Ozarks Environmental and Water Resources Institute (OEWRI)
Missouri State University (MSU)

Standard Operating Procedure for:

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

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Identification of the test method
Escherichia coli (*E. coli*) using the IDEXX Quanti-Tray/2000 System with Colilert reagent (Standard Methods, 9223 B.)

Applicable matrix or matrices
This method is suitable for use with surface water samples.

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

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* and total coliform bacteria.

Standard for *E. coli*
The U.S. Environmental Protection Agency (EPA) and the Missouri Department of Natural Resources (MDNR) have established specific water quality standards for recreational water contact. These standards apply between April 1st and October 31st and for water in losing streams year-round (MDNR 2022). These standards are as follows:

1. For waters designated for whole body contact (WBC) Class A recreation during the recreational season or in losing streams at any time, the geometric mean (GM) for *E. coli* should be less than 126 per 100 milliliters (mL) of water (USEPA 1986, MDNR 2022).
2. For WBC-B designated waters, the GM value should be less than 548 per 100 mL (MDNR 2022).
3. Water designated for secondary contact recreation should not exceed 1,134 *E. coli* per 100 mL of water (MDNR 2022).

In addition to these standards, the EPA has defined a statistical threshold value (STV) for E. coli at 410 colony-forming units (CFU) per 100 mL of water (EPA 2012). This STV is akin to the 90th percentile, meaning it should not be exceeded by more than 10% of the samples taken. To assess compliance with these standards, the EPA recommends conducting weekly sampling and evaluating both the GM and the STV over a 30-day period or relating sampling frequency to the intensity of use of the water body (EPA 1986). Typically, E. coli data is presented in terms of CFU per 100 mL of water or most probable number (MPN), both signifying comparable measurements (MDNR 2014).

Summary of test method

Surface water samples are collected in EPA-accepted Screw-Top Sterile Coliform Polypropylene Water Sample Bottles. 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, 1:1 dilution, or both 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 ± 0.5º C for 24 hours. Results are reported as MPN/100mL.

Definitions

1. Control cultures: For each order of Quanti Trays with different lot numbers, analytical procedures are checked 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.
2. 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.
3. 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.
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.
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.
6. 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%.
7. 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. The detection limit for this analysis is 1 Most Probable Number (MPN) per 100mL of sample.

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.

Health and safety

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.
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.

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.

Equipment and supplies

1. Quanti-Tray Sealer®: catalog number WQTS2X-115. IDEXX Laboratories, Inc., Westbrook, ME
2. Sealed Screw-Top Coliform Polypropylene Water Sample Bottle: 120 mL with 100mL fill line, sterilized and containing 10 mg of sodium thiosulfate. Thermo Fisher Scientific, 168 Third Avenue, Waltham, MA USA 02451.
3. Ultraviolet lamp, long wave for use in testing UV-florescent wells (6-watt, 365-nm UV light).

Reagents and standards

1. Colilert® reagent: for 100 mL samples, catalog number WP200. IDEXX Laboratories, Inc., Westbrook, ME.
2. Quanti-Tray®/2000: 100 trays containing 97 wells each, part number WQT-2K. IDEXX Laboratories, Inc., Westbrook, ME.
3. IDEXX Colilert® and Colilert-18 Quanti-Tray/2000 Comparator: catalog number WQT2KC. IDEXX Laboratories, Inc., Westbrook, ME.
4. Dilution water: sterile DI. Fisher Scientific, Biotech Grade Sterile Water, Cat. No. BP 2485-4, 4-liter PolyPac.

Sample collection, preservation, and storage procedure

1. Arrive at sampling site and record site number, date, and time.
2. Collect a 100 mL surface water sample by placing the sample bottle into the water. Lower bottle into the water, facing upstream, far enough so that the water flows freely into the bottle. Avoid capturing large particulate matter in the bottle.
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.
4. For each sample, the location number, bottle numbers used, and time of collection will also be recorded in the field sample log.
5. The samples will be kept in the possession of OEWRI personnel until transferred to the laboratory with appropriate chain of custody forms, where they become the laboratory's responsibility.
6. Samples will be transported to the laboratory in coolers containing ice. Transport should not take longer than three hours.
7. The samples will be stored at 4ºC until analysis. The maximum hold time is 6 hours; however, they should be processed as soon as possible.

