Media Quality Control

1. GENERAL PROCEDURE

1.1. Test each new batch of prepared media and each new lot of commercially prepared ready to use media before first use with the following:

1.1.1. Positive control: Inoculate with a target organism that is known to produce a positive reaction in the media.

1.1.2. Negative control: Inoculate with a non-target organism which is known to be growth inhibited or produce a negative reaction in the media.

1.1.3. Sterility control: Along with positive and negative controls, incubate an additional tube/plate that has not been inoculated to check for sterility of media.

1.1.4. pH check: Verify pH is within manufacturer specified ranges per DEP SOP MB-03.5.

1.2. Inoculation of Media

1.2.1. For membrane filtration or pour plate media, prepare a known concentration of ATCC/NCTC derived E.coli (per manufacturer's instructions) or use a sterile inoculating needle to add a small amount of the pure control culture to a 99 mL sterile phosphate buffered dilution water bottle (Dilu-Lok). Mix the vial thoroughly and use for the following:

1.2.1.1. For membrane filtration media: Perform a membrane filtration with volumes of this suspension that will yield results in the countable range. (see DEP SOP MB-01.0 or MB-01.1)

1.2.1.2. For pour plate media: Use 0.1 mL of spiked water for pour plate method (see DEP SOP MB-01.5).

1.2.2. For Quanti-Tray media, prepare a known concentration of ATCC/NCTC derived E.coli (per manufacturer's instructions) or use a sterile inoculating needle to add a small amount of the pure control culture to 100 mL of sterile non-buffered water in an IDEXX mixing vessel. Follow the Quanti-Tray procedure (see DEP SOP MB-01.15).

1.2.3. For streak plate media and broth and agar culture tubes, media may be inoculated directly with a small amount of pure control culture.

1.2.4. Incubate controls at the temperature and duration specified for the media.

2. DATA ARCHIVAL

2.1. For membrane filtration media or pour plate media, in LIMS Standard Preparation Tracker, the Media Preparation window contains a section labeled "QC Testing". Enter Date QC Test is set up, analyst, and preparation name. Once the preparation name is entered, the QC positive and negative control and expected results boxes are

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automatically populated with the bacteria that should be used for positive and negative controls. Add the serial number for the cultures used in these boxes after the "S#". When using ATCC/NCTC derived E.coli, make sure to put in the result box for positive control "Universal Microbe CRM S#" instead of the bacteria that is populated.

2.2. For Quanti-Tray media, in LIMS Standard Preparation Tracker, choose **Media/QuantiTray QC Test Setup**. Select the container ID and enter Date Test Setup, Setup By, pH, and serial numbers for the cultures used after the "S#". Include the pH meter serial number in the comments.

2.3. After incubation, check that response to media matches the expected results given in the media preparation module of LIMS Standard Preparation Tracker. If using ATCC/NCTC derived E.coli, check the range of acceptance criteria according to the culture certificate of analysis (COA) for positive growth results. If all QC controls pass, enter the result in LIMS Standard Preparation Tracker. Select **Media/Media QC Pass/Fail** from the menu. Highlight appropriate media batches; enter date, analyst, sterility result and click **Pass**.

2.4. If controls do not produce appropriate responses, retest. If after retest, controls still fail follow step 2.2 to enter results, except click **Fail** instead of **Pass**. Discard media batch. Make a new batch of media and repeat the quality control process.

3. SAFETY/HAZARDOUS WASTE MANAGEMENT

3.1. Empty dehydrated culture media containers can be discarded in the trash.

3.2. Discard expired dehydrated media as hazardous waste.

3.3. Always autoclave any expired or used prepared media for 45 minutes in a hazardous waste autoclave bag before discarding in the trash.

3.4. Refer to the Laboratory Health and Safety Plan for personal protective equipment, use of laboratory hoods, and chemical storage policies.

3.5. Read MSDS information for reagent handling and disposal.

4. REFERENCES

4.1. Standard Methods Online Edition, Section 9020B (2015)

4.2. 2016 TNI Standard Rev 2.1, Module 5, Quality Systems for Microbiological Testing

4.3. FDEP Laboratory Quality Manual https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

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

4.5. DEP SOP MB-03.1 *Maintenance of Bacterial Cultures*

4.6. DEP SOP MB-02.33 *Preparation of Dehydrated Culture Media*

4.7. DEP SOP MB-01.0 *Membrane Filter Method for Total Coliforms*

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4.8. DEP SOP MB-01.1 *Membrane Filter Method for Fecal Coliforms*

4.9. DEP SOP MB-01.15 *Quanti-Tray/2000 Colilert Method for E. Coli and Enterolert Method for Enterococci*

4.10. DEP SOP MB-01.5 *Pour Plate Method for Heterotrophic Plate Count*

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

APPENDIX OF CHANGES

03/18/2009	Updated SOP to new format and included section on documenting results.
03/12/2010	Added version of Standard Methods online being used.
03/17/2011	Updated reference to new version of safety manual.
01/31/2012	Updated reference to Bureau Quality Manual.
01/28/2013	Updated reference from NELAC to TNI Standard
01/13/2015	Added Quanti-Tray media QC and references to SOPs.
12/23/2016	Updated reference for Quanti-Tray SOP, the Quality Manual, and the Health and Safety Plan.
12/18/2017	Included the name “Dilu-lok” for the sterile phosphate buffered dilution water and performed a fragment revision in “Inoculation of Media” section 1.2.1. Corrected the spelling on the word Escherichia in section 4.12. Removed references to DEP SOP MB-01.14 and DEP SOP MB-01.2.
04/26/2019	Corrected spelling of MB-01.1 in section 1.2.1.1. Removed all references to DEP SOP MB 01.4 *Membrane Filter Method for Escherichia coli (E. coli).*
04/28/2020	Updated positive control used for membrane filtration QC.
05/04/2021	Updated positive control used for Quanti-Tray media QC. Updated links for Laboratory Quality Manual and the Health and Safety Plan.
05/19/2022	Updated the link for the Laboratory Health and Safety Manual and added a reference for the Oakton pH and conductivity meter.

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