SOP-C-124b

Determination of Total Coliform & *Escherichia coli* by IDEXX Colilert®

Major Revision
Changes not indicated

Revision 19

Approval:

_____ _____ 8.23.23
Laboratory Manager Date

_____ _____ 8/23/2023
Concurrence Date

Effective date: 8-31-23

Renewal date: _____ Initials: _____

i. Identification of the method
- a. IDEXX Colilert® (2022)
- b. SM 9223 and 9223B (approved 2022)

ii. Applicable matrix or matrices
- a. Nonpotable water and soils

iii. Limits of detection and quantitation
- a. From LOD of 1 up to 2419.6 MPN/100 mL. Range may be extended upward with dilution.

iv. Scope and application, including parameters to be analyzed
- a. Total coliform, *Escherichia coli* (*E. coli*) in freshwater, wastewater and aqueous extracts

v. Summary of the method
- a. Bacteria are reacted with a prepared, specific reagent, sealed in multiple-tube trays, and incubated to allow bacteria to metabolize the chromogenic substrate reagent. Fluorescent and color changes indicate presence of the bacteria. The test is used in multi-well (enumeration) format. Color change from clear to yellow indicate coliform bacteria, while fluorescence of the yellow under a UV light indicates presence of *E. coli*.

vi. Definitions
- a. MPN= Most Probable Number of bacteria enumerated in a specified volume of water sample
- b. ATCC: American Type Culture Collection (1-800-638-6597) sets the industry standard identification for microorganisms.
- c. Refer to QAM-Q-101, “Laboratory Quality Control,” for standard QC definitions.

vii. Interferences
- a. Interferences: more than 2 million heterotrophic bacteria per 100 mL or grams
- b. Sample color interference may be corrected for by comparison to an untreated sample tray filled with sample.
- c. Noncoliform bacteria, such as *Aeromonas, Flavobacterium,* and *Pseudomonas* species, may produce small amounts of the enzyme, but are suppressed and generally will not produce a positive

response within the incubation time unless more than 10^4 colony-forming units (CFU)/mL (10^6 CFU/100 mL) are present.

d. Some strains of *Shigella* and *Salmonella* spp. also may produce a positive fluorescence response.

viii. Safety

a. All aspects of this procedure comply with QAM-S-101 “Laboratory Safety”.
b. Coliform bacteria are indicators of the potential presence of pathogens; therefore, avoid all direct skin contact with sample.
c. All work areas are to be disinfected upon completion of work with a 10% bleach solution or 70% Isopropyl Alcohol.
d. Isopropyl and Ethyl alcohol are flammable. Analysts will take care to avoid igniting the alcohol when disinfecting the biosafety cabinet or other surfaces.
e. Personal protection equipment such as gloves and lab coats should be used when working with samples.

ix. Equipment and supplies

a. Autoclave: A sterilization chamber capable of 121˚C equipped with a pressure gauge and an emergency pressure relief valve. Used for sterilization of applicable equipment. The autoclave is only used for sterilization of waste prior to disposal.
b. Sample bottles: 100 or 250 mL HDPE bottles or equivalent that have been certified sterile by the manufacturer and contain sodium thiosulfate for chlorine removal. Bottles are labeled with an “S” to identify the TIAER container type. The lot number of each sample bottle is recorded on the COC when samples are received.
c. Sample trays: Quanti-Tray®/2000 trays from IDEXX or equivalent.
d. Sample tray sealer: A Quanti-Tray® sealer model 2X or equivalent device capable of sealing the sample trays in preparation for incubating.
e. Incubator: A device with a chamber able to hold a constant temperature of 35˚C±0.5ºC.

f. IDEXX P/A Tray comparator: Used to compare with incubated samples to determine which wells are positive. The ID and expiration date of this comparator are recorded in each run.
g. Pipette: a certified sterile, graduated volume transfer device capable of accurately delivering volumes in increments of 0.1 mL, used in preparing dilutions.
h. UV lamp, long wave (365-366 nm)
i. Dye
j. 10% Bleach: Dilute a household bleach 1:10 in a spray bottle. Prepare fresh weekly.
k. Isopropyl alcohol (also isopropanol)
l. Biosafety cabinet. See QAM-I-121, "Operation and Calibration of the Biological Safety Cabinet."

