Standard Operating Procedure

Enzyme Substrate Test Method for the Detection of Total Coliforms and *Escherichia coli*

Commonwealth of Kentucky
Energy and Environment Cabinet
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Division of Water

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4. Procedures

4A Scope and Applicability

This document provides methodologies for laboratory analysis and quality assurance/quality control (QA/QC) activities for *Escherichia coli* (*E. coli*) and total coliforms samples that are collected by Kentucky Division of Water (KDOW) personnel.

4B Summary of Method

KDOW follows the multi-well enzyme substrate methodology for the enumeration of *E. coli* and total coliforms, as described in *Standard Methods for the Examination of Water and Wastewater*, 20th ed. (APHA 1998). Currently, KDOW exclusively uses IDEXX Laboratories, Inc. Defined Substrate Technology® reagent products to conduct the methods described in this manual. 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, along with a description of the circumstances requiring its use.

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

4C Acronyms

CFU: Colony Forming Unit
COC: Chain of Custody
DEP: Department for Environmental Protection
KDOW: Kentucky Division of Water
MPN: Most Probable Number
PPE: Personal Protective Equipment
QA: Quality Assurance
QC: Quality Control
SOP: Standard Operating Procedures
TSB: Tryptic Soy Broth

4D Health & Safety Warning

4D.1 Job Specific Training

Each employee will be individually trained by his/her supervisor, or designee, to perform assigned job tasks safely. Each employee will be trained in the safety aspects of assigned tasks by the subject matter expert prior to his/her performing the task. Supervisors will document all training provided. Supervisors should use the following methods to increase employee comprehension.

a. Employees will receive verbal instructions and specific directions on how to perform functions safely.

b. Employees will receive a demonstration of job tasks, using known safe work practices.

c. Supervisors, or designee, will observe employees performing the work previously demonstrated. If necessary, remedial instruction will be provided to correct training deficiencies prior to final release to perform unsupervised work.

d. Employees will be given safe operating instructions prior to the use and operation of new equipment or processes.

e. Supervisors shall be responsible for reviewing safe work practices with employees before permitting new, non-routine, or specialized procedures to be performed.

f. Supervisors will make employees aware of Kentucky DEP rules, policies, and procedures.

4D.2 Human Health

Field staff working in and around potentially contaminated surface waters should receive 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.

The use of 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 the pollutants have not been identified. All field staff should 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 laboratory analysis:

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

4E Precautions

The following precautions shall be considered when performing bacteriological analyses

Sample Handling Precautions

- Samples should always be stored in a secure location to ensure that they cannot be tampered with.
- All samples should have appropriate Chain of Custody (COC) documentation and COC documentation must remain with all samples.

Analytical Precautions

- The procedures presented in this SOP are intended for the analysis of samples from ambient waters and should not be used to dictate procedures or QA/QC practices for samples collected for drinking water 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 2007).
- If sample dilutions are made, multiply the most probable number (MPN) value by the dilution factor to obtain the proper quantitative 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 this is seen, consider the sample invalid and discontinue testing.
- Aseptic technique should always be followed when using Colilert®.

4F Interferences

It is important to remain cognizant of potential sources of contamination when performing laboratory analyses. Always disinfect bench tops with anti-bacterial cleaning products. Floors should be cleaned on a regular basis. Wash hands with anti-bacterial soap prior to conducting lab work.

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 resulting in atypical color change should be invalidated and the tests should be repeated on new sample water.

4G Personnel Qualifications / Responsibilities

All personnel involved in microbiological laboratory analysis will meet at least the minimum qualifications for their job classification. In addition, all analysts must be trained in the proper analytical methodologies. Training will continue on-the-job through interaction with experienced personnel and continued outside training when educational opportunities become available. Analysts should, ideally, participate in a three day training course for new analysts performing ambient surfacewater, groundwater and wastewater analyses sponsored by Morehead State University's Kentucky Laboratory Certification Program.

4H 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
Wide-mouth sterile plastic container
Non-corrosive glass bottles w/non-leaking ground glass stoppers or caps
Sterile plastic bags
Chemical Preservatives
Sodium thiosulfate ($Na_2S_2O_3$) tablets
Laboratory Equipment
Water-jacketed incubator
Biological indicator incubator
Autoclave
Refrigerator
IDEXX Quanti-Tray® Sealer
Conductivity meter
pH meter
Balance
Water Purification System
6-watt, 365-nm UV lamp
Laboratory Supplies
IDEXX Quanti-Tray®/2000 multi-well trays
IDEXX Quanti-Tray®/2000 comparator
Colilert® test media
Sterile mixing vessels
Quanti-cult® QC kit
SimPlate® for HPC

Table 1. (cont.) Laboratory Equipment and Supplies

Laboratory Supplies
Tryptic Soy Broth (TSB) (single strength and double strength)
Biological Indicator Capsules
Maximum temperature thermometer
National Institute of Standards and Technology (NIST) calibrated thermometers (with 0.1° increments)
Specific conductivity low-level calibration standard
pH 4, 7 and 10 calibration buffers
Sterile 5mL and 1mL glass pipettes
Pipette bulb/automatic pipetter
1L glass flasks
100mL class A or B graduated cylinder
Glass beakers
Anti-bacterial cleaning wipes
Powderless latex/nitrile gloves
Sterile Water