Quality control

1. Accuracy: Initial analyst demonstration of capability and for each new lot of Quanti-Tray/2000, analyze the following:
    a. 1 replicate of a positive control organism
    b. 1 replicate of a negative control organism
    c. 1 replicate of sterilized water
2. Precision: the analyst should analyze:
    a. Field duplicates (FD): two samples collected at the same time in the field to describe site variability.
    b. Laboratory duplicates (LD): two counts are performed on positive wells to ensure counts are accurate.
    c. Field blank (FB): Used to determine if the field or sample transporting procedures and environments have contaminated the sample.
    d. Laboratory reagent blank (LRB): analyze one LRB per sample batch.

Calibration and standardization

There are no calibration or standardization procedures for this method.

Laboratory procedure

1. If the water sample bottle contains more than 100mL of water, decant the excess water to the 100mL fill line. The bottle containing the water sample should be shaken well just prior to preparation for analysis. For samples known to routinely go over range, prepare a 1:1 or 1:10 dilution.
    a. If the sample was collected during baseflow and is expected to go over range, prepare a 1:1 dilution.
    b. If the sample was collected during stormflow, assume sample will go over range and prepare a 1:10 dilution immediately.
2. Open a Colilert ampule and pour contents into sample bottle. Mix thoroughly and ensure the reagent is completely dissolved. Repeat for the remaining samples.
3. 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).
4. Allow bubbles to settle or dissipate. Failure to do this may result in the wells filling or sealing improperly.
5. Record the sample's site code on the back of the tray for identification purposes.
6. Record the lot number of the reagents and the trays used on the bench sheet in the comments section.
7. Incubate at 35.0 ± 0.5ºC for 24 hours. Record the time and incubation temperature at the start and end of incubation.
8. Count the number of small and large positive wells that are yellow, equal to or greater than the comparator, and refer to the MPN table to find the most probable number for total coliforms. The Colilert Comparator can be used to distinguish a minimal positive from a negative test result.
    a. If well is yellow, but lighter than Colilert Comparator, incubate 4 additional hours and reread results.
    b. Any intensity of yellow color equal to or greater than the comparator is considered a positive test result.
    c. If sample remains less yellow than the comparator, it is negative for total coliforms and *E coli*.
9. Check yellow wells, both large and small, for presence of fluorescence by placing the wells under a blacklight. A well that fluoresces but is not yellow should **not** be marked as an *E.* coli positive well. Refer to the MPN table to determine the *E. coli* concentration.
    a. If the yellow well fluoresces, but less than Colilert Comparator, the well is considered negative.
    b. If the yellow well fluoresces equal to or greater than Comparator, the well is positive.
10. Record results on the bench sheet.

11. The completed bench sheet should be reviewed by the analyst, the laboratory manager, and the OEWRI QA coordinator.

Data acquisition, calculations, and reporting

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.

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

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

For samples for which dilution was required, the concentration in the original water sample is calculated using Equation 2.

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

Where: MPN_{sample} = the concentration in the original water sample
$MPN_{analysis}$ = the concentration of the solution as determined on bench sheet
$V_{aliquot}$ = the volume of the aliquot diluted to 10 mL

Computer hardware and software

IDEXX MPN Generator 3.2: For use in confirming MPN of sample results, as well as calculation of 95% confidence intervals.

Method performance

There are no published method performance data for this method.

Pollution prevention

All waste 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.

Data assessment and acceptable criteria for quality control measures

1. The analyst should review all data for correctness (e.g., use of MPN table).
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 ± 20%.

3. The desired detection limit is 1 MPN/100mL.
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.

Corrective actions for out-of-control or unacceptable data

1. The results for precision and blank data are compared to the acceptable values for this analysis; ± 20% and 1 MPN/100mL, respectively.
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%).
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."
4. The samples cannot be reanalyzed because the sample volume will be depleted after the initial analysis.
5. If data are unacceptable for any reason, the analyst should review their analytical technique prior to conducting this analysis again.

Waste management

The waste generated by this method is not hazardous. Waste 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.

References

1. EPA, Environmental Protection Agency. Water Quality Standards for Coastal and Great Lakes Recreation Waters. 2004.
2. EPA, Environmental Protection Agency. Implementation Guidance for Ambient Water Quality Criteria for Bacteria - Office of Water. Washington, D.C., USA. 1986.
3. EPA, Environmental Protection Agency. Recreational Water Quality Criteria – Office of Water 1.2.1. Washington, D.C., USA. 2012.
4. IDEXX Laboratories, Inc. Westbrook, ME 04092. Instruction manuals for use of: Colilert®, Quanti-Tray®/2000, and Quanti-Tray Sealer®.
5. MDNR, Missouri Department of Natural Resources. Rules of Department of Natural Resources. Division 20 Clean Water Commission. Chapter 7 – Water Quality. 2022. 10 C.S.R. § 20-7.031.
6. MDNR, Missouri Department of Natural Resources. Water Protection Program Fact Sheet, PUB2401. 2014.

