Standard Operating Procedure for:

Quanti-Tray/2000 Colilert-18 Method for *Escherichia coli*, Total coliforms, and Enterolert Method for *Enterococci*

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1. SCOPE AND APPLICATION

1.1 This method is used to measure *Escherichia coli (E. coli)*, Total coliforms (T. coli), and *Enterococci* in non-potable water and is based on Standard Methods SM9223B, EPA Method 1600, and IDEXX Quanti-Tray/2000 Colilert-18 and Enterolert methods. Since *E. coli*, T. coli, and *Enterococci* are commonly found in the intestinal tract of warm-blooded animals, their presence in water samples can indicate the potential presence of fecal pollution and enteric pathogens.

1.2 This procedure is used for samples with Laboratory Information Management System (LIMS) test IDs: ECOLI-18QT, EC-18QT-CL, ENT-24-QT, EN24-QT-CL, TCOLI-18QT.

2. SUMMARY OF METHOD

2.1 A 100-mL volume of sample is mixed with the Colilert-18 or Enterolert snap pack media. After mixing, it is poured into a Quanti-Tray 2000 and is run through the heat sealer to seal the tray. The tray is then incubated at 35.0 ± 0.5°C for 18-22 hours for Colilert-18 or at 41 ± 0.5°C for 24-28 hours for Enterolert. For Colilert-18, any wells that are yellow under ambient light are positive for *T. coli*, and any wells that are yellow under ambient light and fluorescent under a 365 nm UV light are positive for *E. coli*. For Enterolert, any wells exhibiting blue fluorescence under a 365 nm UV light are positive for *Enterococci*. The number of positive wells is counted and used to determine the result by referring to the Most Probable Number (MPN) table or using the IDEXX MPN Generator software. The MPN value for any diluted samples should be multiplied by the dilution factor to obtain the proper result, unless the dilution has been specified in the IDEXX software.

2.2 Marine or brackish water samples and samples with conductivity above 1900 µmhos/cm must be diluted at least tenfold with sterile, non-buffered DI water. *T. coli* samples exhibiting a yellow coloring equal to or greater than the Colilert-18 comparator must also be diluted at least tenfold with sterile, non-buffered DI water.

2.3 Interferences

2.3.1 Media is sensitive to prolonged exposure to light. Make sure to store media in the dark. Discard any media that has changed color.

2.4 Definitions

2.4.1 ***Escherichia coli*** – Bacteria that produce a positive total coliform result and possess the enzyme β-glucuronidase, which metabolizes the MUG fluorogenic

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substrate contained in the media, releasing fluorogen, which produces a blue fluorescence under a long wave 365 nm UV light.

2.4.2 ***Enterococci*** – Bacteria that possess the enzyme β-glucosidase, which metabolizes Enterolert's nutrient-indicator, 4-methyl-umberlliferyl β-D-glucoside and produces a blue fluorescence under a long wave 365 nm UV light. This includes *E. faecalis, E. faecium, E.avium, E. gallinarium* and others from the genus *Enterococcus.*

2.4.3 ***Total coliforms –*** Bacteria that possess the enzyme β-D-galactosidase, which metabolizes the chromogenic substrates ortho-nitrophenyl-β-D-galactopyranoside and chlorophenol red-β-D-galactopyranosidase found in Colilert-18 to produce a yellow color under ambient light.

3. EQUPMENT AND SUPPLIES

Record any equipment repairs or maintenance in LIMS\Laboratory Logbook\Maintenance Log.

1.1 Sterile gloves, latex or nitrile

3.1 120 mL sterile, transparent non-fluorescent IDEXX vessels

3.2 50 mL disposable beakers

3.3 Secondary containers such as 300 mL BOD bottles with caps

3.4 Chlorine test strips

3.5 Quanti-Tray/2000 trays

3.6 UV light, 365 nm, 6 watts

3.7 Colilert-18 Quanti-Tray/2000 comparator for *E. coli* and *T. coli*

3.8 Quanti-Tray rubber insert for Quanti-Tray/2000

3.9 Quanti-Tray Sealer Model Plus (S/N QTP 13215001064 or QTP 13181503550)

3.10 Class A Graduated Cylinder or Class A 100 mL Volumetric Flask

3.11 Oakton PC2700 pH and Conductivity Meter (S/N CP354757) calibrated per DEP SOP MB-03.5 Oakton PC2700 pH and Conductivity Meter Calibration and Use.

