

STANDARD OPERATING PROCEDURE

For

SM 9223B-MPN

(Approved 2004, SM 22nd Edition, 2012)

Enzyme Substrate Coliform Test (Colilert® Test) Most-Probable-Number Procedure for the Analysis of Potable and Non-Potable Water Samples

SOP #: SM 9223B-MPN SOP REVISION #: 1.2 DATE: December 2022

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LIST OF REVISIONS

Rev. #	Date	Description of Revision	Section #
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	10 12 17 Throughout document
1.1	July 2020	Control Document statement added Added year of method approval and Standard Methods Edition (22 nd) Changed division name from Division of Environmental Analysis (DEA) to Division of Environmental Laboratory Sciences (DELS) Made other minor changes to update document. Section 4.5 – Added “Blue Flash” chlorine statement. Sections 6.2 & 11.5 – Added new UV viewing cabinet used Section 7.1 – Added storage specifications for Colilert® P/A Medium. Section 7.2 – Added statement that only ASTM Type II reagent-grade water is required. Section 8.3 – Updated potable and non-potable water sample holding temperature to $\leq 10^{\circ}\text{C}$. Section 8.4 - Updated that potable source water samples (for enumeration of TC/ <i>E. coli</i>) and non-potable water samples must be analyzed within 8 hours of collection (i.e., usually 6 hours maximum in the field and 2 hours maximum in the laboratory). Section 9.2 – Added ATCC # for bacterial cultures used. Sections 9.2 and 9.3 – Added expected results for Quality Control Elements (QCEs). Section 11.6 – Added guidance on the interpretation of questionable results. Section 12.3 – Revised the range of logs (ROL) calculation for a duplicate set to be consistent with <i>Standard Methods</i> . Section 16.0 – Added <i>Standard Methods</i> 22 nd Edition reference.	Title page Title page Title page & header Throughout document



		<p>Section 17.0 – Table 1 – Revised Quality Control Elements (QCE) corrective actions and required sample holding temperature to $\leq 10^{\circ}\text{C}$.</p> <p>Section 17.0 – Table 2 - Revised interpretation of Colilert® Medium Quality Control Reactions to include WinLIMS QCE nomenclature and ATCC numbers.</p> <p>Section 17.0 – Table 3 – Added negative reaction description.</p>	
1.2	December 2022	<p>Section 16.0 – Added references for IDEXX Quanti-Tray and for required preservation temperature during sample transport.</p>	



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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.
- 4.5 A blue flash may be seen when adding Colilert® reagent to a water sample containing excessive chlorine residual. If this is seen, discontinue testing and report data as "Invalidated". The client must be notified immediately with a request that the site be re-sampled.



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 UV viewing cabinet equipped with a long wavelength (366 nm) UV light (6 watt).
- 6.3 Color comparator tray (purchased from manufacturer, IDEXX)
- 6.4 Incubator capable of maintaining $35 \pm 0.5^{\circ}\text{C}$ for 24-28 hours
- 6.5 IDEXX Quanti-Tray® Sealer
- 6.6 IDEXX Quanti-Tray®

7.0 REAGENTS AND STANDARDS

- 7.1 Colilert® Presence-Absence Medium (purchased from manufacturer, IDEXX); stored at $2 - 30^{\circ}\text{C}$ away from light.
- 7.2 Diluent: Sterile ASTM Type I reagent-grade water (Note: Only Type II reagent-grade water is required).

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. Potable and non-potable water samples must be kept at $\leq 10^{\circ}\text{C}$ 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. Potable source water samples (for enumeration of TC/*E. coli*) must be analyzed within



8 hours from the time of collection (i.e., usually 6 hours maximum in the field and 2 hours maximum in the laboratory).

Non-potable water samples must be analyzed within 8 hours from the time of collection (i.e., usually 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 – 1) sterile reagent-grade water alone and sterile reagent-grade water inoculated with the following control cultures: 2) non-fluorescent *Pseudomonas aeruginosa* (ATCC # 27853) (i.e., total coliform & *E. coli* negative); 3) *Klebsiella variicola* (ATCC # 31488) (i.e., total coliform positive and *E. coli* negative), and 4) *E. coli* (ATCC # 25922) (i.e., total coliform & *E. coli* positive) (see Table 2).

9.3 With each batch of samples, run a blank (sterile reagent-grade water); a method negative control (MNC), sterile reagent-grade water spiked with *Pseudomonas aeruginosa*; and two positive control samples [i.e., sterile reagent-grade water spiked with *Klebsiella variicola* (MSC), and sterile reagent-grade water spiked with *E. coli* (MPC)]. Acceptable results are as follows:

1. Method Laboratory Blank Control (MLB) – Total coliform (no yellow color) and *E. coli* (no fluorescence) negative.
2. Method Negative Control (MNC) – Total coliform (no yellow color) and *E. coli* (no fluorescence) negative.
3. Method Special Control (MSC) – Total coliform (yellow color) positive and *E. coli* (no fluorescence) negative.
4. Method Positive Control (MPC) – Total coliform (yellow color) and *E. coli* (fluorescence) positive.

The *P. aeruginosa* MNC can be run with routine batches but must be included for all enforcement or Environmental Strike Force samples. Note: If samples are to be analyzed for *E. coli* only, then only the *E. coli* positive culture control (MPC) is required.

9.4 If sufficient volume of sample is provided, 10% 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 $35 \pm 0.5^{\circ}\text{C}$.

- 11.6 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).



