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Preparation of Culture Media Standard Method 9050

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1 Scope and Application

The Bacteriology Laboratory prepares media for use in the Multiple Tube Method, Heterotrophic Plate Count, Membrane Filtration Method, and various Quality Control procedures. Media prepared include EC Broth, Lauryl Tryptose Broth, EC Medium with MUG, Brilliant Green Broth, Tryptic Soy Broth, Plate Count Agar, MacConkey Sorbitol Agar, and Nutrient Agar. The need for uniformity dictates the use of dehydrated media. Commercially prepared media in liquid form (sterile ampoules, etc.) may be used if known to give equivalent results. When preparing dehydrated media, follow manufacturer's directions for rehydration and sterilization.

2 Definitions

2.1 Refer to Section 3 and Section 4 of the Georgia EPD Laboratory Quality Assurance Manual for Quality Control Definitions. (SOP Reference 13.2)

3 Interferences

3.1 Method interferences may be caused by contaminants in reagents, media, bottles or glassware. To abstain from interferences, all reagents, glassware and bottles are sterilized and tested for sterility prior to use. Each batch of media is aseptically prepared according to manufacturer's instructions, sterilized and tested before use. Each lot of commercially prepared media and reagents are tested prior to use to ensure its quality assurance.

3.2 When preparing culture media and reagents, use only deionized reagent-grade water that has been tested and found free from traces of dissolved metals or inhibitory compounds.

3.3 Glassware must be washed, sterilized, and put in the hot air oven at 180ºC for 2 hours. Glass tubes are sterilized after media is dispensed. A pH check is performed on all batches of glassware using a 0.04% solution of bromothymol blue. After drying and cooling, store glassware in a clean environment to prevent any accumulation of dust or other contaminants.

4 Safety

4.1 Refer to Laboratory Chemical Hygiene Plan and Fire Safety Plan, online revision. (SOP Reference 13.3)

5 Apparatus and Equipment

5.1 Automatic Pipetting Machine
5.2 Autoclave capable of sterilizing at 121°C
5.3 Refrigerator, 2-8°C
5.4 Pan Balance
5.5 pH Meter
5.6 Set of certified ASTM Class 1 Weights or better
5.7 Stirrer/Hot Plate
5.8 Reusable Borosilicate Glass Culture Tube w/screw caps – 25 X 200 mm and 16 X 125mm
5.9 Reusable Borosilicate Glass Culture Tube without Rim – 25 X 200 mm, 12 X 75 mm, 20 X 150 mm, 10 X 75 mm, 16 X 125 mm, and 6 X 50 mm
5.10 Metal or plastic caps: 25 mm and 16 mm
5.11 Glass milk dilution bottles w/caps
5.12 Square – 250 ml Polycarbonate bottles
5.13 Stirring rods – various sizes
5.14 Weigh boats – various sizes
5.15 Spatulas/Scoop – various sizes
5.16 Graduated cylinder – 1000ml and/or 2000ml
5.17 Stainless Steel pot – to dissolve and heat media
5.18 Wire Baskets
5.19 Class A graduated cylinder – 25ml
5.20 Hot Air Oven capable of sterilizing at 180°C

6 Reagents

6.1 Lauryl Tryptose Broth [Single Strength (SS) and Double Strength (DS)]
6.2 EC Broth
6.3 EC Medium with Mug
6.4 Brilliant Green Bile 2%
6.5 MacConkey Sorbital Agar
6.6 Tryptic Soy Broth
6.7 Plate Count Agar
6.8 Nutrient Agar

7 Sample Collection

7.1 Refer to Chapter 5 of the Georgia EPD Laboratory Quality Assurance Manual for Sample Container, Sample Preservation, and Sample Holding Times.

8 Calibration

8.1 Annually, calibration of the balance should be made by a certified vendor. Before each use, verify calibration by using two weights. A full range of weights should be checked monthly.

8.2 The set of class 1 weights or better should be recertified every three years.

8.3 pH meters should be calibrated before each use using 7.0 and 4.0 pH buffers. The thermometer probe in the pH meter should be calibrated yearly against a NIST certified thermometer.

8.4 Maintain sterility with equipment, media, and technique.

9 Quality Control

9.1 Refer to Table 14.1 Quality Control Acceptance Criteria associated with this method.

10 Procedure

10.1 Before daily use, standardize the pH meter with 4.0 and 7.0 buffers. Record results in pH logbook.

10.2 Before daily use, verify balance calibration by checking the weights of two different class 1 weights or better (one above and below the actual weight needed) and record results in the balance logbook. Handle each weight with tweezers and large weights with a white glove only (no bare hands). Check balance "bubble" to make sure it is centered and therefore the balance is leveled. Make sure the balance is clean and free from dust or debris.

10.3 When making media, record all pertinent information in the Media and Reagent Preparation Logbook, such as batch number, lot number, pH, final concentrations, date, initials, etc.

