #1	Port Authority	Total Coliforms	ND
			E. coli	ND
2507005-04	GCXU #2	Port Authority	Total Coliforms	ND
			E. coli	ND
2507005-05	CMAU 4 #1	Port Authority	Total Coliforms	ND
			E. coli	ND
2507005-06	CMAU 4 #2	Port Authority	Total Coliforms	ND
			E. coli	ND
2507005-07	CMAU 4 #3	Port Authority	Total Coliforms	ND
			E. coli	ND
2507005-08	CMAU 7 #1	Port Authority	Total Coliforms	ND
			E. coli	ND
2507005-09	CMAU 7 #2	Port Authority	Total Coliforms	ND
			E. coli	ND
2507005-10	UETU #1	Port Authority	Total Coliforms	ND
			E. coli	ND
2507005-11	UETU #2	Port Authority	Total Coliforms	ND
			E. coli	ND
2507005-12	CMAU 3 #1	Port Authority	Total Coliforms	ND
			E. coli	ND
2507005-13	CMAU 3 #2	Port Authority	Total Coliforms	ND
			E. coli	ND

Guam Environmental Protection Agency
Analytical Services Program

EMAS Laboratory

TODO Y NILALA Y TANO MAN UNO
ALL LIVING THINGS OF THE EARTH ARE ONE

Analysis Method:	**SM 9223 B**	Report No:	**03850**
Analyte:	**Total Coliforms and E. Coli**	Report Date:	**07/28/2025**
Matrix:	**Sand**	Date Analyzed:	**07/26/2025**
Date Collected:	**07/25/2025**	Analysts:	**JF/AC**

Lab Sample ID	Field ID	Location	Analytes	Instanteous Results
2531001-BLK1	Laboratory Blank		Total Coliforms	ND
			E. coli	ND

JF/AC

Analyst

Rudy Paulino

Digitally signed by Rudy Paulino
DN: cn=Rudy Paulino, o=Guam Environmental Protection Agency, ou=Guam EPA EMAS-ASP, email=rudy.paulino@epa.guam.gov, c=US
Date: 2025.08.07 15:03:52 +10'00'

Chemist III or EMAS Division Administrator

Appendix D

Incubator # 2

Daily Temperature Log
(Water Bath: 44.5 ± 0.2°C)

GEPA Log: QC-02-02

GUAM EPA LABORATORY
INCUBATOR # 2 (WATER BATH: 44.5 $\pm$ 0.2°C)
DAILY TEMPERATURE LOG

Thermometer ID: ______________________

	Time		Thermometer Reading (°C)	Correction Factor	Actual Temp (°C)	Acceptable (44.3-44.7°C) (Y/N)	Initials	*If Outside Limits* [illegible] *Up*	*Down*	*Samples Present (Y/N)*	Notes
	am										
	pm										
	am										
	pm										
	am										
	pm										
	am										
	pm										
	am										
	pm										
	am										
	pm										
	am										
	pm										
	am										
	pm										
	am										
	pm										

CRITERIA: Temperature range should be 44.5 $\pm$ 0.2°C and recorded at a minimum of 4 hours apart.
ACTION: If value is greater than criteria contact Supervisor for correction procedure.

Appendix E

Incubator # 3

Daily Temperature Log
(Dry Incubator: 35.0 ± 0.5°C)

GEPA Log: QC-02-03

GUAM EPA LABORATORY

GEPA Log: QC-02-03

INCUBATOR # 3 (DRY INCUBATOR: 35.0 ± 0.5°C)
DAILY TEMPERATURE LOG

Thermometer ID_____(Top)
_____(Bottom)

Date	Time		Thermometer Reading (°C)				Actual Temp (°C)		Acceptable (34.5-35.5°) (Y/N)	Initials	If Outside		Samples Present (Y/N)	Notes
			Top	Correction Factor	Bottom	Correction Factor	Top	Bottom			Up	Down		
	am													
	pm													
	am													
	pm													
	am													
	pm													
	am													
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	pm													
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	pm													

CRITERIA: Temperature range should be 35.0 ± 0.5°C and recorded at a minimum of 4 hours apart.
ACTION: If value is greater than criteria contact Supervisor for correction procedure.

Revision: 2.3 - 11/10/09 esy
File: Incubator # 3 Templog.xls