

Standard Operating Procedure for:

Escherichia coli and Total Coliform using the
IDEXX Quanti-Tray/2000 System with Colilert
Reagent



Karuk Tribe
Water Resources Department

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1 Identification of the test method

Escherichia coli using the IDEXX Quanti-Tray/2000 System with Colilert reagent (Standard Methods, 9223 B.)

2 Applicable matrix or matrices

This method is suitable for use with surface water samples.

3 Detection Limit

The detection limit for this analysis is 1 Most Probable Number (MPN) per 100mL of sample.

4 Scope of the test method

This standard operating procedure describes the test method for the collection and analysis of water samples for the enumeration of *Escherichia coli* (*E. coli*) and Total coliform bacteria.

5 Summary of test method

Surface water samples are collected in 120ml shrink-banded, sterile IDEXX bottles. An undiluted water sample will be analyzed from the sample collected. The Colilert® reagent is added directly to the 100 ml undiluted sample. Both are mixed thoroughly to dissolve the reagent. The sample is transferred to Quanti-Trays®/2000 and sealed using the Quanti-Tray sealer. Samples are incubated at $35.0 \pm 0.5^\circ \text{C}$ for 24 hours. Results are reported as MPN/100mL.

6 Definitions

- 6.1 Analytical batch: The set of samples processed at the same time
- 6.2 Control cultures: For each lot of medium, check analytical procedures by testing with known positive and negative control cultures. For example, *E.coli* is a positive control for this analysis and *Staphylococcus aureus* is a negative control.
- 6.3 Field duplicate (FD): Two samples taken at the same time and place under identical circumstances and that are treated identically throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage as well as laboratory procedures.
- 6.4 Laboratory reagent blank (LRB): An aliquot of sterilized water treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is to determine if the analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.
- 6.5 Laboratory duplicate (LD): Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation or storage procedures.

7 Interferences

Water samples containing humic or other material may be colored. If there is background color, compare inoculated trays to a control tray containing only water (SM, 9223 A.)

8 Health and safety

The analysis involves handling of freshwater samples that may contain live microorganisms and therefore pose some threat of infection. Laboratory personnel who are routinely exposed to such water samples are encouraged to protect themselves from water borne illnesses by wearing clean disposable gloves and washing their hands frequently.

The Colilert® reagent is not hazardous according to the manufacturer's material safety data sheet. The manufacturer does recommend wearing gloves and safety glasses while using this reagent and washing hands after use.

9 Personnel qualifications

Laboratory and field personnel shall have a working knowledge of this analytical procedure and will have received training from a Karuk tribal employee knowledgeable of the proper sample analysis procedures.

10 Equipment and supplies

- 10.1 Sterile, shrink-wrapped 100ml IDEXX bottles.
- 10.2 Quanti-Tray Sealer®: catalog number WQTS2X-115. IDEXX Laboratories, Inc., Westbrook, ME
- 10.3 Incubator

11 Reagents and standards

- 11.1 Colilert® reagent: for 100 ml samples, catalog number WP200. IDEXX Laboratories, Inc., Westbrook, ME.
- 11.2 Quanti-Tray®/2000: 100 trays containing 97 wells each, part number WQT-2K. IDEXX Laboratories, Inc., Westbrook, ME

12 Sample collection, preservation, shipment and storage

- 12.1 Arrive at site and record site number, date and time.
- 12.2 Immerse the thermometer or YSI handheld in the water and leave immersed five minutes before reading temperature. Avoid disturbing the bottom with the thermometer at the sample site.
- 12.3 Label bottle with location (geographic area name and stream or lake name), date, time, water temperature and sampler's initials. Label bottle before immersion using a black permanent marker or pre-printed labels. KARUK TRIBE Bacteria Lab, State Certified Lab, purchases only certified sterile, 100 ml, sealed containers from IDEXX.
- 12.4 Use latex gloves when handling bottles during sampling. Fingers contain contaminants such as nitrates. Bug repellents or sunscreen are particularly

troublesome as contaminants. Once the gloves are on, be careful not to touch your face, the ground, or anything but the bottles.

