US Environmental Protection Agency
Office of Pesticide Programs

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

Determining the Presence of Microbial Contamination in Disinfectant Products Standard Operating Procedures (SOPs)

SOP Number: QC-21-03

Date Revised: 01-29-15

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Title	Determining the Presence of Microbial Contamination in Disinfectant Products
Scope	This SOP describes methodology used to determine the occurrence of microbial contamination of liquid and spray antimicrobial products and is consistent with methodology for conducting the AOAC Use Dilution Method (UDM) or the AOAC Germicidal Spray Products as Disinfectants Test (GSPT), but with sterile carriers. The assays are qualitative and designed to recover and culture bacterial contamination (i.e., spores of *Bacillus* sp.) from a disinfectant sample.
Application	In most instances, the assays will be deemed necessary due to historical evidence of bacterial contamination associated with the sample itself, a previously-collected sample of the same product, or a registrant's product line. Products that show no evidence of product-borne contamination when tested by this procedure are considered adequate for efficacy testing; however, samples with confirmed contamination will be deemed unsuitable for conducting efficacy tests.

	Approval Date
SOP Developer:	_____ Print Name: _____
SOP Reviewer	_____ Print Name: _____
Quality Assurance Unit	_____ Print Name: _____
Branch Chief	_____ Print Name: _____

Date SOP issued:	
Controlled copy number:	
Date SOP withdrawn:	

TABLE OF CONTENTS

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	Refer to SOPs EQ-02 (thermometers), EQ-03 (weigh balances), EQ-04 (spectrophotometers), EQ-05 (timers), and QC-19 (pipettes) for details on method and frequency of calibration.
5. Sample Handling and Storage	Refer to SOP MB-22, Disinfectant Sample Preparation, and SOP COC-01, Chain of Custody Procedures.
6. Quality Control	For quality control purposes, the required information is documented on the appropriate form(s) (see section 14).
7. Interferences	1. Follow Quality control measures for media and reagents used in this evaluation as outlined in SOP MB-10, Media and Reagents Preparation and Quality Evaluation.
8. **Non-conforming Data**	Management of non-conforming data will be specified; procedures will be consistent with SOP ADM-07, Non-Conformance Reports.
9. Data Management	Archive data consistent with SOP ADM-03, Maintaining, Tracking, and Archiving of Records.
10. Cautions	1. Use aseptic techniques to prevent contamination of product and test system. 2. Verify the volume of neutralizer tubes and subculture tubes in advance and adjust accordingly. 3. To avoid introduction of contaminating organisms into the test system and cross-contamination from tube to tube during the assay: a. Autoclave hooks prior to use. b. Ensure that there is sufficient time between transfers (from disinfectant to neutralizer and neutralizer to subculture medium if applicable) to thoroughly flame (to red-hot) the wire hook. c. See additional suggestions in sections 12.1.b and 12.1.c.
11. Special Apparatus	1. Refer to SOP MB-05, AOAC UDM and SOP MB-06, AOAC GSPT for a list of recommended materials.

and Materials	a. Do not use a recirculating chiller/water bath unit during the conduct of the AOAC Use Dilution Method with sterile carriers (section 12.1b). b. Because the assay is performed with sterile carriers, no test microbes or materials related to conducting carrier enumeration will be used.
12. Procedure and Analysis	1. To conduct the assay, follow SOP MB-05, AOAC UDM, for liquid products and SOP MB-06, AOAC GSPT, for spray products, including liquid products to be applied as a spray. 2. Use the test conditions specified for product testing (e.g., water hardness, use-dilution, pH, organic soil, neutralizer, contact time) except for test temperature for the AOAC UDM (see section 12.1b).
12.1 Comments/ Exceptions	a. Do not generate inoculum because test microbes are not used in this assay. b. For liquid products, do not use the recirculating chiller and water bath specified in SOP MB-05 to maintain the temperature of the disinfectant. Place racks of disinfectant-containing tubes directly onto the surface of the biological safety cabinet (BSC). Perform exposure of carriers to disinfectant at room temperature. c. Leave the ultraviolet light (UV) in the BSC on overnight prior to performing the test. Place necessary test equipment (i.e., vortex, hooks, timer) into the BSC prior to turning on the UV light. d. A single assay involves the evaluation of 60 sterile carriers for one product sample. Prepare approximately 65-70 sterile carriers (60 plus extras). e. As a default, use primary tubes only in this assay. f. Do not perform carrier counts.
12.2 Product Sample Preparation	a. Prepare the product according to the test parameters; follow guidelines for disinfectant sample preparation provided in SOP MB-22, Disinfectant Sample Preparation, and SOP COC-01, Chain of Custody Procedures.
12.3 Carrier Preparation	a. Prepare carriers according to the applicable SOP. i. For UDM (stainless steel penicylinders): Follow carrier inoculation (SOP MB-05, section 12.2) except use sterile broth. Add organic soil to the sterile broth as necessary per the test parameters.

	ii. For GSPT (25 × 25 mm glass slide carriers): Follow carrier inoculation (SOP MB-06, section 12.2) except use sterile broth. Add organic soil to the sterile broth as necessary per the test parameters.
12.4 Conducting the Assay - Liquid Products	a. Once drying is complete, conduct the AOAC UDM using the dried, uninoculated carriers according to the test parameters (i.e., product dilution, neutralizer, subculture media, contact time).
12.5 Conducting the Assay - Spray Products	a. Conduct the AOAC GSPT using the dried, uninoculated carriers according to the test parameters (i.e., product dilution, neutralizer, subculture media, contact time).
12.6 Recording Results and Confirmation Testing	a. Report results as (+) for microbial growth or (0) for no growth on the results sheet. In the "Comments" section of the results form, describe the growth in positive tubes (e.g., uniformly turbid, string-like, etc.). Note: Shaking tubes prior to recording results may disrupt the unique physical appearance of typical *Bacillus* sp. growing in liquid media (i.e., string-like, fibrous, not producing uniformly turbid media). b. Confirm at least four positive (showing microbial growth) carriers, if available, using Gram staining, growth on trypticase soy agar (TSA; for initial identification and isolation), and VITEK. If there are less than four positive carriers, confirm each carrier. c. Multiple replications of the assay may be necessary to determine the presence of contaminants in the disinfectant sample.
13. Data Analysis/ Calculations	None.
14. Forms and Data Sheets	Test Sheets. Test sheets are stored separately from the SOP under the following file names: Microbial Contamination Assay: Time Recording Sheet for Carrier Transfers — QC-21-03_F1.docx Microbial Contamination Assay: Test Information Sheet — QC-21-03_F2.docx Microbial Contamination Assay: Results Form — QC-21-03_F3.docx Microbial Contamination Assay: Confirmation Sheet — QC-21-03_F4.docx Microbial Contamination Assay: Processing — QC-21-03_F5.docx

	Sheet
15. References	1. Official Methods of Analysis. Methods 955.15 and 964.02. Posted September 2013. AOAC INTERNATIONAL, Gaithersburg, MD. 2. Official Methods of Analysis. Method 961.02. Posted March 2013. AOAC INTERNATIONAL, Gaithersburg, MD.