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Draft Guidance to Support Registration of Pre-saturated/Impregnated Antimicrobial Towelettes for Disinfection Claims

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Introduction

Existing test methods used to evaluate the efficacy of antimicrobial towelettes were originally designed to test liquid formulations and had to be modified to accommodate towelettes. The standard test method, *ASTM E3363*[1], provides a specific and consistent means to assess the efficacy of towelettes through the combination of chemical inactivation of the test microbe and mechanical removal of inoculum from a surface. This document identifies a test method for evaluating the efficacy of antimicrobial towelettes with disinfectant claims against bacteria as well as regulatory guidance for pesticidal claims for those products. This guidance is draft: ASTM E3363 and the proposed performance standards herein should not be utilized to support product registration until the guidance is finalized. In the meantime, registrants should continue to reference OCSPP 810.2200[2] to support product registration.

This draft guidance is not binding on EPA or any outside parties, and EPA may depart from the guidance where circumstances warrant and without prior notice. Registrants and applicants may propose and submit alternative practices (e.g., modifications to the recommended test methodologies) to the Agency for assessment. The Agency will evaluate any proposed method modifications for appropriateness on a case-by-case basis. This draft guidance may be updated in the future.

These test methods and draft guidance provide a framework for registrants who seek to make a disinfectant claim for antimicrobial towelette products to control bacteria on hard non-porous surfaces.

This document describes:

- Purpose
- Products That May Be Eligible for Disinfectant Towelette Claims
- Test Procedures for Developing Efficacy Data Supporting Disinfectant Claims
- Efficacy Test Criteria
- Gravimetric and Physical Wetness Determination
- Product Chemistry
- Data Submission Procedures for Efficacy Data
- Labeling Guidance

Purpose

EPA is responsible for regulating hospital disinfectants for use on inanimate surfaces and other antimicrobial pesticides used in healthcare and other settings pursuant to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). This guidance addresses efficacy testing for antimicrobial towelettes intended to be used as disinfectants on hard, non-porous surfaces against bacteria.

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[1] *https://secure.astm.org/saml/idp/init?sp=https://www.okta.com/saml2/service-provider/spgeosehwfomoktqepnu&RelayState=https://compass.astm.org/document/?contentCode=ASTM%7CE3363-23%7Cen-US&proxycl=https://secure.astm.org*.

[2] *https://www.regulations.gov/document/EPA-HQ-OPPT-2009-0150-0036*.

Products That May Be Eligible for Disinfectant Towelette Claims Against Bacteria

This document provides testing guidance for pre-saturated/impregnated antimicrobial towelettes with disinfectant claims against bacteria. Antimicrobial towelettes are to be tested with the formulation offered for sale, using the product packaged in the same packaging intended to be marketed. Towelettes are a unique combination of antimicrobial chemical and towelette substrate pre-packaged as a unit in fixed proportions for application. Therefore, the complete product, as packaged in the manner to be offered for sale, must be tested according to the directions for use to ensure efficacy as an antimicrobial towelette.

Formulations beyond pre-saturated/impregnated towelettes may fall outside of the scope of this test guidance. In these cases, registrants are encouraged to consult with the agency prior to conducting efficacy testing. This guidance is not intended to address dry-to-wet towelettes, and/or other deviations from impregnated/pre-saturated towelettes. Those product types will be handled on case-by-case basis. This guidance is not intended for use sites such as drinking glasses, dishes, utensils, cutting boards, soft and porous surfaces.

Test Procedures for Developing Efficacy Data Supporting Antimicrobial Towelette Claims

To evaluate antimicrobial towelette disinfectant claims against bacteria, the EPA recommends use of the most contemporary version ASTM E3363 (Standard Test Method for Quantitative Performance Evaluation of Antimicrobial Towelettes[1]). See Table 1 for a summary of disinfectant claims against bacteria, the required number of batches and carriers required for testing, and the evaluation of success.

Table 1. Summary of Testing for Antimicrobial Towelette Disinfectant Claims Against Bacteria*

Claim	Test Organism	Test Method	No. Batches/Carriers	Evaluation of Success
Limited spectrum disinfectant/hard non-porous surfaces	*Staphylococcus aureus* (ATCC 6538) or *Salmonella enterica* (ATCC 10708)	ASTM E3363	For each organism: 3 batches at the LCL; 5 carriers per batch. Conduct each of the 3 tests per organism on independent test days.	Each independent test should attain a minimum mean 4.5 log reduction (LR) in viable cells.
Broad spectrum disinfectant/hard non-porous surfaces	*Staphylococcus aureus* (ATCC 6538) and *Salmonella enterica* (ATCC 10708) or *Pseudomonas aeruginosa* (ATCC 15442)	ASTM E3363	For each organism: 3 batches at the LCL; 5 carriers per batch. Conduct each of the 3 tests per organism on independent test days.	Each independent test should attain a minimum mean 4.5 LR in viable cells
	Additional bacteria	ASTM E3363	For each organism: 2 batches at the NCL; 5 carriers per batch. Conduct each of the 2 tests per organism on independent test days.	Each independent test should attain a minimum mean 4.5 LR in viable cells.