x. Reagents and standards

a. Tryptic soy broth (TSB) or agar (TSA): a non-selective media, prepared in the lab or purchased pre-sterilized and ready to use
b. Sterile deionized water: Type II ASTM water purchased and certified sterile by the manufacturer. The vendor also supplies a certificate of analysis for chlorine residual, metals, ammonia/organic nitrogen, organic carbon and heterotrophic plate count. This water is stored away from incubators and sample prep area to avoid potential contamination.
c. Reagent snap-packs: Colilert® pre-formulated, buffer reagent pillows, or equivalent. Each batch is tested for positive and negative controls as described below. Store Colilert® in the dark.
d. Standards: Commercial standards are available for bacteria that represent positive and negative controls. Examples are "Quanti-Cult®" from IDEXX stable cultures of *Escherichia coli* (positive for coliform and *E. coli*- ATCC #25922 or 11775), *Pseudomonas aeruginosa* (negative for coliform and *E. coli*-ATCC #10145 or 27853) and *Klebsiella pneumoniae* (positive for coliform, but negative for *E. coli*-ATCC #31488). Prepare controls according to directions enclosed in the kits.

xi. Sample collection, preservation, shipment and storage

a. The TIAER Lab does not collect samples.
b. Samples should be preserved on ice for shipment or delivery to the lab if more than 1 hour transpires from time of collection.
c. Samples can be held at room temperature between receipt and analysis.
d. Holding time is 8 hours total: 6 hours to deliver to lab, then 2 hours more for the lab to complete; solid samples may have longer times on client specifications; certain project QAPPs may allow for other holding times

xii. Quality control

a. In samples with excessive chlorine, a blue flash may be seen when adding Colilert. If this is seen, consider sample invalid and discontinue testing.
b. If a water sample has some background color, compare inoculated Colilert sample to a control blank of the same water sample.
c. Sample bottles, pipets and trays are checked for sterility with non-selective growth media before first use. Record results in the Equipment Prep Log.
d. Sample bottles and trays are checked for auto-fluorescence before first use. Record equipment used and results of test. Record results in the Equipment Prep Log.
e. Sample bottles and pipets are tested for volume before first use. Pipets are verified with an analytical balance. Bottles are verified with Class A glassware. Record all raw data associated with volume checks. Record results in the Equipment Prep Log.
f. Media is checked for sterility, specificity and pH before first use. Record results in the Equipment Prep Log.
g. Media is stored separately from samples, as are other reagents and standards
h. Air quality is tested monthly with non-selective growth media (TSA). A settling plate is placed open on the workspace where sample analysis takes place for 15 minutes. The plate is incubated at 35˚C±0.5ºC for 48 hours. There can be no more than 15 CFUs (no more

than 1 CFU/minute). Record all raw data associated with the Air Quality Test. Log air test in the Maintenance Logbook.

i. The tray sealer is checked monthly. A small amount of dye is mixed into approximately 100 mL of DI and poured into a sample tray. The tray is sealed and each well is visually checked for leakage. Log the Sealer Check in the Maintenance Logbook.
j. Replace the bulb in the UV lamp annually. Verify the intensity of the lamp at least semi-annually with a calibrated light meter. If intensity drops by more than 20% in a 6-month period, replace the lamp. Record results in logbook.
k. All microbiology incubators will have the temperature of at least the top and bottom shelves recorded at least twice a day at least 4 hours apart on each day that samples are analyzed.
l. Initial DOC must be 4 aliquots, prepared and analyzed according to the method, either concurrently or over a period of days.
m. One positive sample is chosen for a duplicate count each month that the test is performed. If 2 or more analysts are trained on this procedure, each analyst counts the typical results. Counts shall be within 10% difference to be acceptable. If only one analyst is trained, the sample is counted twice by the analyst, with no more than 5% difference between counts.

xiii. Calibration and standardization

a. None

xiv. Procedure

a. Disinfect the work surface with 10% bleach for 15 minutes or 70% Isopropyl alcohol for 5 minutes immediately before beginning analysis.
b. Turn on sample tray sealer and allow warm up. The red light on the side of the sealer comes on when the sealer is turned on. The green light will come on when the sealer is ready for use, normally within 10 minutes.