- 12.5 The sample should be taken from flowing, not stagnant water, facing upstream positioned in the thalweg.
- 12.6 Be sure to immerse the bottle completely, 10 cm (4 inches) deep, with mouth of bottle pointing upstream, so no water flows over your hand into the bottle. Be sure the bottle does not get near the bottom of the stream where sediments can be disturbed. Water samples should be collected 6-12 inches below the water surface. Fill bottle, to the 100ml line indicated, on **first immersion**, pour off the excess and cap. Do not under fill or over fill, do not redunk. If too much water is poured off, redo sample with new 100 ml container.
- 12.7 Do not touch bottle mouth or inside of cap. Be careful not to contaminate the sample with surface film, contact with human skin, breathing in/on the bottle or cap, etc. If stream is too shallow to immerse bottle fully, collect as much as possible, being very careful not to touch the bottom. Note depth on field notes.
- 12.8 Collect one "duplicate" sample every two weeks (sampling frequency). Sample sites chosen for duplicate sampling are selected at random among sites sampled. When a duplicate sample is selected for the site, repeat procedures as with normal stream samples. The duplicate is the second sample when two samples are collected. Duplicates document repeatability of individual sample collections and reproducibility of laboratory results.
- 12.9 Samples are analyzed in the Karuk Tribe Microbiology Lab. Keep samples cool while transporting. Store at 4 °C until analysis, but do not freeze. The maximum holding time is 6 hours.
- 12.10 For each sample, the location number, bottle numbers used and time collected will be recorded in the field sample log.
- 12.11 The samples will be kept in the possession of Karuk Tribe personnel who both collect and analyze the samples.

13 Quality control

13.1 **Accuracy:** Initial analyst demonstration of capability and for each new lot of Quanti-Tray/2000, analyze the following:

Check each new lot of Colilert.

- Shine the ultraviolet lamp on the media snap packs. If the lot is fluorescent it will be discarded.
- Dissolve one packet in 100 ml distilled water. Do not incubate. Check for fluorescents.
- Analyze sterile reagent water blank with each batch of samples to verify that there is a negative result from 24-28 hours
- Gravimetrically check each new lot of sterile, transparent, non-fluorescing 100-ml vessels to ensure the 100-mL fill line is accurately represented on the vessel.

Quanti-Cult Procedure

- a) Good laboratory practices will be used for this procedure.
- b) Pre-heat incubator to bring temperature up to 35°C.
- c) Pre-warm rehydration fluid vials to 35°-37°C. Use blue autoclavable foam vial holder to hold vials.
- d) Discard blue cap from rehydration fluid vial.
- e) Remove organism vial from pouch (vial with colorless cap).
- f) Transfer colorless cap onto pre-warmed rehydration vial and discard vial containing the desiccant.
- g) Place rehydration vials into the foam vial holder. Invert and place in incubator for 10 minutes at 35°-37°C.
- h) Fill four sterile IDEXX 100 ml. bottles with distilled water to fill line. Label three bottles with each bacteria name and one bottle “control”. Place in incubator until a temperature of 35° ± 0.5°C is reached.
- i) Remove vial from holder. Hold vial upside down and tap cap gently to mix. Remove cap and look at inside surface to ensure that no un-dissolved black particles are present. Inoculate an additional 10 minutes if present.
- j) Add entire contents of each appropriate bacteria vial to pre-warmed 100 ml. labeled bottles.
- k) Add Colilert reagent to sample bottles including control. Place in incubator and follow incubation instructions outlined in section 7. Do not place in Quanti-Trays.
- l) See figure 5.1 for Quanti-Cult datasheet

Results

The following results should be observed:

Organism	Result
<i>Escherichia coli</i>	Yellow wells, fluorescence
<i>Klebsiella pneumoniae</i>	Yellow wells, no fluorescence
<i>Pseudomonas aeruginosa</i>	Clear wells, no fluorescence
Method Blank	Clear wells, no fluorescence

- m) Disposal
 - i. All materials must be autoclaved prior to disposal and workspaces thoroughly disinfected.
 - ii. Dispose of media in accordance with Good Laboratory Practices.

Bibliography

- IDEXX, “Colisure Granulated Test Kit” product instructions. Number 06-03553-00, undated.
- IDEXX, “Quanti-Tray” product instructions. Number 06-02030-07, undated.

IDEXX, "Quanti-Tray Sealer Model 2X User Manual." Number 06-03128-02, undated.

EPA Region 9 Laboratory Standard Operating Procedure guidance. "Colilert, Colilert-18 and Colisure Total Coliform and E.coli Water Analysis".

Revised July 30, 1998.

Standard Method, "9223 B. Enzyme Substrate Test" 20th ed., rev. 1998.

