Standard Operating Procedure

Enzyme Substrate Test for the Detection of Total Coliforms and *Escherichia coli* in Ambient Waters

Commonwealth of Kentucky
Energy and Environment Cabinet
Department for Environmental Protection
Division of Water

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2. Document Revision History

Date of Revision	Page(s) Revised	Revision Explanation
03/18/2011	Entire Document	New Document
08/27/2018	Entire Document	Reformatted entire document. Added Sample Dilution procedures. Added Computer Software section.

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3. Table of Contents

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4. Scope and Applicability

This document provides laboratory methods and quality assurance/quality control (QA/QC) activities for *Escherichia coli* (*E. coli*) and total coliforms samples collected by Kentucky Division of Water (KDOW) personnel from ambient waters.

5. Summary of Method

KDOW follows the multi-well enzyme substrate method for the enumeration of *E. coli* and total coliforms (APHA 2012). Currently, KDOW exclusively uses IDEXX Laboratories, Inc. Defined Substrate Technology® reagent products to conduct these analyses. The reagent Colilert® contains two nutrient-indicators, ortho-Nitrophenyl-β-galactoside (ONPG) and 4-methyl-umbelliferyl-β -D-glucoside (MUG), which are metabolized by total coliforms and *E. coli.* When total coliforms metabolize ONPG, the sample turns yellow; when *E. coli* metabolize MUG, the sample fluoresces.

On the occasion that KDOW personnel determine that any of the procedures described in this manual are inappropriate, inadequate, or impractical, the variant procedure will be documented in field log books or field observation sheets, as will a description of the circumstances requiring its use.

This manual should be considered a dynamic document that is reviewed and updated as new methods are employed.

6. Acronyms

CFU: Colony Forming Unit
COC: Chain of Custody
DEP: Department for Environmental Protection
KDOW: Kentucky Division of Water
K-WADE – Kentucky Water Assessment Data for Environmental Monitoring
MPN: Most Probable Number
NIST: National Institute of Standards
PPE: Personal Protective Equipment
QA: Quality Assurance
QAPP: Quality Assurance Project Plan
QC: Quality Control
SOP: Standard Operating Procedures
TSB: Tryptic Soy Broth

7. Human Health

Field staff working in and around potentially contaminated surface waters should receive an immunization shot for Hepatitis A in accordance with DEP Policy SSE-708. In addition, staff should receive immunization for Hepatitis B and tetanus, to aid in the prevention of contracting those pathogens. All field staff should also be trained in CPR, First Aid and Blood Borne Pathogens in accordance with DEP Policy SSE-711.

Personal protective equipment (PPE) should be used when analyzing samples from waters known to have the potential for adverse health effects, or in unknown waters that have been determined impaired, but without specifically identified pollutants. Field staff must review “DEPs Personal Protective Equipment Program” document located at the following intranet website: http://eecintra.eppc.pri/DEP/HS/Health/Forms/AllItems.aspx.

The following items are examples of PPE that may be used during analyses:

- Powderless latex or nitrile gloves
- Goggles, or glasses with splash guards
- Lab coats or aprons

8. Cautions and Interferences

Cautions

The following precautions shall be considered when performing bacteriological analyses

Sample Handling Precautions

- All samples must be stored in a secure location to ensure sample integrity.
- All samples must have appropriate Chain of Custody (COC) documentation and COC documentation must remain with all samples throughout analyses.

Analytical Precautions

- These procedures are intended for the analysis of ambient water samples and should not be used for drinking water or wastewater compliance purposes.
- Colilert® results are definitive only after a 24-28 hour incubation period. Positives for both total coliforms and *E. coli* observed before 24 hours and negatives observed after 28 hours are also valid (IDEXX 2013a).
- If sample dilutions are made, multiply the most probable number (MPN) value by the dilution factor to obtain the final result.
- Use only sterile, nonbuffered, oxidant-free water for dilutions.
- In samples with excessive chlorine, a blue flash may be seen when adding Colilert®. If so, consider the sample invalid and discontinue testing.
- Aseptic technique must always be followed when using Colilert®.

Interferences

Staff must remain cognizant of potential contamination sources during laboratory analyses. Always disinfect bench tops with anti-bacterial cleaning products. Floors must be cleaned on a regular basis, and hands must be washed with anti-bacterial soap prior to lab analyses.

Water with high iron or manganese levels in the presence of hydrogen sulfide may react with the Colilert® media to cause an atypical color change (greenish-black or black). Any tests with atypical color changes are invalid and must be repeated on new sample water.

9. Personnel Qualifications

All personnel involved in microbiological laboratory analysis will meet at least the minimum qualifications for their job classification. Analysts should participate in annual sample collection and laboratory training provided by the WQB Microbiology Laboratory Coordinator. On-the-job training will continue through interaction with experienced personnel and outside training, pending availability. Each analyst must read this SOP annually.

