

**United States Department of Agriculture  
Agricultural Marketing Service, Science & Technology  
Microbiological Data Program**

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Title: Microbiological Media		
Revision: 01	Replaces: 04/01/01	Effective: 09/01/03

**1. Purpose:**

To provide standard procedures to ensure that media and reagents used in Microbiological Data Program (MDP) laboratories meet analytical standards for promoting good growth, selectivity, and retention of microorganisms.

**2. Scope:**

This standard operating procedure (SOP) shall be followed by all laboratories conducting microbiological studies for MDP, including support laboratories conducting non-routine activities that may impact the program. All of the procedures and measures required under this SOP must be documented with records maintained.

**3. Outline of Procedure:**

- 5.1 Receipt and inventory of Media and Reagents
- 5.2 Storage and maintenance of Media and Reagents.
- 5.3 Productivity/Selectivity Testing of Laboratory Prepared Selective Media
- 5.4 Productivity/Selectivity Testing of Commercially Prepared Selective media.
- 5.5 Performance and Sterility Testing of Media.
- 5.6 Reagents
- 5.7 *Salmonella* Antisera
- 5.8 Results and Records

**4. References:**

- AOAC International, Quality Assurance Short Course, Quality Assurance for Microbiological Laboratories, 481 North Frederick Ave., Suite 500 Gaithersburg, MD
  - BBL Quality Control and Product Information Manual for Plated Media, January 1996
  - Compendium Of Methods for the Microbiological Examination of Foods, Third Edition, 1992, American Public Health Association, Washington, D.C
  - Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, U.S. Department of Health and Human Services, Public Health Service, May 1999
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- Becton Dickinson and Company/Difco Laboratories, Difco Manual, 11<sup>th</sup> Edition, 1998
- Standard Methods for The Examination Of Dairy Products, 16<sup>th</sup> Edition, 1992, American Public Health Association, Washington, D.C
- USDA, FSIS Microbiology Laboratory Guidebook, 3<sup>rd</sup> Edition, 1998, Washington, D.C
- Quality Control in Microbiology, Department of Health and Human Services, Public Health Service, by J. Michael Miller, May 1993

**5. Specific Procedures:**

All of the procedures required under this SOP must be documented and records maintained.

**5.1 Receipt and Inventory of Dehydrated Media, Commercially Prepared Media and Reagents.**

- 5.1.1 Upon receipt, all containers of media and reagents shall be inspected for damage and condition.
- 5.1.2 Records shall include the following information for each shipment:
  - 5.1.2.1 Name of media, manufacturer and lot number
  - 5.1.2.2 Quantity Received, i.e., size and number of containers
  - 5.1.2.3 Date Received.
  - 5.1.2.4 Preparation date of commercially prepared media (if available).
  - 5.1.2.5 Expiration Date.
  - 5.1.2.6 Storage location of media/reagent.
  - 5.1.2.7 Record date of receipt and initials of person receiving and placing item into storage on each bottle of dehydrated media.
  - 5.1.2.8 Record date of receipt and initials of person receiving on each container/package of commercially prepared media prior to storage.

**5.2 Storage and Maintenance of Media and Reagents**

- 5.2.1 Follow the manufacturer's directions for handling and storage of media/reagents.
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5.2.2 Use older stock first and do not use past expiration date

5.2.3 The following guidelines apply for dehydrated media:

5.2.3.1 Upon opening, each container of dehydrated medium/reagent shall be inspected for volume, tightness of closure, clarity, color, consistency and completeness of label.

5.2.3.2 The date and initials of person opening the dehydrated media/reagent shall appear on the container and in media logbook. Information recorded on any outside boxes or covers shall be transferred to containers when opened.

5.2.4 The following guidelines apply for commercially prepared media:

5.2.4.1 Commercially prepared media shall be inspected prior to use for dehydration or other defects.

5.2.4.2 Document corrective action when media fails to meet acceptance criteria.

**5.3 Productivity/ Selectivity Testing of Selective Laboratory Prepared Media**

5.3.1 Reference Organisms

5.3.1.1 18 to 24 hour pure cultures of reference strains shall be used.

5.3.1.2 Two or more organisms are needed to check the growth characteristics, selectivity, or biochemical reactivity of a medium. Use appropriate references to determine the organisms to be used.

5.3.2. Maintenance of Reference Organisms

5.3.2.1 The laboratory shall maintain three levels of cultures:

5.3.2.1.1 a working set of cultures shall be maintained and kept at 2-8°C.

5.3.2.1.2 a backup set of cultures shall be maintained.

