US Environmental Protection Agency
Office of Pesticide Programs

Office of Pesticide Programs
Microbiology Laboratory
Environmental Science Center, Ft. Meade, MD

Standard Operating Procedure for Media and Reagents: Preparation and Quality Evaluation

SOP Number: MB-10-07

Date Revised: 09-27-19

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Title	Media and Reagents: Preparation and Quality Evaluation
Revisions Made	• Added appropriate autoclaving procedures (e.g., volume of media per container, volume of media per autoclave run, etc.) as Attachment 4. • Added "Media with Reduced Frequency of Performance Assessment Tracking Form." • Minor editorial changes.

SOP Number	MB-10-07
Title	Media and Reagents: Preparation and Quality Evaluation
Scope	Describes the procedures used to log-in, prepare, and evaluate the quality of media and reagents used in microbiological assays by the Microbiology Laboratory Branch (MLB).
Application	For use in the quality evaluation of media and reagents used by MLB.

	Approval Date
SOP Developer:	_____ Print Name: _____
SOP Reviewer	_____ Print Name: _____
Quality Assurance Unit	_____ Print Name: _____
Branch Chief	_____ Print Name: _____

Date SOP issued:	
Controlled copy number:	
Date SOP withdrawn:	

TABLE OF CONTENTS

1. Definitions	1. General growth media = Media which support the growth of a broad range of microorganisms and are used for their general cultivation and maintenance. General growth media are non-selective. Examples include nutrient agar (NA) and tryptic soy agar (TSA) which are used to grow *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella enterica*, etc. 2. Selective media = Media that permit the growth of one type of bacterium while inhibiting the growth of other types. Selective media facilitate the isolation of a desired species. Examples include mannitol salt agar and cetrimide agar. 3. CFU = Colony forming unit. 4. Additional abbreviations/definitions are provided in the text.
2. Health and Safety	Follow procedures specified in SOP MB-01, Laboratory Biosafety. The Study Director and/or lead analyst should consult the Safety Data Sheet for specific hazards associated with chemicals.
3. Personnel Qualifications and Training	Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
4. Instrument Calibration	Refer to SOP QC-13 (autoclaves), EQ-01 (pH meters), EQ-02 (thermometers), and EQ-03 (weigh balances) for details on method and frequency of calibration.
5. Sample Handling and Storage	Store media and reagents as indicated on their Media/Reagent Preparation Sheets.
6. Quality Control	1. Document the required information on the appropriate forms (see section 14). 2. Process re-usable glassware in Miele or Lancer dishwashers and check glassware for detergent residues as noted in SOP QC-03, Glass Washing and Detergent Residues Test. 3. Use de-ionized water to prepare media and reagents. Water must meet the quality indicators noted in SOP-QC-01, Quality Assurance of Purified Water. 4. Use appropriate autoclaving procedures (e.g., volume of media per container, volume of media per autoclave run, etc.) as indicated in Attachment 4 (see section 14).
7. Interferences	1. Discard media if a media preparation number or sterilization batch number is illegible or missing and cannot be determined from the

	Media/Reagent Preparation Sheet or the Daily Sterilization Record Information Log Form (see section 14 and QC-13, respectively). 2. Inspect all pre-sterilized laboratory supplies upon receipt for damage or torn packaging; discard if supplies are damaged. 3. Initiate routine sterility checks of pre-sterilized items if contamination in a test is due to pre-sterilized items.
8. Non-conforming Data	1. Management of non-conforming data (e.g., inadequate media performance/ sterility) will be specified in the test method; procedures will be consistent with SOP ADM-07, Non-Conformance Reports. 2. If a non-conformance involving media/reagents is identified, investigate the cause of the non-conformance and the use of the media/reagents.
9. Data Management	1. Data will be archived consistent with SOP ADM-03, Records and Archives. Examples include media/reagent preparation sheets, sterility/performance forms. 2. Archive certificates of analysis for prepared media purchased by the laboratory (e.g., TSA with 5% sheep's blood).
10. Cautions	1. Do not use materials (e.g., media, reagents, pre-sterilized materials) past the manufacturer's recommended expiration date. 2. Use volumetric glassware as appropriate as indicated on Media Preparation Sheets. 3. Allow hot autoclaved media to equilibrate for a minimum of 30 minutes prior to plating or addition of heat-sensitive additives. If using a water bath, ensure the temperature of the water bath is set to 45-50°C or as appropriate for the media. 4. Reassessment of the sterility of media and reagents not used in a timely manner after preparation and/or after storage for more than one month is strongly recommended prior to use.
11. Special Apparatus and Materials	1. Water baths, for tempering of media. 2. Inoculating loops, for isolation streaks and media inoculation. 3. Volumetric glassware, for preparation of media/reagents as appropriate. 4. Microbial cultures used in the laboratory; refer to Attachment 2. 5. Pre-sterilized filtration units with pore size of 0.2 µm such as Nalgene Analytical Filter Units.