10. Equipment and Supplies

Table 1 contains a list of supplies that may be required for laboratory analysis.

Table 1. Laboratory Equipment and Supplies

Sample Containers	Laboratory Supplies
Wide-mouth sterile plastic container	IDEXX Quanti-Tray®/2000 multi-well trays
Non-corrosive glass bottles w/non-leaking ground glass stoppers/caps	IDEXX Quanti-Tray®/2000 comparator
Sterile plastic bags	Colilert® test media
Chemical Preservatives	Sterile mixing vessels
Sodium thiosulfate ($Na_2S_2O_3$) tablets	Quanti-cult® QC kit
Laboratory Equipment	SimPlate® for HPC
Incubator	Tryptic Soy Broth (TSB) (single & double strength)
Biological indicator incubator	Biological Indicator Capsules
Autoclave	Maximum temperature thermometer
Refrigerator	NIST calibrated thermometers (with 0.1° increments)
IDEXX Quanti-Tray® Sealer	Specific conductivity low-level calibration standard
Conductivity meter	pH 4, 7 and, 10 calibration buffers
pH meter	Sterile 5mL, and 1mL glass pipettes
Balance	Pipette bulb/automatic pipettor
Water Purification System	1L glass flasks
6-watt, 365-nm UV lamp	100mL class A or B graduated cylinder
	Glass beakers
	Anti-bacterial cleaning wipes
	Powderless latex/nitrile gloves
	Sterile Water

11. Step by Step Procedure

11.1 Sample Collection

Sample collection should follow the methods outlined in *Sampling Surface Water Quality in Lotic* Systems (KDOW 2011). Samples must be collected in sterile plastic containers (minimum volume = 120mL). Ideally, at least 2.5cm of headspace should be left in the container after collection to allow for adequate mixing during analysis. If headspace is < 2.6cm after collection **DO NOT** decant the sample to create headspace. Close the container lid and preserve as defined below.

A replicate/split sample is collected in a single sterile plastic container having a minimum volume of 220mL. When collecting duplicate samples, two separate 120mL containers should be used to collect sample water. Duplicate samples should be collected concurrently.

11.2 Sample Handling and Preservation

Sample handling should follow the methods outlined in *Sample Control and* Management (KDOW 2009). Samples must be preserved on ice within 15 minutes of collection and cooled to a holding temperature of ≤10°C. If the source water of the sample is suspected to contain residual chlorine and/or halogen, then 0.1mL of a 10% sodium thiosulfate ($Na_2S_2O_3$) solution should be added to dechlorinate and neutralize residual halogen (40 CFR 136.3, 2017). Samples have a total holding time of 8 hrs, and should only be accepted by the lab if they have been properly preserved and delivered with enough time for analysis to occur within the 8 hour holding time.

If sample analysis cannot be conducted immediately upon lab receipt, the analyst must ensure that a holding temperature of ≤10°C is maintained either by keeping samples in the cooler they were delivered

in or by storing them in a refrigerator that is monitored for temperature. Sample analysis must occur within the 8 hour holding time or discarded.

11.3 Sample Preparation and Analysis

Sample Preparation

Prior to analysis, ensure that all sample bottles contain the required volume (100mL) and at least 2.5 cm of headspace for sufficient mixing by shaking. If the sample volume is < 100mL (or <200mL for replicate/split samples) then discard the sample. If the bottle was over-filled (i.e. < 2.5 cm of headspace), gently shake the bottle ~25 times, and use a sterile pipette to withdraw and discard the excess sample until the appropriate headspace is achieved (2.5 cm).

Analytical Procedures

Analysis using Colilert® Test kits should be performed according to the Quanti-Tray® Enumeration Procedure (Appendix A ; IDEXX 2013a) and user instructions for Quanti-Tray/2000 in (Appendix B; IDEXX 2013b). Aseptic technique MUST be followed during this procedure:

1. Label mixing vessel lids and Quanti-Trays (trays) with the Locale Name/Station ID. The date of analysis must be written on the trays.
2. Turn on Quanti-Tray Sealer (sealer) and allow to warm up. An orange light indicates that the unit is on. A green light will turn on when the sealer is ready to use.
3. Gently shake sample bottle ~25 times. Pour sample to the fill line of a 100mL IDEXX sterile mixing vessel.
4. For replicate/split samples: Gently shake sample bottle ~25 times. Pour sample to the fill line of one sterile mixing vessel. Recap sample bottle and shake bottle ~25 times. Pour remainder of sample to the fill line of a second sterile mixing vessel.
5. Add contents of one Colilert media pack to the vessel. Cap vessel and shake until the media completely dissolves.
6. Allow any foam to settle. Pour sample/media mixture into a tray. Avoid contact with the foil tab and the inside of the tray while pouring.
7. Close the tray by folding the foil tab. Tap the small wells 2-3 times to release any bubbles. Allow any foam to settle.
8. Place the tray in the rubber insert of the sealer. Place the rubber insert and tray onto the feeder tray of the sealer well-side down with the tray's tab facing to the left onto the feeder tray of the sealer.
9. Gently push the tray until the sealer grabs hold. Once the tray is sealed, remove it from the rubber insert and inspect it to ensure that it was properly sealed and all wells contain sample.
10. Invert the trays and incubate in a 35°± 0.5°C incubator for 24 hours.

