

Standard Operating Procedure for:


Escherichia coli and Total Coliform using the
IDEXX Quanti-Tray/2000 System with Colilert reagent
(4010R03 Ecoli IDEXX.doc)

Missouri State University

and

Ozarks Environmental and Water
Resources Institute (OEWRi)

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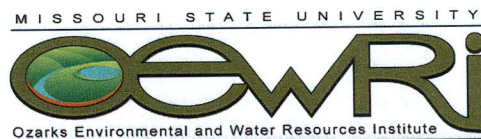
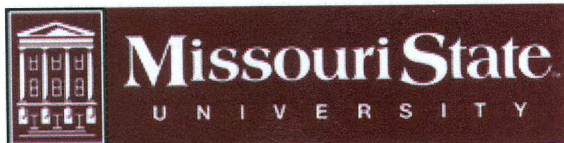


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1 Identification of the test method

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 Standard for E. coli

The U.S. EPA and Missouri's Department of Natural resources require a geometric mean of 126 MPN per 100 mL of water as a water quality standard for recreational contact during the time period of April 1st to October 31st.

6 Summary of test method

Surface water samples are collected in EPA-accepted Whirl-Pak® Coli-Test bags. An undiluted sample will be analyzed from the sample collected. There will be a preliminary sampling event prior to project startup to allow the laboratory to determine if certain sites require dilutions. If a sample requires dilutions a 1:10 dilution of the sample water will be analyzed from the sample collected. The Colilert® reagent is added directly to the 100 mL undiluted sample in the Coli-test bag and to 100 mL of the diluted sample. Both are mixed thoroughly to dissolve the reagent. The samples are transferred to Quanti-Trays®/2000 and sealed using the Quanti-Tray sealer. Samples are incubated at 35.0 ± 0.5° C for 24 hours. Results are reported as MPN/100mL.

7 Definitions

7.1 Analytical batch: The set of samples processed at the same time

7.2 Chain of Custody (COC): used to describe the written record of the collection, possession and handling of samples. Unless the analytical results are expected to be challenged legally, a chain of custody is not required. Sample collection information and forms are adequate.

7.3 Control cultures: For each order of Quanti Trays with different lot numbers, 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.

7.4 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.

- 7.5 Field blank (FB): An aliquot of deionized water treated as a sample in all aspects, including exposure to a sample bottle holding time, preservatives, and all pre-analysis treatments. The purpose is to determine if the field or sample transporting procedures and environments have contaminated the sample.
- 7.6 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.
- 7.7 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.
- 7.8 Method detection limit (MDL): The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.
- To calculate the MDL:
 - Prepare a solution with the concentration of TN near the estimated MDL
 - Analyze seven portions of this solution over a period of at least three days
 - Include all sample processing steps in the determination
 - Calculate the standard deviation (s).
 - From a table of the one-sided *t* distribution select the value of *t* for $7 - 1 = 6$ degrees of freedom at the 99% level. This value is 3.14
 - The product 3.14 times *s* is the desired MDL.
- 7.9 Relative Percent Difference (RPD): calculated as the difference between a sample and duplicate results, divided by the average of the sample and duplicate results, multiplied by 100%.

8 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.)

9 Health and safety

- 9.1 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.

- 9.2 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.

10 Personnel qualifications

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

11 Equipment and supplies

- 11.1 Whirl-Pak® Coli-Test bags: 100 mL, sterilized and containing 10 mg of sodium thiosulfate. Nasco, 901 Janesville Avenue, Fort Atkinson, Wisconsin 53538.
- 11.2 Sampling pole (for whirl-Pak bags): 12 feet long, with retainer rings to hold the bags.
- 11.3 Laboratory glassware as needed: sterilized beaker; place in a 170°C oven for 2 hours, then allow to cool.
- 11.4 Quanti-Tray Sealer®: catalog number WQTS2X-115. IDEXX Laboratories, Inc., Westbrook, ME
- 11.5 Mohr pipets, 10 mL: place in a 170°C oven for 2 hours, then allow to cool.