c. Ensure that the incubator temperature is at the proper level for the test being performed (35˚C±0.5ºC) prior to use.
d. Ensure temperature distribution in incubator has been established. Record the logbook number and page of the distribution confirmation on the incubator, and transfer this information to each subsequent recording of data for this test.
e. If the sample has at least 1 inch (2.5 cm) of headspace, homogenize the sample by shaking 25 times each time the sample is opened. If the sample does not have adequate headspace, pour the entire sample volume into a sterile container large enough to ensure adequate mixing. Homogenize the sample in this container and aseptically transfer appropriate volumes for analysis. For drinking water testing, transfer exactly 100 mL to the original container for analysis.
f. Screen all water samples for chlorine presence in accordance with SOP-C-121, "Determination of Chlorine". Sodium thiosulfate has normally been added prior to sample collection. If chlorine is still detectable, the sample will be discarded and the client notified that resampling should be done. For samples with only 100 mL available and with no dilutions, testing for chlorine may not be possible. Notify the client for such an occurrence as the client may want to resample.
g. Label sterile mixing bottles with sample numbers, including a Method Blank every 20 samples and a duplicate for every 10. A method blank of sterile water is used for a negative control for each batch of 20 samples or less. Note that some project samples may not have enough liquid available for a sample duplicate. In this case, another sample may be chosen out of sequence for the duplicate, or the sample may be flagged as not having enough liquid available for a duplicate. No corrective action is required for this situation.
h. Break the seal on each labeled mixing bottle and add 100 mL of sample. For a method blank, use 100 mL of sterilized DI water.

i. Add the contents of the Colilert snap-pack buffer to the sterile mixing bottle, and shake until dissolved. Ensure that the lot of buffer been tested for positive and negative controls as described below. For each subsequent record of test data, record the logbook number and page where the passing controls can be found along with the lot number. The time of each sample is written on the associated tray and transferred to the personal log when counted the next day.
j. Open a sample tray by holding the well side facing the palm and squeezing so that the tray bends toward the palm. Gently pull the foil tab to separate the foil from the tray (Do not touch the inside of the tray).
k. Pour the sample/reagent mixture directly into the tray. Tap the small wells 2-3 times to release any air bubbles. Allow foam to dissipate.
l. Place the filled sample tray onto the rubber insert for the sealer with the plastic side facing down. Large and small wells are seated in the corresponding insert holes.
m. Push the rubber insert into the sealer with the small wells entering first. When the sealer begins to pull the insert, allow the sealer to feed the insert through.
n. Incubate the filled sample tray for 24-28 (maximum 28) hours at 35ºC±0.5ºC. Incubators may have preheated water in them to be used as heat sinks to prevent rapid temperature changes.
o. Repeat preparation steps for remaining samples and duplicates.
p. Samples that are turbid, highly colored, or that are taken during or just after a period of rainfall may require a dilution to be made. To dilute a sample, begin with the 99 mL of sterile deionized water in a reaction vessel. Use a sterile pipette to add 1 mL of sample to the vessel and dilute to the 100 mL mark for a 1:100 dilution. For a 1:10 dilution, aseptically pipette out 9 mL of dilution water and add 10 mL of sample. Quickly add a reagent -pack to buffer the sample. Shake until dissolved. Diluted sample result will be multiplied by the dilution factor to obtain true value. Other dilution factors may be used as appropriate

to obtain a range of acceptable results. If only 100 mL of sample is available, and the sample appears clear, do not dilute. Use the 100 mL sample for testing.

q. After the incubation period count the wells that are as yellow or more yellow than the IDEXX P/A comparator as positive for Total Coliforms.
r. Count the wells that fluoresce blueish-white under a 6-watt, 365nm, UV light within 5 inches of the sample, in a dark environment, as positive for *E. coli* with Colilert®.
s. If the chromogenic response is questionable, the incubation period may be extended for up to another 4 hours, but never exceed 28 hours maximum. The color comparator is used as needed.
t. For enumeration, use the number of positive small and large wells and the IDEXX Quanti-Tray®/2000 MPN (Attachment 1) table to determine the MPN, or use the manufacturer's computer calculation program. Counts and MPN values are recorded in the analysts' personal logbook.
u. Enter values and quality control information into the QC module of ESDMS or LIMS.
v. For solids extracted into water (not TNI accredited), weigh out a 10 gram portion of the fresh solid sample. A portion can be used to determine Percent Dry Solids in with SOP-C-130, "Determination of Total Solids".
w. Add 90 mL of sterile DI water to the 10 g portion in the mixing bottle.
x. Shake vigorously at least 25 times.
y. Transfer portions of the mixture to other mixing bottles for dilutions to 100 mL with sterile buffer solution. may be 1 mL or 10 mL of the diluted solid brought to 100 .
z. Proceed as done with water samples.
aa. Disinfect the work surface with 10% bleach for 15 minutes or 70% isopropyl alcohol for 5 minutes immediately after analysis.