3.12 Isotemp Refrigerator (S/N 0162177801170112), maintained at 1-5 °C

3.13 Heratherm IMH 60L incubator (S/N 42511812), maintained at 35.0 ± 0.5 °C

3.14 Isotemp 650F Incubator (S/N 303N0034), maintained at 35.0 ± 0.5 °C

3.15 Water baths TSWB 4 (S/N 300471596) and TSWB 5 (S/N 300472033) maintained at 35.0 ± 0.5 °C

3.16 Water baths TSWB 10 (S/N 300613676) and TSWB 7 (S/N 300483022)

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maintained at 41.0 ± 0.5 °C

3.17 Serological disposable pipettes (1, 2, 10, 50 mL as needed), sterile and individually wrapped, with pipette pump. The volume of each new lot is checked per SOP MB-03.3.

4. REAGENTS AND STANDARDS

4.1 Colilert-18 snap pack media (for the detection of *E. coli* and *T. coli*)

4.2 Enterolert snap pack media (for the detection of *Enterococci*).

4.3 Sterile, non-buffered deionized (DI) water (1L)

4.4 IDEXX Antifoam Solution for use with Colilert-18 only

4.5 Control cultures (See SOP MB-03.1.)

4.5.1 *Escherichia coli (E. coli)* ATCC #11775

4.5.2 *Klebsiella aerogenes* (*K. aerogenes*) ATCC #13048

4.5.3 *Klebsiella Variicola (K. variicola), ATCC 31488*

4.5.4 *Serratia marcescens (S. marcescens)* ATCC #43862

4.5.5 *Aerococcus viridans* (*A. viridans*) ATCC #10400

4.5.6 *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC #10145

4.5.7 Universal Microbe CRM, Microgel flash pellet; NSI lab solutions

4.5.8 *Enterococcus faecalis (E. faecalis),* NCTC 775

4.5.9 *Pseudomonas aeruginosa (P. aeruginosa)* Microgel flash pellet; NSI lab solutions NCTC 12951

4.5.10 *Streptococcus bovis (S. bovis)* NCTC #8177

5. CALIBRATION AND STANDARDIZATION

5.1 See the Laboratory Quality Manual.

6. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

See DEP Field SOP FS 1000 for sample collection and preservation.

7. REAGENT AND SAMPLE PREPARATION

7.1 See SOP BB-30 for sample custody and labels.

7.2 To prepare sterile DI water, autoclave 1 L of DI water in a 2 L flask or 2 L glass storage bottle for 15 minutes at 121°C and a minimum of 15 psi. Be careful when

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removing the flask; contents are very hot. Use autoclave tape to ensure that the autoclave reached the proper temperature. Record the reagent in LIMS Autoclave log module located in the Laboratory Logbook. The quantity of sterilized DI water must be specified in the Entry Comments section. The Sterile, non-buffered water should be labeled in the following format: "Sterile DI mmddyy-M#" with the "#" signifying the corresponding reagents made that day. If only a single reagent was made for that date, "M1" would be used. Likewise, if there are multiple reagents for that date, they should be titled accordingly as -M2, -M3, and so forth. Label the 2L flask or bottle appropriately. Multiple flasks or bottles of DI water can be prepared at a time. The sterile, non-buffered water should be used within 7 days of the date it was opened. Record the Open Date on the bottle's label.

7.3 Prior sample analysis, turn on the heat sealer and let it warm up. The amber power light will turn on when the sealer is switched on. The green ready light will turn on when the sealer has warmed up. Place the rubber insert onto the input shelf with the large rectangular cutout facing away from the sealer.

7.4 Retrieve 120 mL sterile IDEXX vessels. For each sample, duplicate and blank, there should be one vessel per analysis. Remove the plastic wrap from the vessel. Place a sample label on each vessel.

Note: Multi-colored permanent markers can be used to circle or highlight the analysis on the label or the tray. Use one colored marker for each test type: red for the Colilert-18 (*E. coli*) test, green for *T. coli*, and purple for the Enterolert (*Enterococci*) test. Keep Test IDs separate and well organized to avoid confusion.