4I Step by Step Procedure

4I.1 Sample Collection

Samples must be collected in sterile plastic or glass containers or sterile plastic bags with a volume of at least 120mL. Sterile plastic and glass containers should not be filled past the 100mL fill line and at least 2.5cm of headspace must be left in the container to allow for adequate mixing during analysis. (APHA 1998)

4I.2 Sample Handling and Preservation

Samples should only be accepted by the analytical laboratory if they have been properly preserved and are delivered within the designated holding time. Samples to be used for *E. coli* analysis MUST be preserved on ice to <10°C within 15 minutes of collection. Samples have a holding time of 6 hours +2 hours for lab processing. Sample analysis MUST occur prior to exceeding an 8 hour holding time, or they should be discarded. If the source water for the sample is suspected of having residual chlorine or halogen, 0.1mL of a 10% sodium thiosulfate ($Na_2S_2O_3$) solution should be added to dechlorinate and neutralize residual halogen within the sample water. (40 CFR 136.3, 2009)

4I.3 Sample Preparation and Analysis

Sample Preparation

Prior to running analyses, check all sample bottles to ensure they contain the required volume of 100mL and at least 2.5 cm of air space in the bottle to allow for sufficient mixing of the sample by shaking. If the sample volume is less than 100mL, discard the sample and cancel the analysis. If the bottle was over-filled resulting in less than 2.5 cm of air space, shake the bottle 25 times and use a sterile pipette to withdraw the excess water from the bottle until the appropriate amount of air space is achieved.

Analytical Procedures
Analysis methods using Colilert® Test kits should be performed according to the Quanti-Tray® Enumeration Procedure in Appendix A (IDEXX 2007) and user instructions for Quanti-Tray/2000 in Appendix B (IDEXX 2010). It is important to follow aseptic technique throughout the following procedure:

1. Shake sample bottle 25 times. Pour sample to the fill line of a 100mL IDEXX sterile mixing vessel.
2. Add contents of one Colilert® media pack to the vessel. Cap vessel and shake until the media is completely dissolved.
3. Allow any foam to settle. Pour sample/media mixture into a Quanti-Tray®/2000. Avoid contact with the foil tab and the inside of the tray while pouring.
4. Close the tray using the foil tab. Tap the small wells 2-3 times to release any bubbles. Allow any foam to settle.
5. Place the tray in the rubber insert of the Quanti-Tray Sealer. Place the rubber insert and tray well side down onto the feeder tray of the sealer.
6. Gently push the tray until the sealer grabs hold. Once the tray is sealed, remove it from the rubber insert and incubate in a 35°± 0.5°C incubator for 24 hours.
7. Results should be read only after the 24 hour incubation time has been reached. Yellow cells indicate a positive result for total coliforms and fluorescing cells indicate a positive result for *E. coli*. *E. coli* results must be read using a 6-watt, 365 UV light within 5 inches of the sample in a dark environment. The following table describes how results should be interpreted. An IDEXX Quanti-Tray®/2000 comparator should be used to aid in the interpretation of results.

Table 2. Colilert® Result Interpretation (from IDEXX 2010)

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

Sample Dilutions
The Colilert® method has an upper quantification range limit of 2419 CFUs for sample water run without dilutions. Dilutions can be made in order to obtain quantifiable results that are >2,419 CFUs.

4I.4 Waste Disposal

All microbiological waste must be placed in a biohazard bag and decontaminated in an autoclave at 119°-124°C for 30-45 minutes prior to being discarded.

4I.5 Troubleshooting

If results are not interpretable after the 24 hour incubation, incubate the trays for an additional 4 hours. Remove trays from the incubator when a 28 hour incubation time has been achieved. Use the comparator to aid in the interpretation results. Any results read after 28 hours should be considered invalid. Any cells that are lighter than the comparator after 28 hours of incubation should be reported as negative.

4I.6 Data Acquisition & Calculations

Record the number of positive large cells and positive small cells for *E. coli* and total coliforms on a laboratory bench sheet (see section 4J.2). Use the IDEXX Quanti-Tray®/2000 MPN table (Appendix B) to calculate the total number of CFUs for each sample. If sample dilutions were made, multiply the MPN value by the dilution factor to obtain the proper quantitative result.

4J Data and Records Management

4J.1 Sampling Records

A COC must accompany every sample collected by the KDOW. Any changes or corrections made to the original entry should be crossed out with a single line, initialed and dated. All original COCs for each project must be maintained permanently in individual project files, according to the DEP State Agency records retention schedule (KDLA 2006).

4J.2 Analytical Records

Analytical results should be recorded using indelible ink in bound logbooks and/or on laboratory bench sheets (Appendix C). The following information must be recorded for each sample:

- Lab sample number
- Collection date, time and collector's initials
- Analysis time and analyst initials
- Sample ID/Sample Source
- Sample volume
- Dilution factor
- Date and time the sample is read and analyst initials
- Large cell and small cell counts
- Result from MPN table
- Calculated MPN based on a dilution factor, if dilutions were made

Any changes or corrections made to the original entry should be crossed out with a single line, initialed and dated. Analytical records produced by each laboratory must be maintained permanently in laboratory files, according to the DEP State Agency records retention schedule (KDLA 2006).

