



**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-02-04

Date Revised: 01-06-09

EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

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for
Tracking of Test Microorganisms

SOP Number: MB-02-04

Date Revised: 01-06-09

Initiated By: _____ Date: ___/___/___

Print Name: _____

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Effective Date: ___/___/___

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1.0 SCOPE AND APPLICATION:

1.1 This protocol provides guidance for establishing receipt and expiration dates for microorganisms used in the microbiology laboratory.

2.0 DEFINITIONS:

2.1 ATCC = American Type Culture Collection

3.0 HEALTH AND SAFETY:

3.1 All manipulations of the test organisms are required to be performed in accordance with biosafety practices stipulated in SOP MB-01, Lab Biosafety.

4.0 CAUTIONS:

4.1 Expired ampules of freeze-dried organisms will not be used and will be discarded appropriately.

5.0 INTERFERENCES: None.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable about and to comply with the laboratory's culturing and disinfectant testing procedures. Documentation of training and familiarization with these procedures can be found in the training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 *Pseudomonas aeruginosa* (ATCC 15442), *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 19659), and *Salmonella enterica* (ATCC 10708); ordered annually and received directly from ATCC.

7.2 *Mycobacterium bovis* (BCG); ordered and received directly from Organon Teknika.

7.3 Other microorganisms used in the microbiology laboratory may be purchased from appropriate vendors when necessary.

8.0 INSTRUMENT OR METHOD CALIBRATION:

8.1 Refer to the laboratory equipment calibration and maintenance SOPs (SOP EQ series) for details on method and frequency of calibration.

9.0 SAMPLE HANDLING AND STORAGE: Not applicable.

10.0 PROCEDURE AND ANALYSIS:

10.1 Supply Control Number

10.1.1 All freeze-dried ampules are given a supply control number upon receipt (see SOP QC-09, Control Numbers).

10.1.2 The supply control number will consist of the date received (R) and the date the ampule expires (E).

For example: For a dehydrated ampule of *S. aureus* received on 01-05-09 and expiring on 03-12-10, the supply control number would be R010509E031210. For a dehydrated ampule of *M. bovis* (BCG) received on 07-24-08 and expiring on 09-04-09, the supply control number would be R072408E090409.

10.2 Microbe Received and Microbe Expiration Number (MRME).

10.2.1 The MRME number will consist of the date the microbe is received (MR) and the date the reconstituted microbe expires (ME). The ME number is not required for *M. bovis* (BCG) because an expiration date is not established.

10.2.1.1 *P. aeruginosa*, *S. aureus*, *B. subtilis*, and *S. enterica*. A culture received on 01-05-09 and reconstituted on 02-04-09 would receive a culture notation of MR010509ME020410, where "MR" represents the date the microbe is received and "ME" represents the date the reconstituted microbe expires.

10.2.1.1.1 Once reconstituted, *P. aeruginosa*, *S. aureus*, *B. subtilis*, and *S. enterica* may only be subcultured for a period of one year.

10.2.1.1.2 Once expired, the stock cultures must be autoclaved and discarded and a new culture initiated from a new unexpired lyophilized

lot from ATCC.

10.2.1.2 *M. bovis* (BCG). *M. bovis* (BCG) is not required to be replaced annually. Therefore, the culture notation will only consist of the MR number.

10.2.1.2.1 Continuous transfers of *M. bovis* (BCG) may be made unless the organism has been compromised.

10.2.2 Additional Culture Notation.

10.2.2.1 The MRME culture notation will have a suffix as follows: for *Staphylococcus* the suffix will be S; for *Pseudomonas* the suffix will be P; for *Bacillus* the suffix will be B; for *Salmonella* the suffix will be SE; for *Mycobacterium* the suffix will be M.

Thus, the final culture notation after reconstitution for *Staphylococcus*, for example, would be MRXXXXXXXXMEXXXXXX-S. The culture notation for *M. bovis* (BCG) would be MRXXXXXXXX-M.

10.2.2.2 Other microorganisms used in the microbiology laboratory will be given culture notations and suffixes consistent with the notations listed in section 10.2.

10.3 Culture Transfer Notation of Test Microbes.

10.3.1 See footnotes for Organism Culture Tracking Form for culture transfer notations for *S. aureus*, *P. aeruginosa*, and *S. enterica* (see section 16.0 in MB-05 and MB-06). For example, *Staphylococcus* test culture notation would be MRXXXXXXXXMEXXXXXX-S-1104TC, where 11 is the monthly culture transfer (number of months since the month of initiation) and 04 is the daily culture transfer (4th daily culture transfer); TC is applied to indicate a 48 hour test culture.

10.3.2 See footnotes for Organism Culture Tracking Form for *M. bovis* (BCG) culture transfer notations (see section 16.0 in MB-06 and MB-07). For *Mycobacterium*, test culture notation would be MRXXXXXXXX-M-1103, 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). TC is applied to identify the test culture. Since test culture transfers typically occur on Monday (or Tuesday), 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 # of Mondays in the month, as either 04 or 05).

11.0 DATA ANALYSIS/CALCULATIONS: None

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data will be recorded promptly, legibly, and in indelible ink on the Test Microbe Confirmation Sheet and the Organism Culture Tracking Form. Completed forms are archived in notebooks kept in secured file cabinets in room D217. Only authorized personnel have access to the secured files. Archived data is subject to OPP's official retention schedule contained in SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL:

13.1 For quality control purposes, the required information is documented on the appropriate record form(s) (see section 16.0 of MB-05, MB-06, and MB-07).

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 Entry errors will be corrected upon discovery.

15.0 REFERENCES: None.

16.0 FORMS AND DATA SHEETS: None.