8. SAMPLE ANALYSIS

8.1 Shake the sample bottle vigorously, approximately 25 times, to uniformly distribute bacteria. Failure to properly mix sample can lead to erroneous results, as bacteria are known to clump together and are therefore not homogeneously distributed throughout sample. Carefully open bottle. Sample should have 1-inch of headspace from the top of the bottle. If the sample bottle is too full, carefully decant sample into a sterile, 500 mL bottle, or BOD bottle, and shake approximately 25 times to uniformly distribute bacteria. Avoid touching the inside of the bottle to prevent contamination of the sample.

Note: Allow samples scheduled for testing to come to room temperature prior to pouring into test vessel.

8.1.1 If the test ID includes –CL, check the sample for chlorine with a chlorine test strip. Pour a small volume of sample into a small container (such as a Durham tube), just enough to submerge the pads at the end of the strip. Swirl the test strip in the sample for 30 seconds. If the pads remain colorless, then the sample has already been dechlorinated and should be analyzed. If the pads

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turn purple, chlorine is present. Do not run the sample. It will be reported as "No Result" with a "Y" qualifier for improper preservation. Record the lot number and result of the chlorine test strip in the **Chlorine Residual** column in Prep Worksheet Manager (e.g. Test Strip lot#; Result=0ppm if no CHL was present). See SOP BB-30 for instructions on using Prep Worksheet Manager.

8.2 If a lab conductivity measurement is needed, pour a small portion of sample into a 30 mL disposable plastic cup. Check the conductivity of the sample (Per *Oakton PC2700 pH and Conductivity Meter* (S/N CP354757) calibrated per *DEP SOP MB-03.5 Oakton PC2700 pH and Conductivity Meter Calibration and Use*) and record in the **Lab Salinity** column in Prep Worksheet Manager. See SOP BB-30 for instructions on using Prep Worksheet Manager.

Note: If the field conductivity of a sample less than 1,900 µmhos/cm but greater than 1,000 µmhos/cm, measure the conductivity and record in the **Lab Salinity** column in Prep Worksheet Manager. Measure conductivity if not measured in the field. Equipment/field blanks always run at 100 mL. If the conductivity is greater than 1,900 µmhos/cm, the sample must be diluted tenfold with sterile, non-buffered DI water. Similarly, samples scheduled for *T. coli* analysis exhibiting a yellow coloring equal to or greater than the Colilert-18 comparator must be diluted at least tenfold with sterile, non-buffered DI water.

8.3 Carefully fill the IDEXX vessel with sample up to the 100-mL line. Check the field ID on the sample container and make sure it matches the field ID on the IDEXX vessel.

8.3.1 If the sample needs to be diluted, use either a Class A graduated cylinder or a sterile serological disposable pipette to measure the desired amount of sample into the IDEXX vessel. Bring the volume up to the 100-mL line on the IDEXX vessel with sterile, non-buffered DI water. Record dilutions in the **Sample Comments 2** column in Prep Worksheet Manager.

8.4 Add 2 drops of IDEXX Antifoam Solution to all Colilert-18 vessels prior to addition of media.

8.5 Gently tap the media snap pack to ensure that the powder settles to the bottom. Open the pack by snapping the top at the score line. Hold the snap pack opening away from face to avoid any residue that comes out when opened. Add the appropriate reagent powder to the sample in the vessel, as indicated by the test ID (Colilert-18 for ECOLI-18QT, EC-18QT-CL, or TCOLI-18QT, and Enterolert for ENT-24-QT or EN24-QT-CL). Cap the vessel and shake vigorously until the powder is dissolved. Record the date and time the reagent powder was added to the sample in the **Date/Time Sample Prepared** column in Prep Worksheet Manager.

8.6 Remove the sample label from the vessel and place it on the paper side of the Quanti-Tray. Unthread the lid of the vessel. Hold the top of the Quanti-Tray in one hand with the wells facing the palm. Squeeze the upper part of the tray, so the wells curve toward the palm and carefully pull the foil tab to open the tray (if there is a tear in the

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tray, discard and obtain a new one). Avoid touching the inside of the tray. Pour the sample/media mixture into the tray. Avoid pouring any sample onto the foil tab. Gently tap the tray a few times to release any trapped air.

