



**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-10-05**

**Date Revised: 06-09-15**

SOP Number	MB-10-05
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	_____	
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Quality Assurance Unit	_____	
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Branch Chief	_____	
	Print Name: _____	

Date SOP issued:	
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<p><b>1. Definitions</b></p>	<p>Additional abbreviations/definitions are provided in the text.</p> <ol style="list-style-type: none"> <li>1. General growth media = Media which support the growth of a broad range of organisms and are used for the general cultivation and maintenance of microorganisms. General growth media are non-selective. Examples include nutrient agar (NA) and tryptic soy agar (TSA).</li> <li>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.</li> <li>3. CFU = Colony forming unit.</li> </ol>
<p><b>2. Health and Safety</b></p>	<p>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.</p>
<p><b>3. Personnel Qualifications and Training</b></p>	<p>Refer to SOP ADM-04, OPP Microbiology Laboratory Training.</p>
<p><b>4. Instrument Calibration</b></p>	<p>Refer to SOP QC-13 (autoclaves), EQ-01 (pH meters), EQ-02 (thermometers), EQ-03 (weigh balances), and EQ-08 (Oxford automatic dispenser) for details on method and frequency of calibration.</p>
<p><b>5. Sample Handling and Storage</b></p>	<p>Not applicable</p>
<p><b>6. Quality Control</b></p>	<ol style="list-style-type: none"> <li>1. Document the required information on the appropriate forms (see section 14).</li> <li>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.</li> <li>3. Use de-ionized water to prepare media. Water must meet the quality indicators noted in SOP-QC-01, Quality Assurance of Purified Water.</li> </ol>
<p><b>7. Interferences</b></p>	<ol style="list-style-type: none"> <li>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).</li> <li>2. Inspect all pre-sterilized laboratory supplies upon receipt for damage or torn packaging; discard if supplies are damaged.</li> <li>3. Initiate routine sterility checks of pre-sterilized items if contamination in a test is due to pre-sterilized items.</li> </ol>

<b>8. Non-conforming Data</b>	Management of non-conforming data will be specified in the standard test method; procedures will be consistent with SOP ADM-07, Non-Conformance Reports.
<b>9. Data Management</b>	Data will be archived consistent with SOP ADM-03, Records and Archives.
<b>10. Cautions</b>	<ol style="list-style-type: none"> <li>1. Do not use products past the manufacturer's recommended expiration date.</li> <li>2. Use volumetric glassware as indicated on Media Preparation Sheets.</li> <li>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.</li> <li>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. See guidance in section 12.6.</li> </ol>
<b>11. Special Apparatus and Materials</b>	<ol style="list-style-type: none"> <li>1. Water baths</li> <li>2. Inoculating loops</li> <li>3. Cultures of <i>Pseudomonas aeruginosa</i> (ATCC #15442), <i>Staphylococcus aureus</i> (ATCC #6538), <i>Salmonella enterica</i> (ATCC #10708), <i>Mycobacterium bovis</i> (BCG) (ATCC #35743), and <i>Mycobacterium terrae</i> (ATCC #15755).</li> <li>4. Spore suspension of <i>Bacillus subtilis</i> (ATCC #19659); a commercial preparation of spores can be purchased. One source for the commercial preparation is Presque Isle Cultures, 3804 West Lake Rd., P.O. Box 8191, Erie PA 16505. In addition, a <i>B. subtilis</i> spore suspension can be generated in-house.</li> <li>5. Spore suspension of <i>Clostridium difficile</i> (ATCC #43598) prepared in-house.</li> <li>6. Any additional microbes used in the laboratory.</li> <li>7. Pre-sterilized filtration unit with pore size of 0.2 µm or 0.45 µm such as Nalgene Analytical Filter Units.</li> </ol>
<b>12. Procedure and Analysis</b>	<ol style="list-style-type: none"> <li>1. Utilize approved media preparation sheets. Approved media preparation sheets are archived on the G-drive.</li> <li>2. Select appropriate media preparation sheet and the required volume.</li> </ol>
12.1 Preparation of Media and Reagents	<ol style="list-style-type: none"> <li>a. Assign a media preparation number for all media, reagents, and carriers prepared in the laboratory. Enter this number into the Media/Reagent Preparation Log Form and the Media/Reagent</li> </ol>

