

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

SOP No: MDP-LABOP-03		Page 1 of 9
Title: Microbiological Media and Reagents		
Revision: 02	Replaces: 09/01/03	Effective: 05/1/06

**1. Purpose**

To provide standard procedures to ensure that media and reagents used in Microbiological Data Program (MDP) laboratories meet the performance requirements specified by their intended use.

**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**

Receipt and Inventory of Media and Reagents	5.1
Storage and Maintenance of Media and Reagents	5.2
Testing of Media	5.3
Reagents	5.4
Antisera	5.5
Results and Records	5.6

**4. References**

- 4.1. Compendium Of Methods for the Microbiological Examination of Foods, Fourth Edition, 2001, American Public Health Association, Washington, D.C
  - 4.2. Miller, J.M. 1987 revision. Quality Control in Microbiology. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, GA.
  - 4.3. Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, 1999. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, GA.
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- 4.4. Difco™ & BBL™ Manual, Manual of Microbiological Culture Media, 2003, Becton Dickinson and Company, Sparks, MD
- 4.5. USDA, FSIS Microbiology Laboratory Guidebook, 3<sup>rd</sup> Edition, 1998, Washington, D.C [http://www.fsis.usda.gov/Science/Microbiological\\_Lab\\_Guidebook/index.asp](http://www.fsis.usda.gov/Science/Microbiological_Lab_Guidebook/index.asp) (last accessed 01/03/06)
- 4.6. Bacteriological Analytical Manual Online. Media Index. US FDA/CFSAN. January 2001. <http://www.cfsan.fda.gov/~ebam/bam-mi.html> - accessed on 12-29-05

## **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**

- 5.1.1. Upon receipt, inspect all containers of media and reagents for damage and condition.
- 5.1.2. Record 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
    - 5.1.2.3.1. Record date of receipt and initials of person who received each bottle of dehydrated media prior to storage.
    - 5.1.2.3.2. Record date of receipt and initials of person who received each container/package of commercially prepared media prior to storage.
    - 5.1.2.3.3. Record date of receipt and initials of person who received each bottle of reagents prior to storage.
  - 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



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## 5.2. Storage and Maintenance of Media and Reagents

5.2.1. Follow the manufacturer's directions for handling and storage of media/reagents.

5.2.2. Use older stock first and ensure the expiration date has not passed.

5.2.3. The following guidelines apply for dehydrated media and reagents:

5.2.3.1. Upon opening, inspect each container of dehydrated medium/reagent for volume, tightness of closure, clarity, color, consistency, and completeness of label. Do not use medium/reagent if it appears abnormal. Document corrective action when media fails to meet acceptance criteria.

5.2.3.2. Enter the date and initials of person opening the dehydrated media/reagent in media logbook. Transfer unique product information recorded on the outside boxes or covers to containers.

5.2.4. The following guidelines apply for commercially prepared media:

5.2.4.1. Prior to use, inspect commercially prepared media for defects.

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

## 5.3. Testing of Media

### 5.3.1. Batch size and testing

5.3.1.1. A batch refers to all tubes, plates, or containers prepared and sterilized at the same time under the same conditions. For small batches of media (less than 100 units), incubate the following (at a minimum): one unit inoculated with a positive control organism and one unit inoculated with a negative control organism. Consult appropriate references to determine the organisms to be tested. Incubate at the temperature and time period specified for the test.

5.3.1.2. For larger batches of media, in addition to testing positive and negative controls, include 3 % of the units for sterility testing.

5.3.1.3. Maintain records of the testing results.

### 5.3.2. Sterility Testing

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- 5.3.2.1. Prior to use, check sterility of media and reagents. Sterility is indicated by a lack of visible growth/turbidity following incubation. This is achieved by incubating one set of uninoculated units at conditions specified by each method.
  - 5.3.2.2. If results of visual examinations are unacceptable, document abnormalities and discard the batch.
  - 5.3.2.3. Discard and autoclave units after incubation. Do not reuse media that have been subjected to sterility testing.
  - 5.3.3. Reference Organisms
    - 5.3.3.1. Use fresh reference cultures (18-24 hours old) for testing media.
    - 5.3.3.2. Consult appropriate references to determine the organisms to be tested. Two or more organisms may be needed to check for growth, selectivity, or the biochemical reactions of a medium.
  - 5.3.4. Preparation of Reference Organisms for Testing
    - 5.3.4.1 From a fresh culture (18-24 hours old), prepare a standardized inoculum to a turbidity equivalent of a 0.5 McFarland standard and inoculate a known amount to test the media.
    - 5.3.4.2 In testing selective media for the ability to inhibit or support bacterial growth, a diluted inoculum is recommended. The dilute suspension provides greater assurance that the performance of media is not limited by a small number of organisms.
    - 5.3.4.3. Media may be tested concurrently with samples by using the appropriate controls.
  - 5.3.5. Performance Testing of Media
    - 5.3.5.1. Each batch of media used in the MDP, whether prepared in the laboratory from dehydrated ingredients or purchased in prepared form, will be examined and tested for performance and sterility prior to use.
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5.3.5.2. Conduct performance testing of laboratory-prepared media as described in appropriate references and SOPs.

5.3.5.3. For purchased, ready-to-use media, performance testing is not necessary if a certificate or other documentation is provided by the manufacturer stating that the media has been properly evaluated.

5.3.5.4. In cases of emergency, where media is prepared and used on the same day, productivity/selectivity testing shall be performed concurrently with sample analyses.

#### 5.4. Reagents

5.4.1. Refer to individual SOPs for the lists of reagents and their usage.

5.4.2 Follow manufacturer's instructions for preparation and storage conditions.

#### 5.5. Antisera

5.5.1 Include in testing positive and negative control strains that carry the appropriate antigens, or specific antigen samples supplied by the manufacturer.

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

#### 5.6 Results and Records

5.6.1 Maintain records of performance testing for each batch of media and reagent tested.

5.6.2 Record all weights, calculations and concentrations used in the preparation of media and reagents.

5.6.3 Other information maintained in the records should include the lot number of media and reagents used, preparation and expiration dates, organisms used, expected reactions, results of sterility tests and visual examinations, problems, and corrective actions. Include the initials of the analyst who performed the tests.

5.6.4 Repeat the test if quality control test results are unacceptable. If the repeat test fails, discard the batch and document all problems and corrective actions taken.



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*Disclaimer: Reference to brand names (kits, equipment, media, reagents etc.) does not constitute endorsement by this agency.*





