Preparation of Dehydrated Culture Media

1. GENERAL PROCEDURE

1.1. Document receipt of new media containers, by selecting **Media/New Media Container(s)** from the menu in the Laboratory Information Management System (LIMS) Standard Preparation Tracker module. Fill out available fields and include Micro-QC_WS# in the comment section of Standard prep tracker. Print a label for each bottle that includes the received date and serial number. Place new containers on the storage shelf. After opening, write the open date and 6-month expiration date on the container, and store in the desiccator with the lid tightly closed. Discard any media that has been open for greater than 6 months. Discard any media that is discolored, caked, or has been damaged by moisture. (Refer to Section 3 for instructions on discarding media.)

1.2. Perform all media preparations in the microbiology hood. Use sterile, clean glassware. If autoclaving, choose a flask with at least twice the volume being prepared. Use deionized water and measure with a Class A graduated cylinder. Use the Mettler MS303S balance (per SOP BB-035) located in the hood to weigh out dehydrated media. Use a magnetic stirrer and hotplate to mix and heat (if required) media before sterilization. Avoid burning or boil-over of media. Overheating can cause nutrient degradation.

1.3. Follow media container label instructions when preparing media. For more details on specific media, refer to the Difco™ & BBL™ Manual (\\Fldep1\dear\Labs\Lab5\Biology\Bench Common\Methods). For prepared media holding times see Table 5.1 (a summary of SM9020:V). Table 5.2 summarizes media commonly used in the lab and includes typical amounts prepared.

1.4. Use autoclave tape and sterilize loosely covered media per container label instructions. Remove media from the autoclave as soon as the chamber pressure reaches zero to avoid overheating or burning the media. If using immediately (or when melting prepared agar), temper media in a water bath at 44-46 °C for a maximum of 3 hours before use. Document sterilization in LIMS **Laboratory/Technical/Laboratory Logbook** *Autoclave_Log*. Include date, items sterilized (use batch#), sterilization time (**Entry Value**), pressure and sterilization temperature (use a max/min thermometer and select appropriate comment in **Comments** box), total run time (use autoclave tape and select the appropriate comment in **Entry Comments**), analyst name, and Micro-QC_WS# in Entry Comments.

1.5. Check final pH of media and adjust as necessary. The media container label instructions include the required pH range. If a specific media is known to require adjustment, it can be pH adjusted before sterilization.

NOTE: The pH of media that needs to be autoclaved should be measured before and after it has been autoclaved. Only record the final pH (after it has been autoclaved).

1.6. Document media preparation in LIMS **Laboratory/Technical/Standard Preparation Tracker**. Select **Group: BIO-MICRO** and then select **Media/Media Preparation** from the menu. Include container serial number, date prepared and date QC setup, prep and QC analyst, media name, format (agar, broth, etc.), final pH, boiled and/or autoclaved, amount of media added, total volume, balance ID, additional ingredients used (**Comments**), Micro-

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QC_WS# (Comments), and serial numbers for QC bacteria. Print barcode labels for stored media and QC checks.

1.7. For membrane filtration (MF) agar, dispense media into clean, sterile petri dishes in 4.5-5.5 mL aliquots, and allow the agar to cool and solidify. Wrap the dishes in aluminum foil and store in the refrigerator. For MF broth, store media in dark amber bottles, to limit light exposure, and refrigerate. For agar tubes, lay rack on its side so that the agar forms a slant when it solidifies. For milk dilution bottles and tubes, allow media to cool, then tighten caps and store in the refrigerator. Attach a printed barcode label to each bag, bottle, or rack of media.

2. QUALITY CONTROL

2.1. Before each use of deionized water, calibrate meters and check pH and conductivity with the Oakton PC2700 pH and conductivity meter per SOP MB-03.5. . Record in LIMS **Laboratory/Technical/Laboratory Logbook** *Lab_Water_QC_Log*.