12 Reagents and standards

- 12.1 Colilert® reagent: for 100 mL samples, catalog number WP200. IDEXX Laboratories, Inc., Westbrook, ME.
- 12.2 Quanti-Tray®/2000: 100 trays containing 97 wells each, part number WQT-2K. IDEXX Laboratories, Inc., Westbrook, ME
- 12.3 Dilution water: sterile DI. Fisher Scientific, Biotech Grade Sterile Water, Cat. No. BP 2485-4, 4 liter PolyPac.

13 Sample collection, preservation, shipment and storage

- 13.1 Arrive at site and record site number, date, time and appearance of water as either cloudy or clear.
- 13.2 Collect a 100 mL surface water sample from the shoreline by placing a Whirl-Pak bag into the retainer attached to a twelve foot pole. Lower the bag into the water facing upstream approximately 6 - 10 feet from the shoreline. Lower the bag into the water far enough so that the water flows freely into the bag. Avoid capturing large particulate matter in the bottle by moving the bag (maintaining the upstream direction).
- 13.3 The sample containers will be pre-labeled with a number representing the sampling sites. For each collection day, the date, stream flow and precipitation within the last 24 hours will be recorded in the field log book.

- 13.4 For each sample, the location number, bottle numbers used and time collected will be recorded in the field sample log.
- 13.5 The samples will be kept in the possession of Missouri State University personnel who both collect until transferring the samples to the laboratory with appropriate chain of custody forms where they become the laboratory's responsibility.
- 13.6 Samples will be transported to the laboratory in coolers containing ice. Transport should not take longer than three hours.
- 13.7 The samples will be stored at 4°C until analysis. The maximum hold time is 6 hours.

14 Quality control

- 14.1 Accuracy: Initial analyst demonstration of capability and for each new lot of Quanti-Tray/2000, analyze the following:
 - a. 1 replicates of a positive control organism,
 - b. 1 replicates of a negative control organism, and
 - c. 1 replicates of sterilized water.
- 14.2 Precision: the analyst should analyze:
 - a. Field duplicates (FD): two samples collected at the same time in the field
 - b. Laboratory duplicates (LD): two replicates taken from the same collection bottle. Analyze at least one LD for every 10 samples collected.
 - c. Calculate Relative Percent Difference (RPD).
- 14.3 Laboratory reagent blank (LRB): analyze one LRB per sample batch.

15 Calibration and standardization

There are no calibration or standardization procedures for this method.

16 Laboratory Procedure

- 16.1 The Whirl-Pak bag containing the water sample is shaken well just prior to preparation for analysis. For samples known to routinely go over range prepare a 1:10 dilution. Remove a 10 mL portion of the sample with a sterilized glass pipet and add it to 90 mL of sterile water in a sterilized 100 mL beaker to make the 1:10 dilution.
- 16.2 Open a Colilert ampule and pour contents into either the sample bag or diluted sample in the beaker. Repeat for the remaining sample.
- 16.3 Mix thoroughly making sure the Colilert reagent is completely dissolved by retying and shaking the bag or by stirring the diluted sample with a sterilized glass stir rod.
- 16.4 Follow manufacturer's instructions for preparation of Quanti-Tray/2000 and use of the Quanti-Tray Sealer (See Appendix A for the manufacturer's instructions).

- 16.5 Allow bubbles to settle or dissipate. Failure to do this may result in the wells filling or sealing improperly.
- 16.6 Record the sample's site code on the back of the well for identification purposes.
- 16.7 Record the lot number of the reagents and the wells used on the bench sheet in the comments section.
- 16.8 Incubate at $35.0 \pm 0.5^{\circ}\text{C}$ for 24 hours.
- 16.9 Count the number of small and large positive wells that are yellow and refer to the MPN table to find the most probable number for Total coliform.
 - a. If sample is yellow, but lighter than Colilert Comparator incubate 4 additional hours.
 - b. Reread results and if color intensifies well is considered positive.
- 16.10 Check yellow wells, both large and small, for presence of fluorescence by placing the wells under a black light. If well fluoresces but less than Colilert Comparator, sample is considered negative. Refer to the MPN table to determine the E. coli concentration.
- 16.11 Record results on the bench sheet.
- 16.12 The completed bench sheet should be reviewed by the analyst, the microbiology faculty supervisor, and the OEWR QA manager.

