EXAMPLE

STANDARD OPERATING PROCEDURE

ANALYTICAL QUANTIFICATION OF *ESCHERICHIA COLI* AND ENTEROCOCCI BACTERIA IN AMBIENT SURFACE WATERS Using AN ENZYME SUBSTRATE TEST (STANDARD METHODS 9223b)

January 2020

Adapted from MassDEP CN 198.0 version February 2007

Prepared by: MassDEP Watershed Planning Program



Contents

[SCOPE & APPLICATION 3](#_Toc31211047)

[SUMMARY OF METHODS 3](#_Toc31211048)

[INTERFERENCES 4](#_Toc31211049)

[LAB SAFETY 4](#_Toc31211050)

[EQUIPMENT AND SUPPLIES 4](#_Toc31211051)

[SAMPLE COLLECTION, PRESERVATION AND STORAGE 6](#_Toc31211052)

[QUALITY CONTROL 6](#_Toc31211053)

[CALIBRATION AND STANDARDIZATION 7](#_Toc31211054)

[DETAILED TEST PROCEDURE 7](#_Toc31211055)

[PREVENTATIVE MAINTENANCE 10](#_Toc31211056)

[DATA INTERPRETATION and ANALYSIS 10](#_Toc31211057)

[METHOD PERFORMANCE 11](#_Toc31211058)

[WASTE MANAGEMENT 11](#_Toc31211059)

[REFERENCES 11](#_Toc31211060)

[APPENDICES 12](#_Toc31211061)

[Appendix A: Lab Notebook Example 13](#_Toc31211062)

[Appendix B: Incubator Temperature Log 14](#_Toc31211063)

[Appendix C: Colilert® QuickGuide 15](#_Toc31211064)

# SCOPE & APPLICATION

This SOP describes the detection and enumeration of total coliform, *Escherichia coli* *(E-coli)* and Enterococci bacteria in surface water samples using the enzyme substrate test, as detailed in **Standard Methods 9223B**. These methods use hydrolyzable substrates for the detection of target bacteria.

The commercially-available supplies developed by IDEXX Laboratories, Inc. were chosen for this procedure. The IDEXX “Colilert” and “Colilert-18” methods detect and enumerate both total coliform and *E-coli* bacteria. The IDEXX “Enterolert” method is used to detect and enumerate Enterococci (fecal streptococci) bacteria.

# SUMMARY OF METHODS

Because pathogenic (disease-causing) organisms are difficult to isolate and identify, fecal coliform and Enterococci bacteria are used to indicate the potential presence of pathogens in water. Coliform and fecal streptococci bacteria are widely distributed in nature and are present in the intestines of warm-blooded animals, including humans. Their presence in surface waters may indicate human or animal fecal contamination. They can be relatively easily identified and enumerated.

Colilert (-24) and Colilert-18:

1. The Colilert reagent is added to a 100-mL volume of freshwater sample, the sample is poured into a multi-well tray, the tray sealed and then incubated for 24 hours (Colilert) at 35° ± 0.5°C. The tray is then checked for color (total coliform) and fluorescent (*E-coli*) reactions. The most probable number (MPN) technique utilizing a multiple well system format is used to determine the number of total coliform and *E. coli* per unit volume (100 mls). The Colilert-18 reagent provides results in only 18-22 hours.
2. Colilert (-24) and Colilert-18 are US EPA-approved for drinking water and source waters. Unlike Colilert (-24) (mainly freshwater use), Colilert-18 can also be used in saltwater, but for *E. coli* only--- not total coliform. NOTE: do at least a 1:10 dilution on Colilert-18 samples if they've been collected in salt/brackish water. <http://www.idexx.com/water/refs/060202711C18.pdf>
3. The method detection limit (MDL) for total coliform and E. coli using Colilert (-24) and Colilert-18 is an MPN of 1 colony forming unit (CFU) per 100 mls.
4. The total coliform group is defined as all bacteria possessing the enzyme B-D-galactosidase. The Colilert reagent contains the nutritive ortho-nitrophenyl-B-D-galactopyranoside (ONPG), which is used to detect the enzyme B-D-galactosidase of total coliforms. The hydrolyzation of the ONPG by the enzyme produces a color change (positive=yellow) at 35.0 ° +/- 0.5° C after an incubation period of 24 to 28 hours. The Colilert method also simultaneously detects the presence of *E. coli* through the hydrolyzation of the fluorogenic, nutritive substrate, 4-methyl-umbelliferyl-B-D-glucuronide (MUG) by the *E. coli* enzyme B-glucronidase. This reaction produces a fluorescent product detectable when viewed under a long-wavelength (365-nm) ultraviolet (UV) light. Non-coliform bacteria cannot metabolize the indicator nutrients.

Enterolert:

1. Similarly, the Enterolert reagent is added to a 100-mL volume of fresh or marine water (diluted 10X) sample, the sample is poured into a multi-well tray, the tray sealed and then incubated for 24 hours at 41° ± 0.5°C. The tray is then checked for blue fluorescent wells indicating enterococci presence. A MPN table is used to determine the most probable number of Enterococci per 100 mls. The MDL for Enterococci is also a MPN of 1 CFU/100 mls.
2. The Enterococci, a sub-group of the fecal streptococci, contain the enzyme B-glucosidase. The Enterolert reagent contains 4-methyl-umbelliferyl-B-D-glucoside, which reacts with the enterococci-produced enzyme B-glucosidase to produce a fluorescent blue color.
3. Enterolert is most applicable for marine/brackish water samples (using the required minimal 10X dilution), but can also be used for fresh water (Note: MA. freshwater surface water quality standard is based on E. coli (Colilert), not Enterococcus). Enterolert is approved by the US EPA as a method for enterococci detection in ambient waters, including fresh, marine or estuarine surface water. It is also an ASTM-approved method (#D 6503-99).