2.2. Calibrate the Oakton PC2700 pH and conductivity meter before use per SOP MB-03.5 and document in the LIMS **Laboratory/Technical/Laboratory Logbook** *pH_Meter_Log*.

2.3. Check balance with NIST-traceable weights prior to use on each working day (per SOP BB-035). Use a high and low weight that brackets the working range. Document balance check in the *Mettler MS303S (micro lab) Daily Use Log.*

2.4. Check the calibration of the media dispensing pipette prior to each use. Set pipette to desired volume for media dispensing.

2.4.1. For agar media dispensed into petri dishes, the volume dispensed should produce a media depth of 4-5 mm. Check the volume setting by dispensing deionized water into a Class A graduated cylinder. Record results in LIMS **Laboratory**/**Technical**/**Laboratory Logbook** *Pipet-Fun_Cal_Log*. If result is not within 0.5 mL of expected volume, do not use pipette. Notify a supervisor.

2.4.2. For broth media dispensed onto a petri pad, check the 2 mL volume setting by dispensing deionized water into a Class A graduated cylinder. Record results in LIMS **Laboratory**/**Technical**/**Laboratory Logbook** *Pipet-Fun_Cal_Log*. If result is not 2 mL to 2.2 mL, do not use pipette. Notify a supervisor.

2.4.3. For agar or broth dispensed into test tubes, check the volume setting by dispensing deionized water into a Class A graduated cylinder. Record results in LIMS **Laboratory**/**Technical**/**Laboratory Logbook** *Pipet-Fun_Cal_Log*. If result is not within 0.5 mL of expected volume, do not use pipette. Notify a supervisor.

2.5. Perform QC on each new batch of media prepared per SOP MB-01.12.

3. SAFETY/HAZARDOUS WASTE MANAGEMENT

3.1. Empty media containers can be discarded in the regular trash.

3.2. Discard expired dehydrated media as hazardous waste. Refer to the Laboratory Waste Management Manual.

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

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

3.5. Read MSDS information for reagent handling and disposal.

4. REFERENCES

4.1. SOP BB-035 Calibration and Use of Mettler MS303S Balance

4.2. SOP MB-03.5 Oackton PC2700 pH and Conductivity Meter

4.3. Standard Methods 9020B (2005) and 9050C (2006)

4.4. SOP MB-01.12, Media Quality Control

4.5. Difco™ & BBL™ Manual \\Fldep1\dear\Labs\Lab5\Biology\Bench Common\Methods

4.6. Bureau Quality Manual https://publicfiles.dep.state.fl.us/DEAR/DEARweb/Labs/lab_qualitymanual_2022.pdf

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

5. TABLES, DIAGRAMS

Table 5.1: Holding Times of prepared media

Media Type	Holding time
MF broth	96 hrs
MF agar	2 weeks
Agar or broth in loose-cap tubes	2 weeks
Agar or broth in screw-cap tubes	3 months
Agar in milk dilution bottles	3 months

Table 5.2: Summary of commonly used media

Media Name	Media Abbrev	Difco Ref#	Total volume (mL)	Media Amount (g)	Boil?	Autoclave?	Autoclave in	Additional Ingredients	pH range
Azide Dextrose Broth	ADB	238710	100	3.47	Y	Y	Milk dilution bottle	-	7.2 ± 0.2
Brilliant Green Lactose Bile Broth	BGLB	273000	350	14	N	Y	Culture tubes w/vial	-	7.2 ± 0.2
Brain Heart Infusion	BHIB	237400	350	12.95	Y	Y	Culture tubes	-	7.4 ± 0.2