5 Quality Control and Quality Assurance Section

5A Quality Control Practices

A rigorous QA/QC program should be established for any lab analyzing bacteriological samples in order to support the validity of analytical data. Table 3 summarizes QC practices that are recommended for laboratories conducting bacteriological analysis for ambient waters. Detailed methods used to perform these tasks can be found in the *Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures Quality Assurance* (USEPA 2005). Each analytical laboratory should develop a QA/QC policy based on the requirements of specific program/project data quality objectives.

Table 3. Quality Control Practices

Laboratory Equipment*	QC Activity	Frequency	Performance Criteria	Corrective Action
pH Meter	Calibration	With 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	With each use	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 Traceable	Calibration	Annually	Must not differ by >1°C from reference thermometer	If calibration fails replace thermometer
	Recertify	Every 5 years	n/a	n/a
Non-NIST	Calibration	Annually	Must not differ by >1°C from reference thermometer	If calibration fails replace thermometer
Water-jacketed 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	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

Table 3. (cont.) Quality Control Practices

Laboratory Equipment*	QC Activity	Frequency	Performance Criteria	Corrective Action
Autoclave	Performance Test	With each use	Must maintain temperature of 119°-124°C	If not functioning correctly perform maintenance
	Timing Check	Quarterly	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	Return unused containers to manufacturer; Use new lot that meets the criteria
	Sterility Test	Each new lot	No growth in 25mL TSB after 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 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

Table 3. (cont.) Quality Control Practices

Laboratory Supplies	QC Activity	Frequency	Performance Criteria	Corrective Action
Colilert Media	Sterility Test	Each new lot	No growth in 50mL double strength TSB and 50mL water after 24 hr. incubation time at 35° ± 0.5°C	Return unused media to manufacturer; Use new lot that meets the criteria
	Performance Test	Each new lot	*E. coli* = yellow and fluorescence; *K. pneumoniae* = yellow and no fluorescence; *P. aeruginose* = clear, no fluorescence	Return unused media to manufacturer; Use new lot that meets the criteria
	Fluorescence Test	Each new lot	No fluorescence	Return unused media to manufacturer; Use new lot that meets the criteria
Quanti-Tray/2000 trays	Sterility Test	Each new lot	No growth in 100mL single strength TSB and 50mL water after 24 hr. incubation time at 35° ± 0.5°C	Repeat on 2-4 more trays from same lot. If growth is observed, return unused trays to manufacturer; Use new lot that meets the criteria
Lab Grade Water	HPC Test	Monthly	<500 CFU/mL	If performance of filtration unit fails perform maintenance/replace filters
	Conductivity	Monthly	<2 micromhos/cm at 25°C	If performance of filtration unit fails perform maintenance/replace filters
	Metals Test (Pb, Cd, Cr, Cu, Ni, Zn)	Annually	Not greater than 0.05 mg/L per contaminant and collectively ≤0.1 mg/L	If performance of filtration unit fails perform maintenance/replace filters
	Total Chlorine Residual	Monthly	<0.1 mg/L	If performance of filtration unit fails perform maintenance/replace filters
	Bacteriological Quality	Annually (if reagent water does NOT meet the criteria for Type II or Medium quality water or better)	Ratio of growth rate 0.8 to 3.0	If performance of filtration unit fails perform maintenance/replace filters
Sterile Water	Sterility Test	Each new batch (lab prepared) or lot (commercially prepared)	No growth in 50mL double strength TSB and 50mL water after 48 hr. incubation time at 35° ± 0.5°C	Discard batch/lot or resterilize, if appropriate

Table 3. (cont.) Quality Control Practices

Laboratory Supplies	QC Activity	Frequency	Performance Criteria	Corrective Action
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.

5B Quality Assurance Practices

QA practices should be based on the requirements of specific program/project data quality objectives. Table 4 provides some examples of QA practices that may be implemented.

Table 4. Quality Assurance Practices

Type of QA	Frequency	Performance Criteria	Corrective Action
Duplicate sample collection	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 8b)	•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 CFU)	• 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 CFU)	•Consider the possibility of field contamination; properly clean all containers used to transport and store samples; censor data as deemed appropriate
Temperature blank	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
Verification of *E. coli* positive results	10% of total samples run	*E. coli* positive cell must be confirmed by adding Kovac's reagent to the medium to detect the presence of indole (the medium should turn red)	•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

5C Quality Assurance/Quality Control Documentation

Analytical laboratories should maintain records of all QA/QC activities relating to microbiological testing. QA/QC logs maintained for equipment should include the make and model number, the annual service date and the vendor who provided the service. QA/QC logs maintained for laboratory supplies should include the manufacturer's name, lot number and expiration date. An example log for QA/QC activities can be found in Appendix D.

