



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

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

**Standard Operating Procedure for
Disinfectant Product Preparation and
Sampling Procedures**

SOP Number: MB-22-03

Date Revised: 10-30-13

SOP Number	MB-22-03
Title	Disinfectant Product Preparation and Sampling Procedures
Scope	This SOP describes procedures for the preparation and sampling of liquid, spray, and towelette disinfectants for efficacy testing.
Application	Procedures are applicable to all disinfectant efficacy test methods performed in the laboratory.

	Approval	Date
SOP Developer:	_____	_____
	Print Name: _____	
SOP Reviewer	_____	_____
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<p>1. Definitions</p>	<ol style="list-style-type: none"> 1. Product sample = Representative container of a disinfectant sample. Multiple containers from the same production lot are all considered to be the same sample. 2. Sampling = Procedure in which part of a product sample is removed from a container for testing. (see section 15.1). 3. Ready-to-use product = Product that requires no activation or dilution. 4. Concentrated liquid product = Liquid product that requires a dilution prior to use. 5. Spray products = trigger, aerosol or pump based products. 6. Towelette products = a pre-moistened wipe-based disinfectant. 7. COC = chain of custody.
<p>2. Health and Safety</p>	<ol style="list-style-type: none"> 1. Follow procedures specified in SOP MB-01, Laboratory Biosafety. The Study Director and/or lead analyst should consult the Material Safety Data Sheet for hazards associated with products. 2. Disinfectants may contain a number of different active ingredients, such as quaternary ammonium compounds, halogens, phenolics, aldehydes, peroxides, and heavy metals. Latex gloves and other personal protective clothing or devices are worn during the handling of disinfectants. 3. A chemical fume hood or other containment equipment, such as a BSC is employed when performing tasks with products.
<p>3. Personnel Qualifications and Training</p>	<p>Refer to SOP ADM-04, OPP Microbiology Laboratory Training.</p>
<p>4. Instrument Calibration</p>	<p>Refer to SOP EQ-03 (weigh balances) and QC-19 (pipettes) for details on method and frequency of calibration.</p>
<p>5. Sample Storage</p>	<ol style="list-style-type: none"> 1. Store disinfectants according to the manufacturer's recommendations if stipulated, or at room temperature, in Room D204. Store flammable products in the flammable cabinet located in Room B204. 2. Activate or dilute disinfectant products within three hours of testing to ensure stability of the product unless test parameters specify otherwise. 3. Follow chain-of-custody (COC) guidelines in SOP COC-01, Disinfectant Sample Log-in, Tracking and Disposal. 4. Store products that require activation and have an extended shelf life prior to testing in a fume hood using secondary containment. Identify the product by name, sample number and preparation number. Establish a new for activated product. Once product is used archive COC seal in the COC laboratory notebook. 5. Use permanent marker to record the test date on the container used for

	<p>testing.</p> <p>6. Prepare a product-specific Media/Reagent Preparation Sheet.</p>
6. Quality Control	Required information is documented on the appropriate record form(s). See section 14.
7. Interferences	None
8. Non-conforming Data	Errors in the preparation of the disinfectant, if not corrected prior to the efficacy test, will result in a repeat of the study.
9. Data Management	Data will be archived consistent with SOP ADM-03, Records and Archives.
10. Cautions	<ol style="list-style-type: none"> a. Strict adherence to the protocol is necessary for the validity of the test results. b. Prepare products inside a BSC and follow aseptic techniques. A fume hood may be necessary for strong samples or caustic materials such as strong acids. c. Do not place a pipette or any other instrument inside the product container.
11. Special Apparatus and Materials	<ol style="list-style-type: none"> a. Sterile 25 × 100 mm tubes – used for testing liquid disinfectants. b. Volumetric glassware (pipettes, flasks, etc.) – to measure liquids for product dilution and for dispensing disinfectants. c. Ethanol – for cleaning the outside of the sample container (70%) and preparation of spray bottles (100%). d. Weigh Balance (Weighs 0 to 5100 g) – to weigh sample containers prior to and after sample removal. e. Sterile spray bottles – used for products requiring application by sprays. f. Forceps – to open sample container or feed towelettes through container aperture. g. Product diluent – Sterile liquid used to make product dilutions. (e.g. tap water, de-ionized water or hard water).
12. Procedure and Analysis	<ol style="list-style-type: none"> a. Prepare a product-specific Media/Reagent Preparation Sheet. Calculate the volume of product needed based on the method in use or as defined in the study protocol and adjust as necessary. b. Retrieve product from sample storage. c. If applicable, remove COC seal and record on COC sample log-in and tracking book. d. For products requiring the use of hard water as the diluent refer to MB-30 (AOAC hard water) or MB-25 (OECD hard water) for instructions.