8.7 Place the filled Quanti-Tray into the rubber insert making sure each well fits into its corresponding slot. Carefully slide the rubber insert with the tray into the sealer with the large rectangular cutout on the insert facing away from the sealer. When the tray has completely gone through the sealer (a part of the tray will remain inside, but the internal rollers will no longer be heard), remove the rubber insert and tray from the sealer. Remove the tray from the rubber insert and place the rubber insert back on the sealer's input shelf.

8.7.1 If the rubber insert is left in the back of the heat sealer for an extended period, the portion that remains in contact with the sealer will be very hot. Handle cautiously to avoid burns.

8.8 Repeat Steps 8.1-8.7 until all trays are sealed.

8.9 Run a blank for each test being analyzed. Fill a sterile Quanti-Tray vessel to the 100-mL line with sterile, non-buffered DI water. Add the appropriate media to the water and proceed as if it were a sample.

8.9.1 Process one blank per test per day. A single tray can be used as a blank for *E. coli* and *T. coli* analyses since they use the same media.

8.9.2 Label each blank with "test name – BLANK", date, start time, and incubation time.

8.10 Incubate the *E. coli* and *T. coli* trays at 35 ± 0.5 °C for 18-22 hours. Incubate the *Enterococci* trays, at 41 ± 0.5 °C for 24-28 hours.

8.10.1 Trays must be stacked in a manner that does not obstruct the water flow in the water bath. Place trays away from the water output source. Maximum sample loads for each water bath and incubator can be found in the "Equipment Temp Distribution and Max Load" excel found here: ..\..\Micro\

8.11 After incubation, remove the trays and count all positive wells. For *T. coli* trays, use ambient light to count the yellow wells that are equal to or greater than the Colilert-18 comparator. For *E. coli* trays, count all wells that are yellow in ambient light and fluorescent under UV light equal to or greater than the Colilert-18 comparator. For *Enterococci*, count all wells that exhibit blue fluorescence. When examining trays for fluorescence, turn off overhead lights and use a 365 nm UV light within 5 inches of the sample to count all wells that are fluorescent.

8.11.1 If any wells are threshold positive, trays may be placed back into the water bath within 10 minutes and removed at the end of the incubation window. This will allow the bacteria more time to metabolize the substrate and produce a stronger positive signal. Use the latest time the sample was removed from the water bath to document the read time.

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8.11.2 If two or more wells on a tray are empty the sample will be reported as "No Result" with an "O" qualifier. A Non-Conformance Report will be submitted, and customer notified.

8.12 Count and record all positive large wells and positive small wells on the excel template **MB-QT-MF-COUNT** found in the folder named "**MF & QT Read** Template" here: X:\Labs\Lab5\Biology\BenchCommon\Micro\MF & QT Read Template

8.12.1 To export the worksheet, log into LIMS on workstation computer. Select Laboratory → Technical → Prep Worksheet Manager. Double click the spreadsheet to be exported: MICRO-QT_xxxx.

8.12.2 Click the 'Export to File…" button on the bottom of the worksheet.

8.12.3 Save the text file as the Worksheet ID in the "MF & QT Read Template" folder. Navigate to the current year's folder. If one has not already been created, create a new folder saved as the current month (ex. 01-JAN).

8.12.4 Open the text file. Select all and copy; then paste it into the "Paste Data Here" sheet of the **MB-QT-MF-COUNT** template.

8.12.5 Data should automatically populate into the "**QT Print**" sheet.

8.12.6 Print sheet. Make sure "Portrait Orientation" and "Fit All Columns on One Page" are selected.

9. DATA ARCHIVAL

9.1 During sample preparation, record preparation and incubation dates/times for each sample on the prep worksheet. Enter media lot numbers, comparator ID, QC record numbers for consumables and equipment used, blanks and dups with their respective Test IDs. Record sterile non-buffered DI water lot number in the **Prep Comments** field. Also, include any pertinent comments and notes. Label the duplicated samples "dup" after the sample number and duplicate their rows.

9.2 Open X:\Labs\Lab5\Biology\BenchCommon\Micro\QuantiTray Data and navigate to the current year's folder. If one has not already been created, create a new folder saved as the current month (ex. 01-JAN).

9.3 Enter well counts in IDEXX MPN Generator. (To install the MPN Generator, go to the IDEXX website. Under IDEXX Businesses, click on **Water Testing Solutions** and go to **Products and Services**. Under Resources & Support, click on **MPN Generator Software**. Under Resources, click on **MPN Generator Software Program**.)