10.4 Carefully weigh the appropriate amount of media on the balance using a weighing boat and spatula/scoop.

10.5 Rinse out the graduated cylinder with deionized water. Then fill cylinder with appropriate amount of deionized water to be used in rehydrating the media. Pour deionized water into the appropriately sized container (plastic, glass or metal), then add the pre-weighed media powder. Mix thoroughly.

10.6 Place appropriately sized stirrers inside the pot. Place pot on Stirrer/Hot Plate and stir and/or warm solution slightly to completely dissolve the broths. Agars should be stirred and boiled to dissolve completely.

10.7 After dissolution, take pH of media by dispensing a small amount into a clean beaker. Record pH in media preparation logbook.

10.8 Check volumetric accuracy using a class A graduated cylinder. Make any necessary adjustments to the pipette machine to obtain the correct volume. Dispense media into proper tubes or bottles using the pipette machine. Tubes containing EC Broth, Lauryl Tryptose Broth, EC Medium with MUG, Brilliant Green Broth should contain inverted fermentation vials, with sufficient medium to cover the inverted vial at least one-third to two-thirds after sterilization. Agars are dispensed into the glass dilution bottles. After dispensing media into proper tubes or bottles, cap or plug them. (Tubes are placed in wire baskets. To permit uniform heating and rapid cooling, pack tubes loosely and in small containers.)

Record the volumetric accuracy check in the media logbook with a check mark. Agars are not checked for volumetric accuracy; therefore, record "NA" in the media logbook.

10.9 Place autoclave tape on each basket of media. Sterilize all media in an autoclave for 15 minutes after the temperature has reached 121°C. When the pressure reaches zero, remove medium from autoclave and cool as quickly as possible to avoid decomposition of sugars by prolonged exposure to heat.

10.10 After media has cooled, pour one tube or bottle of media into a beaker and take the pH. Record results in media preparation logbook. If the pH is inaccurate, then media must be remade.

Media Preparation-Broths

10.11 SS-LSTB (for Stream/Wastewater samples) - Add 356 grams of Lauryl Tryptose Broth to 10 liters of deionized water (35.6g/L). After dissolution, dispense 11 mL into each 20 X 150 mm tube (10 X 75 mm inverted vials). The pH should be 6.8 ± 0.2. Use 178 grams in 5 liters of deionized water if half of a run is desired.

10.12 DS-LSTB (for Drinking Water Samples) – Add 106.8 grams of Lauryl Tryptose Broth to 2 liters of deionized water (53.4g/L). After dissolution, dispense 20 mL into each 25 X 200 mm tube (12 X 75 mm inverted vials). The pH should be 6.8 ± 0.2.

10.13 EC Broth – Add 148 grams to 4 liters of deionized water (37g/L). After dissolution, dispense 5 mL into each 16 X 125 mm tube (6 X 50 mm inverted vials). The pH should be 6.9 ± 0.2. Use 74 grams in 2 liters of deionized water if half of a run is desired.

10.14 Brilliant Green Bile – Add 40 grams to one liter of deionized water. After dissolution, dispense 5 mL into each 16 X 125 mm tube (6 X 50 mm inverted vials). The pH should be 7.2 ± 0.2. Use 20 grams in 500 ml of deionized water if half of a run is desired.

10.15 EC Medium with MUG – Add 37.1 grams to one liter of deionized water. After dissolution, dispense 5 mL into each 16 X 125 mm tube (6 X 50 mm inverted vials). The pH should be 6.9 ± 0.2.

10.16 Tryptic Soy Broth- Add 30 grams to one liter of deionized water. After dissolution, dispense 25 ml into 25X200mm screw-cap tubes for QC on sterility of bottles; or dispense 10 ml in 16X125mm tubes for QC using pure culture bacterial disks. Add 60 grams to one liter of deionized water. After dissolution, dispense 50ml in square polycarbonate bottles for QC of dilution water. The pH should be 7.3 ± 0.2. Store in refrigerator.

Media Preparation-Agars

10.17 Plate Count Agar: Use 23.5 grams per liter of deionized water. Heat with frequent agitation. Boil until dissolved. The pH should be 7.0 ± 0.2. Dispense 100 mL into glass bottles and cap. Store in the refrigerator.

10.18 MacConkey Sorbitol Agar: Use 50 grams per liter of deionized water. Heat with frequent agitation. Boil until dissolved. The pH should be 7.1 ± 0.2. Dispense 100 mL into glass bottles and cap. Store in the refrigerator.

10.19 Nutrient Agar: Use 23 grams per liter of deionized water. Heat with frequent agitation. Boil to dissolve completely. The pH should be 6.8 ± 0.2. Dispense 8 mL into 16 X 125 mm screw-capped tubes and cap. After sterilization, immediately place tubes in an inclined position so that the agar will solidify with a sloped surface. Tighten screw caps after cooling and store in the refrigerator.