<p>12.1 Sampling and Preparation of Liquid Disinfectants</p>	<ul style="list-style-type: none"> a. Weigh the product container and record the weight on the Media/Reagent Preparation Sheet. Gently shake the container of a liquid product prior to opening. 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 (i.e., razor blade, forceps). b. Aseptically pour the necessary amount of product to make the use dilution into a sterile beaker based on the calculations on the Media/Reagent Preparation Sheet. If the sample is not used immediately, cover the beaker with foil. After dispensing, place cap on the product container and secure tightly. c. Weigh the sample container and record weight on the Media/Reagent Preparation Sheet. d. For ready-to-use products dispense 10 ml aliquots directly from the beaker into sterile 25 × 100 mm tubes. e. For concentrated products, aseptically prepare the disinfectant dilution required for the test using the product dispensed in the beaker (see step 12.1.b). Prepare all dilutions using sterile standardized volumetric glassware. f. Examples of disinfectant product dilutions: <ul style="list-style-type: none"> i. If a product requires a 1:10 dilution, 1 part product is added to 9 parts diluent. ii. ½ ounce into gallon of diluent = 1:256 dilution (1 part product + 255 parts diluent) iii. 1 ounce into gallon of diluent = 1:128 dilution (1 part product + 127 parts diluent) iv. ¾ cup (6 oz.) into gallon of diluent = 6:128 dilution (6 parts product + 122 parts diluent) g. For diluted products, use ≥ 1.0 mL or 1.0 g of the product sample to prepare the final solution to be tested. Use v/v dilutions for liquid products and w/v dilutions for solids. Round to 2 decimal places toward a more concentrated product. h. Dispense 10 mL aliquots of the diluted disinfectant into sterile 25 × 100 mm test tubes. Place tubes in a water bath for approximately 10 minutes to allow product to equilibrate to the required temperature. Record the test date on the container using permanent marker. Complete the Media/Reagent Preparation Sheet and COC forms and return the container to the appropriate storage location.
<p>12.2 Sampling and Preparation of</p>	<ul style="list-style-type: none"> a. For spray products which require dilution proceed as described in

<p>Spray Disinfectants</p>	<p>sections 12.1a through 12.1g.</p> <ul style="list-style-type: none"> b. For spray products not supplied with their own spray bottles, prepare a sterile spray bottle used to dispense the test chemical, as follows: c. Working in a BSC, add approximately 10 oz. (~300mL) of ethanol to the spray bottle. Pump the trigger several times to fill the nozzle/sprayer with ethanol. Let stand approximately 10 minutes. d. Spray out approximately 50 mL of ethanol into a sterile beaker. e. Add 10 oz. of sterile DI water to each bottle. Spray out approximately 50 mL of the water into a sterile beaker. Repeat this step one more time. f. Add a small volume (approximately 25 mL) of sterile DI water to the spray bottle and spray approximately 10 mL of the water out into a sterile tube. g. To check sterility, filter 10 mL of water through a 0.45 µm or 0.2 µm filter unit and apply filter to surface of TSA plate. Incubate at 36±1°C for 3-10 days. h. Close nozzle/lid of bottle and leave in the BSC until the efficacy test is completed to ensure sterility. i. Dispense an appropriate amount of product into the spray bottle (s) to conduct the test. Using a permanent marker add the product name and the test date on the spray bottle. Discard remaining product at the end of the test day. A new preparation of the product is required for each test day. j. For aerosol cans, shake the can 25 times prior to use, unless otherwise specified by the manufacturer. k. Spray the product for 10-15 seconds prior to testing to ensure sprayer is operating correctly and product is dispensed properly. l. Record the test date on the spray bottle or container using permanent marker. Complete the Media/Reagent Preparation Sheet and COC forms and return the container to the appropriate storage location.
<p>12.3 Sampling and Preparation of Towelette Disinfectants</p>	<ul style="list-style-type: none"> a. Weigh container and record weight on Media/Reagent Preparation Sheet. b. For towelette canisters, carefully remove the seal attached to the lip of the cover with sterile instruments (i.e., razor blade, forceps). c. Perform all manipulations of the towelettes aseptically. d. Dispense towelette samples as specified on the product label.