13.2 **Precision:** the analyst should analyze:

- a. Field duplicates: one field duplicate per every 10 samples or 10%, randomly selected, taken at the same time
- b. Laboratory duplicates (LD): two replicates taken from the same collection bottle. Analyze at least one LD for every 10 samples collected.
- c. Laboratory reagent blank (LRB): analyze one LRB per sample batch.

13.3 Calculate Relative Percent Deviation (RPD). (Section 16.2)

14 Calibration and standardization

There are no calibration or standardization procedures for this method.

15 Procedure

See Appendix A for the manufacturer's instructions.

- 15.1 The 100ml duplicate water sample is shaken well just prior to preparation for analysis. Samples over the 100 ml mark must not be poured to volume. If there is at least 1" of headspace, the sample may be shaken and excess volume taken out with a sterile pipet. If there is insufficient headspace (<1") for proper mixing, do not pour off and discard a portion of the sample. Rather, pour the entire sample into a larger sterile container, mix properly, and proceed with the analysis.
- 15.2 Open a Colilert ampule and pour contents into either the diluted sample or undiluted sample. Repeat for the remaining sample.
- 15.3 Mix thoroughly, making sure the Colilert reagent is completely dissolved.
- 15.4 Follow manufacturer's instructions for preparation of Quanti-Tray/2000 and use of the Quanti-Tray Sealer.
- 15.5 Allow bubbles to settle or dissipate. Failure to do this may result in the wells filling or sealing improperly.
- 15.6 Record the sample's site code on the back of the well for identification purposes.
- 15.7 Record the lot number of the reagents and the wells used on the bench sheet in the comments section.
- 15.8 Incubate at $35.0 \pm 0.5^{\circ}\text{C}$ for 24 hours.
- 15.9 Count the number of small and large positive wells and refer to the MPN table to find the most probable number for Total coliform.

- 15.10 E. coli results are obtained by placing the wells under a black light and counting the number of fluorescent wells. Refer to the MPN table to determine the E. coli concentration.
- 15.11 Report results on the bench sheet.
- 15.12 The completed bench sheet should be reviewed by the analyst, the laboratory director and the QA manager.

16 Data acquisition, calculations, and reporting

- 16.1 For each sample analyzed, including quality control samples, record the number of small and large positive wells and the MPN in the appropriate places on the bench sheet (see below). Calculate precision for duplicate analyses using equation 1.
- 16.2 Equation 1. Precision (as RPD) = $\frac{(A - B) \times 100\%}{(A + B)/2}$

Where: A = MPN from aliquot A and
B = MPN from aliquot B

17 Computer hardware and software

Word: This document and attached bench sheet are prepared using Microsoft Word. The Word document file name for this SOP is: Standard Operating Procedures for Surface Water_E.coli.doc

18 Method performance

The Karuk Tribe lab must successfully analyze at least one set of PT material once every 12 months for each method for which it is certified. The only method that the Karuk Tribe lab is certified for is an enzyme substrate method using Colilert. The test must contain 10 samples; all shipped at the same time, and must be lyophilized, dehydrated or in aqueous state. The test should contain total coliforms, fecal coliforms, *E. coli*, non-coliforms and at least one blank. An acceptable result is a correct analysis of 9 of the 10 samples, with no false negative results. The WS (Water Supply) Micro Standards and the WP (Water Pollution) Micro Standards from Wibby Environmental should both be used.

- 18.1 Store PT standards in the refrigerator at ~ 4°C. Remember to record the temperature daily until samples are used.
- 18.2 Follow the date of analysis requirements provided by Wibby Environmental.
- 18.3 When ready to use, place the samples in the incubator for 2 hours at ~ 35°C.
- 18.4 Homogenize the samples by shaking vigorously prior to beginning sample analysis
- 18.5 If using the Water Pollution standards, be sure to complete sample dilutions before proceeding with analysis.

- 18.6 The Wibby Environmental WP Microstandard contains total Coliforms, Fecal Coliforms and E.coli within the EPA/NELAC specified concentration range of 20-2400 CFU/100ml
- 18.7 Report results to Wibby Environmental within the specified dates.
- 18.8 Include results in yearly laboratory report.