Sample Dilutions

The Colilert method has an upper quantification range limit of 2420 colony forming units (cfu) for sample water run without dilutions. Dilutions, using sterile non-buffered water, can be made in order to obtain quantifiable results >2,420cfu. Typical dilutions include 1:10, 1:100, and 1:1000. Record the dilution factor on the laboratory bench sheet in the "Dilution Factor" field under the "Sample Info" column. The following steps should be followed:

1. Determine the dilution factor and choose a suitable-sized sterile pipette and pipettor.

2. Open the sterile mixing vessel, placing the lid face up on the counter top. Shake the sample container gently ~25 times in order to homogenize the sample.
3. Open the sample container and carefully insert the pipette being careful not to touch the sides of the container with the pipette or hands. Draw the desired amount of sample into the pipette (e.g. 10mL if dilution factor equals 1:10). Dispense sample into the open sterile mixing vessel. Close the sample container.
4. Open the sterile water container, placing the lid face up on the countertop. Carefully pour the sterile water into the mixing vessel until the volume reaches the 100mL fill line. If the vessel becomes overfilled, repeat step 3 using a new mixing vessel.
5. Add the contents of one Colilert media pack to the mixing vessel. Cap vessel and shake until the media is completely dissolved. Follow steps 3-9 in *Analytical Procedures* above.

11.4 Waste Disposal

Used sterile mixing vessels and trays must be placed in a biohazard bag and sterilized in an autoclave at 119°-124°C for 30-45 minutes prior to disposal. Used sample containers, Colilert media packs, and pipettes can be discarded in a trash. Unused sample water can be poured down a sink drain.

12. Instrument or Method Calibration and Standardization

See Section 17, Table 3 for details pertaining to instrument calibration and standardization requirements and schedules.

13. Troubleshooting

To prevent sample confusion, label both the mixing vessel lids and trays with sample identifiers (e.g. Station ID, Locale Name, Activity Date/Time). When performing multiple analyses, line up labeled trays on the bench top and place the matching mixing vessel (after mixing the sample and media) on top of the tray while allowing foam to settle.

14. Data Acquisition and Calculations

14.1 Interpreting Results

Results should be interpreted using the guidance provided in Table 2. Total coliform results can be read using ambient light. *E. coli* results must be read using a 6-watt, 365 UV light within 5 inches of the sample in a dark environment. An IDEXX Quanti-Tray/2000 comparator should be used to aid in distinguishing a minimal positive from a negative result.

Results must be read after the 24 hour incubation time has been reached. If results are not interpretable after 24 hours, then incubate the trays for up to an additional 4 hours. Remove trays from the incubator when the 24-28 hour incubation is complete. Use the comparator to aid in result interpretation. Any results read after 28 hours are invalid. Any wells that are lighter than the comparator after 28 hours of incubation shall be reported as negative.

Table 2. Colilert® Result Interpretation (from IDEXX 2013b)

Appearance	Result
Less yellow than the comparator*	Negative for total coliforms and *E. coli*
Yellow equal to or greater than the comparator*	Positive for total coliforms
Yellow and fluorescence equal to or greater than the comparator*	Positive for *E. coli*

*IDEXX P/A Comparator, catalog #WP104; Quanti-Tray Comparator #WQTC, or Quanti-Tray/2000 Comparator #WQT2KC

14.2 Determining Most Probable Number (MPN)

Record the positive large well (LW) count and positive small well (SW) count for both total coliforms and *E. coli* in the "Well Counts" columns on a laboratory bench sheet (Appendix C). Use the IDEXX Quanti-Tray/2000 MPN table (Appendix B) to obtain the final result by finding the LW count on the vertical table axis and the SW count on the horizontal table axis. The point where these two axes meet is the final result reported as Most Probably Number (MPN)/100 mL. Final results are rounded to the nearest whole number and recorded in the "MPN" column of the bench sheet.

If sample dilutions were made, multiply the MPN table value by the dilution factor to calculate the final result. For example, if a 1:10 dilution (10mL sample diluted with 90mL dilution water) was analyzed and the resulting MPN is 437.5, then the final result would be 4,375 MPN/100 mL. Record the MPN result in the "MPN" column and the final calculated result in the "Calculated MPN (dilutions)" column on the bench sheet.

