US Environmental Protection Agency
Office of Pesticide Programs

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

Standard Operating Procedure for
Preparation and Sampling Procedures for Antimicrobial Test Substances

SOP Number: MB-22-05

Date Revised: 12-23-19

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Title	Preparation and Sampling Procedures for Antimicrobial Test Substances
Revisions Made	• In section 10, added statement to ensure test substance-specific preparation sheet is approved in advance. • In section 11, clarified selection of volumetric glassware. • In section 12.1, added use of positive displacement pipette for measuring viscous substances. • Minor editorial changes for clarification purposes.

SOP Number	MB-22-05
Title	Preparation and Sampling Procedures for Antimicrobial Test Substances
Scope	This SOP describes procedures for the preparation and sampling of liquid, spray, and towelette substances for testing.
Application	These procedures are used in conjunction with other antimicrobial test methods.

	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Date SOP withdrawn:	

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1. Definitions	1. Test substance = an antimicrobial formulation used in testing. 2. Sampling = procedure in which part of a test substance is removed from a container for testing. 3. Ready-to-use test substance = test substance that requires no activation or dilution. 4. Concentrated liquid test substance = liquid or solid test substance that requires dilution prior to use. 5. Activation = the combination of a base and an activator to prepare the final test substance. 6. Spray test substance = trigger, aerosol, or pump-based test substance. 7. Towelette test substance = a pre-moistened wipe-based test substance. 8. COC = chain of custody.
2. Health and Safety	1. Follow procedures specified in SOP MB-01, Laboratory Biosafety. The Study Director and/or lead analyst should consult the Safety Data Sheet (SDS) for hazards associated with test substances. 2. Test substances may contain a number of different active ingredients, such as quaternary ammonium compounds, halogens, phenolics, aldehydes, peroxides, and heavy metals. Wear appropriate gloves and other personal protective clothing or devices during the handling of test substances as deemed appropriate per the SDS. 3. Use a chemical fume hood or other containment equipment, such as a biological safety cabinet (BSC) when performing tasks with test substances.
3. Personnel Qualifications and Training	Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
4. Instrument Calibration	Refer to SOP EQ-03 (weigh balances) and QC-19 (pipettes) for details on method and frequency of calibration.
5. Sample Storage	1. Store test substances according to the manufacturer's recommendations, if stipulated, or at room temperature. Store flammable test substances in the flammable cabinet located in room B204. 2. Activate or dilute test substances within three hours of testing to ensure stability of the test substance unless test parameters specify otherwise. 3. Follow COC guidelines in SOP COC-01, Chain of Custody Procedures for Antimicrobial Samples, for those test substances requiring COC.

<table>
<tr><td></td><td>4. For test substances not requiring COC, follow recommendations from the Branch Chief, Senior Science Advisor or Quality Assurance Officer to ensure proper storage.
5. Identify the test substance by name, sample number, etc.
6. Archive COC seal(s) in the COC laboratory notebook.
7. For test substances under COC, use permanent marker to record the test date(s) on the container used for testing.</td></tr>
<tr><td>6. Quality Control</td><td>Required information is documented on the appropriate record form(s). See section 14.</td></tr>
<tr><td>7. Interferences</td><td>Do not use test substances beyond the manufacturer’s expiration date.</td></tr>
<tr><td>8. Non-conforming Data</td><td>Errors in the preparation of the test substance sample will result in an invalid study.</td></tr>
<tr><td>9. Data Management</td><td>Data will be archived consistent with SOP ADM-03, Records and Archives.</td></tr>
<tr><td>10. Cautions</td><td>1. Strict adherence to the protocol for preparation of the test substance is necessary for the validity of the test results.
2. Do not place a pipette or any other instrument inside the test substance container – decant the test substance into a sterile container for preparation.
3. Ensure the test substance-specific preparation sheet is approved by the quality assurance unit or designee prior to its use.</td></tr>
<tr><td>11. Special Apparatus and Materials</td><td>1. Sterile glassware – to dispense and prepare test substances and diluents.
2. Volumetric glassware (micropipettes, pipettes, flasks, etc.) and serological pipettes – to measure liquids for test substance preparation, as appropriate.
<table>
<tr><th><u>Measuring Device</u></th><th><u>Volumes Measured</u></th></tr>
<tr><td>Volumetric flask</td><td>≥50 mL</td></tr>
<tr><td>Volumetric pipette</td><td>1-100 mL (based on pipette availability)</td></tr>
<tr><td>Micropipette</td><td>≤ 1 mL</td></tr>
<tr><td>Serological pipette</td><td>2-100 mL</td></tr>
</table>
3. Ethanol – to clean the outside of the sample container (70% v/v) and prepare spray bottles (100%).
4. Calibrated weigh balance – to weigh sample containers prior to and after</td></tr>
</table>