xv. Data analysis and calculations

a. Enumeration: If sample dilutions are made, multiply the MPN value by the dilution factor to obtain the proper

quantitative result. The Control Limit Value (V_L) for duplicate precision is determined as follows:

$$\sum R_{log} = |\log(samp1) - \log(samp2)| + \ldots + |\log(samp\ n-1) - \log(sample\ n)|$$

$$R_{15} = \sum R_{log} / 15$$

$$V_L = 3.27 R_{15}$$

b. Determine the log of each of the duplicate values in the list of 15 data pairs. If either of a set of duplicate values is zero, add 1.0 to both values before calculating the logarithms.
c. Take the absolute value of the difference (range) of each paired log value range (R_{log}).
d. Sum all log value ranges and divide by the total number of pairs (n= 15) to get the average range R_{15}.
e. Multiply the average range R_{15} by 3.27 to get the Control Limit Value (V_L) which will be used to determine duplicate precision acceptability for the next duplicate pair. A rolling average is then used for each entry to calculate a new V_L.
f. Use percent dry calculation and other subsequent dilutions, including 10x (10 g + 90 mL) initial dilution, to calculate bacterial concentration in MPN/gram or MPN/100 gram, or dry depending on client reporting requirements.

xvi. Method performance

a. Method performance, data assessment and acceptance, corrective action: refer to QAM-Q-101, "Laboratory Quality Control"
b. Uncertainty for enumeration is determined at least annually (Table 1) and reported with all data.

xvii. Pollution prevention

a. Waste management and pollution prevention: refer to QAM-W-101, "Disposal of Laboratory Waste"

xviii. Data assessment and acceptance criteria for quality control measures

a. Data assessment and acceptance comply with QAM-Q-101, "Laboratory Quality Control".

b. For enumeration, determine the range of each pair of duplicate values by taking the absolute value of the difference of the two log values, as follows: R_X= |log (samp 1) - log (samp 2)|
c. If the range of logarithms of the pair (Rx) exceeds the Control Limit Value (V_L) described above, initiate corrective action for this nonconformance and the analysis results are considered questionable. If the calculated range is <0.5, sample duplicate values are acceptable, even if the Control Limit Value is exceeded.
d. All results since the last acceptable duplication value are also regarded as questionable and included in the corrective action.
e. Establish initial control limits by having one analyst make duplicates of the first 15 samples, or an equal division of the 15 samples among all analysts trained in the method. All analysts trained in this SOP participate in establishing future control limits based on the rolling recalculation of the Control Limit Value. A method blank acceptance criterion is less than one cell per tray.
f. Positive and negative controls are analyzed with each new lot of snap-buffer pillows.
g. Prepare the bacteria cultures according to the manufacturer's instructions.
h. Analyze each of the three bacteria types in the same manner as a regular sample.
i. The positive and negative controls are counted as follows:
    i. Colilert®- *Escherichia coli* count positive for both yellow and fluoresced wells.
    ii. Colilert®-*Pseudomonas aeruginosa* count negative for both yellow and fluoresced wells.
    iii. Colilert®-*Klebsiella pneumoniae* count positive for yellow wells, but negative for fluoresced wells.
j. Each lot number of sterile pipettes, bottles, dilution water, and trays are tested for sterility once per lot. Test sterile water with an equal volume of double-strength tryptic soy broth. Test supplies by pouring dilution water through each piece of equipment and into double-strength tryptic soy broth media of an equal volume. The broth with

sample is incubated at 35ºC±0.5ºC for 48 hours. A change in color or clarity of the broth indicates presence of nonspecific bacteria that requires corrective action.