15. Computer Software

IDEXX provides a free Windows-based MPN Generator software for calculating and recording results. The software converts the number of positive wells to an MPN with upper and lower 95% confidence limits, and includes a dilution option that calculates the dilution factor. The software includes fields for sample ID and date and creates output files for use in Microsoft Excel and Word. While this software can be used in lieu of the MPN table (Appendix B), analysts must still record all results on the laboratory bench sheet. The software can be downloaded freely (https://www.idexx.com/en/water/resources/mpn-generator/).

16. Data and Records Management

16.1 Chain of Custody Records

A Chain of Custody (COC) should accompany all KDOW samples, and they must contain the following information:

- Station ID
- Activity Date/Time
- Location description (e.g., county, etc.)
- Sample Matrix
- Collection Method (e.g., grab, composite, automated, etc.)
- Analyses Requested

- Preservation
- Signature Blocks - sample custody identification and signature blocks, date and time blocks for relinquishment of samples.

Any changes or corrections made to original entries on COCs should be crossed out with a single line and initialed. All original COCs for each project must be maintained permanently in individual project files following quality assurance project plans (QAPP).

16.2 Analytical Records

Analytical results should be recorded using indelible ink on laboratory bench sheets (Appendix C) or in bound logbooks. Any changes or corrections made to the original entry should be crossed out with a single line and initialed. The following information must be recorded for each sample:

- Lab sample ID (must be unique to analytical laboratory)
- Collection date, time, and collector's initials
- Analysis date/time and analyst initials
- Station ID/Sample Source
- Sample volume
- Dilution factor, if dilution completed
- Date and time the sample is read and analyst initials
- Large well and small well counts (LW and SW)
- Result from MPN table
- Calculated MPN based on a dilution factor, if dilution made

Laboratory bench sheets or logbooks produced by each laboratory must be scanned annually and saved as PDFs. Additionally, they must be maintained permanently in electronic and physical laboratory files, according to QAPP guidelines.

16.3 Data Management

When entering final results into the Kentucky Water Assessment Data for Environmental Monitoring (K-WADE) database, the following parameters should be used: "Total Coliform – Most Probable Number" and "Escherichia coli – Most Probable Number".

17. Quality Control and Quality Assurance

A rigorous QA/QC program must be followed by KDOW laboratories. QA/QC practices are based on guidelines from Section 9020B of *Standard Methods* (APHA 2012) and *Commonwealth of Kentucky Wastewater Laboratory Certification Manual* (KDOW 2013).

Analytical laboratories should maintain records of all QA/QC activities related to microbiological testing. Equipment maintenance logs should include the make and model number, the annual service date, and the vendor who provided the service. Laboratory supply logs should include the manufacturer's name, lot number and expiration date, if any. An example log for QA/QC activities can be found in Appendix D. Electronic versions of the current logs are also available: V:\DOWWQB\Bacteria\QC.log.sheets\KDOW QC Logs.xls.

A laboratory supplies log shall be used to document the receipt and quantity of all laboratory supplies. This ensures that all supply manufacturers and lot numbers can be tracked if needed to implement corrective actions for failed QA/QC tests.

The QA/QC and laboratory supplies logs produced by each laboratory must be scanned annually and saved as PDFs. Additionally, they must be maintained permanently in electronic and physical laboratory files, according to the QAPP guidelines.

17.1 Quality Control Practices

Table 3 summarizes QC practices that to be implemented and the frequency they are to be performed.

