

STANDARD OPERATING PROCEDURE For SM 9223-MPN

Enzyme Substrate Coliform Test Most Probable Number Procedure for Analysis of Potable and Non-Potable Water Samples

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MassDEP

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LIST OF REVISIONS

Rev. #	Date	Description of Revision	Page #
0	April 2004	None	
1.0	February 2010	Section 10.2 added Section 12.3 added Table 4 and Table 5 added Several other minor revisions	7 8 12 - 15 Throughout document



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1.0 SCOPE & APPLICATION

- 1.1 The enzyme substrate test is a technique that utilizes hydrolyzable substrates for the simultaneous detection of total coliform bacteria and *Escherichia coli* enzymes.
- 1.2 The Colilert® method using the Quanti-Tray® option may be used to detect and enumerate total coliform and *E. coli* using the most probable number (MPN) technique.

2.0 SUMMARY OF METHOD

- 2.1 The commercially purchased medium is added to a 100-mL volume of sample, the sample is poured into a multi-well tray, sealed and incubated for 24 hours at $35 \pm 0.5^{\circ}\text{C}$. After incubation, samples are checked for color and fluorescent reactions, the number of positive wells is counted and most probable number results are obtained by referring to the chart provided with the Quanti-Tray® unit.

3.0 DEFINITIONS

- 3.1 When the enzyme substrate technique is used, the total coliform group is defined as all bacteria possessing the enzyme β -D-galactosidase, which cleaves the chromogenic substrate, ortho-nitrophenyl- β -D-galactopyranoside (ONPG) resulting in the release of a chromogen which produces a yellow color.
- 3.2 Using this method, *E. coli* are defined as bacteria giving a positive total coliform response and possessing the enzyme β -glucuronidase which cleaves the substrate 4-methyl-umbelliferyl- β -D-glucuronide (MUG) which produces a fluorescent product when viewed under long wavelength (366 nm) ultraviolet (UV) light.

4.0 INTERFERENCES

- 4.1 Non-coliform bacteria, particularly *Aeromonas* and *Pseudomonas* species, may produce small amounts of the enzyme β -D-galactosidase, but are suppressed and generally will not produce a positive response within the incubation time unless more than 104 colony-forming units (CFU)/mL are present.
- 4.2 *Serratia* species may turn the medium yellow after 24 hours of incubation, but the yellow color is typically brighter than that represented by the color comparator.
- 4.3 Some strains of *Shigella* species may produce a positive fluorescence response. This is not considered a detriment for testing the sanitary quality of water due to the pathogenic nature of *Shigella*.
- 4.4 Some water samples containing humic material may have an innate color. If a water sample has some background color, compare inoculated Colilert® sample to a control blank of the same sample.

5.0 SAFETY

- 5.1 Samples (and positive controls) may contain organisms that are pathogenic to humans. All precautions are to be taken to minimize exposure. All personnel must wear lab coats, safety glasses, and protective gloves while working in the laboratory.



6.0 EQUIPMENT AND SUPPLIES

- 6.1 Sterile Colilert® bottle (purchased from manufacturer) containing sodium thiosulfate
- 6.2 Long wavelength (366 nm) UV light (6 watt)
- 6.3 Color comparator (purchased from manufacturer)
- 6.4 Incubator capable of maintaining $35 \pm 0.5^{\circ}\text{C}$ for 24-28 hours
- 6.5 IDEXX Quani-Tray® Sealer
- 6.6 IDEXX Quanti-Tray®

7.0 REAGENTS AND STANDARDS

- 7.1 Colilert® Presence-Absence medium (purchased from manufacturer)

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

- 8.1 A 100-mL sample must be aseptically collected in a sterile disposable plastic bottle leaving at least 1" (2.5 cm) of headspace to allow for sufficient mixing of the sample prior to analysis. If the sample is chlorinated, make sure that the sample bottle contains the sodium thiosulfate tablet.