	<p>Preparation Sheet (see section 14). The media preparation number consists of two parts:</p> <ol style="list-style-type: none"><li>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.</li><li>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, 2015 would have the media preparation number P-010815-01. 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.</li></ol> <ol style="list-style-type: none"><li>b. Label each preparation of media or reagent clearly with the name of the preparation and the media preparation number.</li><li>c. Strictly follow the specific directions for preparation of media and reagents as listed on the Preparation/Modifications/Notes section of the Media/Reagent Preparation Sheet.</li><li>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.</li><li>e. Instructions for sterilization of media and reagents as listed on the Media/Reagent Preparation Sheet.<ol style="list-style-type: none"><li>i. Refer to QC-13 for generation of sterilization batch number for media and reagents that require autoclaving.</li><li>ii. Record the sterilization batch number in the Sterilization section on the Media/Reagent Preparation Sheet (see section 14).</li><li>iii. For media and reagents that are filter sterilized, record the sterilization mechanism in the <i>Sterilization</i> section on the Media/Reagent Preparation Sheet.</li><li>iv. For media and reagents that do not require sterilization, record N/A in the <i>Sterilization</i> section on the Media/Reagent Preparation Sheet.</li></ol></li><li>f. After autoclaving, 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. When the pH reading is stable, record the final pH on the sheet with the temperature in the <i>Final</i></li></ol>
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	<p><i>pH After Autoclaving</i> section.</p> <ul style="list-style-type: none"> <li>g. Discard the batch of media if the pH falls outside of the desired range as specified on the Media/Reagent Preparation Sheet.</li> <li>h. 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) (see section 14). The temperature recorded must reflect adjustments by the correction factor for the thermometer specified.</li> </ul>
<p>12.2 Storage, Shelf Life and Inspection of Media and Reagents</p>	<p>The storage and shelf life requirements for the media or reagent are listed at the bottom of the Media/Reagent Preparation Sheet. See Attachment 1 for detailed instructions.</p>
<p>12.3 Media Performance Assessment</p>	<p>Conduct the performance verification of media prior to or concurrently with use, <b>preferably with the organism that corresponds to the anticipated use of the medium.</b> Media controls used in neutralization confirmation studies or titer assays may also be used in place of specific media performance tests.</p> <ul style="list-style-type: none"> <li>a. <u>Culture Preparation</u>: Use appropriate daily culture, test culture or standardized spore suspension for the purpose of inoculating media for performance assessment. For media prepared for use in research projects, stock cultures or test cultures generated for use in that project may be used for media performance. Refer to the relevant procedures for culture preparation.</li> <li>b. <u>Inoculation and Incubation of Solid Media</u>: For solid media in plates or tubes (e.g., tryptic soy agar, nutrient agar, Middlebrook 7H11), use 6-8 plates to assess media performance. Spread plate, in duplicate, 3-4 serial ten-fold dilutions of the test microbe.       <ul style="list-style-type: none"> <li>i. Target counts of 30-300 CFU/plate are desirable and should result from at least one of the plated dilutions – 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 or near extinction levels.</li> <li>ii. For solid media in tubes (e.g., nutrient agar slants, Middlebrook 7H11 slants), inoculate a minimum of 2 tubes per batch with an undiluted culture of the test microbe.</li> <li>iii. For <u>selective</u> media in plates or tubes (e.g., mannitol salt agar, cetrimide agar), streak (for plates) or stab (for tubes) inoculate a minimum of 2 tubes or plates with an undiluted</li> </ul> </li> </ul>

	<p>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.</p> <ul style="list-style-type: none"> <li>iv. For <u>selective</u> 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.</li> <li>v. See Attachment 2 for detailed instructions.</li> </ul> <p>c. <u>Inoculation and Incubation of Liquid Media</u>: 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.</p> <ul style="list-style-type: none"> <li>i. Verify CFU/tube by spread plating in duplicate on the appropriate medium. See Attachment 2 for detailed instructions.</li> </ul> <p>d. Frequency of Media Performance</p> <ul style="list-style-type: none"> <li>i. For the following commonly used media – TSA, R2A, tryptic soy broth (TSB), letheen broth – conduct a media performance assessment for every lot of dehydrated medium or every 6 months, whichever comes first.</li> <li>ii. For all other media, conduct a media performance assessment for each in-house preparation of media.</li> </ul>
<p>12.4 Performance Results for Solid Media</p>	<ul style="list-style-type: none"> <li>a. Enumerate the colonies per plate, determine the average CFU/plate and CFU/mL of diluted inoculum, and assess the colony morphology.           <ul style="list-style-type: none"> <li>i. Use whole numbers for the average CFU/plate and CFU/mL, round up when necessary.</li> </ul> </li> <li>b. Record findings on the Performance and Sterility Assessment of Media in Plates form (see section 14).</li> <li>c. If growth occurs and exhibits typical morphology, record a “+”. If no growth is apparent, record a “0”.</li> <li>d. If atypical growth is observed (not the test microbe), record as contaminant.</li> <li>e. If the inoculum titer is significantly below the target of 30-300 CFU, then either the media performance is unsatisfactory or the</li> </ul>