# INTERFERENCES

The following situations represent potential interferences or complications in achieving accurate and precise results when using the Colilert, Colilert-18 and Enterolert methods.

Colilert and Colilert-18:

1. Non-coliform bacteria, particularly *Aeromonas*, and *Pseudomonas* species, may produce small amounts of the enzyme β-D-galactosidase, but are suppressed and generally will not produce a positive response within the incubation time unless more than 104 colony-forming units (CFU)/mL are present.
2. *Serratia* species may turn the medium yellow after 24 hours of incubation but the yellow color is typically brighter than that represented by the color comparator.
3. Some strains of *Shigella* species may produce a positive fluorescence response. This is not considered a detriment for testing the sanitary quality of water due to the pathogenic nature of *Shigella*.
4. Some water samples containing humic material may have an innate color. If a water sample has some background color, compare inoculated Colilert® sample to a control blank of the same sample.
5. Incubation beyond 28 hours may yield a false positive Colilert result, due to cessation of suppression of non-coliform heterotrophic bacteria. The same is true beyond 22 hours for Colilert-18.
6. Presence of free chlorine in the sample may result in a transient blue color upon addition of the Colilert reagent. To avoid this, all bacteria samples should be taken using sample bottles containing sodium thiosulfate.
7. Dilute brackish/marine water samples with sterile fresh water at least ten-fold for Colilert-18 testing.

Enterolert:

1. Dilute marine water samples with sterile fresh water at least ten-fold, per IDEXX Enterolert procedure.

# LAB SAFETY

1. Samples (and positive controls) may contain organisms that are pathogenic to humans, and often the analyst works with water samples of different ranges of contamination. Handle all samples and cultures as if they are infectious. All precautions are to be taken to minimize exposure. These include the use personal protective equipment (lab coats, safety glasses, and protective gloves), keeping the lab work area clean and organized, working at a reasonable pace and using good judgment at all times.
2. It is recommended that all bacteria lab personnel must be immunized against the hepatitis A and B viruses.
3. All bacteria lab personnel must receive on-the-job laboratory safety training.
4. The sealer device is a burn hazard if not properly used and maintained.
5. Material Safety Data Sheets (MSDS) for all chemicals used in this SOP should be kept in the lab.
6. NOTE: *Pseudomonas* sp. and *Klebsiella pneumoniae* used for media batch QC (see page 8-9) are categorized as Class 2 bio-safety hazards, whereas *Enterococcus faecium* and *E. coli* are categorized Class 1. While Class 2 organisms can be used in this SOP, their use presents greater potential risk to analysts should an accident occur, if poor lab techniques were used or if safety protocols were not followed.

# EQUIPMENT AND SUPPLIES

The following lab equipment and supplies are needed to employ the multi-well tray Colilert and Enterolert methods. It is assumed that the necessary field supplies, such as sterile, 120 ml. bacteria sample bottles containing sodium thiosulfate, will be available for use in the field.

Colilert and Colilert-18:

1. Lab disinfectant (e.g., Roccall II 10%, Conflikt detergent) and paper towels (supply room)
2. Disposable plastic gloves and “hot” gloves (incubator and sealer) and lab coats
3. Lab notebook
4. Lab fridge and storage areas for reagents and supplies
5. Quanti-tray sealer
6. Quanti-tray 2000 97-well sample trays
7. Quanti-tray 2000 tray rubber inserts (minimum 2)
8. Colilert reagent [Store in dark at 2-30 deg. C; use within 12 months of manufacture] and Colilert-18 reagant [Store in dark at 2-25 deg. C; use within 12 months of manufacture]
9. Sterile lab sample bottles for raw and diluted samples. Preferably, these bottles should be high clarity (to see end points clearly), graduated for 100 mls (accurate to within 2.5% or approx. 2-3 mls), and be non-fluorescing. IDEXX vessels are preferred.
10. Sterile dilution bottles (90 and 99 ml for 10X and 100X dilutions) pre-filled with sterile, buffered and/or non-buffered dilution water. (**NOTE:** *IDEXX, Inc. stipulates the use of non-buffered, sterile dilution water, since their reagents are already buffered.)* [Store as directed by manufacturer]
11. Sterile pipettes (1, 10, and 50-100 ml sizes)
12. Sterile water (non-buffered). Autoclaved sterile water available in 120-250 ml bottles. Sterile dilution water may be purchased water from IDEXX.[If purchased from IDEXX in sealed, 100 ml bottles, shelf life is 2 years. Store at 2-8 deg. C or at room temperature.]
13. Positive (*E. coli, Enterococcus faecium)* and negative (*Pseudomonas*, other) control cultures (for QC). Difco Bactrol discs are acceptable. [Store at 2-8° C. Discs have 1-year shelf life]
14. Two-shelf, bench-top, mechanical-convection incubator (5-65 deg. C; +/- 1.5 deg. C uniformity; +/- 0.5 deg. C stability) with thermometer. If also running Enterolert, two incubators (at each temperature) are preferred. Incubators should have a temperature log sheet to track internal temperatures several times/day.
15. NIST-certified (or traceable) thermometer
16. 365 nm UV lamp (6 watt) and UV viewing cabinet
17. Colilert color comparator NOTE: The color comparator is the lowest color and fluorescence level at which a result can be considered positive. A typical positive result is much more intense.
18. MPN tables (from IDEXX)
19. Quanti-cult QC kit [Store at 2-8 deg. C until use; use within 18 months of manufacture]
20. Labeled, plastic bio-waste disposal bags, step-on can with cover for temporary waste storage.

