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Enzyme Substrate Coliform Test Standard Method 9223

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1 Scope and Application

1.1 This is the primary method used to detect total coliform bacteria and *E.coli* in drinking water samples. This test involves the addition of a chromo-fluorogenic substrate such as (ONPG+MUG) to 100 ml of a drinking water sample which is then incubated for 24 hours at 35.0 ±0.5 degrees Celsius. The enzyme β-D galactosidase produced by coliform bacteria will metabolize the ONPG yielding a yellow color at the end of the incubation period. All positive samples are also exposed to a UV light to check for fluorescence. Fluorescent positive samples further indicate the presence of *E.coli* caused by the metabolic reaction between the *E.coli* enzyme β-glucuronidase and the substrate MUG.

2 Definitions

2.1 Refer to Section 3 and Section 4 of the Georgia EPD Laboratory Quality Assurance Manual for Quality Control Definitions. (See SOP reference 13.3)

3 Interferences

3.1 Method interferences may be caused by contaminants in reagents, media, bottles or glassware. To abstain from interferences, all reagents and media are tested for sterility prior to use. Also, all bottles and glassware are sterilized and tested prior to usage.

3.1.1 All glassware must be washed, sterilized, and put in the hot air oven at 170 - 180°C for 2 hours. A pH check is performed on all batches of glassware using a 0.04% solution of bromothymol blue. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants.

3.1.2 Each batch of media is aseptically prepared according to manufacturer's instructions, sterilized and tested before being used. Commercially prepared media and reagents are also tested weekly to ensure its quality assurance. Each new lot of water is tested for sterility upon receipt. If the exact pH for commercially prepared media is not provided by the manufacturer, then each lot is checked upon receipt.

3.1.3 Reusable Bottles: All reusable bottles are washed and sterilized prior to use. Sodium thiosulfate is added to sample collection bottles to absorb any chlorine residual that may come from the water sample. Each new lot number is initially checked for volumetric accuracy, and auto-fluorescence.

Disposable Bottles: Each new lot number of sample testing bottles is initially checked for volumetric accuracy, auto-fluorescence and sterility. Sample collection bottles are checked for sterility.

4 Safety

4.1 Refer to Laboratory Chemical Hygiene Plan & Fire Safety Plan, online revision. (See SOP reference 13.4)

5 Apparatus and Equipment

5.1 35° ±0.5°C Incubators
5.2 Reusable bottle -Round 250 ml (8 oz) or 125 ml (4 oz) Polypropylene bottles
5.3 Disposable bottle -Round 125 ml (4 oz) Plastic bottles
5.4 Square - 250 ml Polycarbonate bottles
5.5 Long wavelength (365-366 nm) ultraviolet lamp
5.6 Idexx Quanti-Tray 51 wells or Quanti-Tray/2000 with 97 wells
5.7 IdexxQuanti-Tray Sealer
5.8 44.5° ±0.2°C Water Bath
5.9 pH meter

6 Reagents

6.1 Idexx Colilert Reagent [Packets (P/A)]
6.2 Idexx Colilert 18 Reagent [Packets (P/A)]
6.3 Dilution Water
6.4 Sodium Thiosulfate
6.5 Positive Controls – E. coli (EC) and Klebsiella pneumoniae (Kleb)
6.6 Negative Control - Pseudomonas aeruginosa (PA)
6.7 Color and fluorescence comparator
6.8 Chlorine strips

7 Sample Collection

7.1 Refer to Chapter 5 of the Georgia EPD Laboratory Quality Assurance Manual for Sample Container, Sample Preservation and Sample Holding Times. (See SOP reference 13.3)

8 Calibration

8.1 The term calibration is not totally accurate for this type of test because none of the parameters can be “tweaked”; however, inasmuch as a calibration is a check of equipment performance, the following determinations must be in place to insure accurate results:

- All collection and testing vessels must be sterile.
- The substrate must be used prior to its expiration date and produce desired

results when exposed to known bacteria.

- The incubator should have a constant temperature of 35.0° ±0.5° Celsius.
- The water bath should have a constant temperature of 44.5° ±0.2° Celsius.
- Sterile technique must be used when performing the test.
- Incubation time must not exceed 28 hours for Colilert and 22 hours for Colilert 18.
- Original sample must be colorless.
- Empty test vessel must neither auto-fluorescence nor have any UV block preventing fluorescent samples from exhibiting their fluorescence.
- Adhere to good laboratory practice throughout the test procedure.
- Avoid touching the reagent or the inside of the bottles, trays, or caps.