A supplies log should be used to document the receipt and quantity of all supplies used by the laboratory. This ensures that all supply manufacturers and lot numbers can be tracked in case it is necessary to implement corrective actions for failed QA/QC tests.

6. Reference Section

American Public Health Association (APHA), American Water Works Association, and Water Environment Federation. 1998. *Standard Methods for the Examination of Water and Wastewater*, 20th 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, July 1, 2009 ed.

IDEXX Laboratories, Inc. (IDEXX). 2007. *Colilert Test Kit*. Westbrook, Maine. http://www.idexx.com/pubwebresources/pdf/en_us/water/0612999_c.pdf

---. 2010. *IDEXX Quanti-Tray/2000; Insert and Most Probable Number (MPN) Table*. Westbrook, Maine. http://www.idexx.com/pubwebresources/pdf/en_us/water/0602320.pdf

Kentucky Department for Libraries and Archives (KDLA). 2006. State Agency Records Retention Schedule: Department for Environmental Protection. State Archives and Records Commission, Public Records Division, Frankfort, KY.

U.S. Environmental Protection Agency (USEPA). 2005. *Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures Quality Assurance*, 5th ed. USEPA Office of Water, Cincinnati, OH.

Appendix A. Colilert Test Kit Method Using Quanti-Tray® Enumeration Procedure (IDEXX 2007)

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 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.

Contents
WP020I contains 20 Snap Packs for 100 mL samples
WP200I contains 200 Snap Packs for 100 mL samples
W050I contains 20 Snap Packs for 50 mL samples
W050BI contains 200 Snap Packs for 50 mL samples

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 (50 mL for W050I and W050BI) in a sterile, transparent, nonfluorescing vessel.
2. Cap vessel and shake.
3. Incubate at 35°C ± 0.5°C for 24 hours.
4. Read results according to Result Interpretation table below.

Quanti-Tray® Enumeration Procedure (WP020I and WP200I only)
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°C ± 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*	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.
- Colilert can be run in any multiple tube format. *Standard Methods for the Examination of Water and Wastewater*** 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
The following quality control procedure is recommended for each lot of Colilert:
1. Inoculate 3 sterile vessels filled with 100 mL sterile water with the following:
   A. one with Quanti-Cult™**** *E. coli* or a sterile loop of ATCC**** 25922 or 11775 (*E. coli*)
   B. one with Quanti-Cult *Klebsiella pneumoniae* or a sterile loop of ATCC 31488 (total coliform)
   C. one with Quanti-Cult *Pseudomonas aeruginosa* or a sterile loop of ATCC 10145 or 27853 (noncoliform)
2. Follow the P/A Procedure or Quanti-Tray Enumeration Procedure above.
3. Results should match the Result Interpretation table above.

*IDEXX P/A Comparator, catalog #WP104; Quanti-Tray Comparator #WQTC, or Quanti-Tray/2000 Comparator #WQT2NC
**Eaton AD, Clesceri LS, Greenberg AE. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, 1998, Washington, DC.
*** Quanti-Cult cultures – IDEXX catalog # WKIT-1001
****American Type Culture Collection 1-800-638-6597

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Trousse d'analyse Colilert®

Introduction
Colilert permet la détection simultanée des coliformes totaux et *E. coli* dans l'eau. Ce test est basé sur la technologie brevetée Defined Substrate Technology® (DST®) d'IDEXX. Lorsque les coliformes totaux métabolisent ONPG, l'indicateur de nutriants Colilert, le prélèvement vire au jaune. Lorsque *E. coli* métabolise MUG, l'indicateur de nutriants Colilert, le prélèvement devient fluorescent. Colilert peut détecter simultanément ces bactéries à 1 cfu/100 ml en 24 heures, même en présence de bactéries hétérotrophes d'une concentration de 2 millions par 100 ml.

Contenu
WP020I contient 20 sachets hermétiques pour prélèvements de 100 ml
WP200I contient 200 sachets hermétiques pour prélèvements de 100 ml
W050I contient 20 sachets hermétiques pour prélèvements de 50 ml
W050BI contient 200 sachets hermétiques pour prélèvements de 50 ml

Conditions de Conservation
Conserver entre 2° et 30°C à l'abri de la lumière.

Procédure de Présence/Absence (P/A)
1. Ajouter le contenu d'un sachet dans un prélèvement de 100 ml (50 ml dans le cas du W050I et du W050BI) placé dans un récipient stérile, transparent et non fluorescent.
2. Fermer le récipient et agiter.
3. Incuber à 35°C ± 0,5°C pendant les 24 heures qui suivent.
4. Interpréter les résultats en se référant au tableau d'interprétation des résultats ci-dessous.

Quanti-Tray® Procédure de numération (WP020I et WP200I uniquement)
1. Ajouter le contenu d'un sachet dans un prélèvement de 100 ml d'eau placé dans un récipient stérile.
2. Fermer le récipient et agiter jusqu'à dissolution.
3. Verser le mélange prélèvement/réactif dans un Quanti-Tray ou un Quanti-Tray/2000 et fermer hermétiquement dans un dispositif hermétique Quanti-Tray IDEXX.
4. Placer le plateau hermétiquement fermé dans un incubateur à 35°C ± 0,5°C pendant 24 heures.
5. Interpréter les résultats en se référant au tableau d'interprétation des résultats ci-dessous. Compter le nombre de puits positifs et se référer au tableau MPN fourni avec les plateaux pour obtenir le Chiffre le plus probable (MPN).

