

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

SOP No. MDP-LABOP-03		Page 1 of 8
Title - Microbiological Media, Reagents, Kits and Identification Systems		
Revision 04	Replaces Rev 03 04/01/09	Effective 09/01/11

1. Purpose

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

2. Scope

This standard operating procedure (SOP) shall be followed by laboratories conducting microbiological studies for MDP, including support laboratories conducting non-routine activities that may impact the program. This SOP represents minimum MDP requirements and is presented as a general guideline. Each laboratory shall have written internal procedures that provide specific details concerning how the procedures have been implemented in that laboratory.

3. Outline of Procedure

- 6.1 Reference Organisms
- 6.2 Dehydrated Media
- 6.3 Internally prepared media and reagents
- 6.4 Commercial pre-prepared media and reagents
- 6.5 Kits and Identification systems
- 6.6 Storage and Disposal
- 6.7 Records

4. References

- 4.1 AOAC INTERNATIONAL Guidelines for Laboratories Performing Microbiological and Chemical Analyses of Food and Pharmaceuticals, Appendix B: Food Microbiology, ALACC Criteria, March 2010

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5. Definitions

- 5.1 Batch - A batch refers to all tubes, plates or containers of media prepared at the same time, on the same date, sterilized on the same date and/or with the same lot number when ordered pre-prepared from a manufacturer.
- 5.2 Selective organism – An organism which, grown on a specific media type, produces growth and/or a specific biochemical reaction on the media.
- 5.3 Non-selective organism – An organism which, grown on a specific media type, produces no growth on the media or an atypical biochemical reaction on the media.
- 5.4 Sterility – After incubation no bacterial growth (visible turbidity) is observed in the media or reagent.
- 5.5 MSDS – Material Safety Data Sheet. A form with data regarding the properties and safety precautions for a particular substance.

6. Specific Procedures:

6.1 Reference Organisms

- 6.1.1 Microbiological media shall be verified using reference cultures or well-characterized isolates. See Table 1 for recommended selective and non-selective organisms used to test media commonly used by MDP participating laboratories.
 - 6.1.2 Laboratory shall have internal procedures for storage and handling of organisms.
 - 6.1.3 Laboratories shall have internal written procedures for inoculating media. A dilute inoculum is recommended since it will give greater
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assurance that the medium will support growth of a small number of colonies. Refer to manufacturer's product sheet for recommended organisms not listed.

NOTE: Laboratories may use any suitable organism(s) according to their own internal written procedures.

Table 1- Selective/Non-selective QC Organism Recommendations for Media

<u>Media</u>	<u>Selective Organism</u>	<u>Non-selective Organism</u>
Modified EC + Novobiocin	E. coli O157:H7 (MDP-004)	Non-O157:H7 E. coli (MDP-017)
Levine Eosin Methylene Blue Agar	E. coli (MDP-017)	E. aerogenes or E. coli (MDP-005)
MacConkey Agar	E. coli (MDP-017)	E. aerogenes or E. coli (MDP-005)
Sorbitol MacConkey Agar	E. coli O157:H7 (MDP-004)	Non-O157:H7 E. coli (MDP-017)
Bismuth Sulfite Agar	Salmonella (MDP-014)	E. coli (MDP-017)
Hektoen Enteric Agar	Salmonella (MDP-014)	E. coli (MDP-017)
XLD Agar	Salmonella (MDP-014)	E. coli (MDP-017)
Lysine Iron Agar	Salmonella (MDP-014)	E. coli (MDP-017)
Triple Sugar Iron Agar	Salmonella (MDP-014)	E. coli (MDP-017)
CHROMagar E. coli	E. coli (MDP-017)	E. coli (MDP-017)
CHROMagar O157	E. coli O157:H7 (MDP-004)	Non-O157 E. coli (MDP-017)

6.2 Dehydrated Media

6.2.1 Receipt

Upon receipt of dehydrated media and dehydrated media components, laboratory shall keep records for the following information at a minimum:

- 6.2.1.1 Unique identification (laboratory number)
- 6.2.1.2 Name or description of media/reagent
- 6.2.1.3 Manufacturer's lot number
- 6.2.1.4 Quantity received, (i.e., size and number of containers)

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- 6.2.1.3 Date received
- 6.2.1.4 Manufacturer's Expiration date
- 6.2.1.5 Date opened
- 6.2.1.6 Date prepared for quality control (QC)
- 6.2.1.7 Initials of responsible person

6.2.2 Dehydrated media (containers) shall be labeled with the laboratory number, identification, and date received.

6.2.3 The laboratory shall have internal procedures to prevent the accidental use of expired or defective dehydrated media.