9 Quality Control

9.1 Refer to Table 14.1 for Quality Control Acceptance Criteria associated with this method.

10 Procedure

10.1 **Presence/Absence (P/A) Test Procedure using Colilert**

10.1.1 Assign each sample collection bottle a unique Bac-T Lab number by stamping that number on top of the lid. Also, number the corresponding sterile-testing bottle with the same lab number. (Number each bottle in your run or "batch"). Note: Do not analyze the sample if the volume is less than 100 ml.

10.1.2 Shake the water sample vigorously about 25 times (to ensure proper distribution of bacteria within sample). Be sure lid is on tight.

10.1.3 Pour 100 ml of the water sample into the graduated testing bottle (which has the same corresponding lab number on it). If the sample comes in a bottle that meets the testing bottle requirements (transparent and non-fluorescing), then analyze the sample in that bottle.

10.1.4 Carefully separate one Colilert packet from the strip. Tap the packet to ensure that all the Colilert powder is in the bottom part of the pack (controls dust). Open the packet by snapping back the top at the score line. **Caution: Do not touch the opening of the packet**.

10.1.5 Add one Colilert reagent packet to the water sample. Solution should be colorless.

10.1.6 Aseptically cap and seal the testing bottle.

10.1.7 Shake to mix.

10.1.8 Repeat steps 10.1.2 - 10.1.7 until all lab samples have been analyzed.

10.1.9 Incubate for 24 hours at 35°C ±0.5°C.

10.1.10 Read the results at 24 hours.

- If no yellow color is observed, the test is negative.
- If the sample has a yellow color equal to or greater than the comparator, the presence of total coliforms is confirmed. If color is not uniform, mix slightly, then recheck.
- If the sample is yellow, but lighter than the comparator, it may be incubated an additional 4 hours (but no more than 28 hours total). If the sample is coliform positive, the color will intensify. If it does not intensify, the sample is negative.
- If yellow is observed, check vessel for fluorescence by placing a 6 watt 365-366

nm UV light within five inches of the sample in a dark environment. Be sure the light is facing away from your eyes and towards the vessel. If fluorescence is greater or equal to the fluorescence of the comparator, the presence of E coli is confirmed.

- **NOTE:** If any sample produces an atypical color change (e.g., greenish-black or black) in the absence of yellow color, then invalidate the sample. Request a replacement sample from the same location as the original invalidated sample, and test the sample using the Multiple Tube Fermentation method.

10.1.11 If an inoculated Colilert sample is inadvertently incubated over 28 hours, the following guidelines apply: Lack of yellow color is a VALID NEGATIVE TEST. A corrective action must be completed for valid negative results when incubated beyond 28 hrs. A yellow color after 28 hours is not valid, and a new sample should be requested.

10.1.12 It is important to check the sterile bottle to be sure it can fluoresce, some bottles do not. Check this by exposing an empty bottle to UV light. It should give a slight glow.

10.1.13 Sometimes the Colilert powder gives a slight tint to the water sample. Always note this by writing on the bottle "tint" to alert the analyst so he/she would not interpret the tint as a color change.

10.1.14 Some water samples are too murky to run on the standard Colilert test; these samples must be placed on DS-LSTB.

10.1.15 Run a positive control sample with the first two batches each day. *E.coli* is used with batch #1. Kleb. pneumoniae is used with batch #2. On days with only one run, *E.coli* only will be used. Run a beginning and ending blank sample (negative control) with each batch, unless there are less than six samples in the batch, in which case only run one blank sample. Note: On days with only one batch, regardless of sample size, all quality control must be performed – one positive control sample (use E.coli) and two blank samples (beginning and ending).

Positive Control Sample– Shake one dilution water bottle to check for floating debris or other impurities, and leaks. Pour 100 ml of dilution water into a testing bottle, which is properly labeled. Inoculate with 0.1 ml of *E.coli* or Kleb pneumoniae. Gently swirl to mix. Add one packet of Colilert and shake to mix.

Blank Sample (Sterility Blank) – Same as above, except do not inoculate with any bacteria. Place and incubate with proper run.

10.1.16 Colilert pH check. Pour 100ml of sterile water into the testing vessel. Add a packet of Colilert reagent and shake 25 times. Measure and record reading in the media QC logbook. Refer to SOP 5-010 For pH meter SM4500-H-B. Refer to table 14.1.