Commercially Prepared Media

10.20 Record in the media logbook: the date received, name of media, lot number and pH for each lot.

10.21 Each lot must used on or before the manufacturer's expiration date.

10.22 Each lot of media should be tested for quality prior to use. If the media shelf life is longer than 90 days, then quality testing should be done each quarter. Quality testing includes a positive control, negative control (if applicable) and sterility blank.

Media Storage

10.23 Store dehydrated media (powders) in tightly closed bottles at less than 30°C in an atmosphere of low humidity. Do not use if discolored or caked and lose the character of a free-flowing powder. When a bottle of media is first opened, be sure to write the technician's initials and the date it was opened on the outside of the bottle with a permanent marker.

10.24 After rehydrating a medium, dispense promptly to culture tubes or bottles and sterilize within 2 hours. Do not store nonsterile media.

10.25 Store prepared media out of direct sun to avoid contamination and excessive evaporation. Agars or broths in tightly closed screw-cap bottles can be stored at 4°C for up to 3 months. Broths in loose-fitting closures can be stored a 4° C for up to 2 weeks. Fermentation tubes stored at room temperature (approx. 25° C) cannot be used for more than 2 weeks and discard any tubes with an evaporation loss exceeding 1 ml.

10.26 Label all prepared media correctly before storage. Include date media was prepared, name of media, autoclave number, media lot number, autoclave cycle number, media expiration date, and initials of preparer.

10.27 Discard any expired media promptly. Media must be used on or before its expiration date and pass all quality control measures.

11 Calculations

11.1 The calculations involved in this method include measuring the appropriate amount of media and deionized water.

12 Waste Management

12.1 See GA EPD Laboratory SOP – EPD Laboratory Waste Management Standard Operating Procedures, SOP 6-015, online revision.

13 References

13.1 Standard Methods for the Examination of Water and Wastewater, 20th Edition, American Public Health Association: Washington, D.C., 1998 or later.

13.2 GA EPD Laboratory Quality Assurance Plan, online revision.

13.3 GA EPD Laboratory Safety/Chemical Hygiene Plan & Fire Safety Plan, online revision.

13.4 GA EPD Laboratory SOPs – Initial Demonstration of Capability SOP 6-001, online revision and/or Continuing Demonstration of Capability SOP 6-002, online revision.

13.5 Microbiological Methods for Monitoring the Environment Water and Wastes. EPA-600/8-78-017.

13.6 Manual for the Certification of Laboratories Analyzing Drinking Water, Fifth Edition. EPA 815-R-05-004, January 2005.

14 Practical Quantitation Limits (PQLs) Precision and Accuracy Criteria, and Quality Control Approach

No PQLs associated with this method. Final pH measurements and sterility must be maintained

Table 14.1 Summary of Data Quality Objectives

Method	Parameter	QC Check	Min. Frequency	Accepted Criteria	Corrective Action
SM 9050- Preparation of Culture Media SM9020B	EC Broth	Inoculate with E.coli (EC)	1 tube per lot	Growth	Discard Media
		Inoculate with Klebsiella aerogenes (KA) (formally EA)	1 tube per lot	No Growth	Discard Media
		Inoculate with Staphylococcus aureus (Staph)	1 tube per lot	No Growth	Discard Media
SM 9050- Preparation of Culture Media		No Inoculum	1 basket per lot	No Growth	Discard Media
	Lauryl Tryptose Broth (LSTB) Brilliant Green 2% Bile Broth	Inoculate with E.coli (EC)	1 tube per lot	Growth	Discard Media
		Inoculate with Klebsiella aerogenes (KA) (formally EA)	1 tube per lot	Growth	Discard Media
		Inoculate with Staphylococcus aureus (Staph)	1 tube per lot	No Growth	Discard Media
		No Inoculum	1 basket per lot	No Growth	Discard Media
	EC+MUG	Inoculate with E.coli (EC)	1 tube per lot	Growth and Fluorescence	Discard Media
		Inoculate with Klebsiella pneumoniae (Kleb)	1 tube per lot	No Growth	Discard Media
		Inoculate with Pseudomonas aeruginosa (PA)	1 tube per lot	No Growth	Discard Media

Table 14.1 Summary of Data Quality Objectives

Method	Parameter	QC Check	Min. Frequency	Accepted Criteria	Corrective Action
		No Inoculum	1 tube per lot	No initial fluorescence	Discard Media
		No Inoculum	1 basket per lot	No Growth	Discard Media
	Plate Count Agar and Nutrient Agar Slants	Inoculate with Klebsiella aerogenes (KA) (formally EA)	1 plate or slant per lot	Growth	Discard Media
		No Inoculum	1 plate or slant per lot	No Growth	Discard Media
	MacConkey with Sorbitol Agar and Tryptic Soy Broth	Inoculate with E.coli (EC)	1 plate or tube per lot	Growth	Discard Media
		No Inoculum	1 plate or tube per lot	No Growth	Discard Media

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SOP Update to Previous Version:
Updated online revision.