Enterolert: (in addition to those listed above for Colilert): Enterolert reagent. [Store in dark at 2-30 deg. C]

# SAMPLE COLLECTION, PRESERVATION AND STORAGE

1. New, plastic, disposable, sterile, pre-labeled, 120-mL sample bottles (usually containing a sodium thiosulfate tablet/powder for chlorine neutralization) are used to collect surface water bacteria samples. Examples include Corning locking “flip-top” bottles (preferred) and IDEXX clear, polystyrene “vessels”. See example field collection SOP for detailed method.
2. After the sample is collected, a slight amount is poured off to allow at least 1/2” of headspace in the bottle. This is required to allow for sufficient mixing of the sample at the lab prior to analysis.
3. Samples are placed in a clean plastic bag, which is then placed in a cooler half-filled with wet ice immediately or soon as possible after collection. Samples (at 4 deg. C) are delivered to the lab within 6 hours from collection with the sample tracking/chain-of-custody form filled out by the collector.
4. At the lab, sample analysis must be initiated as soon as possible and within 8 hours of collection and two hours of receipt.

# QUALITY CONTROL

Colilert and Colilert-18:

1. **Media Lot-Specific Quality Control:** used to verify that the Colilert and Colilert-18 media does not provide false positives or false negatives for *E.coli*. Each lot is tested using ***Quanti-cult*** (see Appendix D) cultures or lypholyzed microorganism pellets as follows:
2. Negative Control: sterile water alone, AND
3. Negative Control: sterile water inoculated with non-fluorescent *Pseudomonas* sp. *or Enterococcus faecium* (i.e., total coliform & *E. coli* negative) or with *Klebsiella pneumoniae, Enterobacter aerogenes or* *Enterobacter cloacae*  (i.e., total coliform positive & *E. coli* negative), AND
4. Positive Control: sterile water inoculated with *E. coli* (i.e., total coliform & *E. coli* positive) control cultures.
5. Upon receipt, check reagent packages integrity for proper seal, tears and lack of moisture. When using, inspect appearance of Colilert reagent; it should appear dry, free-flowing and white to off-white in color.
6. Each lot of medium can be checked before use with the UV light to detect auto-fluorescence. If any faint fluorescence is observed, then it should be recorded, discarded and replaced by a new lot.
7. If any media causes the sample to fluoresce prior to incubation, then another lot of medium should be used.
8. **Sample Batch Specific QC:** For each lab batch, run a minimum of one lab blank and one lab duplicate (@ approx. 10% of total samples).
9. For reading and interpretation of results, all analysts should participate in group QC consisting of inter-analyst comparisons to confirm acceptable precision among different readers. This is especially important for the *E. coli* fluorogenic end-point, for which the comparator seems to be less useful.

Enterolert: (in addition or changed from that above for Colilert)

1. **Media Lot-Specific Quality Control**: Similarly, run the following media lot QC samples to verify that the Enterolert media does not provide false positives or false negatives for Enterococci. With each new lot of media purchased, run the following controls:
2. Negative Control: sterile water alone, AND
3. Negative Control: sterile water inoculated with non-fluorescent *Serratia marcescens* (gram -) and *Aerococcus viridans* (gram +), or *E. coli* (gram -), AND
4. Positive Control: sterile water inoculated with fluorescent *Enterococcus faecium*.

# CALIBRATION AND STANDARDIZATION

1. Sample Containers: sample bottles are assumed to meet accuracy limits of < +/- 2.5%.
2. Incubator: The incubator should maintain a temperature of 35° ± 0.5°C for *E. coli*. Monitor temperatures both in air and “totally immersed” in water using total immersion incubator thermometers. Temperature should be recorded for days in use at least twice per day with readings separated by at least 4 hours.
3. Because pre-heated, air‑type incubators may not bring water sample(s) to the specified incubation temperature of 35 °C quickly, false‑negative results could result. Therefore, the time it takes for samples (or a set of samples) to reach 35 °C must be accounted for to ensure that the specified incubation period occurs (typ. >24 hrs.). See also 10.25, 10.29 discussing additional incubation periods.

