



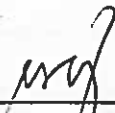
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Title: Total Coliform & E. coli in Water, SM 9223 B
 Number: MB-01-04
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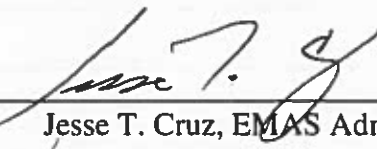
**GUAM ENVIRONMENTAL PROTECTION AGENCY
 EMAS ANALYTICAL PROGRAM**

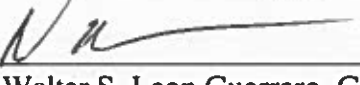
STANDARD OPERATING PROCEDURE

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

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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 based on Standard Method no. SM 9223 B (see ref. no. 11.6). The method meets with the requirements of the National Primary Drinking Water Regulations for public water supplies and the Surface Water Treatment Rule promulgated under the Safe Drinking Water Act.

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 -glucuronidase produced by *E. coli*, a fecal coliform, forms a fluorescent substance when it hydrolyses 4-methylumbelliferyl- β -d-glucuronide (MUG). This combination of substrates allows detection of both coliforms and *E. coli* within 24 hours. The Method Detection Limit (MDL) is 1 CFU/100ml with as many as 2 million heterotrophic bacteria/100 ml present

3. INTERFERENCES

- 3.1. Colilert®/Colilert-18® is a primary water test. Colilert®/Colilert-18® performance characteristics do not apply to samples altered by pre-enrichment or concentration. Do not use Colilert®/Colilert-18® to verify presumptive coliform cultures or membrane filter colonies because the substrate may be overloaded by a heavy inoculum of weak β -galactosidase producing non-coliforms causing false-positive results.
- 3.2. Colilert®/Colilert-18® may give false positive ONPG and MUG reactions in the presence of *Aeromonas hydrophilia* and flavobacterium. Care should be taken when using Colilert®/Colilert-18® for surface waters, particularly those rich in algae. If such samples are positive for coliforms, it may be prudent to confirm the results using other methods such as Multiple Tube Fermentation.
- 3.3. Residual chlorine in the water sample may cause a false negative. To avoid this, sample bottles with sodium thiosulfate is used. The amount of thiosulfate should be sufficient to dechlorinate the sample. For drinking water samples this is 0.1 ml of 3% sodium thiosulfate solution SM 9060A (ref no. 11.1).



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 indicator of the sanitary quality of food and water.
- 4.2. *Escherichia coli* (*E. coli*) – is a Gram-negative, facultative 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 bacterium 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 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 -glucuronidase, 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 in 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. This 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 chomogenic/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.

- 4.8. MPN – is the acronym for Most Probable Number. This method is used to estimate the bacteriological density of a sample. Using statistical tables, the number of wells positive in a sample tray with a known number of wells will give a fairly good estimation of the number of target organism in a sample. IDEXX uses the Quanti-tray and IDEXX-MPN table for this purpose (see Appendix D).

5. HEALTH AND SAFETY WARNINGS

- 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.
- 6.3. If chlorinated water is to be sampled, sterile sample bottles must contained sodium thiosulfate to neutralize any chlorine residual.
- 6.4. At least 100 mL of sample must be collected, allowing at least 1-inch air space to facilitate mixing of sample by shaking.
- 6.5. If a sample bottle is filled too full to allow for proper mixing, do not pour off and discard a portion of the sample. Rather, pour the entire sample into a larger sterile container, mix properly, and proceed with the analysis.



- 6.6 Samplers are encouraged, but not required, to hold drinking water samples at <10°C during transit to the laboratory. Surface water source samples, however, must be held at <10°C during transit. Laboratories should reject samples that have been frozen.
- 6.7 For the analysis of total coliform and *E. coli* in drinking water samples, the time between sample collection and the placement of sample in the incubator must not exceed 30 hours. All samples received in the laboratory must be analyzed on the day of receipt. If the laboratory receives the sample late in the day, the samples may be refrigerated overnight as long as analysis begins within 30 hours of sample collection.
- 6.8 For the analysis of total coliform and *E.coli* in surface water sources, the time between sample collection and the placement of sample in the incubator must not exceed 8 hours.