(Note: provides a final concentration of 100 mg/L $\text{Na}_2\text{S}_2\text{O}_3$)

If tap cleanliness is in question, apply a solution of sodium hypochlorite (100 mg NaOCl/L) to faucet before sampling.

- 8.2 Remove all attachments from the water tap (screens, etc.), open tap, and let run to waste for 2-3 minutes. Reduce the water flow to allow for filling of the bottle without splashing.

Note: Non-potable water samples must be collected according to the applicable program plan.

Keep sample bottle closed until it is to be filled, fill the bottle without rinsing, replace cap immediately, and secure the top with the attached plastic "lock".

- 8.3 Samples must be accompanied by a sample tracking/chain-of-custody form filled out by the collector. Non-potable water samples must be kept at $< 10^{\circ}\text{C}$ from the time of collection to the time of analysis.
- 8.4 Potable water samples must be analyzed as soon as possible but no longer than 30 hours after collection. Non-potable water samples must be analyzed within 8 hours of collection (i.e. 6 hours maximum in the field and 2 hours maximum in the laboratory)

9.0 QUALITY CONTROL

- 9.1 A summary of all the quality control elements for this method is shown in Table 1.
- 9.2 With each new lot of medium purchased, run 4 controls – sterile water alone and sterile water inoculated with: 1) non-fluorescent *Pseudomonas* spp. (i.e., total coliform & *E. coli* negative); 2) *Klebsiella pneumoniae*, *Enterobacter aerogenes* or *Enterobacter cloacae* (i.e., total coliform positive & *E. coli* negative); and 3) *E. coli* (i.e., total coliform & *E. coli* positive) control cultures (Table 2).



- 9.3 With each batch of samples, run a blank (sterile water) and two positive control samples (i.e., sterile water spiked with *Klebsiella pneumoniae*, *Enterobacter aerogenes* or *Enterobacter cloacae*, and sterile water spiked with *E. coli*). Note: If samples are to be analyzed for *E. coli* only, then only the *E. coli* positive culture control is required.
- 9.4 If sufficient volume of sample is provided, 5% of samples should be analyzed in duplicate to assess method precision.

10.0 CALIBRATION AND STANDARDIZATION

- 10.1 The calibration of each lot of sample containers used to measure sample volume must be checked by ensuring a mass (g) to measured volume (mL) ratio of one for reagent water at 4°C.
- 10.2 The Quanti-Tray® sealer needs to be checked with each analytical batch by adding a dye (e.g., crystal violet) to the water. If dye is observed outside the wells, either perform maintenance or use another sealer.
- 10.3 Refer to Laboratory Quality Assurance Plan for calibration and standardization procedures of laboratory equipment used for this analysis.

11.0 PROCEDURE

- 11.1 Shake the sample well (25 times) and aseptically fill the Colilert® bottle to the 100 mL mark.
- Note: If dilution of non-potable water samples is desired, the diluent must be sterile reagent water, not buffered water. The Colilert® reagent includes a buffer. The final volume of sample + diluent must be 100 mL.
- 11.2 Without touching the perforated section of the Colilert® medium snap-pack, tap the medium down into the bottom of the snap-pack and open the pack by snapping along the perforations.
- 11.3 Empty the entire packet of medium into the bottle containing the sample. Cap the sample and shake. Gently pull foil tab to separate foil from the tray. Avoid touching the inside of foil or tray. Pour sample into the tray, avoiding contact with the foil tab. Tap the small wells two to three times to release any air bubbles. Allow foam to settle.
- 11.4 Seal the tray using the Quanti-Tray® sealer:
- 11.4.1 Turn the power switch on. The power light should be illuminated.
- 11.4.2 Allow the sealer to warm up and the green ready light to come on (up to 10 minutes).
- 11.4.3 Place an empty Quanti-Tray® rubber insert on the input shelf with the large cutout facing away from the sealer. Use rubber insert appropriate for trays selected (Quanti-Tray® or Quanti-Tray® 2000).
- 11.4.4 Place the tray filled with sample and reagent onto the rubber insert, making sure that the tray is properly seated in the rubber insert, and with each well of the tray in its corresponding rubber insert hole.
- 11.4.5 Slide the rubber insert with tray into the sealer until the motor grabs the rubber insert and begins to draw it into the sealer.