9.3.1 Open the IDEXX MPN Generator located in the folder: X:\Labs\Lab5\Biology\BenchCommon\Micro\QuantiTray Data. Click on the **Options** icon. In the space labeled **default directory to save files:** paste the

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address above. Select the **Date Format: MM/DD/YYYY**, **Decimal Format: 0.00,** and **File Format: csv**. Check the box next to **Use Dilution Mode**. Save changes.

9.3.2 In the blank space at the top of the main MPN Generator page, enter the **File Name** in the format "**QTmmddyy-QT"**, where "mmddyy" is the date the samples were processed. In the **Sample Date** box, enter the analysis date. Fill in the **Analyst** (using LIMS ID), **Method** (Colilert-18 or Enterolert), and **Analyte** (*E. coli*, *T. coli*, or *Enterococci*). Enter the volume of undiluted sample as **100.**

9.3.3 Enter the **Sample ID**, the number of **Quanti-Tray®/2000 Positive Large Wells (0-49)** and the number of **Quanti-Tray®/2000 Positive Small Wells (0-48)**. Click **Calculate** and then **Log**.

9.3.3.1 If a sample was diluted, change the volume of undiluted sample before calculating.

9.3.3.2 *E. coli*/*T. coli* samples share one tray and will need to be read and entered twice. Once for Colilert-18, analyte "Coliforms" for *T. coli* and again for Colilert-18 for *E. coli.*

9.3.4 Repeat Step 9.3.3. until all the Quanti-Trays have been entered for that date. Change the File Name and Sample Date to reflect a new date being entered.

9.3.4.1 The MPN Generator creates an Excel file in the folder selected in Step 9.3.1. The name of the Excel file is the same as the File Name entered in the MPN Generator, i.e., the date the samples were processed in the format "**QTmmddyy-QT"**.

9.4 Print the Excel file created by the MPN Generator.

9.4.1 Open the Excel file. Change the name by removing the first "QT" leaving the name as "**mmddyy-QT**". Hide columns F, I and J. In Print Preview, select Page Setup. Change the Orientation to **Landscape**. In the Header/Footer tab, select **Custom Header…**. In the Center section, type in the File Name. In the Sheet tab, select **Gridlines**. Press OK and Print. Do not save changes.

9.5 Perform a two-person verification on the Excel printout. (Refer to SOP BG-01.)

9.6 Upload results to QC Manager per SOP BB-31.

9.7 Refer to SOP BB-32 for data authorization.

10. QUALITY CONTROL

10.1 Sample Replicates

10.1.1 Analyze at least one laboratory replicate per analysis per day.

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10.1.2 Maintain a control chart of duplicate results. Convert results to logarithms and calculate the range (difference of logarithms of a replicate set). The range should be < 3.27 times the mean range of the last 15 replicate sets.

10.1.3 Corrective Actions: Report laboratory replicate results that do not meet the acceptance criteria with a "J" qualifier.

10.2 Method Blanks

10.2.1 Add 100 mL of sterile, non-buffered DI water to a mixing vessel and add the appropriate media (Colilert-18 for *E. coli* and *T. coli* and Enterolert for *Enterococci*). Shake until dissolved, add to a Quanti-Tray, and seal. Label the tray as a blank and include the test ID, date, start time and incubation time. Incubate blanks with samples for 18-22 hours at 35 ± 0.5 °C for Colilert -18 media, or 24 - 28 hours at 41.0 ± 0.5 °C for Enterolert media.

10.2.2 Absence of growth in method blanks demonstrates that media, mixing vessels, Quanti-Trays, and sterile non-buffered DI water are free of contamination of the target organism from improper handling, inadequate sterilization, or environmental exposure.

10.2.3 Corrective Actions: If method blanks have growth, determine the cause of contamination. The media, mixing vessels, Quanti-Trays, and sterile non-buffered DI water batch can be checked for sterility. If the cause of contamination has also affected samples, report samples in the batch that are greater than MDL with a "V" qualifier and the comment "Result is V-qualified due to control blank failure."