Table 3. Quality Control Practices

Laboratory Equipment*	QC Activity	Frequency	Performance Criteria	Corrective Action
pH Meter	Calibration	Before each use	Must meet or exceed instrument accuracy specs.	Re-calibrate to within allowable specs.
	Slope Calculation	Monthly	95% - 105%	If slope is not within range perform maintenance on electrode following manufacturer's instructions
	Performance Test	Annually	Must meet or exceed instrument accuracy specs.	If performance fails repair or replace meter
Conductivity Meter	Standardization	At least monthly	Must meet or exceed instrument accuracy specs.	Re-calibrate to within allowable specs.
	Calibration	Annually	Must meet or exceed instrument accuracy specs.	If calibration fails repair or replace meter
Balance	Calibration	Monthly	Must meet or exceed instrument accuracy specs.	Re-calibrate to within allowable specs.
	Serviced	Annually	Must meet or exceed instrument accuracy specs.	If instrument fails repair or replace balance
Thermometers: NIST and non-NIST Traceable	Calibration	Annually	Must not differ by >1°C from reference thermometer	If calibration fails replace thermometer
Incubator	Serviced	Annually	Must meet or exceed instrument accuracy specs.	If instrument fails repair or replace incubator
	Temperature Check	Twice daily (when in use)	35° ± 0.5°C	Adjust temperature settings as necessary
Bioindicator Incubator (aka Block Incubator)	Serviced	Annually	Must meet or exceed instrument accuracy specs.	If performance fails repair or replace incubator
	Temperature Check	Once daily (when in use)	55°- 60°C	If not functioning correctly perform maintenance
Autoclave	Performance Test	Monthly	Must maintain temperature of 119°-124°C	If not functioning correctly perform maintenance
	Timing Check	Monthly	An entire cycle must be completed within 45 minutes when a 15 minute sterilization period is used	If not functioning correctly perform maintenance
	Bioindicator Test	Monthly	Spore strips or spore ampules must show negative growth after 48 hr. incubation time	If not functioning correctly perform maintenance
Quanti-Tray/2000 Sealer	Sealer Check	Monthly	No dye should be observed outside of wells	If not functioning correctly perform maintenance
Ultraviolet Lamp	Fluorescence	Quarterly	Fluorescence should be within 70% of original reading of bulb	If reading fails replace bulb
Refrigerator	Temperature Check	Once daily (when in use)	1° - 5°C	Adjust temperature settings as necessary
Laboratory Supplies	**QC Activity**	**Frequency**	**Performance Criteria**	**Corrective Action**
Sample Containers	Volume Check	Each new lot	Volume at fill line is 100mL ± 2.5mL Volume at fill line is 200mL ±5mL	Return unused containers to manufacturer; Use new lot that meets the criteria
	Sterility Test	Each new lot	No growth in 25mL TSB after 24 and 48 hr. incubation time at 35° ± 0.5°C	Return unused containers to manufacturer; Use new lot that meets the criteria
	Fluorescence Test	Each new lot	No fluorescence	Return unused containers to manufacturer; Use new lot that meets the criteria
IDEXX Sterile Mixing Vessels	Volume Check	Each new lot	Volume at fill line is 100mL ± 2.5mL	Return unused vessels to manufacturer; Use new lot that meets the criteria
	Sterility Test	Each new lot	No growth in 25mL TSB after 24 and 48 hr. incubation time at 35° ± 0.5°C	Return unused vessels to manufacturer; Use new lot that meets the criteria
	Fluorescence Test	Each new lot	No fluorescence	Return unused vessels to manufacturer; Use new lot that meets the criteria
Sterile Water	Sterility Test	Each new batch (lab prepared) or lot (commercially prepared)	No growth in 50mL double strength TSB and 50mL water after 24 and 48 hr. incubation time at 35° ± 0.5°C	Discard batch/lot or sterilize, if appropriate
Tryptic Soy Broth (TSB)	pH Check	Each new batch (lab prepared) or lot (commercially prepared)	7.3 ± 0.2	Return unused broth to manufacturer; Use new lot that meets the criteria
	Sterility Test	Each new batch (lab prepared) or lot (commercially prepared)	No growth	Discard batch/lot or resterilize, if appropriate
Pipettes	Volume Check	Each new batch	Certificate of calibration from vendor is acceptable	n/a
Graduated Cylinders	Volume Check	Each new lot	Must meet Class B tolerance; Certificate of calibration from vendor is acceptable	n/a

*It is recommended that annual calibration and repair of equipment be performed by professional service technicians.

17.2 Quality Assurance Practices

QA practices should be based on program and project data quality objectives outlined in QAPP documents. Table 4 provides some examples of QA practices that may be required. Types of QA samples are described below:

Replicate/Split: A type of replicate sample in which a 200mL volume sample is split into two 100mL subsamples and analyzed using identical methods. This type of sample measures analysis variability.

Sterile water blank: A sterile water solution that is subjected to all aspects of laboratory analysis as an environmental sample, and is used to determine analytical bias.

Field blank: A blank solution (e.g. deionized water) that is subjected to all aspects of sample collection, preservation, transportation, and laboratory handling as an environmental sample. This sample quantifies bias in the field collection and transport to the laboratory.

Temperature blank: A blank solution that is subjected to all aspects of preservation as an environmental sample. This sample quantifies temperature related bias.

Comparison Counting: A second analyst replicates counting the number of positive wells on a randomly chosen tray. This sample quantifies bias in individuals in determining positive vs negative results.