k. In the CAR 08-0233 file is a study done by an outside consultant lab showing that airborne contamination may be caused in the TSB by *Bacillis sphaericus*. This bacterium is not of interest in any of the methods tested for by TIAER Lab. However, all incidences of sterility check failures are documented by a CAR.
l. Record all reagents prepared in the Reagent Log. Record preparation of standards (controls) in the Standards Log.
m. No washable labware are used for this procedure. All supplies and sterile water for dilutions are purchased pre-sterilized.
n. Positives observed before 24 hours and negatives observed after 28 hours are valid readings.
o. For each lot number used, maintain copies of all manufacturers' certificates for sterility checks of containers and water in non-selective media, analysis of the water meeting ASTM Type II specifications for metals and heterotrophic bacteria. These records are maintained for a minimum of five years after the end of a project for which these data are collected.
p. Verify the volume mark of each lot of dilution water bottles, sample collection bottles and disposable pipettes by testing one bottle from each lot. Record the logbook and page number of verification on the box containing the lot confirmed for volume. On each subsequent recording of data, list the confirmation logbook number and page for that lot used.
q. Avoid prolonged exposure of the substrate media to direct sunlight. Discard colored media.

xix. Corrective actions for out-of-control data

a. All aspects of this procedure comply with QAM-Q-101, "Quality Control" and QAM-Q-105 "Corrective Action".

xx. Contingencies for handling out-of-control or unacceptable data

a. All aspects of this procedure comply with QAM-Q-101, “Quality Control”.
b. If holding time has expired and data is not acceptable, resampling is the next option, if practical, as decided by the Program Manager.

xxi. Waste management

a. Waste management and pollution prevention: refer to QAM-W-101, "Disposal of Laboratory Waste".
b. Used IDEXX trays and other bacterial contaminated material are sterilized by autoclave prior to disposal and recorded in the Autoclave Log.
c. See QAM-I-110, “Operation and Calibration of the Autoclave.”

xxii. References

a. Colilert®, publication 06-12999-10, IDEXX Laboratories, Inc., 2022.
b. Quanti-Tray®, publication 06-02320-14, IDEXX Laboratories, Inc., 2013.
c. Quanti-Cult® Procedure, publication 06-01964-06, IDEXX Laboratories, Inc. (2013)
d. Standard Methods for the Examination of Water and Wastewater, (approved 2022), Methods 9223 & 9223B. QA/QC under SM 9020 (approved 2022).
e. National Environmental Laboratory Accreditation Conference (TNI, The NELAP Institute) standard, 2016.
f. CAR 08-0233, airborne contamination study
g. Appendix J, Uncertainty, FDA Screening and Testing Group, 2006.

xxiii. Any tables, diagrams, flowcharts and validation data

a. Determination of Uncertainty, Table 1
b. IDEXX Quanti-Tray®/2000 MPN (From publication 06-02320-14), Table 2
c. Signage to inform clients of headspace requirements, Diagram 1

Table 1. Determination of Uncertainty

Determine the square root of the variance of at least 7 replicates of BioBall® measurements on at least an annual basis. Report all measurements by this method with an uncertainty of plus or minus the value determined.

Example:

Replicate	Measurement
1	30
2	31
3	24
4	35
5	29
6	26
7	20
Variance (v)= 24.48	sq. root of v= 4.95

Replicate 1 reported as 30 ± 4.95
4.95 is the Standard Uncertainty

Table 2. IDEXX Quanti-Tray/2000 MPN Table

IDEXX Quanti-Tray*/2000 MPN Table

# Large Wells Positive	# Small Wells Positive																								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.3	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	17.5	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	61.8	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	93.5	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	93.6	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	90.8	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	366.4	387.3	410.6	435.2

06-03202-03 1/15

*Quanti-Tray is either a trademark or a registered trademark of IDEXX Laboratories, Inc. in the United States and/or other countries. Covered by U.S. Patent Numbers 4,925,789 ; 5,429,933 ; 5,518,892. Other patents pending.

Table 2. IDEXX Quanti-Tray/2000 MPN Table (cont.)

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	73.3	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	85.1	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	77.5	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	1988.3	2419.6	>2419.6

06-03202-03 2/15

Diagram 1: Signage to inform clients of headspace requirements