- 11.7 If the sample is total coliform-positive as indicated in Section 11.6, expose the sample to UV light in the UV viewing cabinet equipped with a 366-nm UV light (6 watt). 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) (see Table 3 for interpretation of Colilert medium reactions). 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.
- 11.8 If the sample results are questionable after 24 hours of incubation, (i.e., a yellow color change but less intense than the yellow color of the comparator), the sample may be incubated for an additional 4 hours (total of 28 hours) and rechecked for color and fluorescent reactions. If the yellow color intensifies, to match or exceed that of the comparator, 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 (ROL) for the duplicate MPNs as follows (*Standard Methods for the Examination of Water and Wastewater*, 22nd Edition, 2012, Section 9020-9e, Page 9-18):

$$\text{ROL for a Duplicate Set} = \text{Log}_{10} (\text{MPN } 1) - \text{Log}_{10} (\text{MPN } 2)$$

If either result of a duplicate set is < 1, add 1 to both values before calculating the logarithms as follows:

$$\text{ROL for a Duplicate Set} = \text{Log}_{10} [(\text{MPN } 1) + 1] - \text{Log}_{10} [(\text{MPN } 2) + 1]$$

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

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*, 22nd Edition, 2012. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.
- 16.2 IDEXX Colilert® Test Kit Procedure (2019 IDEXX Technical Insert).
- 16.3 IDEXX Quanti-Tray® 51 & 2000 Insert and Most Probable Number (MPN) Table (2013 IDEXX Technical Insert).
- 16.4 IDEXX Quanti-Tray® Sealer Model 2X Users-Manual.
- 16.5 References for required preservation temperature during sample transport:
 - Finished Drinking Water: Revised Total Coliform Rule – 40 CFR Part 141.852(a)(3)
 - Drinking Source Water: 40 CFR Part 141.74(a)(1) Footnote 2
 - Ground Water Rule: 40 CFR Part 141.402(c) Footnote 1
 - Wastewater, Ambient Water, and Sewage Sludge: 40 CFR Part 136.3(e) Table II



17.0 TABLES

TABLE 1. Quality Control Elements, Frequency, Acceptance Criteria, and Corrective Actions for the Enzyme Substrate Total Coliform/*E. coli* Test, Most-Probable-Number Procedure for the Analysis of Water Samples by SM 9223B Using Colilert Quanti-Tray®

QC Elements	Frequency	Acceptance Criteria	Corrective Action
Sample storage	Every sample	Potable water samples are analyzed ASAP and no later than 30 hours from the time of collection. Potable source water and non-potable water samples must be analyzed within 8 hours of collection (i.e., usually max 6 hours in the field and 2 hours in the laboratory). Potable and non-potable water samples must be stored at $\leq 10^{\circ}\text{C}$ from time of collection to time of analysis.	Qualify data (H or J) as estimated value (exceeded holding time or holding temperature, respectively) and contact sample collector to obtain new sample
Method Negative Control (MNC) (sterile reagent water spiked with <i>Pseudomonas aeruginosa</i>)	With each batch of 20 or fewer samples ($\geq 5\%$)	Total coliform and <i>E. coli</i> negative	Qualify data (J) as estimated value (<i>E. coli</i> and/or TC positive for the MNC – media or other failure) and contact sample collector to obtain new sample
Method Positive Control (MPC - equivalent to LCS-LFB; sterile reagent water spiked with <i>E. coli</i>)	With each batch of 20 or fewer samples ($\geq 5\%$)	Positive for both total coliform (yellow color) and <i>E. coli</i> (fluorescent)	Qualify data (J) as estimated value (<i>E. coli</i> and/or TC negative for the MPC – media or other failure) and contact sample collector to obtain new sample
Method Special Control (MSC - equivalent to LCS-LFB; sterile reagent water spiked with <i>Klebsiella variicola</i>)	With each batch of 20 or fewer samples ($\geq 5\%$)	Positive for total coliform (yellow) and negative for <i>E. coli</i> (non-fluorescent)	Qualify data (J) as estimated value (<i>E. coli</i> positive and/or TC is negative for the MSC – media or other failure) and contact sample collector to obtain new sample
Method Laboratory Blank Control (MLB - equivalent to LRB; sterile reagent water)	With each batch of 20 or fewer samples ($\geq 5\%$)	Total coliform and <i>E. coli</i> negative	Qualify data (B) as estimated value (<i>E. coli</i> and TC positive or only TC positive for a field sample, and for the MLB – laboratory contamination) and contact sample collector to obtain new sample.
Method Duplicate	10% (if sufficient sample volume is provided)	Range of Logs within current calculated acceptance criteria.	Qualify data (J) – unacceptable precision.



TABLE 2. Interpretation of Colilert® Medium Quality Control Reactions

Quality Control Organism	Yellow	Fluorescent
Method Laboratory Blank Control (MLB) (Equivalent to LRB) – Sterile reagent-grade water	No	No
Method Negative Control (MNC) (<i>Pseudomonas aeruginosa</i> , ATCC # 27853) (non-fluorescent strain)	No	No
Method Control Special (MCS) (<i>Klebsiella variicola</i> , ATCC # 31488)	Yes	No
Method Positive Control (MPC) (<i>E. coli</i> , ATCC # 25922)	Yes	Yes

TABLE 3. Interpretation of Colilert® Medium Reactions

Reaction	Result
Yellow	Total Coliform Positive
Fluorescent	<i>E. coli</i> Positive
Less intense yellow than the comparator	Total Coliform and <i>E. coli</i> Negative



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	22.1	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	22.5	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	57.5
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	29.9	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	60.4	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	42.5	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	95.7	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	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	90.6	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	127.6	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						



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	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.1	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	48.8	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	73.3	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	74.8	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	93.7	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	110.1	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	234.6	240.2	245.8	251.5	257.2	263.1
42	163.3	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	284.1	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	437.3
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	1966.3	2419.6	>2419.6