	<p>starting inoculum was substandard; media performance assessment should be repeated. For <u>selective</u> media, verify the performance per the appropriate media reactions (e.g. agar turning fluorescent green for cetrimide agar) or colony characteristics, see corresponding method SOPs MB-05 and MB-07. Complete the form (see section 14) under the “Performance Assessment” caption by checking either Satisfactory or Unsatisfactory per the observations.</p> <p>f. For media in tubes, record the presence or absence of growth on the Performance and Sterility Assessment of Media in Tubes form (see section 14).</p> <p>g. For <u>plates</u>, 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, or MB-28 for colony characteristics). Record findings on the Performance and Sterility Assessment of Media in Plates form (see section 14).</p> <p>h. For <u>tubes</u>, record the presence or absence of growth. Record findings on the Performance and Sterility Assessment of Media in Tubes form (see section 14).</p>
<p>12.5 Performance Results Liquid Media</p>	<p>a. Record findings on the Performance and Sterility Assessment of Liquid Media form (see section 14).</p> <p>b. For each tube, record a “+”if growth is observed (indicated by turbidity or growth) or a “0” if growth is not observed.</p> <p>c. If atypical growth is observed (not the test microbe), record as contaminant.</p> <p>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.</p> <p>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 CFUs 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.</p>
<p>12.6 Sterility Verification of Solid and</p>	<p>Verify sterility on a minimum of 2% of each preparation of solid and liquid media.</p> <p>a. Place plates or tubes of media, for use with organisms other than</p>

<p>Liquid Media</p>	<p><i>M. bovis</i> (BCG), in a 36±1°C incubator for 3-10 days.</p> <p>b. Incubate solid and liquid media used for the growth of <i>M. bovis</i> (BCG) at 36±1°C for 17-21 days.</p> <p>c. Following incubation if no growth on solid media or in liquid media 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.</p> <p>d. For solid media, 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, examine the remaining plates of the preparation in question prior to use to determine the extent of the contamination, and perform an additional sterility assessment. If any growth is observed in the second assessment, discard the medium.</p>
<p>12.7 Sterility Verification of Reagents (not used to support microbial growth)</p>	<p>a. If a reagent is dispensed into multiple bottles, evaluate at least one bottle per preparation for sterility.</p> <p>b. Use a pre-sterilized 0.45 µm or 0.2 µm filter unit and aseptically filter approximately 2% of the volume from one bottle of reagent.</p> <p>c. Do not place anything such as a pipette into the reagent bottle.</p> <p>d. Aseptically transfer the filter to a TSA, TSA with 5% sheep’s blood (TSAB), or NA plate, incubate for 3-10 days at 36±1°C, and assess filter for presence of microbial growth.</p> <p>e. For reagents that are dispensed in tubes, aseptically filter the number of tubes that represents approximately 2% of the total volume prepared through one pre-sterilized 0.45 µm or 0.2 µm filter unit, plate the filter on TSA, TSAB, or NA and incubate for 3-10 days at 36±1°C.</p> <p>f. For example, suppose 1 L of dilution water is prepared in 9 mL aliquots. 2% of 1 L is 20 mL; therefore filter the contents of 3 tubes (27 mL).</p> <p>g. Following incubation of 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 Sterility Verification for Reagents form (see section 14).</p>
<p>12.8 Sterility of Carriers</p>	<p>a. For stainless steel and porcelain carriers or other small carriers (e.g., 5×5×1 mm glass coupons), place one carrier per autoclaved</p>