Interprétation des Résultats

Aspect	Résultat
Moins jaune que le comparateur*	Négatif pour les coliformes totaux et *E. coli*
Aussi jaune ou plus jaune que le comparateur*	Positif pour les coliformes totaux
Couleur jaune et fluorescence égales ou supérieures au comparateur*	Positif pour *E. coli*

- Évaluer la fluorescence avec une ampoule UV de 6 watts et 365 nm placée à 13 cm du prélèvement dans l'obscurité. Orienter la lumière vers le prélèvement, dans la direction opposée à celle des yeux de l'opérateur.
- Les résultats de Colilert doivent être lus entre 24 et 28 heures. En outre, les résultats positifs pour les coliformes totaux et *E. coli* notés avant 24 heures, de même que les resultats negatifs notés après 28 heures sont également valides.

Remarques Concernant la Procédure
- Cette notice peut différer des réglementations en vigueur dans votre pays. Pour tout test de conformité, suivre les procédures réglementaires appropriées.
- Colilert peut être effectué en format de tubes multiples. Utiliser des méthodes standards et les tableaux MPN pour le contrôle des eaux et eaux usées** afin de déterminer les Chiffres les Plus Probables (MPN).
- Si un prélèvement d'eau présente une couleur de fond, comparer le prélèvement inoculé avec Colilert à un contrôle neutre du même prélèvement d'eau.
- Si les prélèvements sont dilués, multiplier la valeur MPN par le facteur de dilution pour obtenir le résultat quantitatif correct.
- Utiliser uniquement de l'eau stérile, non tamponnée et sans oxydant pour les dilutions.
- Colilert est avant tout un test pour eau. Les caractéristiques de performance de Colilert ne s'appliquent pas aux prélèvements altérés par tout enrichissement préalable ou toute concentration.
- Avec les prélèvements présentant un excédent de chlore, il peut se produire une rapide lueur bleuâtre lors de l'ajout de Colilert. Si tel est le cas, le prélèvement n'est pas valide et il faut cesser le test.
- Utiliser systématiquement des techniques aseptiques dans l'emploi de Colilert. Mettre au rebut conformément aux Bonnes pratiques de laboratoire.

Procédures de contrôle de qualité
Il est recommandé d'adopter la procédure de contrôle de qualité suivante pour chaque lot de Colilert :
1. Inoculer 3 récipients stériles remplis de 100 ml d'eau stérile comme suit:
   A. un avec Quanti-Cult™ *** *E. coli* ou une boucle stérile d'ATCC**** 25922 ou 11775 (*E. coli*)
   B. un avec *Klebsiella pneumoniae* Quanti-Cult ou une boucle stérile d'ATCC 31488 (coliformes totaux)
   C. un avec *Pseudomonas aeruginosa* Quanti-Cult ou une boucle stérile d'ATCC 10145 ou 27853 (non coliforme)
2. Suivre la procédure P/A ou la procédure de numération Quanti-Tray ci-dessus.
3. Les résultats doivent correspondre aux résultats du tableau d'interprétation ci-dessus.

*Comparateur P/A IDEXX, réf. n° WP104 ; Comparateur Quanti-Tray n° WQTC ou Quanti-Tray/2000 Comparateur n° WQT2NC
**Eaton AD, Clesceri LS, Greenberg AE. *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, 1998, Washington, DC.
*** Cultures Quanti-Cult - IDEXX réf. n° WKIT-1001
**** American Type Culture Collection 1-800-638-6597

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Kit di analisi Colilert®

Introduzione
Colilert rileva simultaneamente i coliformi totali e l'*E. coli* nell'acqua. Si basa su una tecnologia di substrato definito (Defined Substrate Technology) brevettata IDEXX® (DST®). Quando i coliformi totali metabolizzano l'indicatore di nutrienti del Colilert, ONPG, il campione diventa giallo. Quando l'*E. coli* metabolizza l'indicatore di nutrienti del Colilert, MUG, il campione diventa fluorescente. Il Colilert è in grado di rilevare simultaneamente questi batteri in concentrazioni di 1 cfu/100 ml entro 24 ore anche se sono presenti addirittura 2 milioni di batteri eterotrofici per 100 ml.

Contenuto
WP020I contiene 20 pacchetti Snap per campioni da 100 ml
WP200I contiene 200 pacchetti Snap per campioni da 100 ml
W050I contiene 20 pacchetti Snap per campioni da 50 ml
W050BI contiene 200 pacchetti Snap per campioni da 50 ml

Conservazione
Conservare a 2°–30°C lontano dalla luce.

