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 print a label for each bottle that includes the received date and serial#. 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 PCR-2.1) 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™ Manuals (located in \\fldep1\DEAR\Labs\Lab5\BenchCommon\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**), and analyst name.

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.

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 #, 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)**, 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. Place the dishes in plastic bags, and

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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 with the Accumet Excel XL 25 pH meter per SOP MB-03.4 and conductivity with the Fisher Scientific Conductivity Meter per Fisher Scientific Traceable® Bench Conductivity Meter w/RS-232 Output Instructions and SOP TA-6.25. Record in LIMS **Laboratory/Technical/Laboratory Logbook** *Lab_Water_QC_Log*.

2.2. Calibrate the Accumet Excel XL 25 pH meter before use per SOP MB-03.4 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 PCR-2.1). 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.

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.

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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 PCR-2.1 Calibration and Use of Mettler MS303S Balance

4.2. SOP MB-03.4 Accumet Excel XL 25 pH Meter Calibration and Use

4.3. Fisher Scientific Traceable® Bench Conductivity Meter w/RS-232 Output Instructions

4.4. SOP TA-6.25 Calibration and Use of Mettler Toledo SevenMulti for Measuring Conductivity, Salinity and TDS

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

4.6. SOP MB-01.12, Media Quality Control

4.7. Difco™ & BBL™ Manual \\fldep1\DEAR\Labs\Lab5\BenchCommon\Methods

4.8. Bureau Quality Manual http://www.dep.state.fl.us/labs/library/progplan.htm

4.9. Laboratory Health and Safety Plan https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

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	N	Y	Milk dilution bottle	-	7.2 ± 0.2
Bile Esculin Agar	BEA	Remel R452361 or R452362	350	22.4	Y	Y	Culture tubes	-	6.8 ± 0.2
Brilliant Green Lactose Bile Broth	BGLB	273000	350	14	N	Y	Culture tubes w/vial	-	7.2 ± 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	BHIB	237400	350	12.95	Y	Y	Culture tubes	-	7.4 ± 0.2
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
Esculin Iron Agar	EIA	248810	500	8.25	Y	Y	Flask	-	7.1 ± 0.2
Eosin Methylene Blue Agar	EMB	211221	500	18.7	Y	Y	Milk dilution bottle	-	7.1 ± 0.2
Heterotrophic Plate Count	HPC	275220	1000	60	Y	Y	Milk dilution bottle		7.1 ± 0.2
KF Streptococcus Agar	KF Strep	249610	1000	76.4	Y	Y	Flask	-	7.2 ± 0.2
Luria-Bertani Broth	LB Broth Miller	244620	1000	25	N	Y	Culture tubes	-	7.0 ± 0.2
Lauryl Tryptose Broth	LTB	224140	350	12.46	N	Y	Culture tubes w/vial	-	6.8 ± 0.2
m Enterococcus Agar	mEnt	274610	500	21	Y	N	Flask	-	7.2 ± 0.2
mEI Agar	mEI	214885	1000	72	Y	Y	Flask	0.24 g of Nalidixic acid (BP908-25) (In 5 mL DI water. Add a few drops of 0.1 N NaOH to dissolve.) and 0.02 g of TTC (264310)	7.1 ± 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	-	7.4 ± 0.2
mFC agar	mFCA	288320	1000	37	Y	N	Flask	15 g of Agar (214010)	7.4 ± 0.2
mTEC Agar	mTEC	CAT#3062 1020-I	500	22.65	Y	Y	Flask	-	7.3 ± 0.2
Modified mTEC Agar	Modified mTEC	214884	500	22.8	Y	Y	Flask	-	7.3 ± 0.2
Simmon's Citrate Agar	SCA	211620	350	8.47	Y	Y	Culture tubes	-	6.9 ± 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

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Media Name	Media Abbrev	Difco Ref#	Total volume (mL)	Media Amount (g)	Boil?	Autoclave?	Autoclave in	Additional Ingredients	pH range
double strength Tryptic Soy Broth	TSB 2X	211825	1000	60	N	Y	Milk dilution bottle	-	7.3 ± 0.2
Tryptone Broth	TB	ACROS 61184-1000	350	5.25	N	Y	Culture tubes	-	7.3 ± 0.2
Urea substrate	US	Fisher U15	100	2	N	N	Milk Dilution Bottle	10 mg Phenol red (417240050), use w/in 1 week	3-4

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.

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

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