# DETAILED TEST PROCEDURE

Colilert and Colilert-18 (and Enterolert, subject to changes/additions, as specified herein; see below):

1. Coordinate analytical work regarding the quantity and timing of sample delivery.
2. Preheat the Quanti-Tray sealer (usually takes around 10 minutes). Sealer is ready to use when green light is on.
3. Prepare all work areas by cleaning with lab disinfectant. Prepare counter work area with the necessary dilution bottles, reagents, trays, pipettes, etc., leaving sufficient space to prepare samples for incubation.
4. Fill out lab notebook with required information. Include the date that the media lot was tested and passed.
5. Check incubator to ensure it is ON, maintaining the proper temperature and has sufficient space for sample trays when they are ready. Record incubator temps to the nearest 0.5 deg. C.
6. When samples arrive, transfer custody of the samples by removing them from the plastic bag in the ice chest and checking off each sample on the COC form. When all samples have been transferred, sign the COC form and record the time. Discuss whether any samples are suspected of containing high bacterial counts.
7. Optional: Depending on the number of samples, perform sample preparation /incubation in groups resulting in approx. 30-minute incubation intervals, keeping other samples on ice until needed. In all cases, keep the total incubation time the same for all samples.
8. Mark the Lab Notebook with date, sample ID, lab identification number, date and time of collection, date and time of receipt, date and time of analysis, volume of original sample analyzed (dilution rate), lab QC sample information, start/end times of incubation, and the analyst’s name. It is at this point that the dilution scheme for each sample is identified.
9. Use a Sharpie pen to mark all sample bottles suspected of having high counts.
10. With a waterproof wax pencil (so as not to tear the paper on the back of the Quanti-Tray) gently mark the Quanti-Trays with the lab identification number of each sample.
11. For each sample (including dilutions), invert sample bottle 20-25 times to ensure complete mixing of the sample. DO NOT TOUCH THE INSIDES OF CONTAINERS OR SAMPLE WATER. Inspect each IDEXX vessel for inside cleanliness; if particles or other contamination is observed, place the container aside with other contaminated, new bottles (i.e., DO NOT USE).
12. The preferred order for sample analysis is to start with the “oldest” samples first and proceed in the order in which they were collected. QC samples (e.g., lab blank, lab duplicates, QC samples) and dilutions can be done at any time during the analysis.
13. **NO DILUTION SAMPLES:** If no dilutions are to be performed on the sample, mix sample (as above) and immediately pour the sample into a new, sterile IDEXX vessel up to the 100 ml line. If over the line, pour off to meet the 100 ml. meniscus, or use a sterile 1-10 ml pipette to pipette off to the 100 ml line. (If IDEXX vessel is used to collect sample in the field, simply mix and then pour off/pipette off to the 100 ml line). Cap sample and proceed to 10.17. See Table 3 below for dilution summary.
14. **10 ML DILUTION SAMPLES**: (e.g., for marine/brackish Colilert-18 samples; known or suspected “HOT” samples to be quantified at a level > 2420 org./100 ml.). Pour 100 mls of sterile water into a new, sterile IDEXX vessel to the 100 ml. line (and pipette off as needed to 100 mls using a sterile 10 ml pipette). Then, pipette off 10 mls to achieve 90 mls of sterile water. Use the same pipette to pipette 10 mls of the original, mixed sample into the 90 mls of sterile water. Cap and mix as above. See Table 3 below for dilution summary.
15. **100 ML DILUTION SAMPLES**: (e.g., for marine/brackish Colilert-18 samples; for known or suspected “VERY HOT” samples to be quantified at a level >> 2420 org./100 ml). Pour 100 mls of sterile water into a new, sterile IDEXX vessel to the 100 ml line (and pipette off as needed to 100 mls using a sterile 1 ml pipette). Now, using the same pipette, pipette off 1 ml to achieve 99 mls. Then use the same pipette to pipette 1 ml of the original, mixed sample into the 99 mls of sterile water. Cap and mix as above. See Table 1 below for dilution summary.
16. Tap each reagent packet before opening to ensure complete dispensing. Open Colilert reagent packet and add entire contents to the 100-ml sample (or diluted sample). Cap and invert the bottle > 25 times until all the reagent is dissolved. Let any foam settle. Some stubborn particles may remain; these may dissolve during incubation.
17. After complete mixing, dispense sample into the Quanti-Tray. Use one hand to hold the Quanti-Tray upright with the well side facing the palm. Squeeze upper part of the Quanti-Tray so that it bends towards the palm. Open the Quanti-Tray by pulling the foil tab away from the well side. Avoid touching the inside of the foil or tray. (NOTE: If Quanti-Tray is believed to have been contaminated, discard the contaminated Quanti-tray and set up a new one and note in notebook). Pour the Colilert-treated sample directly into the Quanti-Tray while avoiding contact with the foil tab. Tap the small wells to release any air bubbles.
18. Place the sample-filled Quanti-Tray onto the rubber tray carrier of the Quanti-Tray Sealer with well side (plastic) of the Quanti-Tray facing down to fit into the carrier.
19. Seal the Quanti-Tray by moving the rubber tray into the sealer. The sealer will automatically take the rubber tray to disperse the sample into the wells and seal the Quanti-Tray. Remove the Quanti-Tray, assuring that it is fully sealed. (NOTE: If the Sealer stops in the middle of processing a Quanti-Tray sample, use the black button (with the arrows) on the front of the Sealer to reverse the direction of the Quanti-Tray in the Sealer. Allow the green light on the Sealer to go on before attempting to seal the sample again.) If the sealer does not easily “accept” the tray, lift up the end of the tray slightly while continuing to push it into the sealer, so that the leading edge clears the opening of the sealer.
20. **Reagent Lot QC samples.** Run QC samples (test organisms can be purchased from IDEXX) for each new lot of reagents when reagents are first received. Rehydrate organisms per manufacturer’s instruction. Pour 100 mls sterile water into an IDEXX bottle (as above), add the QC organisms, add Colilert reagent, and proceed with sealing and incubation as above. Controls include:
    1. **Negative Control use** *P. aeruginosa.*
    2. **Positive Control use** *E. coli*.
21. **Batch QC samples.** For each lab batch of samples, Prepare **LAB BLANK** as follows (minimum one per batch): Pour 100 mls. of sterile water into an IDEXX vessel, add reagent, cap and mix, pour into tray, seal and incubate. Prepare **LAB DUPLICATE** as follows (minimum one per batch): Using 250 ml. sample: Mix sample as above and pour two 100-ml aliquots into two separate IDEXX vessels, add reagents, cap and mix, pour into trays, seal and incubate. Using a 120 ml. sample: Mix sample (each time) and pipette two 10-ml aliquots into two separate IDEXX vessels containing 90 mls of sterile water, add reagents, cap and mix, pour into trays, seal and incubate. Multiply results by 10. Provided 100 mls is left in the sample container, run “raw”, 100-ml (undiluted) sample also.
22. Incubate all prepared Colilert Quanti-Trays in the incubator at **35 ±0.5 oC** for 24 - 28 hours. If Colilert-18 is used, incubate for 18-22 hours at **35 ±0.5 oC**. Record the dates and times in the lab notebook. Avoid any opening and closing the incubator during use in order to maintain stable proper temperature.
23. After sample trays have been placed in the incubator, thoroughly clean work area. Place all “bio-waste” (items that have been in contact with sample water, including pipettes, sample bottles, dilution bottles, etc.) in pre-labeled, plastic bio-waste bags. Temporarily store in the designated bio-waste storage area in the lab. Recycle or dispose (as appropriate) other materials that have not touched the samples. Once the work area is clear, disinfect the work area.
24. After 24 hours (18 hours for Colilert-18), remove the Colilert trays from the incubator. Record the time in the lab notebook. To determine the total coliform result: for each tray, count the number of wells that turn yellow (at least as strong as the yellow color of the comparator). Note in the lab notebook the number of positive (yellow) wells. Use the MPN table (Appendix A) to find the total coliform value as MPN/100 ml sample.
25. To determine the *E. coli* result, expose each sample to 365-nm UV light in the UV viewing cabinet (or in a dark room wearing UV-protective glasses with a hand-held UV lamp held within 5 inches of the sample). If the sample fluoresces (compare to comparator), then the sample is positive for *E. coli.* 
    1. Count the number of fluorescent wells and record in the lab notebook.
    2. Use the MPN table (Appendix a) to find the confirmed *E. coli* value as MPN/100 mL sample in the lab notebook.