12. Procedure and Analysis	1. Utilize media/reagent preparation sheets approved by the Quality Assurance Unit or designee; electronic versions of the preparation sheets are archived on the shared network drive. 2. On the electronic media/reagent preparation sheet, enter the total volume of media/reagent to be prepared – the media/reagent preparation sheet will automatically adjust the mass/volume of the ingredients. 3. Verify that the amounts of ingredients specified on the media/reagent preparation sheet are accurate. For example, if a 2 L batch of FTM is made, the prep sheet should accurately reflect the calculation of 29.8 g for 1 L multiplied by 2: 29.8 × 2 = 59.6 g dehydrated medium required to prepare 2 L of FTM.
12.1 Preparation of Media and Reagents	a. Assign a media preparation number for all media, reagents, and carriers prepared in the laboratory. Record this number on the Media/Reagent Preparation Log Form and the Media/Reagent Preparation Sheet (see section 14). The media preparation number consists of two parts: i. The first seven digits represent the date the medium or reagent was prepared: P-MMDDYY where P=prepared, MM=month, DD=day and YY=the last two digits of the calendar year. ii. The suffix, where the digits after the dash act as a counter for the number of preparations made on the same date. For example, the first preparation made on January 8, 2019 would have the media preparation number P-010819-01. The next item prepared on that same day would have a suffix of -02; the third preparation made on that same day would have a suffix of -03, etc. b. Label each preparation of media or reagent clearly with the name of the preparation and the media preparation number. c. Strictly follow the specific directions for preparation of media and reagents as listed on the Media/Reagent Preparation Sheet. d. Complete all fields on the media preparation sheet with the appropriate information for that section. If a section is not applicable to the item being prepared, place N/A (not applicable) in that section. e. Sterilization of media and reagents (listed on the Media/Reagent Preparation Sheet).

	i. Refer to QC-13 to generate sterilization batch numbers for media and reagents that require autoclaving. ii. Record the sterilization batch number in the *Sterilization* section on the Media/Reagent Preparation Sheet (see section 14). iii. For media and reagents that are filter sterilized, record the sterilization mechanism in the *Sterilization* section on the Media/Reagent Preparation Sheet (see section 14). iv. For media and reagents that do not require sterilization, record N/A in the *Sterilization* section on the Media/Reagent Preparation Sheet (see section 14). v. To ensure proper sterilization of media and reagents, follow the Recommended Media/Reagent Sterilization Procedures in QC-13. f. Verify the pH of the media as specified on the Media/Reagent Preparation Sheet (e.g., before and/or after autoclaving, once per lot, etc.). g. Record the pH of the media or reagent at room temperature (20°C to 25°C) unless otherwise specified on the Media/Reagent Preparation Sheet. h. Discard the batch of media if the pH falls outside of the desired range as specified on the Media/Reagent Preparation Sheet. i. If using a water bath to equilibrate media, record the temperature of the water bath at the time the media is used (e.g., just prior to pouring in plates) on the Water Bath Temperature Record form (see section 14). The temperature recorded must reflect adjustments by the correction factor for the thermometer specified.
12.2 Storage, Shelf Life and Inspection of Media and Reagents	a. The storage and shelf life requirements for the medium or reagent are listed at the bottom of the Media/Reagent Preparation Sheet. See Attachment 1 for detailed instructions.
12.3 Media Performance Assessment	Conduct the performance verification of media prior to or concurrently with use, **preferably with the organism that corresponds to the anticipated use of the medium.** Media controls used in neutralization confirmation studies or titer assays may also be used in place of specific media performance tests.

