Maintenance of Bacterial Cultures

1. SCOPE AND APPLICATION

    1.1. Bacterial cultures are grown from certified reference stocks for use in quality control (QC) tests. Cultures are used as positive and negative controls to verify the response of each new batch of media. Other QC tests that require the use of cultures include buffered water check, water suitability, and the Use Test.

2. EQUIPMENT AND SUPPLIES – Record any equipment repairs or maintenance in LIMS Laboratory Logbook *Maintenance_Log*

    2.1. Heratherm IMH 60 (S/N 42511812), maintained at 35˚C ± 0.5°C

    2.2. Isotemp 60L (S/N 43315553), maintained at 35˚C ± 0.5°C

    2.3. Biology Refrigerator, maintained at 1˚C – 5˚C

    2.4. Sterile inoculating loops or sterile cotton swabs.

    2.5. Parafilm®

3. REAGENTS AND STANDARDS

    3.1. TSA Plates

        3.1.1. Prepare TSA per SOP MB-02.33. If prepared on day of use, pour autoclaved media directly into plates and allow to cool. If using refrigerated media, melt agarusing a boiling water bath on day of use. Pour melted agar into petri dishes and let cool. Discard unused melted agar.

    3.2. Microbiologics® Kwik-Stik™ Reference Culture Microorganisms

        3.2.1. *Enterobacter aerogenes* ATCC 13048

        3.2.2. *Pseudomonas aeruginosa* ATCC 10145

        3.2.3. *Escherichia coli* ATCC 11775

        3.2.4. *Aerococcus viridans* ATCC 10400

        3.2.5. *Enterococcus faecalis* ATCC 19433

        3.2.6. *Enterococcus faecium* ATCC 35667

        3.2.7. *Serratia marcescens* ATCC 43862

4. REFERENCE CULTURE RECONSTITUTION

    4.1. Allow Kwik-Stik™ to warm to room temperature before removing from pouch.

    4.2. Document cultures in LIMS Standard Preparation Tracker (**Laboratory/Technical/Standard Preparation Tracker**). Enter the expiration date as 90 days from date of preparation. Make sure to include media batch# and incubation temperature and duration in the description. Print two labels for each strain. Initial each label and mark the first set as “Stock” and the second set as “Working”. On the stock

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labels, record the next transfer date (maximum of two weeks from the read date). Attach labels on TSA plates.

4.3. Remove the Kwik-Stik™ from the pouch. Tear off the pull-tab label and place on the "Stock" dish. Squeeze the cap of the Kwik-Stik™ to break the ampoule and release the hydrating fluid. Tap the bottom of the Kwik-Stik™ to help move the fluid down to the pellet. Once hydrated, pinch the bottom of the Kwik-Stik™ to crush the pellet and mix it with the fluid.

4.4. Use the saturated swab from the Kwik-Stik™ to streak one-quarter of the "Stock" TSA plate, Using the same swab, streak one-quarter of the "Working" TSA plate. Using a sterile inoculating loop, or if unavailable, a sterile cotton swab,, streak three additional areas of decreasing concentration, using a fresh loop or swab for each area. This ensures the growth of isolated colonies. Incubate at 35 °C ± 0.5 °C for 20-48 hours. After incubation, seal with Parafilm® and store in the Biology Refrigerator. Record both plates as culture name KS (KWIKSTIK) Stock and Working in LIMS **Standard Preparation Tracker**.

4.5. Always use the "Working" dishes for quality control procedures. The "Stock" dishes should remain untouched and be used exclusively for subculture transfers. "Working" dishes must not be used to replace "Stock" dishes through sub-culturing." dishes.

5. WORKING STOCK SUBCULTURES

5.1. Within two weeks after reference culture reconstitution, transfer a well isolated "Stock" colony onto two new TSA plates. Record this as subculture #1 in LIMS **Standard Preparation Tracker**. The expiration date should remain 90 days from the preparation date to avoid issues when linking cultures in the LIMS Preparation Tracker.. Label the plates as "Stock" and "Working",and manually write out the expiration date on the plate as 14 days from read date on the label.

5.2. For each subsequent subculture use the "Stock" plate to transfer growth to two new TSA plates (one "Stock" and one "Working"). Increment the subculture number when documenting in LIMS. After five transfers (subculture #4), start with a new reference culture as in Section 4. Limiting transfers to no more than five prevents the growth of lab strains that may produce different phenotypic characteristics from the reference strain.

6. QUALITY CONTROL

6.1. After every transfer, check the TSA spread plate for purity and viability. Observe for growth and uniform colonies. Document the purity and viability of the colonies by adding the comment: "Pure growth and uniform isolated colonies observed" in the Sample comment section of LIMS Preparation LogBook-Micro_QC worksheets.

7. SAFETY/HAZARDOUS WASTE MANAGEMENT

7.1. Always autoclave any expired or unused bacterial cultures for 45 minutes before discarding in the trash.

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7.2. Refer to the Laboratory Health and Safety Plan for safety equipment, use of laboratory hoods, and chemical storage policies.

7.3. Refer to MSDS information and the Laboratory Health and Safety Plan for reagent handling and disposal.

8. REFERENCES

8.1. Standard Methods Online Edition (2017), 9020B

8.2. NELAC Quality Systems Manual Appendix D.3.7

8.3. Microbiologics® Kwik-Stik™ Product Insert

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

8.5. FDEP Laboratory Health and Safety Plan

https://floridadep.sharepoint.com/dear/Shared%20Documents/FDEP%20Health%20%20Safety%20Plan%20B%20-%20June%202021.pdf

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

APPENDIX OF CHANGES

11/05/2009 Updated documentation procedure for QC of cultures.

10/28/2010 Updated reference to health and safety plan.

10/22/2012 Made more specific references to incubators; added Parafilm to Equipment and Supplies; corrected spelling of "Standard" in Sec 4.2; corrected subculture transfer from slant tubes to broth tubes and procedure for viability/purity checks.

10/14/2013 Updated procedure to include a Stock TSA plate for subcultures.

10/24/2014 Clarified instructions in section 4.4 and added sterile cotton swabs to the Equipment and Supplies section.

01/22/2015 Formatted for 508 compliance.

12/23/2016 Updated incubator information and references to the Quality Manual and Healthand Safety Plan.

12/14/2017 Revised information in section 5.

01/03/2019 Updated list of bacteria in section 3, and 8.1 from 1997 to 2017 version.

01/29/2020 Modified biology refrigerator used.

12/20/2021 Updated incubator and refrigerator information. Updated links to Quality Manualand FDEP Laboratory Health and Safety Plan. Section 6.2 was removed due to procedure

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no longer used as a quality control check.

12/15/2022 Added the Fisher Isotemp Incubator to the Equipment and Supplies section. Changed the expiration date of all cultures to 90 days in LIMS, and mentioned to manually write out the expiration date on the label, updated link to FDEP Laboratory Health and Safety Plan.

12/08/2023 Removed BHI (Brain Heart Infusion) Broth from Reagents and Standards as reagent is no longer used; Changed instances of transfer expiration dates from “preparation date” to “read date;” Revised information in section 4.11/22/2024 Updated link to Quality Manual. Updated section 2.2 to remove the Fisher Isotemp Incubator 650 F and to add new incubator with the new serial number. Updated section 4.4 to correct incubation times. Additionally, the language and structure were refined to enhance clarity and readability

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