10.1.17 Colilert media fluorescence check. Pour 100ml of sterile water into the testing vessel. Add a packet of Colilert reagent and shake 25 times. Place a 6-watt 365 -366 nm UV light within five inches of the sample in a dark environment. Be sure the light is facing away from your eyes and towards the vessel. Record the reading in the media QC logbook. Refer to table 14.1.

10.2 **Presence/Absence Test Procedure using Colilert 18**

10.2.1 Assign each sample collection bottle a unique Bac-T Lab number by stamping that number on top of the lid. Also number the corresponding sterile testing bottle with the same lab number. (Number each bottle in your run or "batch").

10.2.2 Shake the water sample vigorously about 25 times (to ensure proper distribution of bacteria within sample). Be sure lid is on tight.

10.2.3 Pour 100 ml of the water sample into the graduated sterile testing bottle (which has the same corresponding lab number on it).

10.2.4 Carefully separate one Colilert 18 packet from the strip. Tap the packet to ensure that all the Colilert 18 powder is in the bottom part of the pack (controls dust). Open the packet by snapping back the top at the score line. **Caution: Do not touch the opening of the packet**.

10.2.5 Add one Colilert 18 reagent packet to the water sample. Solution should be colorless.

10.2.6 Aseptically cap and seal the testing bottle.

10.2.7 Shake to mix.

10.2.8 Repeat steps 10.2.2 - 10.2.7 until all lab samples have been analyzed.

10.2.9 Incubate in the water bath for 7-10 minutes at 44.5° ±0.2° Celsius.

10. 2.10 Remove samples from water bath and incubate for 18 hours at 35°C ±0.5°C.

10. 2.11 Read the results at 18 hours.

- If no yellow color is observed, the test is negative.
- If the sample has a yellow color equal to or greater than the comparator, the presence of total coliforms is confirmed. If color is not uniform, mix slightly, then recheck.
- If the sample is yellow, but lighter than the comparator, it may be incubated an additional 4 hours (but no more than 22 hours total). If the sample is coliform positive, the color will intensify. If it does not intensify, the sample is negative.
- If yellow is observed, check vessel for fluorescence by placing a 6 watt 365 nm UV light within five inches of the sample in a dark environment. Be sure the light is facing away from your eyes and towards the vessel. If fluorescence is greater or equal to the fluorescence of the comparator, the presence of E coli is confirmed.
- **NOTE:** If any sample produces an atypical color change (e.g., greenish-black or black) in the absence of yellow color, then invalidate the sample. Request a replacement sample from the same location as the original invalidated sample, and test the sample using the Multiple Tube Fermentation method.

10. 2.12 If an inoculated Colilert 18 sample is inadvertently incubated over 22 hours, the following guidelines apply: Lack of yellow color is a VALID NEGATIVE TEST. A corrective action must be completed for valid negative results when incubated beyond 22 hrs. A yellow color after 22 hours is not valid, and a new sample should be requested.

10.2.13 It is important to check the sterile testing bottle to be sure it can fluoresce, some bottles do not. Check this by exposing an empty bottle to UV light. It should give a slight glow.

10.2.14 Sometimes the Colilert 18 powder gives a slight tint to the water sample. Always note this by writing on the bottle "tint" to alert the analyst so he/she would not interpret the tint as a color change.

10.2.15 Some water samples are too murky to run on the standard Colilert 18 test; these samples must be placed on DS-LSTB.

10.2.16 Run a positive control sample with the first two batches each day. E.coli is used with batch #1. Kleb. pneumoniae is used with batch #2. On days with only one run, *E.coli* only will be used. Run a beginning and ending blank sample (negative control) with each batch, unless there are less than six samples in the batch, in which case only run one blank sample. Note: On days with only one batch, regardless of sample size, all quality control must be performed – one positive control sample (use E.coli) and two blank samples (beginning and ending).

Positive Control Sample – Shake one dilution water bottle to check for floating debris or other impurities, and leaks. Pour 100 ml of dilution water into a testing bottle, which is properly labeled. Inoculate with 0.1 ml of *E.coli* or Kleb. pneumoniae. Gently swirl to mix. Add one packet of Colilert 18 and shake to mix.

Blank Sample (**Sterility Blank)** – Same as above, except do not inoculate with any bacteria. Place and incubate with proper run.

10.2.17 Colilert 18 pH check. Pour 100ml of sterile water into the testing vessel. Add a packet of Colilert 18 reagent and shake 25 times. Measure and record reading in the media QC logbook. Refer to SOP 5-010 For pH meter SM4500-H-B. Refer to table 14.1.