10.3 Negative Controls

10.3.1 For each lot of media, transfer a small amount of plated *P. aeruginosa, or a Pseudomonas aeruginosa (P. aeruginosa)* Microgel flash pellet, for Colilert- 18 media, or plated *S. marcescens* and *A. viridans* cultures for Enterolert media into separate sterile vessels containing 100 mL of sterile, non-buffered DI water, and label with the species and culture serial number. Microgel flash pellets of *E. coli* and *S. bovis* can also be used for Enterolert negative controls. Shake vigorously. Add the appropriate media snap pack to the corresponding vessel and mix. Add the control sample to a Quanti-Tray labeled with the media lot number, the species, culture serial number, and "N" for negative control and heat seal. Incubate Colilert -18 media at 35 ± 0.5 °C for 18-22 hours and Enterolert media at 41.0 ± 0.5 °C for 24 - 28 hours.

10.3.2 All wells on the Quanti-Tray/2000 should be negative.

10.3.3 Corrective Actions: If there are any positive wells on the negative control tray, repeat the test. If the repeat test is positive, do not use the lot for analysis, and contact supervisor.

10.4 Record results in LIMS Standard Preparation Tracker per SOP MB-01.12.

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10.5 Positive Controls

10.5.1 Test each lot of media with a known concentration of Universal Microbe CRM or *E. faecalis* for Enterolert media per manufacturer instructions. A ATCC or NCTC microgel flash pellet derived E. coli and *K. variicola* can also be used for Colilert-18 media. Add the appropriate media snap pack to the corresponding vessel and mix. Add the control sample to a Quanti-Tray labeled with the media lot number, the species, culture serial number, and "P" for positive control and heat seal. Incubate Colilert-18 media at 35 ± 0.5 °C for 18-22 hours. and Enterolert media at 41.0 ± 0.5 °C for 24-28 hours.

Note: *K. variicola* is the positive control used for T. coli. Universal Microbe is the positive control for both *E. coli* and *T. coli*.

10.5.2 Count all positive wells and record results in the comments section of a Micro-QC preparation worksheet. Growth results in the range of acceptance criteria according to the culture COA indicate that the media supports growth of the target organism. Refer to steps 8.11-8.12 for how to read wells.

10.5.3 Corrective Actions: If the trays do not show the correct positive result, repeat the test. If the repeat test is not producing the expected result, do not use the lot for analysis, and contact supervisor.

10.5.4 Record results in LIMS Standard Preparation Tracker per SOP MB-01.12.

10.6 pH Check

10.6.1 For each lot of media, add the appropriate media snap pack to a sterile vessel containing 100 mL of sterile, non-buffered DI water. Check the pH of the media per SOP MB-03.4.

10.6.2 pH should be 7.0-7.6 for Colilert-18 and 7.1-7.9 for Enterolert. Enter results in the pH column of the Micro-QC preparation worksheet prepared for the media QC. If the pH is not within range, do not use the lot for analysis and contact a supervisor.

10.6.3 Record results in LIMS Standard Preparation Tracker per SOP MB-01.12.

10.7 Volume Check

10.7.1 For each lot of Quanti-Tray mixing vessels, perform a volume check on one vessel. Fill a mixing vessel with DI water up to the 100 mL line. Pour the water into a Class A To Contain (TC) graduated cylinder. Check the volume on the graduated cylinder. The meniscus should fall within 2.5% of the expected, 100 mL volume. If the meniscus falls outside of 2.5% of the expected volume, the QC fails and the check should be redone. If the volume check fails again, do not use the lot and notify the supervisor. Record the volume observed on the graduated cylinder and "Pass" or "Fail" in LIMS Laboratory/Technical/Laboratory Logbook *Pipet- Fun_Cal_Log.*

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10.8 Sterility Checks

10.8.1 Check sterility on each lot of sample containers, mixing vessels, and trays with TSB 2X (see SOP MB-02.33). If there is growth (turbidity), recheck lot or batch. If recheck also fails (shows growth), do not use lot, and contact supervisor. Record results in LIMS Laboratory/Technical/Laboratory Logbook *Lab_Supplies_QC.*

10.8.1.1 Sample containers and mixing vessels: Add a small amount of TSB 2X (10-20 mL) to the container to be checked. Cap and swirl/shake the media so that it touches every surface area in the container. Incubate at 35 ± 0.5 °C for 24 hours.