*NOTE: Empty wells do not affect the test interpretation as long as the entire sample is in the tray. The effect on the Most Probable Number (MPN) table is statistically insignificant. An empty or partially filled well is interpreted the same way as a full well.*

1. For added QC or if the sample results are questionable after 24 hours of incubation (18 hours for Coliert-18), the sample may be incubated for an additional 4 hours (total of 28 hours for Colilert; 22 for Colilert-18), and rechecked for color and fluorescent reactions. If the color intensifies, the sample is total coliform positive; if it does not, the sample is negative. If an inoculated test is inadvertently incubated over 28 hours (22 for Colilert-18), the following guidelines apply:
   1. lack of yellow color is a valid negative test
   2. a yellow color after 28 hours is not valid
2. Colilert and Colilert-18 results are definitive at 24-28 and 18-22 hours, respectively. Any positives for both total coliform and *E.coli* observed before the minimum time and negatives observed after the maximum time are also valid. Positive results after the maximum time are not valid.
3. After all MPN results have been recorded, review the lab notebook to ensure that all required data and metadata have been recorded completely and accurately.
4. Place used Colilert trays and other waste in “bio-waste” bags, tie securely when only ½ full and temporarily store in the lab in the designated, secured location.

Enterolert: (in addition to or changes from that above for Colilert)

1. Incubate all prepared Enterolert Quanti-Trays in the incubator at **41 ±0.5 oC** for 24 - 28 hours.
2. Prepare negative and positive controls as indicated above.
3. Dilute marine (brackish to salt) water samples with sterile fresh water at least ten-fold, per IDEXX Enterolert procedure.
4. There is no fluorescent end-point comparator for Enterolert. Compare fluorescence in sample trays to negative control.

**General Dilution Schemes** (depending on sampling bottle used and without pre-filled dilution bottles):

Table 1: Summary of General Dilution Procedures

| Sample bottle used | Flip-top locking HDPE (or other) | IDEXX vessel PS |
| --- | --- | --- |
|  | Procedures | |
| 0X dilution MIX bottle | Mix, pour 100 mls into clear PS IDEXX vessel, pour off/pipette off to 100 mls if necessary, add reagent | Mix, pour off/pipette off for necessary dilutions and to 100 mls, then add reagent |
| 10X dilution bottle | Pour 100 mls. of sterile DI into IDEXX vessel and pour off/pipette off to 100 ml. line as necessary. Pipette off 10 mls. Pipette 10 mls. of mixed sample into the 90 mls. in the IDEXX vessel. Add reagent | Pour 100 mls. of sterile DI into a new IDEXX vessel and pour off/pipette off to 100 ml. line as necessary. Pipette off 10 mls. Mix sample and pipette off 10 mls into 90 ml IDEXX vessel. Add reagent |
| 100 X dilution bottle | Same as above for 10X but using 1 ml. Add reagent | Same as above for 10X but using 1 ml. Add reagent |
| \* These procedures assume that the IDEXX vessel 100 ml line can be used as an accurate (+/- 2.5%) measure. | | |

# PREVENTATIVE MAINTENANCE

1. Sealer: Trained personnel (only) must inspect, clean and maintain the sealer according to the manufacturer’s recommendations (Appendix C).
2. Rubber Inserts: Rinse with clean water; autoclave or clean with isopropyl alcohol or bleach.