Note: If at any time you wish to reverse the motor drawing the rubber insert into the sealer (for example, if a misaligned tray is accidentally fed into the sealer), press and hold the reverse button. Do not reverse the motor once the rubber insert has been drawn fully into the input slot.

11.4.6 In approximately 15 seconds, the tray will be sealed and partially ejected from the rear of the sealer. Remove the rubber insert and tray from the rear of the sealer.

11.4.7 Sealer must be turned off when not in use.

11.5 Incubate trays for 24 hours at $35 \pm 0.5^{\circ}\text{C}$.

11.4 After incubation, check the sample wells for a yellow color change that is at least as strong as the yellow color of the comparator. A yellow color that is equal to or deeper than that of the color comparator verifies that the sample is positive for total coliforms (Table 3). Count the number of positive sample wells (record the number of positive large and small wells separately if the Quanti-Tray® 2000 is used). Refer to the MPN table provided with the Quanti-Tray® to determine the Most Probable Number (MPN) of total coliforms (see Tables 4 and 5).

11.5 If the sample is total coliform-positive as indicated in 11.4, expose the sample to a long wavelength (366 nm) UV light using the hand-held UV light in the dark microscopy room (Table 3). Count the number of positive (fluorescing) sample wells (record the number of positive large and small wells separately if the Quanti-Tray® 2000 is used). Refer to the MPN table provided with the Quanti-Tray® to determine the Most Probable Number (MPN) of *E. coli* (see Tables 4 and 5). Non-yellow wells which fluoresce are not considered *E. coli* and should not be counted.

11.6 If the sample results are questionable after 24 hours of incubation, the sample may be incubated for an additional 4 hours (total of 28 hours), and rechecked for color and fluorescent reactions. If the color intensifies, the sample is total coliform positive; if it does not, the sample is negative. If an inoculated test is inadvertently incubated over 28 hours, the following guidelines apply:

- Lack of yellow color is a valid negative test.
- A yellow color after 28 hours is not valid and must be repeated.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 The most probable number (MPN) of organisms per 100 mL is read directly from the Table supplied with the Quanti-Tray® (see Tables 4 and 5). Be sure to use the Table appropriate for the tray in use (i.e., Quanti-Tray or Quanti-Tray® 2000).

12.2 The MPN tables provide the most probable number of organisms assuming the use of 100 mL volume of sample. If samples were diluted prior to pouring into the tray, the MPN number must be multiplied by the dilution factor to obtain the MPN/100mL.

12.3 Calculation of Precision QC Criterion – Determine the range of logs for the duplicate MPNs as follows (*Standard Methods for the Examination of Water and Wastewater*, 1998, Page 9-10):

$$\text{Range of Logs for Duplicate Set} = \text{Log}[(\text{MPN } 1) + 1] - \text{Log}[(\text{MPN } 2) + 1]$$

$$\text{Precision QC Criterion} = 3.27 \text{ (Mean Range of Logs for 15 Most Recent Duplicate Sets)}$$



13.0 METHOD PERFORMANCE

- 13.1 The detection limit of this method is < 1 MPN per sample volume or dilution tested.
- 13.2 The Quanti-Tray® 2000 method may be used to quantify results for samples with an MPN of <1 to 2419 organisms per 100 mL sample analyzed. The range of the method can be expanded by using multiple trays and sample dilution.

14.0 POLLUTION PREVENTION

- 14.1 The quantity of media and chemicals purchased should be based upon expected usage during its shelf life.
- 14.2 Actual preparation volumes should reflect anticipated usage and stability.
- 14.3 Wastes are collected and disposed of properly.

15.0 WASTE MANAGEMENT

- 15.1 Dispose of all positive samples by discarding in autoclave bags and autoclaving for a minimum of 30 minutes.