19 Data assessment and acceptable criteria for quality control measures

- 19.1 The analyst should review all data for correctness (e.g., use of MPN table).
- 19.2 Precision values are calculated for pairs of duplicate analyses.
- 19.3 Record the precision values as RPD on the bench sheet.
- 19.4 The desired precision is $\pm 20\%$ (RPD).
- 19.5 The desired detection limit is 1 MPN/100mL
- 19.6 The completed bench sheet is reviewed by the analyst's supervisor or the Karuk Tribe Lab Director

20 Corrective actions for out-of-control or unacceptable data

- 20.1 The results for precision and blank data are compared to the acceptable values for this analysis; $\pm 20\%$ and 1 MPN/100mL, respectively.
- 20.2 If a precision value exceeds 20% then the analyst should write in the comments section of the bench sheet: "These data are associated with an out-of-control duplicate analysis. The UCL = 20%." Note: "UCL" is the Upper Control Limit (i.e., 20%).
- 20.3 If a blank value exceeds 1 MPN/100mL then the analyst should write in the comments section of the bench sheet: "These data are associated with a blank value that exceeds the detection limit of 1 MPN/100mL."
- 20.4 The samples cannot be reanalyzed because the sample volume will be depleted after the initial analysis.
- 20.5 If data are unacceptable for any reason, the analyst should review their analytical technique prior to conducting this analysis again.

21 Waste management

The wastes generated in this method are not hazardous. The water can be discarded in the laboratory sink. Quanti-Trays are hand delivered to *Basic Lab* in Redding, CA where they are autoclaved and then discarded with the trash.

22 References

- 22.1 IDEXX Laboratories, Inc. Westbrook, ME 04092. Instruction manuals for use of: Colilert®, Quanti-Tray®/2000, and Quanti-Tray Sealer®.
- 22.2 Standard Methods for the Examination of Water and Wastewater. Method 9223 B., APHA, 21st Edition, 2005.

23 Reporting

23.1 Procedure for notification of clients for drinking water positives:

It is required to formally notify water suppliers for Total or *E.coli* positive test results as follows:

- a) Formally document notification- see attachment data sheet
- b) Notify an officially designated contact person with the water supplier.
- c) Arrange for re-sampling within 24 hours. Important: the notification must be with a “live voice” The message should not be left on an answering machine when notifying the water supplier.

23.2 A final report will be written by the Project Coordinator and QC Officer as well as sent to any funders, local Boards, EPA, and other interested parties. The final report will include the table and graphs that were developed for the web site and media, and it will describe the program's goals, methods, quality control results, data interpretation, and recommendations. Following notification of the Tribal Council, the Karuk Water Resources Program would then inform the North Coast Regional Water Quality Control Board staff and work cooperatively with that agency for abatement of problems.

24 Tables, diagrams, flowcharts and validation data

24.1 See Appendix A for MPN tables and Quanti-Tray/2000 instructions.

24.2 See below for the bench sheet. The analyst should make a copy of this form for each batch of samples analyzed.

24.3 The following validation procedures will be established throughout the project: Equipment will be calibrated at the start of the season and checked before each collection; blind field replicates will be submitted to the laboratory, which will also analyze lab duplicates, blanks, and Quanti-Cult new lot checks, chain of custody will be maintained; field sheets and data entry will be checked by the QC Officer; descriptive statistics and graphs will be produced.



Quanti-Tray[®]

Insert & MPN Table



Quanti-Tray Certificate of Sterility

This certifies that the enclosed Quanti-Trays have been sterilized with ethylene oxide.
For further information or documentation, contact IDEXX Laboratories, Inc.

IDEXX Laboratories
One IDEXX Drive, Westbrook, Maine 04092 USA

Phone 1-800-321-0207
Fax 207-856-0630

IDEXX

06-02030-09

Quanti-Tray®

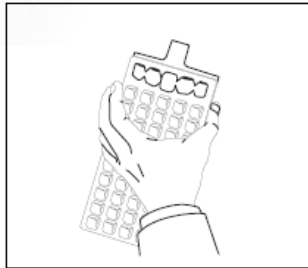
Introduction

IDEXX Quanti-Trays are designed to give quantitated bacterial counts of 100 ml samples using IDEXX Defined Substrate Technology* reagent products. Add the reagent/sample mixture to a Quanti-Tray, seal it in a Quanti-Tray Sealer and incubate per the reagent directions. Then count the number of positive wells and use the MPN table attached to determine the Most Probable Number (MPN).

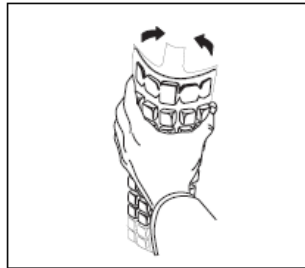
Contents

This package contains 100 sterile, 51-well Quanti-Trays.