# DATA INTERPRETATION and ANALYSIS

For each sample batch analysis, draft sample and quality control data are reviewed, interpreted and validated using the following definitions and criteria.

Data Report: The lab analyst shall count the number of positive Quanti-Tray cells for each sample/tray, and use the MPN table to obtain the Most Probable Number (MPN) per 100 mls. MPN results will be multiplied by the dilution factor as needed to obtain the final draft result.

Peer Review and Preliminary Approval: A separate lab report reviewer shall be designated to evaluate the accuracy and completeness of the lab report. The reviewer shall discuss any problems, concerns and issues with the lab analyst as needed, and if all data appear OK, preliminarily approve the MPN results as DRAFT data.

Table 2: Colilert (and Colilert-18 Reactions)

| * 1. Reaction | * 1. Result or interpretation |
| --- | --- |
| 1. Yellow | * 1. Total Coliform positive |
| 1. No color or indeterminant | * 1. Negative |
| * 1. Fluorescent (yellow) | * 1. *E. coli* positive |
| * 1. Yellow color intensity at or near the comparator color | 1. Inconclusive; re-incubate an additional 4 hours |
| * 1. > 28 (22) hours, positive | 1. Not valid |
| * 1. > 28 (22) hours, negative | 1. Valid |
| * 1. < 24 (18) hours, positive | 1. Valid |
| * 1. < 24 (18) hours, negative | 1. Not valid |

Table 3: Enterolert Reactions

|  |  |
| --- | --- |
| * 1. Reaction | * 1. Result |
| * 1. Blue fluorescence in 24-28 hours | * 1. Enterococci positive |
| * 1. No fluorescence in 24-28 hours | * 1. Enterococci negative |
| * 1. Blue fluorescence < 24 hours | * 1. Enterococci positive |
| * 1. No fluorescence beyond 28 hours | * 1. Enterococci negative |

Table 4: QC Interpretations (Colilert and Enterolert)

|  |  |  |
| --- | --- | --- |
| 1. Quality Control Organism | * 1. Yellow | * 1. Fluorescent |
| 1. Pseudomonas sp. 2. (Non-fluorescent strain) | 1. No | 1. No |
| 1. Klebsiella pneumonia, Enterobacter aerogenes or Enterobacter cloacae | 1. Yes | 1. No |
| 1. E. coli | 1. Yes | 1. Yes (yellow) |
| 1. Enterococcus faecium | 1. --- | 1. Yes (blue) |
| 1. Serratia marcescens | 1. --- | 1. No |

# METHOD PERFORMANCE

The method detection limit for Colilert, Colilert-18 and Enterolert has been determined to be a MPN of 1 colony forming unit (CFU) per sample volume or dilution tested.

# WASTE MANAGEMENT

All materials that have come in contact with samples (trays, bottles, pipettes, etc.) shall be bagged as “bio-waste” and temporarily stored in 32 gallon, plastic cans in a designated, secured location at DWM, prior to transport to the MADEP WES Lab in Lawrence, MA. for proper disposal (autoclave sterilization, plastics recycling and solid waste disposal).

The used Colilert trays and other non-recyclable trash to be autoclaved shall be placed in separate, labeled “bio-waste” bags at DWM for transport to WES for sterilization and solid waste disposal. The sample bottles and reagent bottles shall be bagged as “bio-waste” separately from the trays.

# 

# REFERENCES

1. *Standard Methods for the Examination of Water and Wastewater*, 20th Edition, 1998. (SM 9323B) American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC
2. <http://www.idexx.com/>