7. APPARATUS AND MATERIALS

- 7.1. Colilert® / Colilert -18® dry media in “Snap-Packs,” stored in the dark at 4-30°C
- 7.2. IDEXX Quanti-Tray®/2000 MPN trays
- 7.3. IDEXX Quanti-Tray® Sealer
- 7.4. Water bath incubator at 44.5 ± 0.2°C
- 7.5. Incubator at 35 ± 0.5°C
- 7.6. 6 watt, 365 nm UV lamp
- 7.7. Colilert® MPN Tables
- 7.8. Colilert® color comparator

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:

<u>Organisms</u>	<u>Expected Results</u>
<i>E. coli</i> (fecal coliform)	Yellow, fluorescent
Coliform, fecal (e.g. <i>Klebsiella pneumoniae</i>)	Yellow, non fluorescent
Coliform, non-fecal (e.g. <i>Enterobacter aerogenes</i>)	Yellow, non fluorescent
Non-coliform (e.g. <i>Pseudomonas aeruginosa</i>)	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. Carefully separate one Colilert® or Colilert-18® Snap Pack from the strip taking care not to accidentally open adjacent pack.
- 9.1.2. Tap the Snap Pack to ensure all of the powder is in the bottom of the pack.
- 9.1.3. Open the pack being careful not to touch the opening of the pack.
- 9.1.4. Aseptically transfer the powder from the pack to the water sample in a sterile, non-fluorescent 120 ml bottle.
- 9.1.5. Aseptically cap and seal the bottle
- 9.1.6. Shake until dissolved.
- 9.1.7. When using the Colilert -18®, pre warm the sample in $44.5^{\circ} \pm 0.2^{\circ}\text{C}$ water bath for 7-10 minutes before incubating in the $35^{\circ} \pm 0.5^{\circ}\text{C}$ air-type incubator.
- 9.1.8. For the regular Colilert®, incubate directly in the incubator.
- 9.1.9. 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 $35 \pm 0.5^{\circ}\text{C}$.
- 9.1.10. 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.
- 9.1.11. 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, test is negative.



- 9.1.12. 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. MPN Enumeration Test Procedure

- 9.2.1. Quantification of coliforms using the Colilert[®] method can be done using the Quanti-Tray[®]/2000.
- 9.2.2. For the MPN enumeration, follow the steps 9.1.1 to 9.1.6 above then follow the steps below (use only the regular Colilert[®] snap caps):
- 9.2.3. Pour the sample-reagent mixture from step 9.1.6 into a Quanti-Tray[®]/2000 while avoiding contact with the foil tab.
- 9.2.4. Seal the tray according to the instructions of the Quanti-Tray[®] sealer.
- 9.2.5. Be sure to use the correct insert for the Quanti-Tray[®]/2000.
- 9.2.6. Incubate for 24 hours at 35 ± 0.5°C
- 9.2.7. Follow the same interpretation guidelines in 9.1.11. The large well on top of the tray is considered a large well.
- 9.2.8. Refer to the Quanti-Tray[®] MPN table to determine the Most Probable Number of total coliform (yellow wells) and *E. coli* (yellow and fluorescing wells). The color and intensity of fluorescence may vary.
- 9.2.9. One single yellow well should be interpreted as positive for total coliform for that sample.

9.3. Procedural Notes

- 9.3.1. If an inoculated Colilert[®] sample is inadvertently incubated more than 28 hours, and for 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.
- 9.3.2. Some water samples containing humic material may have an innate color. If a water sample has some background color, compare the inoculated Colilert[®]/Colilert-18[®] sample to a control blank of the same water sample.
- 9.3.3. Colilert[®]/Colilert-18[®] is already buffered and does not require the use of buffered water for dilutions.
- 9.3.4. 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 Record (Appendix A) is properly filled out. The Chain of Custody Record should contain the following information:
- Project name
 - Sampler's name and signature
 - Date and time of sample collection
 - Field sample ID
 - Source of sample (including name, location and sample type)
 - Analyses required

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

- 10.2 The results for presence/absence and quantification of total coliform and *E. coli* are determined according to 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-02-01), Appendix B; and presence/absence in the LIMS. An analytical results report is generated from the LIMS (Appendix C). If IDEXX Quanti-Tray[®]s are used, any positive wells indicate presence and sample is recorded as positive. The number of positive wells are counted (first yellow wells for total coliforms and then yellow and fluorescent wells for *E. coli*) and the results are converted to the MPN for total coliform and *E. coli* using the appropriate Quanti-Tray[®] MPN table (Appendix D). The MPNs are then recorded into the corresponding LIMS data sheet.
- 10.3 Laboratory data reports for Colilert[®]/Colilert-18[®] results will include presence/absence for total coliforms and *E. coli* for each samples as well as MPN if Quanti-Trays[®] were used. 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: $44.5 \pm 0.2^{\circ}\text{C}$), GEPA Log: QC-02-02 (Appendix E) if using Colilert-18[®], and in the Incubator # 3 Daily Temperature Log (Dry Incubator: $35.0 \pm 0.5^{\circ}\text{C}$), GEPA Log: QC-02-03 (Appendix F) 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.