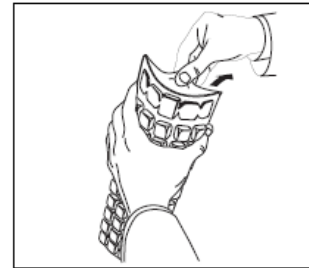
User Instructions



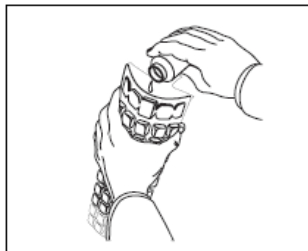
1. Use one hand to hold a Quanti-Tray upright with the well side facing the palm.



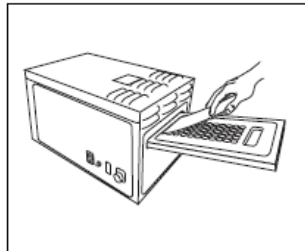
2. Squeeze the upper part of the Quanti-Tray so that the Quanti-Tray bends towards the palm.



3. Open the Quanti-Tray by pulling the foil tab away from the well side. Avoid touching the inside of the foil or tray.



4. Pour the reagent/sample mixture directly into the Quanti-Tray avoiding contact with the foil tab. Allow foam to settle.



5. Place the sample-filled Quanti-Tray onto the rubber tray carrier of the Quanti-Tray Sealer with the well side (plastic) of the Quanti-Tray facing down to fit into the carrier.

6. Seal according to Sealer instructions.

7. Incubate according to reagent directions.

8. Count positive wells and refer to the MPN table on the back of this instructions sheet to find the Most Probable Number (MPN).

9. Dispose of media in accordance with Good Laboratory Practices.

**For Technical Assistance, visit www.idexx.com/water, or
in the U.S. and Canada, call 1-800-321-0207 or 207-856-0496**

IDEXX Laboratories, Inc. One IDEXX Drive, Westbrook, Maine 04092 USA

* Quanti-Tray and Defined Substrate Technology are trademarks or registered trademarks of IDEXX Laboratories, Inc. in the United States and/or other countries. US Patent Numbers 4,925,789 ; 5,429,933 ; 5,518,992. Other patents pending.