	a. Culture Preparation: Use appropriate daily culture, test culture, stock culture or standardized spore suspension for the purpose of inoculating media for performance assessment. Refer to the relevant procedures for culture preparation (for example, SOPs MB-05, MB-07, MB-25, etc.). b. Inoculation and Incubation of Solid Media: For solid media in plates or tubes (e.g., TSA, NA, Middlebrook 7H11 (M7H11)), use 6-8 plates or tubes to assess media performance. Spread plate, in duplicate, 3-4 serial ten-fold dilutions of the test microbe. i. Target counts of 30-300 CFU/plate are desirable and should result from at least one of the dilutions plated – this dilution will serve as the basis for determining media performance. The dilutions should result in plate counts which are too numerous to count (TNTC) through extinction (no CFU) or near extinction levels. ii. For solid media in tubes (e.g., NA slants, M7H11 slants), inoculate a minimum of 2 tubes per batch with an undiluted culture of the test microbe. iii. For selective media in plates or tubes (e.g., mannitol salt agar, cetrimide agar), streak- (for plates) or stab- (for tubes) inoculate a minimum of 2 plates or tubes with an undiluted culture of the appropriate target organism (i.e., the organism the media is designed to identify). Perform an isolation streak on selective media in plates to aid in the assessment of the medium's reaction and organism's colony characteristics. iv. For selective liquid media (e.g., reinforced clostridial media (RCM), Middlebrook 7H9 broth with 15% glycerol (MADC)), inoculate a minimum of 2 tubes with an undiluted culture of the appropriate organism. v. See Attachment 2 for detailed instructions. c. Inoculation and Incubation of Liquid Media: For liquid media (e.g., letheen broth, nutrient broth, Modified Proskauer Beck broth), evaluate 6-8 tubes for performance testing. Inoculate tubes in duplicate with 0.1 mL aliquots of 3-4 serial ten-fold dilutions of the appropriate culture. i. Verify CFU/tube by spread plating in duplicate on the appropriate medium. See Attachment 2 for detailed

	instructions. d. Frequency of Media Performance i. For commonly used media (see Attachment 3), conduct a media performance assessment for each lot of dehydrated medium or every 6 months, whichever comes first. ii. For all other media, conduct a media performance assessment for each in-house preparation of media.
12.4 Performance Results for Solid Media	a. Enumerate the colonies per plate, determine the average CFU/plate and CFU/mL of diluted inoculum, and assess the colony morphology. i. Use whole numbers for the average CFU/plate and CFU/mL, round up when necessary. b. Record findings on the Performance and Sterility Assessment of Media in Plates form (see section 14). c. If growth occurs and exhibits typical morphology, record a "+." If no growth is apparent, record a "0." d. If atypical growth is observed (not the test microbe), record as contaminant. e. If the inoculum titer is significantly below the target of 30-300 CFU, then either the media performance is unsatisfactory or the starting inoculum was substandard; repeat the media performance assessment. f. For selective media, verify the performance per the appropriate media reactions (e.g., agar turning fluorescent green for cetrimide agar) or colony characteristics; refer to the appropriate method SOP. Complete the appropriate form (see section 14) under the "Performance Assessment" caption by checking either Satisfactory or Unsatisfactory per the observations. g. For solid media in tubes, record the presence or absence of growth on the Performance and Sterility Assessment of Media in Tubes form (see section 14). h. For plates, enumerate the colonies per plate, determine the average CFU/plate and CFU/mL of diluted inoculum, and assess the colony morphology (refer to SOP MB-05, MB-07, MB-15, MB-28, or MB-34 for colony characteristics). Record findings on the Performance and Sterility Assessment of Media in Plates form (see section 14).

12.5 Performance Results Liquid Media	a. Record findings on the Performance and Sterility Assessment of Liquid Media and Solid Media in Tubes form (see section 14). b. For each tube, record a "+" if growth is observed (indicated by turbidity or growth) or a "0" if growth is not observed. c. If atypical growth is observed (not the test microbe), record as contaminant. d. Following incubation, assess the performance of each medium and record observations on the Performance and Sterility Assessment of Media in Tubes form (see section 14). Read plate counts to determine the CFU/tube. e. Performance is judged to be satisfactory when at least one of the two tubes in a dilution set that received a sufficiently low challenge (1-50 CFU/tube) of the test microbe shows growth. The number of CFU delivered to each tube in a set is based on the corresponding averaged plate counts for that dilution. All tubes in dilution sets receiving greater than the targeted challenge should show growth as well. Based on this criterion under the "Performance Assessment" caption on the form, check either Satisfactory or Unsatisfactory.
12.6 Sterility Verification of Solid Media	a. Verify sterility on a minimum of 2% of each preparation of media. b. Place plates or tubes of solid media in an incubator for 3-10 days. Use an incubation temperature and environment (e.g., aerobic or anaerobic) consistent with the anticipated organism of use. If necessary, place agar plates in sterile plastic bags during incubation to prevent dehydration. i. If desired, allow agar plates to remain at room temperature prior to use to monitor their sterility. c. Following incubation, if no growth is observed, record a "0" (satisfactory). If growth is observed, record a "+" (unsatisfactory) on the appropriate Performance and Sterility Assessment form (see section 14). On each form, fill in "Sterility Assessment" by indicating either Satisfactory or Unsatisfactory per the observations. d. A satisfactory result for sterility is typically based on no microbial growth observed in the plates or tubes following incubation. Although infrequent, an occasional bacterial or fungal colony may appear on the surface of the agar; in these