10.8.1.2 Trays: In a sterile 120 mL mixing vessel , combine approximately 50 mL TSB 2X with 50 mL sterile, non-buffered DI water up to the 100 mL line. Pour the mix into the tray and seal. The tray should be full, and all surface areas should have had contact with the media. Incubate at 35 ± 0.5 °C for 48 hours.

10.9 Autofluorescence Check

10.9.1 For each new lot of Quanti-Tray/2000, Enterolert, and Colilert-18 received, hold a 365 nm UV light within 5 inches over the media packet or empty Quanti-Tray in subdued light to check for any autofluorescence. If any autofluorescence is detected, do not use the lot, and contact the manufacturer. Record results in the MICRO-QC worksheet designated for the QC of the media or Quanti-Tray.

10.10 Sealer Performance Check

10.10.1 Once a month, add one to two drops of food coloring to 100 mL of DI water. Add mixture to a tray and seal. Check each well for leaks. If leaks are found, repeat the check. If repeat check also fails, do not use the sealer, and contact a supervisor. Record results as “Pass-No Leak” or “Fail-Leak” in LIMS Laboratory/Technical/Laboratory Logbook *Instrument_Log.*

10.11 Sodium Thiosulfate Dechlorination Check

10.11.1 Check each lot of sample containers that have a sodium thiosulfate tablet for adequate dichlorination.

10.11.1.1 Dilute bleach to 0.1%. Check the concentration with the Hach Colorimeter II per SOP TA-06.22 and record in Standard Preparation Tracker.

10.11.1.2 Dilute the 0.1% solution to 16 mg/L Cl_2 solution to the sample container with the tablet that is being tested. Shake until the tablet is dissolved. Use a chlorine test strip to determine if the solution has been dechlorinated. If the bleach solution is not dechlorinated, the lot fails, and the check should

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be redone. If the dechlorination check fails again, do not use the lot. Notify supervisor. In LIMS lab logbook **Lab_Supplies_QC** enter the lot # of the container in the lot # box. Select "dilute bleach" in **Entry type** box. In **Entry Description** select "NaTS de-chlorination check". Record the date and analyst. Enter the SN of the 16 mg/L Cl2 solution in the **batch ID** box and enter the final concentration of the solution after dechlorinating in the **entry value** box. Enter the lot number of the chlorine test strips in **data source** box.

10.12 Well Counting Variability

10.12.1 Each trained analyst must compare well counts against another trained analyst from one positive sample per analyte monthly. Counts should be within 10% of each other. Comparisons must be performed by all analysts performing this test within one hour after the Quanti-Tray has been pulled from incubation. When comparing counts, each trained analyst will write their count on the back of the Quanti-Tray along with their initials. The counts will then be recorded in MB-PARCOUNTLOG.

11. MAINTENANCE OF QUANTI-TRAY SEALER & UV LIGHT

11.1 Clean the Quanti-Tray Sealer at least monthly or more often as needed. Refer to the Quanti-Tray PLUS User Manual pdf file located in X:\Labs\Lab5\Biology\BenchCommon\Micro\QuantiTray Data\Manuals for instructions.

11.2 Annually replace the bulb in the 365 nm UV light. Record the bulb replacement in LIMS laboratory logbook **MAINTENANCE_LOG**.

11.2.1 Record UV light bulbs into the LIMS laboratory logbook **LAB_SUPPLIES_LOG** when new light bulbs are received.

12. INCUBATOR TEMPERATURE AND RECOVERY

12.1 Corrective action: If the incubator or water bath goes out of the method specified temperature range while under test conditions, notify management. Create an NCR and J-qualify the data. Determine the cause of the temperature fluctuations.

