



**US Environmental Protection Agency
Office of Pesticide Programs**

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

**Standard Operating Procedure for
Tracking of Test Microorganisms**

SOP Number: MB-02-07

Date Revised: 07-13-15

SOP Number	MB-02-07
Title	Tracking of Test Microorganisms
Scope	Provides guidance for establishing receipt and expiration dates for microorganisms used in the Microbiology Laboratory Branch (MLB) as well as denotation and tracking of those microorganisms.
Application	Assigning supply and organism control numbers, culture transfer notation, and VIM barcodes to microorganisms as per SOP guidance allows the laboratory to track their use, on paper and electronically (where applicable).

	Approval	Date
SOP Developer:	_____	_____
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Date SOP issued:	
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1. Definitions	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 products.
3. Personnel Qualifications and Training	Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
4. Instrument Calibration	Not applicable.
5. Sample Handling and Storage	Not applicable.
6. Quality Control	For quality control purposes, the required information is documented on the appropriate record form(s) (see section 14 of this SOP as well as relevant test method SOPs.
7. Interferences	None
8. Non-conforming Data	Entry errors will be corrected upon discovery.
9. Data Management	Data will be archived consistent with SOP ADM-03, Records and Archives.
10. Cautions	Expired microorganisms will not be used and will be destroyed by autoclaving.
11. Special Apparatus and Materials	<ol style="list-style-type: none"> 1. See Attachment 1 (section 14) for a list of microorganisms currently in use in the laboratory. 2. Microorganisms used in the laboratory may be purchased from appropriate vendors or received from other federal agencies for special studies.
12. Procedure and Analysis	Procedures for generating stock cultures for organisms maintained on slants and stabs or as frozen stock cultures can be found in relevant test method SOPs. Refer to Attachment 2 (section 14) for an overview on tracking of microorganisms used in the laboratory.
12.1 Supply Control Number	<ol style="list-style-type: none"> a. All purchased organisms are given a supply control number upon receipt (see SOP QC-09, Control Numbers).

	<p>b. Record the supply control number on the packing slip and place a copy of the packing slip into the Biological Inventory Logs record book (see section 12.7).</p>
<p>12.2 Organism Control Number: Overview</p>	<p>a. Assign an organism control number to all laboratory generated cultures (e.g., frozen stock cultures, transfer cultures, test cultures, etc.) except for spore suspensions and carriers inoculated with spore suspensions. The organism control number consists of the date the microbe expires (ME) and a two- or three-letter suffix denoting the organism. The organism control number may also include any culture transfer notation depending upon the type of culture (refer to section 12.5 for culture transfer notation).</p> <p>i. Spore suspensions (e.g., <i>B. subtilis</i>, <i>C. difficile</i>) generated in the laboratory, as well as carriers inoculated with the spore suspensions, currently receive a preparation number rather than an organism control number. See section 12.6.</p> <p>b. Assign an organism control number in the MEXXXXXX format. For example, notate a culture expiring on 12/31/17 as ME123117.</p>
<p>12.3 Organism Control Number: Expiration Dates</p>	<p>a. Expiration dates for laboratory-generated cultures may vary depending upon the microorganism and type of culture. Refer to the information below and other relevant SOPs for guidance.</p> <p>b. For organisms maintained as lab-generated frozen stock cultures: Once frozen, these organisms may be used for eighteen months from the date that the culture was frozen. For example, assign an ME number of ME080316 to stocks frozen on 02-03-15. Any cultures, including stock cultures, daily transfers, and test cultures are valid for up to 18 months after the stock cultures are frozen.</p> <p>c. For organisms maintained on slants and stabs (e.g., <i>B. subtilis</i>): Once reconstituted, these organisms may be used for one year. Any cultures, including stock cultures, daily transfers, and test cultures, are valid for up to 12 months after reconstitution of the lyophilized culture.</p> <p>d. For <i>Mycobacterium bovis</i> (BCG): Upon storage of established slants (approximately 6-7 weeks after being reconstituted), cultures may be used for 18 months.</p> <p>e. Once expired, autoclave and discard the stock cultures and initiate a new culture from a new unexpired lyophilized/frozen lot from ATCC.</p>
<p>12.4 Organism</p>	<p>a. The two or three letters in the suffix of the organism control number</p>

<p>Control Number: Suffixes</p>	<p>denote the genus and species of the organism (for example, Pa for <i>P. aeruginosa</i>, Sa for <i>S. aureus</i>, Bs for <i>B. subtilis</i>, Se for <i>S. enterica</i>, Cd for <i>Clostridium difficile</i>, Mt for <i>M. terrae</i>, Mb for <i>M. bovis</i>, and FCV for Feline Calicivirus). Other microorganisms used in the microbiology laboratory will be given two- or three-letter suffixes consistent with the notation listed above.</p> <p>b. Refer to section 12.5 for culture transfer notation.</p>
<p>12.5 Culture Transfer Notations of Test Microbes</p>	<p>a. See the Organism Culture Tracking Forms for guidance and appropriate culture transfer notations. The forms are found in relevant test method SOPs. Several specific examples of culture transfer notation are provided below.</p> <p>b. For cultures initiated from frozen stock cultures (no daily transfers required): a <i>Staphylococcus</i> test culture notation would be MEXXXXXX-Sa-2, where 2 is the vial number of the frozen stock culture.</p> <p>c. For frozen stock cultures used in efficacy testing under the Antimicrobial Testing Program:</p> <p>i. An example of a <i>Staphylococcus</i> daily transfer notation would be MEXXXXXX-Sa-2-D3, where 2 is the vial number of the frozen stock culture and D3 is applied to indicate the third 24 hour daily transfer. Up to 5 daily transfers into 10 mL of broth may be conducted prior to the inoculation of the final test cultures.</p> <p>ii. An example of a <i>Staphylococcus</i> test culture notation would be MEXXXXXX-Sa-2-D3TC, where 2 is the vial number of the frozen stock culture, D3 indicates that the test culture was inoculated using the third 24 hour daily transfer, and TC is applied to indicate a 48 hour test culture.</p> <p>d. For <i>Mycobacterium bovis</i> (BCG):</p> <p>i. The test culture notation would be MEXXXXXX-Mb-1103SL, where 11 represents the month of culture transfer (the month of the year) and 03 represents the week of the month for that transfer (the 3rd week of the month). The weeks of each month are numbered consecutively starting with the 1st Monday of the month (as 01) and ending with the last Monday of the month (depending on the number of Mondays in the month, as either 04 or 05).</p> <p>ii. SC is applied to identify a stock culture (e.g., a transfer from</p>