7. MDNR, Missouri Department of Natural Resources. *E.Coli* Monitoring at the Lake of the Ozarks, Environmental Services Program/Water Protection Program fact sheet. 2011.
8. Standard Methods for the Examination of Water and Wastewater. Method 9223 B., APHA, 21st Edition. 2005.

Tables, diagrams, flowcharts and validation data

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

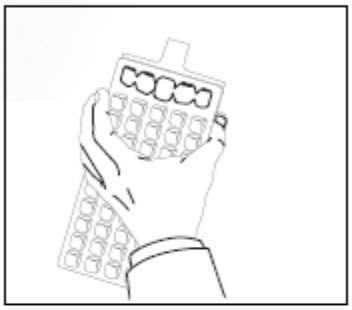
Introduction

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).

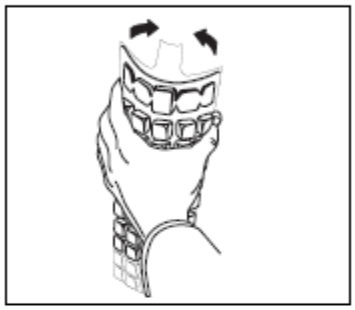
Contents

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

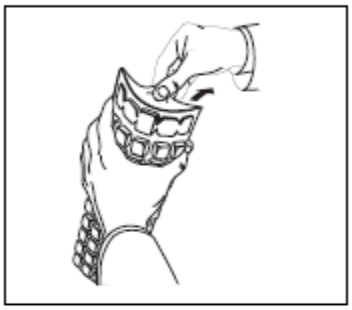
User Instructions



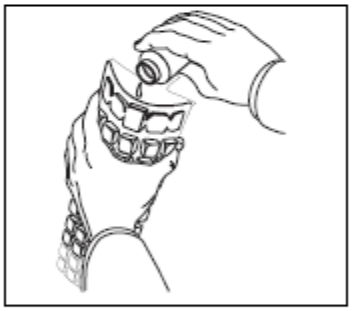
1. Use one hand to hold a Quanti-Tray upright with the well side facing the palm.



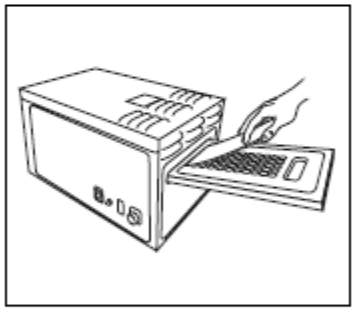
2. Squeeze the upper part of the Quanti-Tray so that the Quanti-Tray bends towards the palm.



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

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	12.2	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	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	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	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	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	365.4	387.3	410.6	435.2

*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.

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	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7
28	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.9	112.0	114.2	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4
31	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.7	156.4
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4
35	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7
38	127.9	130.8	133.8	136.8	139.9	143.0	146.2	149.4	152.6	155.9	159.2	162.6	166.1	169.6	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.7	203.7	207.7
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.1
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.1	224.2	229.4	234.8	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	1966.3	2419.6	>2419.6

Total Coliform and *E. coli* Bench Sheet

Missouri State University
Ozarks Environmental and Water Resources Institute

Analyst: _____ Project: _____ Date analyzed: _____

Incubator Data:

Start Day/Time: _____ Start Temperature (ºC): _____

End Day/Time: _____ End Temperature (ºC): _____

Data reviewed by:	

Sample Data		**Total Coliform (yellow)**							***E. coli* (yellow & fluorescent)**						
		Large Well Positive Count		Small Well Positive Count		Most Probable Number **Coliform** (MPN/100mL)* [Mean of A + B]	95% Confidence Limit		Large Well Positive Count		Small Well Positive Count		Most Probable Number ***E. coli*** (MPN/100mL)* [Mean of A + B]	95% Confidence Limit	
Sample Identification	Date Collected	Replicate		Replicate			-	+	Replicate		Replicate			-	+
		A	B	A	B				A	B	A	B			

Comments:_____

*See MPN table