10.2.18 Colilert 18 media fluorescence check. Pour 100ml of sterile water into the testing vessel. Add a packet of Colilert 18 reagent and shake 25 times. Place a 6-watt 365-366 nm UV light within five inches of the sample in a dark environment. Be sure the light is facing away from your eyes and towards the vessel. Record the reading in the media QC logbook. Refer to table 14.1.

10.3 **MPN Procedures**

Colilert and Colilert 18 can be used for multiple tube MPN analysis. Samples that need a number count, such as source approvals, can be placed on Quanti-Tray.

10.3.1 **Quanti-Tray (MPN) Enumeration Test Procedure**

10.3.2 Refer to steps 10.1.1-10.1.7 above. Note: When using Colilert 18 refer to Section 10.2.

10.3.3 Pour the sample reagent mixture above directly into the tray avoiding contact with the foil tab, and then seal the tray using the Quanti-Tray sealer. Note: Turn on sealer to warm, once the red light turns green the sealer is ready for use.

10.3.4 Label the tray with the appropriate Bac-T Lab number.

10.3.5 Incubate inoculated tray for 24 hours at 35° ± 0.5°C for Colilert and 18 hrs.35° ± 0.5°C for Colilert 18. Note: The pre-warming process is not required when using Colilert 18 reagent.

10.3.6 Follow the same interpretation directions from 10.1.10 – 10.1.11 to count the number of positive wells using Colilert and 10.2.10 – 10.2.11 using Colilert 18. Refer to the MPN table to determine the Most Probable Number (MPN) of total

coliforms (yellow wells) and *E.coli* (fluorescent wells) in your sample. The color and fluorescence intensity of positive wells may vary.

11 Calculations

11.1 The Revised Total Coliform Rule informs us that the presence of one coliform bacterium in a 100 ml sample renders it positive and requires that system to submit additional samples. The enzyme substrate test is sensitive enough to detect the one bacterium; therefore, there are no calculations involved for presence/absence analysis. For MPN analysis use the Reference Tables 11.1 and 11.2 provided by the product manufacturer. Results are posted as positive or negative for presence/absence analysis and a number is reported for MPN analysis for total coliform and/or *E. coli.*

Table 11.1

IDEXX
51-Well Quanti-Tray
MPN Table

No. of wells giving positive reaction per 100 mL sample	Most Probable Number MPN	95% Confidence Limits Lower	Upper
0	<1	0	3.7
1	1.0	0.3	5.6
2	2.0	0.6	7.3
3	3.1	1.1	9.0
4	4.2	1.7	10.7
5	5.3	2.3	12.3
6	6.4	3.0	13.9
7	7.5	3.7	15.5
8	8.7	4.5	17.1
9	9.9	5.3	18.8
10	11.1	6.1	20.5
11	12.4	7.0	22.1
12	13.7	7.9	23.9
13	15.0	8.8	25.7
14	16.4	9.8	27.5
15	17.8	10.8	29.4
16	19.2	11.9	31.3
17	20.7	13.0	33.3
18	22.2	14.1	35.2
19	23.8	15.3	37.3
20	25.4	16.5	39.4
21	27.1	17.7	41.6
22	28.8	19.0	43.9
23	30.6	20.4	46.3
24	32.4	21.8	48.7
25	34.4	23.3	51.2
26	36.4	24.7	53.9
27	38.4	26.4	56.6
28	40.6	28.0	59.5
29	42.9	29.7	62.5
30	45.3	31.5	65.6
31	47.8	33.4	69.0
32	50.4	35.4	72.5
33	53.1	37.5	76.2
34	56.0	39.7	80.1
35	59.1	42.0	84.4
36	62.4	44.6	88.8
37	65.9	47.2	93.7
38	69.7	50.0	99.0
39	73.8	53.1	104.8
40	78.2	56.4	111.2
41	83.1	59.9	118.3
42	88.5	63.9	126.2
43	94.5	68.2	135.4
44	101.3	73.1	146.0
45	109.1	78.6	158.7
46	118.4	85.0	174.5
47	129.8	92.7	195.0
48	144.5	102.3	224.1
49	165.2	115.2	272.2
50	200.5	135.8	387.6
51	>200.5	146.1	infinite

Table 11.2

IDEXX Quanti-Tray*/2000 MPN Table – Page 1

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	42.9	44.2	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	45.9	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	57.6
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	29.9	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	69.8	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	95.8	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	105.9	108.6	111.2	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	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4	233.3
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	231.0	240.0	249.5	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	209.8	218.7	228.2	238.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
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	410.6	435.2