	<p>preparation into a 10 mL tube of Fluid Thioglycollate Medium (FTM) or Letheen Broth (LB) and incubate for 3-10 days at 36±1°C.</p> <p>b. For glass slide carriers, place one carrier per autoclaved batch into a 20 mL tube of FTM or LB and incubate for 3-10 days at 36±1°C.</p> <p>c. Following incubation, if no growth is observed, record a “0” and if growth is observed record a “+” on the appropriate form. Record the assay results and observations on the Sterility Verification of Official Carriers form (see section 14).</p>														
<p><b>13. Data Analysis/ Calculations</b></p>	<ol style="list-style-type: none"> <li>Verify that the amounts of ingredients specified in the recipe are accurate. For example, if 2 L of FTM are made, the prep sheet should accurately reflect the calculation of 29.8 g for 1 liter multiplied by 2: <math>29.8 \times 2 = 59.6</math> g dehydrated medium required to prepare 2 liters of FTM.</li> <li>Calculate the percent difference due to evaporation or loss during autoclaving. The percent difference is determined using the formula:        Percent Difference = <math>100\% - [(Measured\ Volume/Target\ Value) \times 100]</math></li> </ol>														
<p><b>14. Forms and Data Sheets</b></p>	<ol style="list-style-type: none"> <li>Attachment 1: Storage and Inspection of Media and Reagents</li> <li>Attachment 2: Inoculation and Incubation of Solid and Liquid Media</li> <li>Test Sheets. Test sheets are stored separately from the SOP under the following file names:           <table border="0" data-bbox="483 1255 1409 1682"> <tr> <td>Media/Reagent Preparation Log Form</td> <td>MB-10-05_F1.docx</td> </tr> <tr> <td>Blank Media/Reagent Preparation Sheet</td> <td>MB-10-05_F2.xlsx</td> </tr> <tr> <td>Water Bath Temperature Record</td> <td>MB-10-05_F3.docx</td> </tr> <tr> <td>Performance and Sterility Assessment of Solid Media in Plates</td> <td>MB-10-05_F4.xlsx</td> </tr> <tr> <td>Performance and Sterility Assessment of Liquid and Solid Media in Tubes</td> <td>MB-10-05_F5.xlsx</td> </tr> <tr> <td>Sterility Verification of Reagents</td> <td>MB-10-05_F6.xlsx</td> </tr> <tr> <td>Sterility Verification of Official Carriers</td> <td>MB-10-05_F7.xlsx</td> </tr> </table> </li> </ol>	Media/Reagent Preparation Log Form	MB-10-05_F1.docx	Blank Media/Reagent Preparation Sheet	MB-10-05_F2.xlsx	Water Bath Temperature Record	MB-10-05_F3.docx	Performance and Sterility Assessment of Solid Media in Plates	MB-10-05_F4.xlsx	Performance and Sterility Assessment of Liquid and Solid Media in Tubes	MB-10-05_F5.xlsx	Sterility Verification of Reagents	MB-10-05_F6.xlsx	Sterility Verification of Official Carriers	MB-10-05_F7.xlsx
Media/Reagent Preparation Log Form	MB-10-05_F1.docx														
Blank Media/Reagent Preparation Sheet	MB-10-05_F2.xlsx														
Water Bath Temperature Record	MB-10-05_F3.docx														
Performance and Sterility Assessment of Solid Media in Plates	MB-10-05_F4.xlsx														
Performance and Sterility Assessment of Liquid and Solid Media in Tubes	MB-10-05_F5.xlsx														
Sterility Verification of Reagents	MB-10-05_F6.xlsx														
Sterility Verification of Official Carriers	MB-10-05_F7.xlsx														
<p><b>15. References</b></p>	<ol style="list-style-type: none"> <li>Official Methods of Analysis. Revised 2013. 18<sup>th</sup> Ed., AOAC INTERNATIONAL, Gaithersburg, MD, (Methods 955.14, 955.15, 964.02, 961.02, and 966.04).</li> <li>Official Methods of Analysis. Revised 2012. 18<sup>th</sup> Ed., AOAC INTERNATIONAL, Gaithersburg, MD, (Method 965.12).</li> </ol>														

