



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 Microorganisms

SOP Number: MB-02-09

Date Revised: 09-27-21

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Title	Tracking of Microorganisms
Revisions Made	<ul style="list-style-type: none">• Minor editorial changes for clarification purposes.• Section 12.2, added an option to assign an additional suffix (-1 or -01) to the Virus Stock Number to indicate the order of the infection event on a given day.• Section 12.3, added a statement to indicate that virus frozen stock cultures do not have expiration dates.• Section 12.6, added an additional exception to cultures tracked in the biological inventory: “Exceptions also include laboratory-generated stock cultures placed in storage while awaiting verification of acceptability criterion (e.g, titer, biochemical/antibiotic susceptibility testing). Stock cultures ultimately deemed acceptable will be entered into the VIM database. Cultures deemed unacceptable will be discarded.”• Section 12.7, added instructions for recording, in the Biological Inventory Disposal Log, partial use of a frozen stock culture vial.

SOP Number	MB-02-09
Title	Tracking of 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).

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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.
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, document the control numbers (see sections 12.1 and 12.2) 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	<ol style="list-style-type: none"> 1. Manage non-conforming data consistent with SOP ADM-07, Non-Conformance Reports. 2. Correct entry errors upon discovery.
9. Data Management	<ol style="list-style-type: none"> 1. Archive data consistent with SOP ADM-03, Records and Archives. 2. MLB utilizes the biological module of the Vertere Inventory Management System (VIM) to electronically track, from receipt to disposal, microorganisms maintained in the laboratory.
10. Cautions	Do not use expired microorganisms.
11. Special Apparatus and Materials	<ol style="list-style-type: none"> 1. See Attachment 1 (section 14) for a list of microorganisms currently in long term storage in the laboratory. 2. Microorganisms used in the laboratory are purchased from appropriate vendors or received from other federal agencies.
12. Procedure and Analysis	Procedures for generating stock cultures for long term storage are 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. Assign a supply control number to organisms upon receipt (see SOP QC-09, Control Numbers). 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 sections 12.6 and 12.7).

<p>12.2 Organism Control Number: Overview and Exceptions</p>	<ul style="list-style-type: none"> a. Assign an organism control number to all laboratory generated cultures, except those specified in section 12.2.b and c. <ul style="list-style-type: none"> i. Assign an organism control number in the following format: ii. The organism control number consists of the date the microbe expires (ME; see Attachment 1) in XXXXXX format and a two- or three-letter suffix denoting the organism. For example, notate a <i>Staphylococcus aureus</i> culture expiring on 12/31/24 as ME123124-Sa. iii. See Attachment 1 for the assigned suffix for each organism. b. Assign a media preparation number (see SOP MB-10, Media and Reagents), rather than an organism control number, to the following: <ul style="list-style-type: none"> i. Spore and conidial suspensions (e.g., <i>B. subtilis</i>, <i>C. difficile</i>, <i>T. interdigitale</i>) generated and stored in the laboratory. ii. Carriers inoculated with spore suspensions and stored for later use in efficacy testing. c. Frozen stock cultures of viruses receive a Virus Stock Number rather than an organism control number. Assign a Virus Stock Number in the following format: Use a two- or three-letter suffix denoting the organism followed by the date in XXXXXX format, where XXXXXX denotes the infection date for the virus (i.e., the day virus was added to cell culture to initiate infection for stock generation purposes). For example, on 12/31/21, an analyst infects a cell line with FCV Virus. Upon harvest of the virus, regardless of the harvest date, the Virus Stock Number for FCV is FCV-123121. <ul style="list-style-type: none"> i. Analysts may elect to assign an additional suffix (e.g., -1 or -01) to the Virus Stock Number to indicate the order of the infection event on a given day. For example, the FCV frozen stock culture which originated from the first virus infection event on 12/31/21 may be designated FCV-123121 (no suffix), FCV-123121-1, or FCV-123121-01. The second virus infection event on 12/31/21 would be designated FCV-123121-2 or FCV-123121-02. ii. See Attachment 1 for the assigned suffix for each virus.
<p>12.3 Expiration Dates</p>	<ul style="list-style-type: none"> a. Assign expiration dates for laboratory-generated cultures. Refer to relevant SOPs and Attachment 1 for guidance. b. Virus frozen stock cultures do not have expiration dates. c. Once expired, autoclave and discard the stock cultures and initiate a