13. SAFETY/HAZARDOUS WASTE MANAGEMENT

13.1 All media/bacteria must be autoclaved for 45 minutes before disposal.

13.2 Refer to the Laboratory Health and Safety Plan.

13.3 Read MSDS information for reagent handling and disposal.

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14. REFERENCES

14.1 Standard Methods 9223 B-2016

14.2 Standard Methods 9222 B-2017

14.3 Standard Methods 9020-2017

14.4 EPA Method 1600

14.5 IDEXX Quanti-Tray/2000 Colilert-18 method instructions

14.6 IDEXX Quanti-Tray/2000 Enterolert method instructions

14.7 IDEXX Quanti-Tray Sealer PLUS Manual

14.8 NELAC Quality Systems Manual

14.9 FDEP Laboratory Quality Manual
https://publicfiles.dep.state.fl.us/DEAR/DEARweb/Labs/lab-qualitymanual-2024.pdf

14.10 FDEP Laboratory Health and Safety Plan
https://www.floridadep.net/resources/health-safety-manual

14.11 DEP SOP-001/01 FS 1000, *General Sampling Procedures*

14.12 DEP SOP MB-01.5, *Pour Plate Method for Heterotrophic Plate Count*

14.13 DEP SOP MB-02.33, *Preparation of Dehydrated Culture Media*

14.14 DEP SOP MB-03.1, *Maintenance of Bacterial Cultures*

14.15 DEP SOP MB-03.4, *Accumet Excel XL 25 pH Meter Calibration and Use*

14.16 DEP SOP MB-03.5 Oakton PC2700 pH and Conductivity Meter Calibration and Use

14.17 DEP SOP BB-30, *Sample Custody, Preparation Labels and Worksheet Instructions for Bench Biology Samples*

14.18 DEP SOP BB-31, *LIMS QC Manager Data Uploading Instructions for Bench Biology and Microbiology Samples*

14.19 DEP SOP BB-32, *Test Level Authorization of Uploaded Results for Bench Biology Samples*

APPENDIX OF CHANGES

01/16/2015	Combined Colilert and Enterolert analyses; added chlorine check and analysis blanks; updated Data Archival to reflect the use of the IDEXX MPN Generator; under Quality Control, added volume and sterility checks; added a Maintenance Section; added Quanti-Tray/2000 manuals to References section.
02/26/2015	Changed all tlhlab5 and tlhlab3 network server locations to fldep1 network.

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03/16/2017	Updated equipment information, clarified counting procedures, added comparator to equipment list and instructions for use, added instructions for threshold positive wells, added method blanks section to Section 10, and updated hyperlinks.
03/16/2018	Added IDEXX Antifoam Solution and new Control Cultures to Section 4. Added instructions to Section 8. Added new Control Cultures along with instructions to Section 10.
04/29/2019	Updated serial numbers for Quanti-Tray/2000 sealers under equipment. Updated serial number for incubators and added backup incubators. Updated name/ATCC# for E. aerogenes to Klebsiella aerogenes. Added comment in sample analysis section.
01/02/2020	Added new incubator. Changed SM9222B and SM9020 to correct version.
02/13/2020	Updated section 4.4.1. Added section 10.10 Well Counting Variability.
04/23/2020	Updated format of Chlorine Test Strip result, updated units in section 8.2 and revised information in section 9.4.1.
02/08/2021	Added a new water bath and HRT 60 L incubator. Removed 625 F and Heratherm IMH incubators that are out of service. Updated incubation instructions in the Sample Analysis section. Updated the positive media QC section to include instructions for quantitative testing. Updated LIMS documentation in dechlorination section. Added a corrective action section for failing incubators.
02/09/2022	Added Total coliforms (T.coli) analysis by Quanti-Tray to SOP including Definition, Preparation, and Data Archival. Updated links for FDEP Laboratory Quality Manual and FDEP Laboratory Health and Safety Plan
07/27/2022	Modified conductivity measurement requirements to an as needed basis. Added culture references to section 4.6, updated hyperlinks and equipment. Edited sections 8, 9.4.1 and 10.6 (reworded for clarity). Added SOP reference for the Oakton PC2700 pH and conductivity meter.
08/01/2022	Revised sections 3.9, 3.12 and 3.17 to specify new equipment.
09/22/2023	Updated section 4.4.1 to reflect modification in sterile, non-buffered water expiration. Edited section 8.2 to further clarify conductivity measurements. Updated section 10 to mention the usage of Universal Microbe and TSB in media QC.
05/06/2024	Added Table of Contents. Minor updates made to equipment list. Added cultures to section 4.6. Added section 8.13 to include new procedure for recording Quanti-Tray results using excel template. Updated section 10.3 to include other culture options. Updated section 10.6 for procedure and recording of volume checks. Added autofluorescence check to section 10. Updated section 10.9 to record “Leak/No Leak” as part of the Quanti-Tray Sealer check. Updated section 11 to include UV light maintenance. Updated language and formatting throughout the

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document to eliminate typos and improve the consistency and coherence of the instructions. Updated the link to the DEP Laboratory Assurance Manual 2023.

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