Attachment 1. Storage and Inspection of Media and Reagents

<b>Media Type</b>	<b>Storage</b>	<b>Inspection</b>
Liquid Media in Tubes with Morton Closures	Store at room temperature for up to 2 months in plastic bags.	<p>Prior to use, inspect liquid media in tubes with Morton Closures stored longer than one month.</p> <p>Check the fluid level in approximately 5% per batch and/or per rack of media to be used in testing to determine if more than 5% of the fluid has evaporated.</p> <p>If less than 5% of the solution has evaporated, the media can be used; otherwise discard the media.</p>
Liquid Media in Screw-Capped Tubes or Bottles	Store for up to 3 months (tightly capped).	<p>Prior to use, inspect liquid media in tubes with screw caps stored longer than two months.</p> <p>Check the fluid level in approximately 5% per batch and/or per rack of media to be used in testing to determine if more than 5% of the fluid has evaporated.</p> <p>If less than 5% of the solution has evaporated, the media can be used; otherwise discard the media.</p>
Reagents	<p>If not labile or harmed by long storage periods, use for up to one year from the date of preparation.</p> <p>If a recommended shelf life is known, use within that time.</p> <p>Do not use commercially prepared reagents beyond the manufacturer's expiration date.</p>	<p>Prior to use, inspect reagents for any sign of contamination and discard appropriately if necessary.</p> <p>For dilution blanks, determine if more than 2% of the fluid level has evaporated. If more than 2% of the fluid has evaporated, adjust the volume in each tube prior to use or prepare a new batch as necessary.</p>

<b>Media Type</b>	<b>Storage</b>	<b>Inspection</b>
Solid Media in Plates	Store in plastic bags at 2-5°C or at room temperature, agar-side up.  Puncture the plastic bags in a few areas to allow air exchange and to reduce excessive condensation.  Store both selective and non-selective media for up to 3 months.	Prior to use, look for evidence of contamination, uneven filling or bubbles on the surface of agar, color changes, hemolysis and signs of dehydration such as shrinking, cracking and loss of volume. Discard any defective plates.
Solid Media in Tubes	Store both selective and non-selective media at 2-5°C for up to 3 months.	Prior to use, look for evidence of contamination, uneven filling, color changes, hemolysis and signs of dehydration such as shrinking, cracking and loss of volume. Discard any defective tubes.
Commercially Purchased Media (Plates)	Do not use commercially prepared media beyond the manufacturer's expiration date.	Prior to use, look for evidence of contamination, uneven filling, color changes, hemolysis and signs of dehydration such as shrinking, cracking and loss of volume. Discard any defective media tubes or plates.

Attachment 2. Inoculation and Incubation of Solid and Liquid Media

Test Microbe	Inoculation of Solid and Liquid Media	Incubation of Solid and Liquid Media
<i>S. aureus</i> , <i>P. aeruginosa</i> , <i>S. enterica</i> and <i>B. subtilis</i>	Inoculate tubes and/or plates with 0.1 mL of the $10^{-5}$ to $10^{-7}$ dilutions. The dilutions plated assume an initial titer of approximately $10^8$ to $10^9$ CFU/mL.	Incubate plates and/or tubes at $36\pm 1^\circ\text{C}$ for 24-48 h.
<i>C. difficile</i>	Inoculate tubes of selective liquid media with an undiluted culture of <i>C. difficile</i> .	Incubate at $36\pm 1^\circ\text{C}$ for 24 h under anaerobic conditions (e.g. Gas-Pak anaerobic environment generation system or anaerobic chamber incubator).
<i>M. bovis</i> (BCG)	Inoculate tubes and plates with 0.1 mL of the $10^{-2}$ , $10^{-3}$ , $10^{-4}$ and $10^{-5}$ dilutions of the standardized culture. The dilutions plated assume an initial culture titer of $10^7$ CFU/mL of standardized culture.  <i>Note:</i> The lowest dilution ( $10^{-2}$ and $10^{-3}$ ) will likely show CFU levels which are TNTC.	Incubate plates at $36\pm 1^\circ\text{C}$ for 17-21 days.  Incubate tubes at $36\pm 1^\circ\text{C}$ for up to 30 days.
<i>M. terrae</i>	Inoculate tubes of selective liquid media with an undiluted culture of <i>M. terrae</i> .  Inoculate plates with 0.1 mL of the $10^{-4}$ , $10^{-5}$ and $10^{-6}$ dilutions of the test culture. The dilutions plated assume an initial titer of approximately $10^7$ to $10^8$ CFU/mL.	Incubate tubes of selective liquid media at 150 rpm and $36\pm 1^\circ\text{C}$ for 7-10 days.  Incubate plates at $36\pm 1^\circ\text{C}$ for 17-21 days.