Procedura Relativa a Presenza/Assenza (P/A)
1. Unire il contenuto di un pacchetto ad un campione da 100 ml (50 ml per W050 e W050B) in un a provetta sterile, trasparente e non fluorescente.
2. Incappucciare la provetta ed agitarla.
3. Incubare a 35°C ± 0,5°C per 24 ore.
4. Leggere i risultati secondo la tabella di Interpretazione dei risultati qui sotto.

Procedura di Enumerazione Quanti-Tray® (solo WP020I e WP200I)
1. Unire il contenuto di un pacchetto ad un campione di acqua da 100 ml in una provetta sterile.
2. Chiudere la provetta e agitarla fino a dissoluzione.
3. Versare la miscela campione/reagente in un vassoietto Quanti-Tray o Quanti-Tray/2000 e sigillarlo in un Sigillatore Quanti-Tray IDEXX.
4. Mettere il vassoietto sigillato in un'incubatrice a 35°C ± 0,5°C per 24 ore.
5. Leggere i risultati secondo la tabella di Interpretazione dei risultati qui sotto. Contare il numero di pozzetti positivi e consultare la tabella MPN fornita insieme ai vassoietti per ottenere il numero piu probabile.

Interpretazione dei Risultati

Aspetto	Risultato
Meno giallo rispetto al colore di confronto*	Negativo per coliformi totali ed *E. coli*
Giallo uguale o più intenso rispetto al colore di confronto*	Positivo per coliformi totali
Giallo e fluorescenza uguali o più intensi rispetto al colore di confronto*	Positivo per *E. coli*

- Individuare la fluorescenza con una luce a raggi ultravioletti da 6 watt, 365 nm, entro circa 13 cm dal campione, in ambiente buio. Dirigere la luce verso il campione, in direzione opposta ai propri occhi.
- I risultati del Colilert sono definitivi a 24-28 ore. Inoltre, i risultati positivi sia per i coliformi totali che per l'*E. coli* osservati prima di 24 ore ed i risultati negativi osservati dopo 28 ore sono anch'essi validi.

Note Sulla Procedura
- Questo inserto informativo potrebbe non riflettere le normative locali. Per i test sulla conformità, assicurarsi di seguire le procedure normative corrispondenti.
- Il Colilert si può eseguire in qualsiasi formato a provetta multipla. I metodi standard per l'esame delle tabelle MPN dell'acqua e delle acque di scarico** vanno usati per ottenere i Numeri Più Probabili (MPN).
- Se un campione di acqua dovesse presentare della colorazione di sfondo, confrontare il campione Colilert inoculato con controllo vuoto dello stesso campione di acqua.
- Se il prodotto viene diluito, moltiplicare il valore MPN per il fattore di diluizione per ottenere la quantità giusta.
- Per le diluizioni usare solo acqua sterile, non tamponata, priva di ossidanti.
- Il Colilert è un test primario per l'acqua. Le caratteristiche di prestazione del Colilert non sono applicabili a campioni alterati da qualsiasi pre-arricchimento o da concentrazione.
- In campioni con cloro eccessivo, quando si aggiunge il Colilert si potrebbe vedere un lampo azzurro. In questo caso, considerare il campione non valido e interrompere l'analisi.
- Quando si usa il Colilert va sempre seguita la tecnica asettica. Eliminare secondo le buone pratiche di laboratorio.

Procedure di Controllo della Qualità
Si raccomanda la seguente procedura di controllo della qualità per ogni lotto di Colilert:
1. Inoculare con quanto segue 3 provette sterili contenenti 100 ml di acqua sterile:
   A. una con Quanti-Cult™ *** *E. coli* o un'ansa sterile di ATCC**** 25922 o 11775 (*E. coli*)
   B. una con Quanti-Cult *Klebsiella pneumoniae* o un'ansa sterile di ATCC 31488 (coliformi totali)
   C. una con Quanti-Cult *Pseudomonas aeruginosa* o un'ansa sterile di ATCC 10145 o 27853 (non-coliforme)
2. Seguire la procedura P/A o la procedura di enumerazione Quanti-Tray descritte sopra.
3. I risultati devono corrispondere a quelli della tabella di Interpretazione dei risultati indicata sopra.

*Comparatore P/A IDEXX, codice di catalogo WP104; Comparatore Quanti-Tray N. WQTC o Quanti-Tray/2000 Comparatore N. WQT2NC
**Eaton AD, Clesceri LS, Greenberg AE. *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, 1998, Washington, DC.
***Colture Quanti-Cult™ N. di catalogo IDEXX WKIT-1001
****American Type Culture Collection 1-800-638-6597

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Colilert® Testkit

Einführung
Colilert ist zum gleichzeitigen Nachweis von Gesamtcoliformen und *E. coli* im Wasser bestimmt. Es basiert auf der patentierten Defined Substrate Technology® (DST®) von IDEXX. Wenn die Gesamtcoliformen den Nährstoff-Indikator ONPG von Colilert metabolisieren, verfärbt sich die Probe gelb. Wenn *E. coli* den Nährstoff-Indikator MUG von Colilert metabolisieren, fluoresziert die Probe. Colilert kann diese Bakterien gleichzeitig im Bereich von 1 CFU/100 ml innerhalb von 24 Stunden nachweisen, selbst wenn 2 Mio. heterotrophe Bakterien pro 100 ml vorhanden sind.