Hospital or healthcare disinfectant/hard non-porous surfaces	*Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 15442)	ASTM E3363	For each organism: 3 batches at the LCL; 5 carriers per batch. Conduct each of the 3 tests per organism on independent test days.	Each independent test should attain a minimum mean 4.5 LR in viable cells.
	Additional bacteria	ASTM E3363	For each organism: 2 batches at the NCL; 5 carriers per batch. Conduct each of the 2 tests per organism on independent test days.	Each independent test should attain a minimum mean 4.5 LR in viable cells.

*Use the most contemporary version of ASTM E3363.

Note, this method is only intended to support registration for disinfection claims against bacteria. For all other disinfectant claims (e.g., those against viruses, *C. difficile*, *C. auris*), registrants should continue to follow existing efficacy guidance and test methods. See Table 2 for a summary of antimicrobial claims, their associated test methods, the required number of batches and carriers required for testing, and the evaluation of success.

Table 2. Summary of Testing for Antimicrobial Towelette with Other Disinfectant Claims*

Claim	Test Organism	Test Method	No. Batches/Carriers	Evaluation of Success
Broad spectrum disinfectant/hard non-porous surfaces	Viruses	ASTM E1053	2 batches at the LCL for hardest to kill strain. For all additional viruses, two batches at the nominal concentration.	Attain a minimum mean 3.0 LR in viable virus in each test.
Hospital or healthcare disinfectant/hard non-porous surfaces	Viruses	ASTM E1053	2 batches at the LCL for claimed virus or approved surrogate.	Attain a minimum mean 3.0 LR in viable virus in each test.
Antimicrobial towelette claims against spores of *C. difficile*	*Clostridioides difficile*	ASTM E2839 and ASTM E3218	3 batches at the LCL; 10 carriers per batch. Conduct each of the 3 tests on independent test days.	Attain a minimum mean 6.0 LR in viable cells in each independent test.
Antimicrobial towelette claims against drug resistant *C. auris*	*Candida auris*	EPA MLB SOP MB-35	2 batches at the LCL; 5 carriers per batch. Conduct each of the 2 tests on independent test days.	Attain a minimum mean 5.0 LR in viable cells in each independent test.

*Use the most contemporary version of the identified methods.

Efficacy Test Criteria

For applicants submitting data using ASTM E3363, apply the following criteria:

Culture Production: Stock cultures and test cultures should be produced following ASTM E3363. When the recommended culturing methods from ASTM E3363 are modified by the

applicant to support bacteria not identified in ASTM E3363, the applicant should submit the complete testing protocol, identifying and describing each modification to the Agency for review and evaluation prior to the initiation of the tests. All materials and procedures employed in the testing should be fully described.

Number of Batches and Test Carriers per Batch: Refer to Table 1. See the *810.2000 product performance guideline*[3] for LCL requirements. Conduct each test per organism on independent test days (tests against more than one organism may be conducted on the same day). Evaluate five carriers per microbe against the product and three carriers as controls. Conduct testing at a single laboratory.

Neutralizer Confirmation: Conduct neutralization testing to confirm and document the effectiveness of the product neutralizer. Refer to ASTM E3363.

Contact Time: The contact time for the product should not exceed 10 minutes based on the gravimetric and physical wetness determination test.

Inoculum Soil Load: Include the three-part soil load in the test inoculum as specified in ASTM E3363.

Control Carrier Counts: Each of the three control carriers should exhibit counts of a minimum of 1.0×10^5 to a maximum of 1.5×10^6 colony forming units (CFUs) per carrier.

Evaluation of Success: To be deemed effective, the product must attain a minimum mean 4.5 log reduction (LR) in viable cells for each independent test.

Modifications to the Test Procedures: When recommended methods are modified by the applicant to support specific claims for a product, the applicant should submit the complete testing protocol, identifying and describing each modification to the Agency for review and evaluation prior to the initiation of the tests. All materials and procedures employed in the testing should be fully described.

Gravimetric and Physical Wetness Determination: In addition to efficacy testing, conduct physical and gravimetric wetness tests on the antimicrobial towelettes in an environmental chamber as described below. Conduct the physical and gravimetric wetness test with each batch of product assessed for efficacy testing using the longest contact time prescribed for the claim. Use towelettes formulated using the substrate with the greatest binding affinity for the active ingredient.