5.3.2.1.3 a stock culture maintained at -70°C or some other long-term storage mechanism.

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5.3.2.2 Cultures from slants shall not be transferred more than five times. Revive a stock culture from long-term storage to start a fresh culture every 3 to 4 months or more frequently.

5.3.3 Preparation of Reference Organisms for Testing

5.3.3.1 Use fresh cultures (18-24 hours old) for testing media.

5.3.3.2 For selective media requiring tests for ability to inhibit organisms or when determining the ability to support growth, a dilute inoculum will be needed. The dilute suspension will give greater assurance that the medium will support growth of a small number of organisms or inhibit the reference organism.

5.3.3.3 Prepare a standardized inoculum to a turbidity equivalent of a 0.5 McFarland standard and use a 3-millimeter loop to deliver a known amount to test the media.

5.3.3.4 Media may be tested inline with samples by streaking the controls.

5.3.4 Growth Performance/Efficiency testing of Solid Selective Media prepared in the laboratory.

5.3.4.1 Obtain certificate of analysis or other documentation from manufacturer that media meets required specifications for selectivity.

5.3.4.2 Optionally, conduct media growth performance/efficiency test as described in USDA, FSIS Microbiology Laboratory Guidebook, 3<sup>rd</sup> Edition 1998, Washington DC. The reference is listed in item 4. of this SOP.

**5.4 Productivity/Selectivity Testing of Commercially Prepared Selective Media**

5.4.1 Purchased, ready to use, selective solid media need not be tested for Growth Performance/Efficiency if a certificate or other documentation is obtained from the manufacturer stating that the media has been properly evaluated.

5.4.2 Laboratories that purchase prepared media are required to maintain back-up supplies of media and reagents. In cases of emergency, where media is



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prepared and used on the same day, productivity/selectivity testing shall be performed concurrently with sample analyses.

**5.5 Performance and Sterility Testing of Media.**

5.5.1 Each batch of media used in the MDP, whether prepared in the laboratory from dehydrated ingredients or purchased in prepared form shall be examined prior to use and tested for performance and sterility. Tests shall include sterility, ability to support growth, selective and/or inhibitory growth characteristics, and the biochemical reactivity of media in response to an appropriate culture.

5.5.2 A batch refers to all tubes, plates, or containers prepared at the same time and sterilized in the same autoclave using the same conditions. On small batches of media (less than 100 units) incubate (at a minimum) the following: one unit inoculated with a positive control organism, and one unit inoculated with a negative control organism.

Incubate at the usual temperature and time period required in the specific analysis. For larger batches of media, test 2% of the units as specified above. Maintain records.

Sterility is indicated by lack of visible growth/turbidity following incubation.

5.5.3 Discard and autoclave units after incubation. Do not reuse sterility test media.

**5.6 Reagents**

5.6.1 The laboratory shall record all weights, calculations, and concentrations used in the preparation of reagents in QA records.

5.6.2 All sterile reagents shall be labeled with the name of the reagent and date prepared. Reagents shall be stored appropriately.

**5.7 *Salmonella* Antisera**

5.7.1 Before use or concurrently each lot of *Salmonella* antisera shall be checked with a positive and negative control antigen with records maintained.



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5.7.2 Store antisera and prepare working solutions according to manufacturer's directions. Discard any secondary dilutions of poly H antisera at the end of the workday.

**5.8 Results and Records**

5.8.1 Records of media performance must be maintained for each batch of media tested.

5.8.2 Records shall include the lot number of media used and expiration date, the date media was prepared, organisms used, expected reactions, obtained reactions, results of sterility tests and visual examinations, problems, and corrective actions. This information shall include the initials of the analyst performing the observations.

5.8.3 If quality control test results are unacceptable, repeat the test. If the repeat test is unacceptable, discard the batch and document all problems and corrective actions taken.

5.8.4 If results of visual examinations are unacceptable, document abnormalities and discard media.



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Revision 01

May 16, 2003

Technical Advisory Committee

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- Renumbered document for consistency with other SOPs
  - Added reference for media efficiency testing procedures to section 4
  - Modified subsection 5.1.1 to require inspection for condition of media and reagents
  - Revised subsection 5.2 media and reagent storage requirements
  - Modified subsection 5.3.2 requirements for maintenance of reference organisms
  - Added provision for testing media inline with samples by streaking the controls as subsection 5.3.4
  - Revised subsection 5.3.4 growth performance/efficiency testing requirements
  - Modified subsection 5.6 reagent storage and labeling requirements
  - Modified subsection 5.7.2 antisera storage requirements
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