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Media Name	Media Abbrev	Difco Ref#	Total volume (mL)	Media Amount (g)	Boil?	Autoclave?	Autoclave in	Additional Ingredients	pH range
Brain Heart Infusion with 6.5% NaCl	BHI 6.5	237400	350	12.95	Y	Y	Culture tubes	21g of NaCl	7.4 ± 0.2
Brain Heart Infusion Agar	BHIA	241820	350	18.2	Y	Y	Culture tubes	-	7.4 ± 0.2
EC medium	EC	231420	350	12.95	N	Y	Culture tubes w/vial	-	6.9 ± 0.2
Heterotrophic Plate Count	HPC	275220	1000	60	Y	Y	Milk dilution bottle		7.1 ± 0.2
Plate Count Agar	PCA	247930	1000	23.5	Y	Y	Milk dilution bottle	-	7.0±0.2
Lauryl Tryptose Broth	LTB	224140	350	12.46	N	Y	Culture tubes w/vial	-	6.8 ± 0.2
mEndo broth MF	mEndo	274920	500	24	Y	N	Flask	10 mL of 190 proof Ethanol	7.2 ± 0.1
mEndo Agar	mEndoA	274920	1000	48	Y	N	Flask	20 mL of 190 proof Ethanol and 15 g of Agar (214010)	7.2 ± 0.1
mFC broth base	mFC	288320 or 288330	500	18.5	Y	N	Flask	5mL of 1% solution of *Rosalic Acid in 0.2N NaOH	7.4 ± 0.2
mFC agar	mFCA	288320	1000	37	Y	N	Flask	15 g of Agar (214010) 10mL of 1% solution of *Rosalic Acid in 0.2N NaOH	7.4 ± 0.2
Tryptic Soy Agar	TSA	236940	1000	40	Y	Y	Milk dilution bottle	-	7.3 ± 0.2
Tryptic Soy Broth	TSB	211825	1000	30	N	Y	Milk dilution bottle	-	7.3 ± 0.2
double strength Tryptic Soy Broth	TSB 2X	211825	1000	60	N	Y	Milk dilution bottle	-	7.3 ± 0.2

*To make 1% Rosolic Acid pour 100 mL of 0.2N NaOH (usually located by the pH meter) into a 100 mL TD Class A graduated cylinder. Once NaOH has been measured, pour a little out into a cup (like the ones we used to calibrate the meters) and pour the rest into an amber bottle. Take 1 g of Rosolic Acid (located in the Micro refrigerator) and pour into the amber bottle. Take a plastic pipette dropper into the cup that contains NaOH and rinse out whatever is leftover in the vial into the amber bottle. Once you feel that all the powder from the vial has been discarded, pour the rest of the NaOH into the amber bottle. Place a stir bar into the amber bottle and mix for at least 10 mins or when the powder has fully dissolved before adding it into the mFC. Do not add the Rosolic Acid when the mFC has already boiled; add it along with the agar (if making mFC agar) so it can all heat up and boil together. Shelf life for 1% Rosolic acid is 7 days from the day it's been made. Do not use Rosolic Acid for mFC media after it has exceeded its shelf life.

APPENDIX OF CHANGES

08/13/2010 Updated to new version of Health and Safety Plan

05/30/2012 Updated hyperlink to lab quality manual.

05/14/2013 Updated to reflect new balance model.

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05/13/2014	Removed SOP for pH meter no longer in use. Updated SOP reference for balance MS303S
01/28/2015	Formatted for 508 compliance.
10/13/2017	Updated links and Prep Worksheet Manager instructions
04/26/2019	Edited Table 5.2 to reflect media currently in use.
04/23/2020	Edited Table 5.2 to include new media currently in use
02/02/2021	Edited Table 5.2 to reflect media currently in use. Included steps on how to prepare 1% Rosolic Acid. Updated to reflect Micro-QC_WS as well as taking the final pH.
01/28/2022	Corrected links to the Difco™ & BBL™ Manuals in BenchCommon/Methods. Updated link to Bureau Quality Manual.
02/17/2022	Updated pH and conductivity meter to the Oakton PC2700. Removed references to the old pH and conductivity meters. Updated the SOP numbers.

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