16.0 REFERENCES

- 16.1 *Standard Methods for the Examination of Water and Wastewater*, 20th Edition, 1998. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC
- 16.2 IDEXX Colilert® Procedure.
- 16.3 IDEXX Quanti-Tray® Sealer Model 2X Users Manual



17.0 TABLES

TABLE 1. Quality Control Elements and Acceptance Limits for the Enzyme Substrate Coliform Test, Most Probable Number Procedure for Analysis of Water Samples by SM9223 using Quanti-Tray

QC Elements	Frequency	Acceptance Criteria	Corrective Action
Sample storage	Every sample	Potable Water samples are analyzed within 30 hours of collection; non-potable water samples are analyzed within 8 hours of collection (6 hr in the field + 2 hr in the laboratory) and are stored at $\leq 10^{\circ}\text{C}$ prior to analysis	Qualify data (H or J) as estimated value (exceeded holding time or holding temperature, respectively) and contact sample collector to obtain new sample
Positive Culture Control (<i>Klebsiella</i> or <i>Enterobacter</i> sp.)	One per analytical batch	Total coliform-positive <i>E. coli</i> -negative	Qualify data
Positive Culture Control (<i>E. coli</i>)	One per analytical batch	Positive	Qualify data
Negative Control (sterile water)	One per analytical batch	Negative	Qualify data
Method Duplicate	10% (if sufficient sample volume is provided)	Range of Logs within current calculated acceptance criteria.	Qualify data



TABLE 2. Interpretation of Colilert® Medium Quality Control Reactions

Quality Control Organism	Yellow	Fluorescent
<i>Pseudomonas</i> sp. (Non-fluorescent strain)	No	No
<i>Klebsiella pneumonia</i> , <i>Enterobacter aerogenes</i> or <i>Enterobacter cloacae</i>	Yes	No
<i>E. coli</i>	Yes	Yes

TABLE 3. Interpretation of Colilert® Medium Reactions

Reaction	Result
Yellow	Total Coliform Positive
Fluorescent	<i>E. coli</i> Positive



TABLE 4. 51-Well Quanti-Tray® MPN Table

No. of wells giving positive reaction per 100 ml sample	Most Probable Number	95% Confidence Limits Lower	Upper
0	<1	0.0	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 5. Quanti-Tray® 2000 MPN Table

See next two pages.



IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)
Small Wells Positive

# Large 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	26.4	27.5	28.6	29.7
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	28.2	29.3	30.4	31.5	32.6	33.7
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	28.5	29.6	30.7	31.8	32.9	34.0	35.1
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.5	18.6	19.7	20.8	21.9	23.0	24.1	25.2	26.3	27.4	28.5	29.6	30.7	31.8	32.9	34.0	35.1	36.2
10	11.0	12.1	13.2	14.3	15.4	16.5	17.6	18.7	19.8	20.9	22.0	23.1	24.2	25.3	26.4	27.5	28.6	29.7	30.8	31.9	33.0	34.1	35.2	36.3	37.4
11	12.2	13.4	14.5	15.6	16.7	17.8	18.9	19.1	20.2	21.4	22.5	23.7	24.8	25.9	27.0	28.1	29.2	30.3	31.4	32.5	33.6	34.7	35.8	36.9	38.0
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	39.4	40.6	41.8
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	37.4	38.6	39.8	41.0	42.2	43.4
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	44.0	45.2
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.1	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	51.1
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	44.6	46.0	47.4	48.8	50.2	51.6	53.0	54.4	55.8	57.2	58.6	60.0	61.4
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	60.1	61.6	63.1	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	54.5	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	88.5	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	129.1	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	98.9	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	143.8	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						



IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)

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	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	35.3	36.4	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	53.8	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	79.1	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	97.1	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	78.6	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	85.0	86.7	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	160.3	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	221.2	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	195.6	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	284.3	293.3	302.6	312.1	321.9	332.1	342.5	353.0	363.9	374.9	386.2	397.8	409.6	421.5	433.4
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	1298.7	1413.6	1553.1	1732.9	1966.3	2419.6	>2419.6