	<p>a slant to a slant).</p> <p>iii. For cultures grown quiescently, SL is applied to identify a test culture (this indicates a transfer from a slant culture to a liquid culture).</p> <p>iv. For cultures grown with agitation, a 1° (representing a primary culture) or 2° (representing a test culture) designation is added after the month and week for the transfer (see SOP MB-07).</p>
12.6 Culture Tracking for Spore Suspensions and Inoculated Carriers	<p>a. Assign a preparation number to laboratory generated spore suspensions (e.g., <i>B. subtilis</i>, <i>C. difficile</i>).</p> <p>b. Assign a preparation number to a set of carriers inoculated with spore suspensions.</p> <p>c. Consult relevant test method SOPs for the appropriate storage conditions, including storage time, temperature, etc.</p>
12.7 Biological Inventory Management: Overview	<p>a. MLB utilizes the biological module of the Vertere Inventory Management System (VIM) to electronically track, from receipt to disposal, microorganisms maintained by MLB:</p> <p>i. Vendor-supplied vials (e.g., ATCC culture)</p> <p>ii. Stock cultures in long term storage at -70°C or below</p> <p>iii. Spore suspensions</p> <p>iv. Carriers inoculated with spores</p> <p>v. Other sources of cultures (e.g., CDC-supplied)</p> <p>b. Exceptions include cultures stored and maintained on slants (e.g., <i>M. bovis</i> (BCG) and <i>B. subtilis</i>), daily transfers for test culture generation, and test cultures. These cultures are generally short-lived, and their generation is recorded separately on the appropriate Organism Culture Tracking Form. See relevant test method SOPs.</p> <p>c. As an adjunct to the electronic VIM database, the laboratory maintains a Biological Inventory Logs record book. See Biological Inventory Disposal Log form (section 14).</p>
12.8 Biological Inventory Management: Implementation	<p>a. When cultures are received from outside sources (e.g., purchased) or trackable cultures are generated from within the laboratory for long term storage (see section 12.7), the MLB VIM inventory manager or designee will:</p> <p>i. Assign a supply control number to purchased</p>

	<p>microorganisms/biologicals as per section 12.1.</p> <ul style="list-style-type: none"> ii. Generate the Biological Inventory Disposal Log form. iii. Assign VIM barcodes to each individual stored culture (e.g., vial, tube, plate of carriers, etc.) entered on the Biological Inventory Disposal Log form. iv. For purchased microorganisms, record the supply control number and VIM barcode on the packing slip and place a copy of the packing slip into the Biological Inventory Logs record book. Store the biological in the appropriate location. v. Place VIM barcodes directly onto the Biological Inventory Disposal Log form in the “VIM #” box. vi. Enter the barcode number and appropriate information (e.g., microorganism, supply control number, organism control number, preparation number, etc.) into the VIM system. <ul style="list-style-type: none"> b. To assist in the tracking process, analysts must notify the MLB VIM inventory manager when generating trackable cultures (section 12.7) and provide relevant information (e.g., organism, control number, number of vials, expiration date, etc.). c. Analysts may access stored cultures as needed for their work. d. Upon removal of a tracked culture from storage, analysts must date and initial the “Date Consumed” and “User Initials” blocks on the appropriate page of the Biological Inventory Disposal Log. <ul style="list-style-type: none"> i. If the tracked culture is returned to storage rather than being consumed, no entry is necessary. e. The MLB VIM inventory manager disposes the culture barcode in the VIM system and enters the date in the “Date Removed from VIM” box on the appropriate page of the Biological Inventory Disposal Log. f. On a quarterly basis, the Branch Chief and the MLB VIM inventory manager check the laboratory space, freezers, and refrigerators to reconcile the electronic inventory with the actual physical inventory and record findings on the Biological Inventory Inspection Log (see section 14).
<p>13. Data Analysis/ Calculations</p>	<p>None</p>

14. Forms and Data Sheets	Test Sheets. Test sheets are stored separately from the SOP under the following file names: Biological Inventory Disposal Log MB-02-07_F1.docx Biological Inventory Inspection Log MB-02-07_F2.docx Attachment 1: Microorganisms Used by the OPP Microbiology Laboratory MB-02-07_F3.docx Attachment 2: Overview of Tracking of Microorganisms in the Laboratory MB-02-07_F4.docx
15. References	None