		instances, perform an additional sterility assessment. In addition, examine the remaining plates of the preparation in question prior to use to determine the extent of the contamination. If any growth is observed in the second assessment, discard the medium.
12.7	Sterility Verification of Liquid Media and Reagents	a. If a medium or reagent is dispensed into multiple bottles, evaluate at least one bottle per preparation for sterility. b. Use a pre-sterilized 0.2 µm filter unit and aseptically filter approximately 2% of the total volume of medium or reagent. c. Do not place anything such as a pipette into the reagent bottle. d. Aseptically transfer the filter to a TSA, TSA with 5% sheep's blood (BAP), or NA plate, incubate for 3-10 days, and assess filter for presence of microbial growth. Use an incubation temperature and environment (e.g., aerobic or anaerobic) consistent with the anticipated organism of use. If necessary, place agar plates in sterile plastic bags during incubation to prevent dehydration. e. Following incubation of the filter, if no growth is observed, record a "0" (satisfactory). If growth is observed, record a "+" (unsatisfactory). Record the assay results and observations on the appropriate form (see section 14). f. For media that cannot be filtered because of their viscosity (e.g., fluid thioglycollate medium (FTM), Kirchner's medium, Middlebrook 7H9 broth), dispense at least 2% of the total volume prepared into 10-20 mL aliquots in sterile tubes and incubate the tubes for 3-10 days. Use an incubation temperature consistent with the anticipated organism of use.
12.8	Prepared Media and Reagents	a. An in-house media performance and/or sterility assessment of prepared media and reagents acquired from commercial vendors that are accompanied by a certificate of quality/analysis is optional.
12.9	Sterility of Carriers	b. For small carriers (e.g., 1 cm steel disks, steel or porcelain penicylinders), place one carrier per autoclaved preparation into a 10 mL tube of FTM, letheen broth (LB), or tryptic soy broth (TSB) and incubate for 3-10 days at 36±1°C. c. For glass slide carriers, place one carrier per autoclaved batch into a 20 mL tube of FTM, LB, or TSB and incubate for 3-10 days at 36±1°C.

	d. Following incubation, if no growth is observed, record a "0" and if growth is observed record a "+" on the Sterility Verification of Carriers form (see section 14).
13. Data Analysis/ Calculations	1. Calculate the percent difference due to evaporation or loss during autoclaving (see Attachment 1). The percent difference is determined using the formula: Percent Difference = 100% − [(Measured Volume/Target Value) × 100]
14. Forms and Data Sheets	1. Attachments. Attachments are stored separately from the SOP under the following file names: Storage and Inspection of Media and Reagents — MB-10-07_A1.docx Inoculation and Incubation of Solid and Liquid Media — MB-10-07_A2.docx List of Media with Reduced Frequency of Performance Assessment — MB-10-07_A3.docx Recommended Media Sterilization Procedures — MB-10-07_A4.docx 2. Test sheets. Test sheets are stored separately from the SOP under the following file names: Media/Reagent Preparation Log Form — MB-10-07_F1.docx Example Media/Reagent Preparation Sheet — MB-10-07_F2.xlsx Water Bath Temperature Record — MB-10-07_F3.docx Performance and Sterility Assessment of Solid Media in Plates — MB-10-07_F4.xlsx Performance and Sterility Assessment of Liquid Media and Solid Media in Tubes — MB-10-07_F5.xlsx Sterility Verification of Reagents — MB-10-07_F6.xlsx Sterility Verification of Carriers — MB-10-07_F7.xlsx Media with Reduced Frequency of Performance Assessment Tracking Form — MB-10-07_F8.docx
15. References	1. Official Methods of Analysis. Revised 2013. 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, (Methods 955.14, 955.15, 964.02, 961.02, and 966.04). 2. Official Methods of Analysis. Revised 2012. 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, (Method 965.12).