# APPENDICES

Appendix A: Lab Notebook Example

Appendix B: Incubator Temperature Log

Appendix C: Colilert® QuickGuide

# Appendix A: Lab Notebook Example

Analysis Start Date: 6/21/19

Test: Colilert

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Analyst | Project | Sample  ID | Sample Date | Sample Time | Analysis Date | Analysis Start Time | Media Lot # | Dilution | Incubation Start Time | Incubation Start Temp | Incubation End Time & Temp | Incubation End Temp | UV Reader | # of large yellow wells | # of small yellow wells | Total Colifrm MPN | # of large fluores wells | # of small fluores wells | E. coli MPN |
| J. Bush | River A | 51-587 | 2/12/04 | 12:45 | 2/12/04 | 14:24 | 368-01 | 0 | 16:35 | 35.5 | 16:45 | 35.0 | RC | 14 | 14 | 67 | 7 | 7 | 34 |
| J. Bush | River A | 51-587 | 2/12/04 | 12:45 | 2/12/04 | 14:26 | 368-01 | 10 | 16:35 | 35.5 | 16:45 | 35.0 | RC | 2 | 2 | 8 | 1 | 1 | 5 |
| J. Bush | River A | 51-588 | 2/12/04 | 12:55 | 2/12/04 | 14:29 | 368-01 | 0 | 16:35 | 35.5 | 16:45 | 35.0 | RC | 37 | 37 | 287 | 26 | 26 | 211 |
| J. Bush | River A | 51-588 | 2/12/04 | 12:55 | 2/12/04 | 14:31 | 368-01 | 10 | 16:35 | 35.5 | 16:45 | 35.0 | RC | 4 | 4 | 27 | 4 | 4 | 27 |
| J. Bush | River A | 51-588 | 2/12/04 | 12:55 | 2/12/04 | 14:35 | 368-01 | 100 | 16:35 | 35.5 | 16:45 | 35.0 | RC | 0 | 0 | 0 | 0 | 0 | 0 |
| J. Bush | River A | 51-589 | 2/12/04 | 13:25 | 2/12/04 | 14:45 | 368-01 | 0 | 16:35 | 35.5 | 16:45 | 35.0 | RC | 9 | 9 | 49 | 5 | 5 | 32 |
| J. Bush | River A | 51-590 | 2/12/04 | 13:25 | 2/12/04 | 14:47 | 368-01 | 10 | 16:35 | 35.5 | 16:45 | 35.0 | RC | 2 | 2 | 8 | 2 | 2 | 8 |

# Appendix B: Incubator Temperature Log

TEMPERATURE LOG

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Date | Time | Incubator TEMP, deg. C | TEMP (other) | Temp Dial Setting | Initials | Comments |
| 4/5/04 | 9:15 | 35.5 | 35.0 | 1.6 | rc |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |

\* Record before, during and after incubations.