	<ul style="list-style-type: none"> e. For dispenser-fed towelettes in canisters, using sterile gloves or flame sterilized forceps start towelette feed. Thread a corner of the first towelette, from the center of the roll, through the container dispenser, if applicable, and pull out first towelette. The remaining towelettes should automatically feed through the dispenser. Remove and discard 2-3 towelettes. f. For canisters, invert 3-4 times or roll container to distribute the liquid before removing towelettes for the wiping procedure. g. Close the lid of the towelette dispenser when not actively removing towelettes. h. Products formulated as single towelettes in packets are sampled from the product box, opened, and the towelette removed aseptically (using sterile gloves or flame-sterilized forceps). i. After completion of the test or study, weigh the towelette canister and record the weight. j. Record the test date on the container using permanent marker. Complete the Media/Reagent Preparation Sheet and COC forms and return the container to the appropriate storage location. 								
<p>12.4 Documentation on Media/Reagent Preparation Sheet</p>	<ul style="list-style-type: none"> a. Record all required information on the Media/Reagent Preparation Sheet (refer to SOP QC-15, Media Prep and Sterilization Run Numbers). 								
<p>13 Data Analysis/ Calculations</p>	<p>None</p>								
<p>14 Forms and Data Sheets</p>	<p>1. Media/Reagent Preparation Sheets. Sheets are stored separately from the SOP under the following file names:</p> <table border="0" style="width: 100%;"> <tr> <td style="padding-left: 40px;">Media/Reagent Preparation Sheet for Liquid Products</td> <td style="text-align: right; padding-left: 20px;">MB-22-03_F1.xlsx</td> </tr> <tr> <td style="padding-left: 40px;">Media/Reagent Preparation Sheet for Spray Products</td> <td style="text-align: right; padding-left: 20px;">MB-22-03_F2.xlsx</td> </tr> <tr> <td style="padding-left: 40px;">Media/Reagent Preparation Sheet for Towelette Products</td> <td style="text-align: right; padding-left: 20px;">MB-22-03_F3.xlsx</td> </tr> <tr> <td style="padding-left: 40px;">Media/Reagent Preparation Sheet for Sterile Spray Bottle</td> <td style="text-align: right; padding-left: 20px;">MB-22-03_F4.xlsx</td> </tr> </table>	Media/Reagent Preparation Sheet for Liquid Products	MB-22-03_F1.xlsx	Media/Reagent Preparation Sheet for Spray Products	MB-22-03_F2.xlsx	Media/Reagent Preparation Sheet for Towelette Products	MB-22-03_F3.xlsx	Media/Reagent Preparation Sheet for Sterile Spray Bottle	MB-22-03_F4.xlsx
Media/Reagent Preparation Sheet for Liquid Products	MB-22-03_F1.xlsx								
Media/Reagent Preparation Sheet for Spray Products	MB-22-03_F2.xlsx								
Media/Reagent Preparation Sheet for Towelette Products	MB-22-03_F3.xlsx								
Media/Reagent Preparation Sheet for Sterile Spray Bottle	MB-22-03_F4.xlsx								
<p>15 References</p>	<p>1. ISO/IEC: 17025 (2005): Section 5.7 Sampling.</p>								