Table 4. Quality Assurance Practices

Type of QA	Frequency	Performance Criteria	Corrective Action
Replicate/Split sample	10% of total samples or one sample per test run	Range of logs must be less than a calculated precision criterion value (SM 9020B, Section 9e)	•Review all analytical results since last precision check and censor as dictated by project specific data quality objectives •Identify and resolve the analytical problem before making further analyses
Sterile water blank	One per test run	Results should not exceed the minimum reporting limit for *E. coli* or total coliforms (<1 MPN/100mL)	• Repeat on 2-4 more trays from same lot. If a positive result is observed, return unused trays to manufacturer; Use new lot that meets the criteria •Consider the possibility of analyst error or non-aseptic conditions; review procedure and lab cleaning requirements; censor data as deemed appropriate
Field blank	One per day of sampling	Results should not exceed the minimum reporting limit for *E. coli* or total coliforms (<1 MPN/100mL)	•Consider the possibility of field contamination; properly clean all containers used to transport and store samples; censor data as deemed appropriate
Temperature blank or Temperature check using IR thermometer	One per day of sampling in each container used to transport and store samples	Temperature <10°C	•Discard all samples that do not meet a holding temperature of <10°C
Comparison Counting of Quanti-Trays	Monthly	Replicate counts by the same analyst should agree within 5%, those between analysts should agree within 10%	•Review tray reading procedures; censor data as deemed appropriate

18. References

American Public Health Association (APHA), American Water Works Association, and Water Environment Federation. 2012. *Standard Methods for the Examination of Water and Wastewater*, 22nd ed. APHA Washington, DC.

"Guidelines Establishing Test Procedures for the Analysis of Pollutants; Analytical Methods for Biological Pollutants in Wastewater and Sewage Sludge: Final Rule" *Code of Federal Regulations (CFR)* Title 40, Pts. 136.3, September 12, 2017 Ed.

IDEXX Laboratories, Inc. (IDEXX). 2013a. Colilert *Test Kit*. Westbrook, Maine. https://www.idexx.com/resource-library/water/colilert-procedure-en.pdf

---. 2013b. IDEXX *Quanti-Tray/2000; Insert and Most Probable Number (MPN) Table*. Westbrook, Maine. https://www.idexx.com/resource-library/water/quanti-tray-2000-procedure-en.pdf

Kentucky Division of Water (KDOW). 2013. Commonwealth of Kentucky Wastewater Laboratory Certification Manual. Environmental and Public Protection Cabinet, Department for Environmental Protection, Division of Water. Frankfort, KY.

---. 2011. Sampling the Surface Water Quality in Lotic Systems. Revision 2. Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky.

---. 2009. Sample Control and Management. Kentucky Department for Environmental Protection, Frankfort, Kentucky.

Colilert* Test Kit

Introduction

Colilert* simultaneously detects total coliforms and *E. coli* in water. It is based on IDEXX's patented Defined Substrate Technology* (DST*). When total coliforms metabolize Colilert's nutrient-indicator, ONPG, the sample turns yellow. When *E. coli* metabolize Colilert's nutrient-indicator, MUG, the sample also fluoresces. Colilert can simultaneously detect these bacteria at 1 cfu/100 mL within 24 hours even with as many as 2 million heterotrophic bacteria per 100 mL present.

Storage

Store at 2–30°C away from light.

Presence/Absence (P/A) Procedure

1. Add contents of one pack to a 100 mL sample in a sterile, transparent, nonfluorescing vessel.
2. Cap vessel and shake.
3. Incubate at 35±0.5°C for 24 hours.
4. Read results according to Result Interpretation table below.

Quanti-Tray* Enumeration Procedure

1. Add contents of one pack to a 100 mL water sample in a sterile vessel.
2. Cap vessel and shake until dissolved.
3. Pour sample/reagent mixture into a Quanti-Tray* or Quanti-Tray*/2000 and seal in an IDEXX Quanti-Tray* Sealer.
4. Place the sealed tray in a 35±0.5°C incubator for 24 hours.
5. Read results according to the Result Interpretation table below. Count the number of positive wells and refer to the MPN table provided with the trays to obtain a Most Probable Number.

Result Interpretation

Appearance	Result
Less yellow than the comparator[1]	Negative for total coliforms and *E. coli*
Yellow equal to or greater than the comparator	Positive for total coliforms
Yellow and fluorescence equal to or greater than the comparator	Positive for *E. coli*

- Look for fluorescence with a 6-watt, 365-nm UV light within 5 inches of the sample in a dark environment. Face light away from your eyes and towards the sample.
- Colilert results are definitive at 24–28 hours. In addition, positives for both total coliforms and *E. coli* observed before 24 hours and negatives observed after 28 hours are also valid.

Procedural Notes

- This insert may not reflect your local regulations. For compliance testing, be sure to follow appropriate regulatory procedures. For example, samples run in other countries are incubated at 36±2°C for 24–28 hours.
- Colilert can be run in any multiple tube format. *Standard Methods for the Examination of Water and Wastewater*[2] MPN tables should be used to find Most Probable Numbers (MPNs).
- If a water sample has some background color, compare inoculated Colilert sample to a control blank of the same water sample.
- If sample dilutions are made, multiply the MPN value by the dilution factor to obtain the proper quantitative result.
- Use only sterile, nonbuffered, oxidant-free water for dilutions.
- Colilert is a primary water test. Colilert performance characteristics do not apply to samples altered by any pre-enrichment or concentration.
- In samples with excessive chlorine, a blue flash may be seen when adding Colilert. If this is seen, consider sample invalid and discontinue testing.
- Aseptic technique should always be followed when using Colilert. Dispose of in accordance with Good Laboratory Practices.