Inhalt
WP020I enthält 20 Snap Packs für 100 ml Proben
WP200I enthält 200 Snap Packs für 100 ml Proben
W050I enthält 20 Snap Packs für 50 ml Proben
W050BI enthält 200 Snap Packs für 50 ml Proben

Lagerung
Bei 2°–30°C und nicht im Licht lagern.

Presence/Absence (P/A) Test
1. Den Inhalt einer Packung zu einer 100 ml Probe (50 ml für W050I und W050BI) in einem sterilen, transparenten, nicht fluoreszierenden Gefäß hinzugeben.
2. Das Gefäß verschließen und schütteln.
3. Für den verbleibenden 24-Stunden-Zeitraum bei 35°C ± 0,5°C inkubieren.
4. Die Ergebnisse gemäß der nachstehenden Ergebnisauswerte-Tabelle ablesen.

Quanti-Tray® Auszähl-Methode (nur WP020I und WP200I)
1. Den Inhalt einer Packung zu einer 100 ml Wasserprobe in einem sterilen Gefäß hinzugeben.
2. Das Gefäß verschließen und so lange schütteln, bis der Inhalt aufgelöst ist.
3. Die aus Probe und Reagenz bestehende Mischung in ein Quanti-Tray oder Quanti-Tray/2000 gießen und in einem IDEXX Quanti-Tray Sealer fest verschließen.
4. Das verschlossene Tray 24 Stunden in einen Inkubator im Temperaturbereich von 35°C ± 0,5°C stellen.
5. Die Ergebnisse anhand der nachstehenden Ergebnisauswerte-Tabelle ablesen. Die Anzahl der positiven Vertiefungen zählen und die wahrscheinlichste Zahl (MPN; Most Probable Number) anhand der MPN-Tabelle, die den Trays beiliegt, ermitteln.

Ergebnisauswertung

Aussehen der Probe	Mögliche Ergebnisse
Geringere Gelbfärbung als der Comparator *	Negativ für Gesamtcoliforme und *E. coli*
Gleiche oder stärkere Gelbfärbung als der Comparator *	Positiv für Gesamtcoliforme
Gelbfärbung und Fluoreszenz gleich oder stärker als die des Comparators *	Positiv für *E. coli*

- Prüfung auf Fluoreszenz mit einer 6-Watt, 365 nm UV-Lampe aus einem Abstand von 13 cm in einer dunklen Umgebung. Dabei die Lampe nur auf die Probe, nicht auf die Augen, richten.
- Colilert Ergebnisse sind nach 24–28 Stunden definitiv. Außerdem sind positive Ergebnisse für Gesamtcoliforme und *E. coli*, die vor Ablauf von 24 Stunden beobachtet werden, und negative Ergebnisse, die nach 28 Stunden beobachtet werden, gültig.

Verfahrenshinweise
- Diese Packungsbeilage gibt möglicherweise nicht Ihre örtlichen Vorschriften wieder. Bei den Qualitätstests sind die anwendbaren aufsichtsbehördlichen Verfahren zu befolgen.
- Das Colilert Verfahren kann in jedem Multiple-Tube-Format durchgeführt werden. Zur Ermittlung der MPNs (wahrscheinlichste Zahlen) sollten MPN-Tabellen für Standardverfahren zur Untersuchung von Wasser und Abwasser** verwendet werden.
- Wenn eine Wasserprobe etwas Hintergrundfarbe aufweist, ist die inokulierte Colilert Probe mit einer Kontrollprobe derselben Wasserprobe zu vergleichen.
- Bei Probenverdünnungen den MPN-Wert mit dem Verdünnungsfaktor multiplizieren, um das korrekte quantitative Ergebnis zu erhalten.
- Nur steriles, nicht gepuffertes, keine Oxidantien enthaltendes Wasser zur Verdünnung verwenden.
- Colilert ist ein primärer Wassertest. Die Leistungsmerkmale von Colilert gelten nicht für Proben, die durch Voranreicherung oder Konzentration modifiziert wurden.
- In Proben mit übermäßigem Chlorgehalt wird bei der Zugabe von Colilert u.U. ein blaues Aufleuchten beobachtet. In diesem Fall ist die Probe als ungültig zu betrachten und der Test abzubrechen.
- Bei der Verwendung von Colilert ist ein aseptisches Vorgehen vorgeschrieben. Entsorgung gemäß Standard-Laborpraktiken.

Qualitätskontrollverfahren
Das folgende Qualitätskontrollverfahren wird für jedes Colilert Los empfohlen:
1. Drei sterile, mit 100 ml sterilem Wasser gefüllte Gefäße folgendermaßen inokulieren:
   A. ein Gefäß mit Quanti-Cult™*** *E. coli* oder sterile Probenschleife mit ATCC**** 25922 oder 11775 (*E. coli*)
   B. ein Gefäß mit Quanti-Cult *Klebsiella pneumoniae* oder eine sterile Probenschleife mit ATCC 31488 (Gesamtcoliform)
   C. ein Gefäß mit Quanti-Cult *Pseudomonas aeruginosa* oder eine sterile Probenschleife mit ATCC 10145 oder 27853 (Nichtcoliform)
2. Das oben beschriebene P/A-Verfahren oder das Quanti-Tray Auszählverfahren befolgen.
3. Die Ergebnisse sollten mit der Ergebnisauswerte-Tabelle oben übereinstimmen.