- Use three pre-cleaned 12-inch × 12-inch glass or 12-inch × 12-inch stainless-steel carriers or 150 × 20 mm glass Petri plates to represent the surface to be treated.
- Pre-clean each carrier surface with 70% ethanol, rinse in deionized water, and air dry.
- Record the weight (weight #1: dry and untreated).
- Distribute the liquid in the canister or package; remove and discard the first 3-5 towelettes.

[3] *https://www.regulations.gov/document/EPA-HQ-OPPT-2009-0150-0034*.

- For each carrier, follow the prescribed wiping pattern identified in the most current version of ASTM E3363.
- Re-weigh; record the results (weight #2: wet and treated).
- Allow carriers to sit horizontally with the lid off for the contact time in an environmental chamber set at 35±5% relative humidity and 20-25°C. Do not use a fume hood or biological safety cabinet.
- Record the final weight (weight #3: post contact time).
- After recording the final weight, use a single dry sheet of Kim Wipe (e.g., 11 cm × 21 cm) to verify the physical presence of wetness; each Kim Wipe should show visible absorption of liquid. Document the presence of visible wetness in the report for each carrier along with the gravimetric data.
    - The data must show the presence of free liquid on the treated surface by weight and physical observations (i.e., presence of wetness on the Kim Wipe).
    - The weight difference for this test may fall approximately in the range of 0.1 g – 3.0 g.
    - The treated carriers should appear wet without flooding and be reasonably representative of the product at its point-of-use.
- The contact time on the label should not exceed the time during which wetness is observed during the gravimetric and physical wetness test.

Product Chemistry Considerations for Towelette Formulations

Confidential Statement of Formula (CSF)

- The trade name (if components unknown) must be stated on the CSF. The trade name/components must be in column 10 of the CSF or attached. Each attachment must be part of the CSF and must be reflected in box “B” of the CSF. The supplier’s name and address must be in column 11 of the CSF or as part of the attachment referenced above. The attachment must have a header that contains the following information:
    - EPA Registration Number
    - Name of the product
    - Basic formulation or alternate formulation (see box “A” of the CSF form)
    - Page number
- Each component in the formulation must be listed in box 10, which is representative of the bulk liquid formulation applied to the towelette.
- For each component of the formulation listed in box 10, determine the amount of the component in the formulation. Place this value in box 13a.
- In box 13b, determine the percentage by weight of the product for each component. In box 14a and 14b, determine the upper and lower certified limits based on the percent by weight reported in box 13b.
- The active ingredients will be represented by two concentrations in boxes 13b, 14a, and 14b. The precent by weight of the active ingredient(s) in box 13b will list the %w/w of the bulk liquid as the first concentration.
- The second concentration in box 13b will represent the %w/w of the expressed liquid (i.e., at Day 1 (D1) as previously defined). The %w/w of the expressed liquid will be

distinguished by [brackets] directly below the first concentration of the bulk liquid. The expressed liquid refers to the amount of liquid that is extracted from the towelette using the Agency's Enforcement Analytical Method (EAM) at D1. Calculate the upper and lower certified limits for bulk and expressed liquids in boxes 14a and 14b according 40 CFR 158.350.

- List the impregnated material as a component in the formulation in box 10 and include it in the total weight of formulation (box 17). Place the impregnated material's purpose in formulation in box 15.
- Active Ingredient input plus expressed in brackets, upper and lower certified on expressed limits, expressed nominal is on label,
- Expressed Liquid is based off EAM that is produced by the lab based on the type of fabric (substrate/matrix). If fabric (substrate/matrix) changes, determine EAM for that specific fabric (substrate/matrix). The substrate/matrix is not part of the total weight of the formulation. Include the substrate/matrix purpose on CSF.

Storage and Stability

- Guideline 830.6317 will be fulfilled by testing the concentration of active ingredient(s) in the expressed liquid. The registrant will have the option of completing an accelerated study according to the 2012 memo or the one-year study.

Data Submission Procedures for Efficacy Data

To assist in the proper review and evaluation of product performance, submit complete descriptions of the test employed and the results obtained to the Agency *following the data submission guidelines*[4]. All product performance data must be developed in compliance with the Good Laboratory Practice Standards in 40 CFR part 160 (view the GLPs)[5].

Labeling Guidance

Example of Label Claim: Kills 99.9% of [insert tested bacteria] on treated hard, non-porous surfaces in [insert contact time] minutes when used according to disinfection instructions.

[4] *https://www.epa.gov/pesticide-registration/pesticide-registration-manual-chapter-15-submitting-data-and-confidential#procedures*.

[5] *https://www.ecfr.gov/current/title-40/chapter-I/subchapter-E/part-160*.