IDEXX Quanti-Tray*/2000 MPN Table – Page 2

IDEXX Quanti-Tray*/2000 MPN Table

# Large Wells Positive	# Small 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	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	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	26.6	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	27.9	29.0	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	37.9	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	52.9	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	48.8	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	50.6	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	44.9	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	73.4	74.8	76.2	77.6	79.1	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	97.2	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	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	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	97.4	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	80.8	82.6	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	91.6	93.6	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	146.6	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	114.6	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	139.9	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	196.8	201.0	205.3	209.6	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	241.1
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	219.1	224.2	229.4	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	193.6	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	293.3	302.6	312.3	322.3	332.5	343.0	353.8	364.9	376.2	387.9	399.8	412.0	424.5	437.4
46	241.5	250.0	258.9	268.2	277.8	287.8	298.1	308.8	319.9	331.4	343.3	355.5	368.1	381.1	394.5	408.3	422.5	437.1	452.0	467.4	483.3	499.6	516.3	533.5
47	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.8	616.7	640.5	665.3	691.0
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.6	501.2	524.7	549.3	574.8	601.5	629.4	658.6	689.3	721.5	755.6	791.5	829.7	870.4	913.9	960.6	1011.2
49	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	866.4	920.8	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1986.3	2419.6	>2419.6

12. Waste Management

12.1 See GA EPD Laboratory SOP-EPD Laboratory Waste Management Standard Operating procedures, SOP 6-015, online revision.

13. References

13.1 Standard Methods for the Examination of Water and Wastewater, 20th Edition, American Public Health Association: Washington, D.C., 1998.

13.2 Manual for the Certification of Laboratories Analyzing Drinking Water, EPA/815-R-05-004, January 2005.

13.3 GA EPD Laboratory Quality Assurance Plan, online revision.

13.4 GA EPD Laboratory Safety/Chemical Hygiene Plan & Fire Safety Plan, online revision.

13.5 GA EPD Laboratory SOPs – Initial Demonstration of Capability SOP 6-001, online revision and/or Continuing Demonstration of Capability SOP 6-002, online revision.

13.6 SOP for pH Meter, SM4500-H+B, SOP 5-010, online revision.

14. Practical Quantitation Limits (PQLs), Precision and Accuracy Criteria and Quality Control Approach

Table 14.1 Summary of Data Quality Objectives

Method	Applicable Parameter	QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria
Standard Method 9223	Chlorine Detection	Chlorine test strips.	Each sample that smells of chlorine.	Sample should remain clear.	If indicator shows color change, sample is discarded, and repeat sample requested.	Excess Chlorine
	Incubator Temperature	35.0 degrees Celsius	Twice Daily	±0.5 degrees Celsius	Adjust temperature	
	Water Bath Temperature	44.5 degrees Celsius	Twice Daily	±0.2 degrees Celsius	Adjust temperature	
	Colilert or Colilert 18 Accuracy	Negative Control -- Pseudomonas aeruginosa (PA) _____ Pos./Neg. Control -- Klebsiella pneumoniae (Kleb) _____ Pos./Pos. Control -- E. coli (EC)	Weekly	Clear _____ Yellow/No Fluorescence _____ Yellow/ Fluorescence	Retest. Check Bacteria Stock. Discard whichever is errant	
		pH Check	Each Lot	7.0 – 7.6	Re-check pH. Consult the manufacturer.	
		Fluorescence Check (no inoculum)	Each Lot	No Fluorescence	Do not use. Consult the manufacturer.	
	Accuracy of Run	Beginning and Ending Blank Sample (Sterility Blanks)	Each Run	Clear Sample	Check sterility records of dilution water, bottles, and samples to determine source of contamination.	

					Reanalyze all samples in run, if necessary.	
		Positive Control Sample (fluorescence) _____ Total Positive Control (yellow)	1st Run Daily – use E.coli _____ 2nd Run Daily – use Kleb pneumoniae	Positive Yellow with Fluorescence _____ Positive Yellow without Fluorescence	Check Colilert or Colilert 18 and Bacteria Stock. Discard whichever is errant. Reanalyze all samples in run, if necessary.	
	Drinking Water Sample		Each	Clear _____ Yellow/No Fluorescence _____ Yellow/ Fluorescence	None	Neg _____ pos/neg _____ pos/pos

15. Updates

15.1 Updated for online revision