Quality Control Procedures

1. One of the following quality control procedures is recommended for each lot of Colilert:
   A. IDEXX-QC Coliform and *E.coli*[3]: *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*
   B. Quanti-Cult*[4]: *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.
   C. Fill three sterile vessels with 100 mL of sterile nonbuffered oxidant-free water and inoculate with a sterile loop of ATCC[5] strains, *Escherichia coli* ATCC 25922 or 11775, *Klebsiella pneumoniae* ATCC 31488 and *Pseudomonas aeruginosa* ATCC 10145 or 27853.
2. Follow the P/A Procedure or Quanti-Tray Enumeration Procedure above.
3. Results should match the Result Interpretation table above.

1. IDEXX P/A Comparator, catalog # WP104; Quanti-Tray Comparator #WQTC, or Quanti-Tray/2000 Comparator #WQT2KC
2. Eaton, AD, Clesceri, LS, Greenberg, AE, Rice, EN. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, 2005. Washington, DC.
3. IDEXX-QC Coliform and *E. coli*—IDEXX Catalog # UN3373-WQC-TCEC
4. Quanti-Cult* cultures—IDEXX catalog # WKIT-1001
5. American Type Culture Collection 1-800-638-6597 atcc.org

Quanti-Tray*/2000

Insert and Most Probable Number (MPN) Table

IDX 33/02 – 06/12
WATER ANALYSIS METHODS
www.afnor-validation.org

Quanti-Tray* Certificate of Sterility

This certifies that the enclosed Quanti-Tray/2000 trays have been sterilized with ethylene oxide.

For technical support, please call:

North/South America: +1 207 556 4496 or 1 800 321 0207
Europe: +00800 4339 9111
UK: +44 01638 676800
China: +86 21 61279528
Japan: +81 422 71 5921
Australia: +1800 443 399

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The method Colilert*-18/Quanti-Tray*/2000 for water analysis is granted NF Validation by AFNOR Certification as an alternative method to the standard ISO 9308-3 for detection and enumeration of *Escherichia coli* ß-glucuronidase positive in bathing water, under the Certificate number: IDX 33/02 – 06/12 For more information about end of validity, please refer to the certificate NF Validation available on website mentioned above

La méthode Colilert*-18 / Quanti-Tray*/2000 pour le contrôle des eaux est certifiée NF Validation par AFNOR Certification comme méthode alternative à la norme NF EN ISO 9308-3 pour le dénombrement des *Escherichia coli* ß-glucuronidase positive dans les eaux de baignades sous le n° d'attestation: IDX 33/02 – 06/12. La date de fin de validité de la certification NF Validation est précisée sur l'attestation, disponible auprès d'IDEXX ou d'AFNOR Certification.

One IDEXX Drive
Westbrook, Maine 04092 USA

Introduction

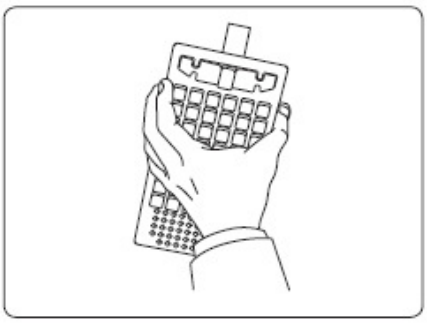
IDEXX Quanti-Tray*/2000 is designed to give quantitated bacterial counts of 100 mL samples using IDEXX reagent products. Add the reagent/sample mixture to a Quanti-Tray/2000, seal it in a Quanti-Tray* Sealer and incubate per the reagent instructions. Count the number of positive large and small wells and use the Most Probable Number (MPN) Table attached to determine the MPN.

Contents

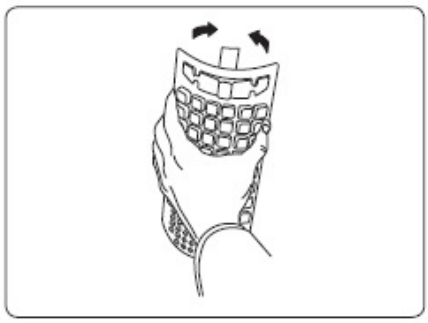
This package contains sterile Quanti-Tray/2000 trays.

User Instructions

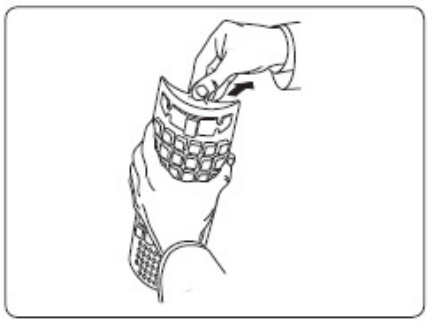
1. Use one hand to hold a Quanti-Tray* upright with the well side facing the palm.