6.3 Internally prepared media and commercially purchased media

6.3.1 Each new lot received from manufacturer shall be examined for suitability before use or concurrently with samples. If concurrent testing with samples is performed and QC fails, sample results may be invalid. An investigation shall be performed following the laboratory's internal procedures.

6.3.2 Preparation records shall include:

6.3.2.1 Preparation (recipes)

6.3.2.2 pH

6.3.2.3 Sterilization records

6.3.2.3.1 Autoclave records shall show date, run number, autoclave number if applicable, nature of material/load, time into autoclave, time at desired temperature, time out of autoclave, responsible person.

6.3.2.3.2 If other sterilization is used, records shall indicate the method and responsible person.

6.3.2.3.3 Autoclaves shall be verified for temperature, time and

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sterility each day of use; each load sterile control all media; weekly spore check.

6.3.2.4 Fill volumes (if appropriate)

6.3.2.5 Batch size

6.3.2.6 Quantity

6.3.3 Suitability testing (QC) for internally prepared or purchased prepared media shall include:

6.3.3.1 Productivity (+ culture)

6.3.3.2 Sterility

6.3.3.3 Appearance of media (internally prepared)

6.3.3.4 The person who approves or rejects

6.3.4 New lots or batches of commercially pre-prepared media or reagents.

Each new lot received from manufacturer shall be examined for suitability. Initial suitability may be satisfied through a Certificate of Analysis (CoA). The CoA shall state that the media has been properly evaluated for productivity (+ culture); sterility; and appearance. The CoA is a record and shall be maintained according to Records Section 6.7. Alternatively, in-house laboratory suitability testing may satisfy suitability requirements.

6.3.5 The laboratory shall have internal procedures to prevent the accidental use of expired or defective commercial pre-prepared media and reagents.

6.4 Kits, Quality Critical Reagents and Identification systems

6.4.1 Every lot of material shall be approved using the laboratory's internal procedures. Records shall be sufficient to establish an audit trail, including the date approved and traceability to the person approving or rejecting the material.

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6.4.2 Internally prepared reagents shall be labeled with

- 6.4.2.1 Substance
- 6.4.2.2 Strength
- 6.4.2.3 Solvent (if not water)
- 6.4.2.4 Special precautions or use restrictions
- 6.4.2.5 Date of preparation
- 6.4.2.6 Date of expiration
- 6.4.2.7 The responsible person

6.4.3 Serological tests shall include a positive control and a saline negative control.

6.5 Laboratory Grade Water

The laboratory shall have written internal procedures to ensure the laboratory water is fit for use in microbiological methods.

6.6 Records

- 6.6.1 Records shall be traceable to the original manufacturer and lot.
- 6.6.2 Records shall be available for on-site inspections or desk audit reviews
- 6.6.3 Records shall be maintained for at least the time specified in MPO ADMIN 5.4.3.

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

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Revision 04 May 2011 Microbiological Data Program

- Revised entire SOP to conform to ISO 17025 and AOAC requirements
- Non-selective organism challenge is “optional” in March 2010 ALACC guidelines
- Deleted disposal traceability for media, reagents, kits
- Added requirement for traceability of person approving or rejecting media and reagents.
- Reformatted/renumbered SOP
- Added requirements for sterilization and lab grade water.
- Provided allowance for in-line testing.

Revision 03 March, 2009 Monitoring Programs Office

- Re-wrote entire SOP to conform to ISO 17025 and AOAC requirements

Revision 02 March, 2006 Monitoring Programs Office

- Added “reagents” in title
- Updated references
- Condensed sections 5.3, 5.4, and 5.5 to one section
- Added sterility testing

Revision 01 May 16, 2003 Technical Advisory Committee

- 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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