# Appendix C: Colilert® QuickGuide (these procedures also generally apply to Enterolert)

1. Two hours prior to anticipated delivery of samples, turn on incubator and record temperature to verify 35.0 deg. C (=/- 0.5 C).
2. Prepare all work areas by cleaning with lab disinfectant.
3. When samples arrive, transfer custody of samples from crew to analyst using sign-off procedures (COC form)
4. Allow samples to warm up to near room temperature.
5. Turn on PC and open E.coli EDD template. Fill out electronic lab notebook with sample information and per instructions in the “read me” tab. This will generate the paper lab notebook page.
6. Prepare counter work area with the necessary dilution bottles, reagents, trays, pipettes, etc., leaving sufficient space to prepare samples for incubation. With a waterproof wax pencil (so as not to tear the paper on the back of the Quanti-Tray) gently mark the Quanti-Trays with the lab identification number of each sample. Inspect each IDEXX vessel for inside cleanliness; if particles or other contamination is observed, place the container aside with other contaminated, new bottles (i.e., DO NOT USE), and discuss with DWM QC Analyst.
7. Turn on Quanti-Tray sealer to allow preheating (usually takes around 10 minutes). Sealer is ready to use when green light is on.
8. For each sample (including dilutions), invert sample bottle 20-25 times to ensure complete mixing of the sample prior to taking aliquots (for dilutions). DO NOT TOUCH THE INSIDES OF CONTAINERS OR SAMPLE WATER. The preferred order for sample analysis is to start with the “oldest” samples first and proceed in the order in which they were collected. QC samples (e.g., lab blank, lab duplicates, QC samples) and dilutions can be done at any time during the analysis.
9. **NO DILUTION SAMPLES:** If no dilutions are to be performed on the sample, mix sample (as above) and immediately pour off sample water to the 100 ml line (bottom of meniscus). Tap each reagent packet before opening to ensure complete dispensing. Open Colilert reagent packet and add entire contents to the 100 ml. sample (or diluted sample). Mix thoroughly until no more reagent particles. Pour into pre-labeled tray. Seal in sealer.
10. **10 ML DILUTION SAMPLES**: Pour 100 mls. of sterile DI water (sterilized WES-DI water) into a new, sterile IDEXX vessel to the 100 ml. line (and pipette off as needed to 100 mls using a sterile 10 ml pipette.). Now, using the same pipette, pipette off 10 mls to achieve 90 mls. of sterile water. Then use the same pipette to pipette 10 mls. of the original, mixed sample into the 90 mls. of sterile water. Cap and mix. Add reagent. Mix thoroughly until no more reagent particles. Pour into pre-labeled tray. Seal in sealer.
11. **100 ML DILUTION SAMPLES**: Pour 100 mls. of sterile DI water into a new, sterile IDEXX vessel to the 100 ml. line (and pipette off as needed to 100 mls using a sterile 1 ml pipette.). Now, using the same pipette, pipette off 1 ml. to achieve 99 mls. Then use the same pipette to pipette 1 ml. of the original, mixed sample into the 99 mls. of sterile water. Cap and mix. Add reagent. Mix thoroughly until no more reagent particles. Pour into pre-labeled tray. Seal in sealer.
12. Use of Trays. After complete mixing as stated above, dispense sample into the Quanti-Tray. Use one hand to hold the Quanti-Tray upright with the well side facing the palm. Squeeze upper part of the Quanti-Tray so that it bends towards the palm. Open the Quanti-Tray by pulling the foil tab away from the well side. Avoid touching the inside of the foil or tray. Tap the small wells to release any air bubbles.
13. Use of Sealer. Place the sample-filled Quanti-Tray onto the rubber tray carrier of the Quanti-Tray Sealer with well side (plastic) of the Quanti-Tray facing down to fit into the carrier. Seal the Quanti-Tray by moving the rubber tray into the sealer. The sealer will automatically take the rubber tray to dispense the sample into the wells and seal the Quanti-Tray. Remove the Quanti-Tray, assuring that it is fully sealed. (NOTE: If the Sealer stops in the middle of processing a Quanti-Tray sample, use the black button (with the arrows) on the front of the Sealer to reverse the direction of the Quanti-Tray in the Sealer. Allow the green light on the Sealer to go on before attempting to seal the sample again.) If the sealer does not easily “accept” the tray, lift up the end of the tray slightly while continuing to push it into the sealer, so that the leading edge clears the opening of the sealer.
14. **Reagent Lot QC samples (DWM QA Analyst only).** Run the following *E. coli* QC samples for each new lot of reagents: 1) **Negative Control.** Prepare *P. aeroginosa (or other)* samples as for *E. coli* below. Cap/tray/seal/incubate. 2) **Positive Control.** Prepare a positive QC sample by pouring 100 mls into an IDEXX vessel (as above), then adding an *E. coli* pellet according to manufacturer’s directions, then adding Colilert reagent. Cap/tray/seal/incubate. Confirm correct analytical end-points.
15. **Batch QC samples (Lab analysts).** For each lab batch of samples, Prepare **LAB BLANK** as follows (minimum one per batch): Pour 100 mls. of sterile, deionized water into an IDEXX vessel, add reagent, cap and mix, pour into tray, seal and incubate. Prepare **LAB DUPLICATE** as follows (minimum one per batch): Using 250 ml. sample: Mix sample as above and pour two 100 mls. aliquots into two separate IDEXX vessels, add reagents, cap and mix, pour into trays, seal and incubate. Using a 120 ml. sample: Mix sample (each time) and pipette two 10 mls. aliquots into two separate IDEXX vessels containing 90 mls. of sterile, deionized water, add reagents, cap and mix, pour into trays, seal and incubate. Multiply results by 10. Provided 100 mls. is left in the sample container, run “raw”, 100 ml (undiluted) sample also. NOTE: Previous to 2011, lab analysts also ran positive and/or negative QC for each lab batch, but this was discontinued in 2011.
16. Incubate all prepared Colilert Quanti-Trays in the incubator at **35 ±0.5 oC** for 24 - 28 hours. If Colilert-18 is used, incubate for 18-22 hours at **35 ±0.5 oC**. Record the dates and times in the lab notebook.
17. After sample trays have been placed in the incubator, thoroughly clean work area. First, place all “bio-waste” (items that have been in contact with sample water, including pipettes, sample bottles, dilution bottles, etc.) in pre-labeled, plastic bio-waste bags. Keep the plastic sample and reagent bottles separate from other waste (separate bags). Tie the bag(s) when only half-full and temporarily store in the designated bio-waste storage area in the lab. Recycle or dispose (as appropriate) other materials that have not touched the samples. Once the work area is clear, disinfect the work area.
18. After 24 hours (18 hours for Colilert-18), remove the Colilert trays from the incubator (and shut off). Record the time in the lab notebook. For each tray, count the number of wells that turn yellow (at least as strong as the yellow color of the comparator). A yellow color that is equal to or deeper than that of the color comparator verifies that the sample is positive for total coliforms. Note in the lab notebook the number of positive (yellow) wells in the Quanti-Tray.
19. To determine the *E. coli* result, expose each sample to 365 nm UV light by placing them one-by-one into the UV light viewing cabinet (or in a dark room wearing UV-protective glasses with a hand-held UV lamp held within 5 inches of the sample. If the sample fluoresces (compare to comparator), then the sample is positive for *E. coli.* Count the number of fluorescent wells and record in the lab notebook.
20. Colilert and Colilert-18 results are definitive at 24-28 and 18-22 hours, respectively. Any positives for both total coliform and *E.coli* observed before the minimum time and negatives observed after the maximum time are also valid. Positive results after the maximum time are not valid.
21. After all MPN results have been recorded and entered into the electronic EDD, review the lab notebook to ensure that all required data and metadata have been recorded completely and accurately. Save (as lab batch ID) and print EDD sheet. Give printout to QC Analyst. Keep working printout with manually-recorded results in paper lab notebook.
22. Place used Colilert trays and other waste in “bio-waste” bags, tie securely when only ½ full and temporarily store in the lab in the designated, secured location.

Colilert (and Colilert-18 Reactions)

| Reaction | Result or interpretation |
| --- | --- |
| Yellow | Total Coliform positive |
| No color or indeterminant | Negative |
| Fluorescent (yellow) | *E. coli* positive |
| Yellow color intensity at or near the comparator color | Inconclusive; re-incubate an additional 4 hours |
| > 28 (22) hours, positive | Not valid |
| > 28 (22) hours, negative | Valid |
| < 24 (18) hours, positive | Valid |
| < 24 (18) hours, negative | Not valid |

QC Interpretations (Colilert and Enterolert)

|  |  |  |
| --- | --- | --- |
| Quality Control Organism | Yellow | Fluorescent |
| Pseudomonas sp.  (Non-fluorescent strain) | No | No |
| Klebsiella pneumonia, Enterobacter aerogenes or Enterobacter cloacae | Yes | No |
| E. coli | Yes | Yes (yellow) |
| Enterococcus faecium | --- | Yes (blue) |
| Serratia marcescens | --- | No |