	new culture.
12.4 Culture Transfer Notations of Test Microbes	<p>a. If use of a frozen vial requires transfers (e.g., 24 or 48 hr), add the vial number to the end of the organism control number. For example, a <i>Staphylococcus</i> test culture notation is MEXXXXXX-Sa-2, where 2 is the vial number of the frozen stock culture.</p> <p>b. For frozen stock cultures used in testing which requires multiple transfers, assign a daily transfer (D) or test culture (TC) notation as follows:</p> <p>i. An example of a <i>Staphylococcus</i> daily transfer notation is MEXXXXXX-Sa-2-D1, where 2 is the vial number of the frozen stock culture and D1 is applied to indicate the first 24-hour daily transfer.</p> <p>ii. An example of a <i>Staphylococcus</i> test culture notation is 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>c. For <i>Mycobacterium bovis</i> (BCG):</p> <p>i. Assign test culture notation as follows: 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 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>
12.5 Culture Tracking for Spore	<p>a. Consult relevant test method SOPs for the appropriate storage conditions, including storage time, temperature, etc.</p>

<p>Suspensions and Inoculated Carriers</p>	
<p>12.6 Biological Inventory Management: Overview</p>	<ul style="list-style-type: none"> a. MLB utilizes the biological module of VIM to electronically track, from receipt to disposal, microorganisms stored in the laboratory: <ul style="list-style-type: none"> i. Vendor-supplied cultures (e.g., ATCC culture, Culti-Loops) ii. Stock cultures in long term storage at approximately -80°C iii. Other sources of cultures (e.g., CDC-supplied) b. Exceptions include spore/conidial suspensions, inoculated carriers, 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 either on Media/Reagent Preparation Sheets or the appropriate Organism Culture Tracking Form. See relevant test method SOPs. <ul style="list-style-type: none"> i. Exceptions also include laboratory-generated stock cultures placed in storage while awaiting verification of acceptability criterion (e.g, titer, biochemical/antibiotic susceptibility testing). Stock cultures ultimately deemed acceptable will be entered into the VIM database. Cultures deemed unacceptable will be discarded. 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>12.7 Biological Inventory Management: Implementation</p>	<ul style="list-style-type: none"> 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 12.6), the MLB VIM inventory manager or designee will: <ul style="list-style-type: none"> i. Assign a supply control number to the microorganisms/biologicals as per section 12.1. ii. Generate the Biological Inventory Disposal Log form. iii. Assign VIM barcodes to each individual stored culture (e.g., vial) entered on the Biological Inventory Disposal Log form. iv. 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 microorganism in the appropriate location.

	<ul style="list-style-type: none"> 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, etc.) into the VIM system. b. To assist in the tracking process, analysts must notify the MLB VIM inventory manager when generating trackable cultures (section 12.6) 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. Some studies (e.g., virology) may require partial use of a frozen stock culture vial, in which a vial is removed from storage, an aliquot removed, and the vial returned to storage for later use. In this event, in the “Date Consumed” block, record the date/initials/“returned” (i.e., vial returned to freezer). 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 will check the laboratory space, freezers, and refrigerators to reconcile the electronic inventory with the actual physical inventory. <ul style="list-style-type: none"> i. Record discrepancies on the Biological Inventory Inspection Log (see section 14). 				
13. Data Analysis/ Calculations	None				
14. Forms and Data Sheets	<p>Test Sheets. Test sheets are stored separately from the SOP under the following file names:</p> <table border="0" style="width: 100%;"> <tr> <td style="width: 70%;">Biological Inventory Disposal Log</td> <td>MB-02-09_F1.docx</td> </tr> <tr> <td>Biological Inventory Inspection Log</td> <td>MB-02-09_F2.docx</td> </tr> </table>	Biological Inventory Disposal Log	MB-02-09_F1.docx	Biological Inventory Inspection Log	MB-02-09_F2.docx
Biological Inventory Disposal Log	MB-02-09_F1.docx				
Biological Inventory Inspection Log	MB-02-09_F2.docx				

	Attachment 1: Microorganisms in Long Term Storage in the OPP Microbiology Laboratory	MB-02-09_F3.docx
	Attachment 2: Overview of Tracking of Microorganisms in the Laboratory	MB-02-09_F4.docx
15. References	None	