IDEXX

IDEXX Quanti-Tray™/2000 MPN Table

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	23.2	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	17.2	18.2	19.2	20.3	21.3	22.3	23.3	24.4	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	13.2	14.3	15.3	16.3	17.3	18.3	19.3	20.4	21.4	22.4	23.4	24.5	25.7	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	17.5	18.5	19.5	20.6	21.6	22.6	23.6	24.7	25.8	27.0	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.5	15.6	16.6	17.6	18.6	19.6	20.7	21.7	22.7	23.7	24.8	25.9	27.0	28.1	29.3
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.8	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	20.0	21.1	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0	32.1	33.2	34.3	35.4	36.5	37.6	38.7
12	13.5	14.6	15.7	16.8	17.9	19.0	20.1	21.2	22.3	23.4	24.5	25.6	26.7	27.8	28.9	30.0	31.1	32.2	33.3	34.4	35.5	36.6	37.7	38.8	40.0
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	43.6
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	36.6	37.8	39.0	40.2	41.4	42.6	43.8	45.2
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.8	32.0	33.2	34.4	35.6	36.8	38.0	39.2	40.4	41.6	42.8	44.0	45.2	46.5
16	18.9	20.1	21.3	22.5	23.7	24.9	26.1	27.3	28.5	29.7	30.9	32.1	33.3	34.5	35.7	36.9	38.1	39.3	40.5	41.7	42.9	44.1	45.3	46.5	47.8
17	20.3	21.6	22.8	24.1	25.3	26.5	27.7	28.9	30.1	31.3	32.5	33.7	34.9	36.1	37.3	38.5	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.4
18	21.8	23.1	24.3	25.5	26.7	27.9	29.1	30.3	31.5	32.7	33.9	35.1	36.3	37.5	38.7	39.9	41.1	42.3	43.5	44.7	45.9	47.1	48.3	49.5	50.8
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	38.9	40.2	41.5	42.8	44.1	45.4	46.7	48.0	49.3	50.6	51.9	53.2	54.5
20	24.9	26.2	27.5	28.8	30.1	31.4	32.7	34.0	35.3	36.6	37.9	39.2	40.5	41.8	43.1	44.4	45.7	47.0	48.3	49.6	50.9	52.2	53.5	54.8	56.1
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.8	37.1	38.4	39.7	41.0	42.3	43.6	44.9	46.2	47.5	48.8	50.1	51.4	52.7	54.0	55.3	56.6	57.9
22	28.2	29.5	30.8	32.1	33.4	34.7	36.0	37.3	38.6	39.9	41.2	42.5	43.8	45.1	46.4	47.7	49.0	50.3	51.6	52.9	54.2	55.5	56.8	58.1	59.4
23	29.9	31.2	32.5	33.8	35.1	36.4	37.7	39.0	40.3	41.6	42.9	44.2	45.5	46.8	48.1	49.4	50.7	52.0	53.3	54.6	55.9	57.2	58.5	59.8	61.1
24	31.7	33.1	34.5	35.9	37.3	38.6	40.0	41.3	42.6	43.9	45.2	46.5	47.8	49.1	50.4	51.7	53.0	54.3	55.6	56.9	58.2	59.5	60.8	62.1	63.4
25	33.6	35.0	36.4	37.8	39.2	40.6	42.0	43.4	44.8	46.2	47.6	48.9	50.3	51.7	53.0	54.4	55.8	57.2	58.6	60.0	61.4	62.8	64.2	65.6	67.0
26	35.5	36.9	38.3	39.7	41.1	42.5	43.9	45.3	46.7	48.1	49.5	50.9	52.3	53.7	55.1	56.5	57.9	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1
27	37.4	38.9	40.4	41.8	43.2	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	62.8	64.2	65.6	67.0	68.4	69.8	71.2
28	39.5	41.0	42.4	43.8	45.2	46.6	48.0	49.4	50.8	52.2	53.6	55.0	56.4	57.8	59.2	60.6	62.0	63.4	64.8	66.2	67.6	69.0	70.4	71.8	73.2