17 Data acquisition, calculations, and reporting

- 17.1 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.

$$\text{Equation 1. Relative Percent Difference (RPD)} = \left[\frac{(A - B)}{(A + B)/2} \right] 100$$

Where: A = original sample MPN
B = duplicate sample MPN

- 17.2 Calculation of water sample concentrations, corrected for dilution: For samples for which dilution was required, the concentration in the original water sample is calculated using equation 2.

$$\text{Equation 2: } \text{MPN}_{\text{sample}} = \text{MPN}_{\text{analysis}} \times (10.0 \text{ mL} / V_{\text{aliquot}})$$

Where: $\text{MPN}_{\text{sample}}$ = the concentration in the original water sample,
 $\text{MPN}_{\text{analysis}}$ = the concentration of the solution as determined in (15.11)
 V_{aliquot} = the volume of the aliquot diluted to 10 mL in (15.1).

- 17.3 The evaluation of MDL and precision require calculation of standard deviation. Standard deviations should be calculated as indicated below, where n = number of samples, x = concentration in each sample. Note: This is the sample standard deviation calculated by the STDEV function in Microsoft Excel.

$$s = \left(\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1} \right)^{1/2}$$

18 Computer hardware and software

- 18.1 Word: This document and attached bench sheet are prepared using Microsoft Word. The Word document file name for this SOP is: 4010R01 Ecoli IDEXX.doc.
- 18.2 IDEXX MPN Generator 3.2: For use in confirming MPN of sample results, as well as calculation of 95% confidence intervals.

19 Method performance

There are no published method performance data for this method.

20 Pollution prevention

All wastes from these procedures shall be collected and disposed of according to existing waste policies within the MSU Biology Department. Volumes of reagents made should mirror the number of samples being analyzed. These adjustments should be made to reduce waste.

21 Data assessment and acceptable criteria for quality control measures

- 21.1 The analyst should review all data for correctness (e.g., use of MPN table).
- 21.2 Precision values are calculated for pairs of duplicate analyses. Record the precision values as a percent on the bench sheet. The desired precision is $\pm 20\%$.
- 21.3 The desired detection limit is 1 MPN/100mL.
- 21.4 The completed bench sheet is reviewed by the analyst's supervisor or the OEWRI QA coordinator. Using IDEXX MPN Calculator 3.2 MPN results are compared and 95% confidence intervals are generated.

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

- 22.1 The results for precision and blank data are compared to the acceptable values for this analysis; $\pm 20\%$ and 1 MPN/100mL, respectively.
- 22.2 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%).

- 22.3 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.”
- 22.4 The samples cannot be reanalyzed because the sample volume will be depleted after the initial analysis.
- 22.5 If data are unacceptable for any reason, the analyst should review their analytical technique prior to conducting this analysis again.

23 Waste management

The wastes generated in this method are not hazardous. They can be discarded in the following manner: the water can be discarded in the laboratory sink and Quanti-Trays are autoclaved and then can be discarded with the paper trash.

24 References

- 24.1 IDEXX Laboratories, Inc. Westbrook, ME 04092. Instruction manuals for use of: Colilert®, Quanti-Tray®/2000, and Quanti-Tray Sealer®.
- 24.2 Missouri Department of Natural Resources. E. Coli Monitoring at the Lake of the Ozarks, Environmental Services Program/Water Protection Program fact sheet. 2011.
- 24.3 EPA, Environmental Protection Agency. Water Quality Standards for Coastal and Great Lakes Recreation Waters. 2004.
- 24.4 Standard Methods for the Examination of Water and Wastewater. Method 9223 B., APHA, 21st Edition, 2005.