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Appendix A

Chain of Custody Record



Guam EPA Laboratory
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Appendix B

Analytical Results Logbook

(GEPA Log: MB-02-01)



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Appendix C

LIMS Analytical Results Report



Guam EPA Laboratory
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Appendix D

MPN Table

IDEXX Quanti-Tray/2000 MPN Table

# Large Wells Positive	# Small Wells Positive																								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.8	44.1	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.8	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.5
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.8	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.7	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	106.0	108.6	111.3	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.6	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	166.8	172.2	177.8	183.6	189.6	195.8	202.2	208.8	215.6	222.6	230.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	230.9	239.6	248.5	259.5	270.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.6	218.7	228.2	238.2	248.6	259.3	270.3	281.5	293.7	313.0	329.2
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.6	325.5	344.8	365.4	387.3	410.0	435.2

IDEXX Quanti-Tray/2000 MPN Table

Small Wells Positive

# Large Wells Positive	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
0	25.3	28.4	27.4	28.4	29.5	30.5	31.5	32.0	33.0	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.5
1	20.0	27.7	28.7	29.8	30.8	31.9	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2
2	27.8	20.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.8	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.9	54.0	55.1	56.3
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.9	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.8	54.1	55.2	56.4	57.6	58.7	59.9
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.9	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.3	59.4	60.6	61.8
8	36.6	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.5	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.3	67.5	68.8	70.1
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4
13	44.8	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6
16	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.0	80.5	82.0	83.5	84.9
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7
20	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.7	92.2	93.8
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
23	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.4	104.1
24	68.9	70.5	72.1	73.7	75.3	77.0	78.8	80.5	81.9	83.6	85.2	86.8	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.6	104.5	106.1	107.9
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.9
26	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.3	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7
28	80.6	82.0	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.9	112.0	114.2	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4
31	91.4	93.0	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.8	149.1
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.7	156.4
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4
35	109.7	112.2	114.8	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7
38	127.9	130.8	133.8	136.8	139.8	143.0	146.2	149.4	152.6	155.9	159.2	162.6	166.1	169.6	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.7	203.7	207.7
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.7	200.6	204.6	208.6	214.0	218.5	223.0
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.0
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	218.1	224.2	228.4	234.8	240.2	245.8	251.5	257.2	263.1
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	238.1	244.5	251.0	257.7	264.6	271.7	278.9	286.3	293.8	301.5	309.4	317.4	325.7
44	192.8	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.8	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4
45	214.1	220.9	227.9	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.1	302.3	311.7	321.3	331.1	341.0	351.2	361.6	372.2	383.0	393.9	405.0	424.5	437.4
46	241.5	250.0	259.0	268.2	277.6	287.3	297.1	307.1	317.3	327.6	338.1	348.8	359.7	370.8	382.1	393.6	405.3	417.2	429.2	441.4	453.8	466.4	480.3	494.5
47	280.9	292.4	304.4	316.9	330.0	343.0	357.0	371.2	385.7	400.4	415.4	430.6	446.1	461.8	477.7	493.8	510.1	526.6	543.3	560.2	577.3	594.6	612.1	630.0
48	344.1	360.0	376.4	393.8	410.0	428.0	446.8	465.8	485.0	504.2	524.7	545.3	566.1	587.1	608.4	630.0	651.8	673.8	696.0	718.4	741.0	763.8	786.8	810.0
49	481.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	865.8	918.4	974.3	1033.6	1096.4	1163.8	1235.7	1312.1	1393.1	1478.8	1569.3	1664.6	1765.0



Guam EPA Laboratory
B-15-6101 Mariner Ave.
Tiyán, Barrigada
Guam 96921

Title: Total Coliform & E. coli in Water, SM 9223 B
Number: MB-01-04
Date: 03/13/2019
Rev. no. 003

Appendix E

Incubator # 2

Daily Temperature Log
(Water Bath: $44.5 \pm 0.2^{\circ}\text{C}$)

GEPA Log: QC-02-02

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

Thermometer ID: _____

	Time	Thermometer Reading (°C)	Correction Factor	Actual Temp (°C)	Acceptable (44.3-44.7°C)	Initials	If Outside Limits			Notes
					(Y/N)		Adjusted		Samples Present (Y/N)	
							Up	Down		
	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 ± 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 F

Incubator # 3

Daily Temperature Log (Dry Incubator: $35 \pm 0.5^{\circ}\text{C}$)

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°C) (Y/N)	Initials	If Outside			Notes
		Top	Correction Factor	Bottom	Correction Factor	Top	Bottom			Adjusted		Samples Present (Y/N)	
										Up	Down		
	am												
	pm												
	am												
	pm												
	am												
	pm												
	am												
	pm												
	am												
	pm												
	am												
	pm												
	am												
	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.