29	41.7	43.2	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	62.8	64.2	65.6	67.0	68.4	69.8	71.2	72.6	74.0	75.4
30	43.9	45.5	47.1	48.7	50.2	51.7	53.2	54.7	56.2	57.7	59.2	60.7	62.2	63.7	65.2	66.7	68.2	69.7	71.2	72.7	74.2	75.7	77.2	78.7	80.2
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.0	59.7	61.4	63.1	64.8	66.5	68.2	69.9	71.6	73.3	75.0	76.7	78.4	80.1	81.8	83.5	85.2	86.9
32	48.7	50.4	52.1	53.8	55.5	57.2	58.9	60.6	62.3	64.0	65.7	67.4	69.1	70.8	72.5	74.2	75.9	77.6	79.3	81.0	82.7	84.4	86.1	87.8	89.5
33	51.2	53.0	54.8	56.5	58.3	60.1	61.9	63.7	65.5	67.3	69.1	70.9	72.7	74.5	76.3	78.1	79.9	81.7	83.5	85.3	87.1	88.9	90.7	92.5	94.3
34	53.9	55.7	57.5	59.4	61.3	63.1	65.0	66.9	68.8	70.6	72.5	74.4	76.3	78.2	80.1	82.0	83.9	85.8	87.7	89.6	91.5	93.4	95.3	97.2	99.1
35	56.8	58.6	60.5	62.4	64.3	66.2	68.1	70.0	71.9	73.8	75.7	77.6	79.5	81.4	83.3	85.2	87.1	89.0	90.9	92.8	94.7	96.6	98.5	100.4	102.3
36	59.8	61.7	63.7	65.6	67.5	69.4	71.3	73.2	75.1	77.0	78.9	80.8	82.7	84.6	86.5	88.4	90.3	92.2	94.1	96.0	97.9	99.8	101.7	103.6	105.5
37	62.9	64.9	66.9	68.9	70.9	72.9	74.9	76.9	78.9	80.9	82.9	84.9	86.9	88.9	90.9	92.9	94.9	96.9	98.9	100.9	102.9	104.9	106.9	108.9	110.9
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.5	90.8	93.1	95.4	97.7	100.0	102.3	104.6	106.9	109.2	111.5	113.8	116.1	118.4	120.7
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	85.9	88.4	90.8	93.2	95.6	98.0	100.4	102.8	105.2	107.6	110.0	112.4	114.8	117.2	119.6	122.0	124.4	126.8
40	73.8	76.2	78.5	80.9	83.3	85.7	88.1	90.5	92.9	95.3	97.7	100.1	102.5	104.9	107.3	109.7	112.1	114.5	116.9	119.3	121.7	124.1	126.5	128.9	131.3
41	78.0	80.5	83.0	85.5	88.0	90.5	93.0	95.5	98.0	100.5	103.0	105.5	108.0	110.5	113.0	115.5	118.0	120.5	123.0	125.5	128.0	130.5	133.0	135.5	138.0
42	82.6	85.2	87.8	90.4	93.0	95.6	98.2	100.8	103.4	106.0	108.6	111.2	113.8	116.4	119.0	121.6	124.2	126.8	129.4	132.0	134.6	137.2	139.8	142.4	145.0
43	87.6	90.4	93.2	96.0	98.8	101.6	104.4	107.2	110.0	112.8	115.6	118.4	121.2	124.0	126.8	129.6	132.4	135.2	138.0	140.8	143.6	146.4	149.2	152.0	154.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.1	125.5	128.9	132.3	135.7	139.1	142.5	145.9	149.3	152.7	156.1	159.5	162.9	166.3	169.7	173.1
45	99.3	102.5	105.7	109.0	112.3	115.6	118.9	122.2	125.5	128.8	132.1	135.4	138.7	142.0	145.3	148.6	151.9	155.2	158.5	161.8	165.1	168.4	171.7	175.0	178.3
46	106.3	109.8	113.4	117.0	120.6	124.2	127.8	131.4	135.0	138.6	142.2	145.8	149.4	153.0	156.6	160.2	163.8	167.4	171.0	174.6	178.2	181.8	185.4	189.0	192.6
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	144.8	149.5	154.2	159.0	163.8	168.6	173.4	178.2	183.0	187.8	192.6	197.4	202.2	207.0	211.8	216.6	221.4	226.2
48	123.9	128.1	132.1	136.3	140.6	145.0	149.4	153.8	158.2	162.6	167.0	171.4	175.8	180.2	184.6	189.0	193.4	197.8	202.2	206.6	211.0	215.4	219.8	224.2	228.6
49	135.5	140.8	146.4	152.3	158.5	165.0	171.7	178.4	185.1	191.8	198.5	205.2	211.9	218.6	225.3	232.0	238.7	245.4	252.1	258.8	265.5	272.2	278.9	285.6	292.3