* IDEXX P/A Comparator, Best.-Nr. WP104; Quanti-Tray Comparator WQTC oder Quanti-Tray/2000 Comparator WQT2NC
** Eaton AD, Clesceri LS, Greenberg AE. *Standard Methods for the Examination of Water and Wastewater* (Standardverfahren zur Untersuchung von Wasser und Abwasser). American Public Health Association, 1998, Washington, DC.
*** Quanti-Cult Kulturen - IDEXX Best.-Nr. WKIT-1001
**** American Type Culture Collection 1-800-638-6597

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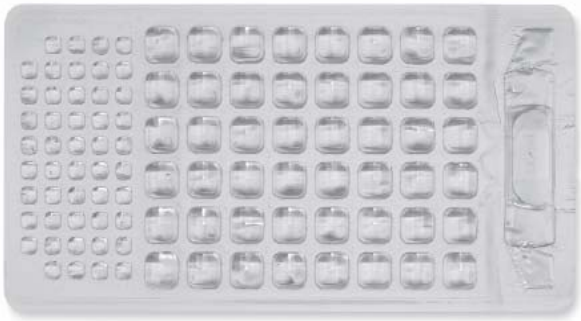
Appendix B. Quanti-Tray/2000 Instruction Manual

IDEXX **Quanti-Tray*/2000**

Insert and Most Probable Number (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.

North/South America: 1-207-556-4496/1-800-321-0207

Europe: 00800 4339 9111

UK: +44 (0) 1638 676800

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Japan: +81 422 71 5921

Australia: 1800 655 978

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IDEXX

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www.idexx.com/water

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06-02320-11

IDEXX **Quanti-Tray*/2000**

English Version

Introduction

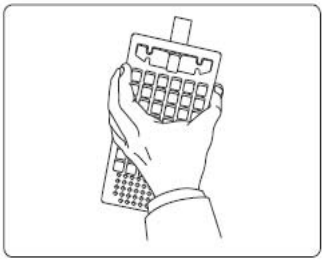
IDEXX Quanti-Tray/2000 is designed to give quantitated bacterial counts of 100 mL samples using IDEXX Defined Substrate Technology* reagent products. Add the reagent/sample mixture to a Quanti-Tray/2000, seal it in a Quanti-Tray Sealer and incubate per the reagent instructions. Count the number of positive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

Contents

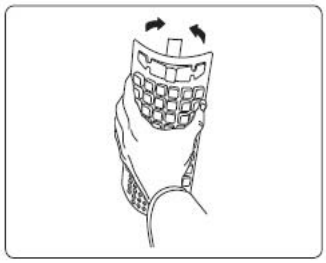
This package contains sterile Quanti-Tray/2000s.

User Instructions

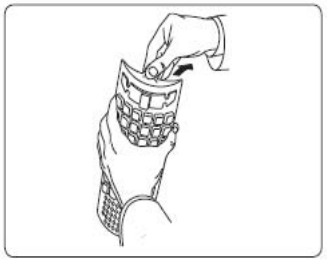
1. Use one hand to hold a Quanti-Tray upright with the well side facing the palm.



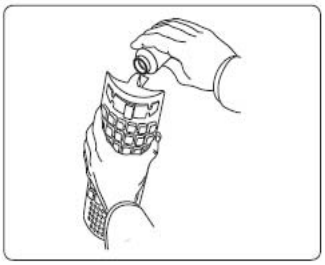
2. Squeeze the upper part of the Quanti-Tray so that the Quanti-Tray bends toward the palm.



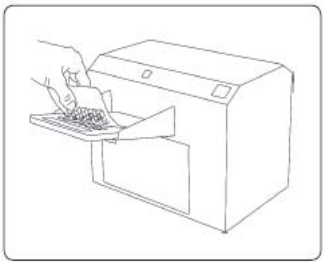
3. Gently pull foil tab to separate the foil from the tray. Avoid touching the inside of the foil or tray.



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
U.S./Canada 1-800-321-0207 or 1-207-556-4496
www.idexx.com/water

IDEXX

[†] Download IDEXX's MPN generator software for automated Quanti-Tray results at www.idexx.com/mpngenerator.

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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 and Defined Substrate Technology are 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

# 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	

Revision: 1.0

Appendix C. Laboratory Bench Sheet

Month_____	Year_____		Page ____ of ____			TOTAL COLIFORM				E. coli			
						24 Hrs				24 Hrs			
Sample Number and Date	Collector Time/Initials	Analyst Time/Initials	Sample ID/Sample Source	Volume/ Dilution	Date/Time Read/Initials	LC	SC	Number per 100 mL	Calculated Number per 100 mL	LC	SC	Number per 100 mL	Calculated Number per 100 mL

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