	sample removal, as required. 5. Sterile spray bottles – to apply spray formulations. 6. Forceps – to open sample container or feed towelettes through container aperture. 7. Test substance diluent – sterile liquid used to make test substance dilutions (e.g., tap water, de-ionized water, or hard water).
12. Procedure and Analysis	1. Prepare a test substance-specific Media/Reagent Preparation Sheet (refer to section 14) according to the test substance use-directions or as defined in the study protocol and have it approved by the quality assurance unit or designee prior to use. 2. Retrieve test substance from sample storage. 3. For test substances requiring COC, remove COC seal and record in COC sample log-in and tracking book. Weigh the test substance container and record the weight on the Media/Reagent Preparation Sheet both prior to and after removing a sample. After use, replace seals, complete COC paperwork, and return the test substance to its appropriate storage location.
12.1 Sampling and Preparation of Liquid Test Substances	a. Gently shake the container of a liquid test substance prior to opening, thoroughly clean the area around the cap and spout using 70% ethanol, and allow to dry. Remove the cap. Do not touch the inside surface of the cap. If present, carefully remove the seal attached to the lip of the spout with sterile instruments (e.g., razor blade, forceps). b. Aseptically pour the appropriate volume into a sterile vessel and securely re-cap the test substance. If the sample is not used immediately, cover the vessel with sterile foil. c. For concentrated test substances, aseptically prepare the test substance use-dilution required for the test using the appropriate sterile glassware or pipettes. i. Add the test substance to the diluent. ii. For viscous test substances, use a positive displacement pipette to measure the test substance. Alternatively, use a serological, volumetric, or micropipette and rinse the pipette (repeat pipetting) using the previously measured diluent to ensure complete delivery of the test substance. d. For test substances requiring the use of hard water as the diluent, refer to MB-30 (Preparation of hard water and other diluents for

	preparation of antimicrobial products) for instructions. e. For concentrated test substances, use ≥ 1.0 mL or 1.0 g of the test substance sample to prepare the final solution to be tested. Use v/v dilutions for liquid test substances and w/v dilutions for solid test substances. f. Examples of test substance dilutions: i. 1:10 dilution = 1 part test substance + 9 parts diluent. ii. ½ ounce into gallon of diluent = 1:256 dilution (1 part test substance + 255 parts diluent) iii. 1 ounce into gallon of diluent = 1:128 dilution (1 part test substance + 127 parts diluent) iv. ¾ cup (6 oz.) into gallon of diluent = 6:128 dilution (6 parts test substance + 122 parts diluent) g. Dispense the test substance as required by the test method. If required, place the test substance in a water bath for approximately 10 minutes to allow the test substance to equilibrate to the required temperature. h. Complete the Media/Reagent Preparation Sheet. i. Follow the appropriate test method for conducting the assay. j. Discard the remaining test substance at the end of the test day.
12.2 Sampling and Preparation of Spray Test Substances	a. For aerosol cans and trigger or pump sprayers, shake the can 25 times prior to use, unless otherwise specified by the manufacturer. b. Spray the test substance for 10-15 seconds prior to testing to ensure sprayer is operating correctly and test substance is dispensed properly. c. For spray test substances which require dilution, proceed as described in sections 12.1a through 12.1f. d. For spray test substances not supplied with their own spray bottles, prepare a sterile spray bottle (a previously unused spray bottle) to dispense the test substance as follows: i. Working in a BSC, add ~300mL of 100% ethanol to the spray bottle. Pump the trigger several times to fill the nozzle/sprayer with ethanol. Let stand ~10 minutes. ii. Spray out ~50 mL of ethanol into a sterile beaker.

	iii. Add ~300 mL of sterile DI water to each bottle. Spray out ~50 mL of the water into a sterile beaker. Repeat this step. Aseptically remove the remaining sterile water in the spray bottle. iv. Add a small volume (~25 mL) of sterile DI water to the spray bottle and spray ~10 mL of the water out into a sterile vessel. v. Check sterility of the spray bottle by filtering ~10 mL of water through a 0.2 µm filter unit. Apply the filter to surface of a TSA or TSA with 5% sheep's blood agar plate and incubate at 36±1°C for 3-10 days. vi. Aseptically remove the remaining DI water. Close nozzle/lid of bottle and leave in the BSC. e. For spray test substances not supplied with their own spray bottles, dispense an appropriate amount of test substance into a sterile spray bottle(s) to conduct the test. Label the spray bottle with the test substance name and the test date. Discard the remaining test substance at the end of the test day. The same spray bottle may be used for the same test substance over multiple test days. f. Prior to testing, wipe the spray nozzle using 70% ethanol and sterile gauze and allow to dry. g. Follow the appropriate test method for conducting the assay.
12.3 Sampling and Preparation of Towelette Test Substances	a. Wipe the outside of the towelette container using 70% ethanol and allow to air dry prior to opening. b. If present, carefully remove any seals with sterile instruments (i.e., razor blade, forceps). c. Perform all manipulations of the towelettes aseptically. d. Dispense towelette samples as specified on the test substance label. i. For dispenser-fed towelettes in canisters: using sterile gloves or sterile forceps, thread a corner of the first towelette from the center of the roll through the container dispenser, if applicable, and pull out the first towelette. The remaining towelettes should automatically feed through the dispenser. Remove and discard 3-5 towelettes. e. For canisters, invert 3-4 times or roll container to distribute the liquid before removing towelettes for the wiping procedure. f. Close the lid of the towelette container when not actively removing

	towelettes. g. Aseptically remove individually packaged towelettes from their packaging using sterile gloves or sterile forceps. h. Follow the appropriate test method for conducting the assay.
13. Data Analysis/ Calculations	None.
14. Forms and Data Sheets	1. Media/Reagent Preparation Sheets. Sheets are stored separately from the SOP under the following file names: Media/Reagent Preparation Sheet for Liquid Test Substances — MB-22-05_F1.xlsx Media/Reagent Preparation Sheet for Spray Test Substances — MB-22-05_F2.xlsx Media/Reagent Preparation Sheet for Towelette Test Substances — MB-22-05_F3.xlsx Media/Reagent Preparation Sheet for Sterile Spray Bottle — MB-22-05_F4.xlsx
15. References	None.