IDEXX Quanti-Tray™/2000 MPN Table

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	22.6	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	17.7	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	22.3	23.4	24.5	25.6	26.7	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	18.6	19.7	20.8	21.9	23.0	24.1	25.2	26.3	27.4	28.5	29.6	30.7	31.8	33.0	34.1	35.3	36.4
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	34.2	35.4	36.6	37.8
11	12.0	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	14.9	16.3	17.7	19.1	20.5	21.9	23.3	24.7	26.1	27.5	28.9	30.3	31.7	33.1	34.5	35.9	37.3	38.7	40.1	41.5	42.9	44.3	45.7	47.1
13	14.8	16.0	17.1	18.3	19.5	20.8	22.1	23.3	24.5	25.8	27.1	28.4	29.7	31.0	32.3	33.6	34.9	36.2	37.5	38.8	40.1	41.4	42.7	44.0	45.3
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.8	44.1	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.8	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.8	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	38.9	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	48.3	49.7	51.1	52.5	53.9	55.3	56.7	58.1	59.5
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	36.9	38.3	39.7	41.1	42.5	43.9	45.3	46.7	48.1	49.5	50.9	52.3	53.7	55.1	56.5	57.9	59.3	60.7	62.1	63.5
24	31.7	33.1	34.5	35.9	37.3	38.7	40.1	41.5	42.9	44.3	45.7	47.1	48.5	49.9	51.3	52.7	54.1	55.5	56.9	58.3	59.7	61.1	62.5	63.9	65.3
25	33.6	35.0	36.4	37.8	39.2	40.6	42.0	43.4	44.8	46.2	47.6	49.0	50.4	51.8	53.2	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2
26	35.5	36.9	38.3	39.7	41.1	42.5	43.9	45.3	46.7	48.1	49.5	50.9	52.3	53.7	55.1	56.5	57.9	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1
27	37.4	38.8	40.2	41.6	43.0	44.4	45.8	47.2	48.6	50.0	51.4	52.8	54.2	55.6	57.0	58.4	59.8	61.2	62.6	64.0	65.4	66.8	68.2	69.6	71.0
28	39.5	41.0	42.4	43.8	45.2	46.6	48.0	49.4	50.8	52.2	53.6	55.0	56.4	57.8	59.2	60.6	62.0	63.4	64.8	66.2	67.6	69.0	70.4	71.8	73.2
29	41.7	43.2	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	62.8	64.2	65.6	67.0	68.4	69.8	71.2	72.6	74.0	75.4
30	43.9	45.5	47.1	48.7	50.3	51.9	53.5	55.1	56.7	58.3	59.9	61.5	63.1	64.7	66.3	67.9	69.5	71.1	72.7	74.3	75.9	77.5	79.1	80.7	82.3
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	66.8	68.5	70.2	72.0	73.7	75.4	77.1	78.9	80.6	82.3	84.0	85.7	87.4
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.1	69.9	71.7	73.5	75.3	77.1	78.9	80.7	82.5	84.3	86.1	87.9	89.7	91.5
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.5	69.4	71.3	73.2	75.1	77.0	78.9	80.8	82.7	84.6	86.5	88.4	90.3	92.2	94.1	96.0
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.7	74.6	76.5	78.4	80.3	82.2	84.1	86.0	87.9	89.8	91.7	93.6	95.5	97.4	99.3
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.3	80.3	82.3	84.3	86.3	88.3	90.3	92.3	94.3	96.3	98.3	100.3	102.3	104.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.6	75.6	77.6	79.6	81.6	83.6	85.6	87.6	89.6	91.6	93.6	95.6	97.6	99.6	101.6	103.6	105.6	107.6
37	62.9	64.9	66.9	68.9	71.0	73.0	75.0	77.0	79.0	81.0	83.0	85.0	87.0	89.0	91.0	93.0	95.0	97.0	99.0	101.0	103.0	105.0	107.0	109.0	111.0
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.8	86.0	88.2	90.4	92.6	94.8	97.0	99.2	101.4	103.6	105.8	108.0	110.2	112.4	114.6	116.8	119.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.8	93.2	95.6	98.0	100.4	102.8	105.2	107.6	110.0	112.4	114.8	117.2	119.6	122.0	124.4	126.8
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.6	93.0	95.4	97.8	100.2	102.6	105.0	107.4	109.8	112.2	114.6	117.0	119.4	121.8	124.2	126.6	129.0	131.4
41	78.0	80.5	83.0	85.5	88.0	90.5	93.0	95.5	98.0	100.5	103.0	105.5	108.0	110.5	113.0	115.5	118.0	120.5	123.0	125.5	128.0	130.5	133.0	135.5	138.0
42	82.6	85.2	87.8	90.5	93.2	95.9	98.6	101.3	104.0	106.7	109.4	112.1	114.8	117.5	120.2	122.9	125.6	128.3	131.0	133.7	136.4	139.1	141.8	144.5	147.2
43	87.6	90.4	93.2	96.0	98.9	101.9	104.8	107.8	110.8	113.8	116.8	119.8	122.8	125.8	128.8	131.8	134.8	137.8	140.8	143.8	146.8	149.8	152.8	155.8	158.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.5	133.1	136.7	140.3	143.9	147.5	151.1	154.7	158.3	161.9	165.5	169.1	172.7	176.3
45	99.3	102.5	105.8	109.2	112.6	116.0	119.4	122.8	126.2	129.6	133.0	136.4	139.8	143.2	146.6	150.0	153.4	156.8	160.2	163.6	167.0	170.4	173.8	177.2	180.6
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	141.8	146.1	150.4	154.7	159.0	163.3	167.6	171.9	176.2	180.5	184.8	189.1	193.4	197.7	202.0	206.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.0	160.0	165.0	170.0	175.0	180.0	185.0	190.0	195.0	200.0	205.0	210.0	215.0	220.0	225.0	230.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.6	171.5	177.4	183.3	189.2	195.1	201.0	207.0	213.0	219.0	225.0	231.0	237.0	243.0	249.0	255.0	261.0
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.4	213.3	222.4	231.5	240.6	249.7	258.8	267.9	277.0	286.1	295.2	304.3	313.4	322.5	331.6
50	14																								

Karuk Tribe Microbiology Laboratory
Log for formally notifying client of a Total or E. coli
Positive Test Result

Name of Water Supplier	Phone Number	Date of Sample	Date of Notification	Time of notification	Date scheduled for Re-Sample	Staff Initials Performing Notification