25 Tables, diagrams, flowcharts and validation data

- 25.1 See Appendix A for MPN tables and Quanti-Tray/2000 instructions.
- 25.2 See below for the bench sheet. The analyst should make a copy of this form for each batch of samples analyzed.

Appendix A



Quanti-Tray[®]/2000

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

06-02320-07

Quanti-Tray[®]/2000

Introduction

IDEXX Quanti-Tray/2000 is 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/2000, seal it in a Quanti-Tray Sealer and incubate per the reagent directions. Then count the number of positive large and small wells and use the MPN table attached to determine the Most Probable Number (MPN).

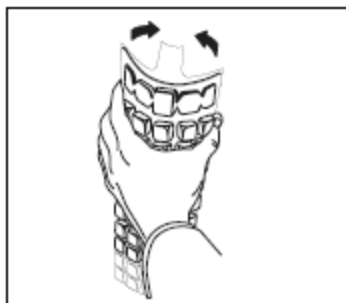
Contents

This package contains 100 sterile Quanti-Tray/2000s.

User Instructions



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



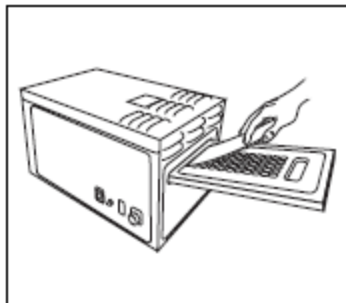
2. Squeeze the upper part of the Quanti-Tray so that the Quanti-Tray bends towards the palm.



3. Gently pull foil tab to separate the foil from the tray. 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. Tap the small wells 2-3 times to release any air bubbles. Allow foam to settle.



5. Place the sample-filled Quanti-Tray onto the Quanti-Tray/2000 rubber insert of the Quanti-Tray Sealer with the well side (plastic) of the Quanti-Tray facing down.

6. Seal according to Sealer instructions.
7. Incubate according to reagent directions.
8. Count large and small positive wells and refer to the Quanti-Tray/2000 MPN table 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 1-207-856-0496.

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

* Quanti-Tray and Defined Substrate Technology are either trademarks or registered trademarks 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.

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	22.7	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	22.3	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	57.6
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	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	116.8	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	177.8	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	155.2	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	172.1	178.6	185.3	192.4	200.0	208.2	216.7	225.6	234.9	244.8	255.2	266.0	277.3	289.1	301.5	324.2
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							

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	38.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.5	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	42.8	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	93.7
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	77.6	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	91.6	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	302.8	312.3	322.3	332.5	343.0	353.8	364.9	376.2	387.8	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	419.6	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

Missouri State University
Ozarks Environmental and Water Resources Institute
Springfield, Missouri
Escherichia coli IDEXX System

Analyst: _____

Project: _____

Date analyzed: _____

Data reviewed by:	

Incubator Data: Start Day/Time: _____
End Day/Time: _____

Start Temperature (°C): _____
End Temperature (°C): _____

Sample Data		Large well Positive count		Small well Positive count		Most Probable Number (MPN/100mL) * [Mean of A + B]
Sample Identification	Date Collected	Replicate		Replicate		
		A	B	A	B	

Comments: _____

*See MPN tables.

Missouri State University
 Ozarks Environmental and Water Resources Institute
 Springfield, Missouri
Total coliform IDEXX System

Analyst: _____

Project: _____

Date analyzed: _____

Data reviewed by:	

Incubator Data: Start Day/Time: _____
 End Day/Time: _____

Start Temperature (°C): _____
 End Temperature (°C): _____

Sample Data		Large well Positive count		Small well Positive count		Most Probable Number (MPN/100mL) * [Mean of A + B]
Sample Identification	Date Collected	Replicate		Replicate		
		A	B	A	B	

Comments: _____

* See MPN tables.