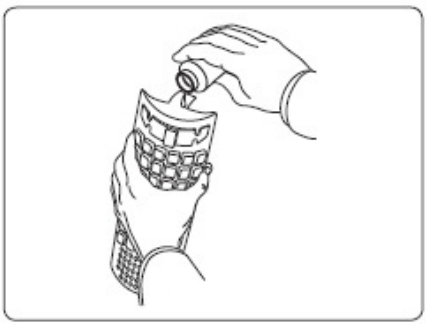
2. Squeeze the upper part of the Quanti-Tray so that the Quanti-Tray bends toward the palm.



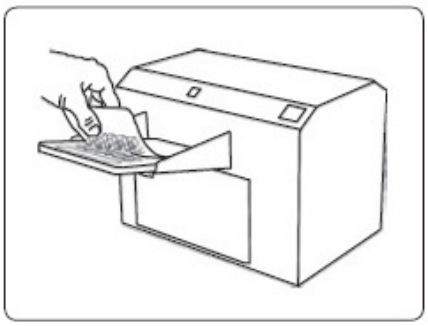
3. Gently pull foil tab to separate the foil from the tray. Avoid touching the inside of the foil or tray.



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 the Quanti-Tray Sealer instructions.
7. Incubate according to reagent instructions.
8. Count large and small positive wells and refer to the Quanti-Tray/2000 MPN table to find the MPN.†
9. Dispose of media in accordance with good laboratory practices.

For technical support, please call:

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idexx.com/water

†Download the IDEXX MPN generator software for automated Quanti-Tray results at idexx.com/mpngenerator.

*Quanti-Tray is a trademark or registered trademark of IDEXX Laboratories, Inc. in the United States and/or other countries.

IDEX

One IDEXX D
Westbrook, Maine 04092

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

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	1986.3	2419.6	>2419.6

Appendix C. Laboratory Bench Sheet

DOW/FRANKFORT MICROBIOLOGY LAB BACTERIOLOGICAL ANALYSIS BENCH SHEET

Month/Year:		Method: SM9223	Page ___ of ___				TOTAL COLIFORM			E. coli			Calculated cfu/100mL (dilutions)
							24 Hrs			24 Hrs			
Collection Info	Collector (initials)	Analysis Info	Sample ID/Sample Source	Sample Info	Date/Time Read	Analyst (initials)	LC	SC	cfu/100 mL	LC	SC	cfu/100 mL	
Sample #: 1514803 Coll. Date: Coll. Time:		Date: Time: Analyst:		Volume: Dilution Factor:	Date: Time:								
Sample #: 1514804 Coll. Date: Coll. Time:		Date: Time: Analyst:		Volume: Dilution Factor:	Date: Time:								
Sample #: 1514805 Coll. Date: Coll. Time:		Date: Time: Analyst:		Volume: Dilution Factor:	Date: Time:								
Sample #: 1514806 Coll. Date: Coll. Time:		Date: Time: Analyst:		Volume: Dilution Factor:	Date: Time:								
Sample #: 1514807 Coll. Date: Coll. Time:		Date: Time: Analyst:		Volume: Dilution Factor:	Date: Time:								
Sample #: 1514808 Coll. Date: Coll. Time:		Date: Time: Analyst:		Volume: Dilution Factor:	Date: Time:								
Sample #: 1514809 Coll. Date: Coll. Time:		Date: Time: Analyst:		Volume: Dilution Factor:	Date: Time:								
Sample #: 1514810 Coll. Date: Coll. Time:		Date: Time: Analyst:		Volume: Dilution Factor:	Date: Time:								
Sample #: 1514811 Coll. Date: Coll. Time:		Date: Time: Analyst:		Volume: Dilution Factor:	Date: Time:								

revised 05/01/2015 by JS

Appendix D. Example QA/QC Log

IDEXX MIXING VESSELS AND SAMPLE BOTTLES STERILITY, VOLUME, FLUORESCENCE CHECK

(To Be Performed For Each New Lot)

Lab: KDOW Microbiology Laboratory			**Year: 2011**			**Page #: 1**		
Date Received	Lot # of Media/Supplies	Manufacturer	Sample bottles/Mixing Vessels +25mL SS TSB*		Volume** (check with Class B graduated cylinder)	Fluorescence		Analyst Initials
			Growth	No Growth		Positive	Negative	

Note Action Taken if results are unacceptable

*SS = Single Strength, Incubate at 35º±0.5ºC for 48 hours.

**Sample container volume should = 120 mL ±2.5mL;

Mixing vessel volume should = 100mL ± 2mL at the fill line