

Guidance for the Efficacy Evaluation of Products with Sporicidal Claims Against *Clostridium difficile* (June 2014)

This document provides an update to the Agency's interim guidance for the efficacy evaluation of antimicrobial pesticides that are labeled for treating hard non-porous surfaces in healthcare settings contaminated with spores of *Clostridium difficile* (*C. difficile*).

The Agency's goal is to utilize the most technically sound approach and best practices for the development of antimicrobial product efficacy data. Based on the results of two collaborative studies, research conducted by the Agency and stakeholders, as well as input received during laboratory workshops, the Agency is adding specificity to the procedures for evaluating the efficacy of products against spores of *Clostridium difficile*. To further standardize testing, the Agency is recommending the use of purified spores of one clinically-relevant test strain (ATCC #43598) with specific storage conditions for the final spore suspension. Specific instructions and details for use of reduced media, incubation conditions for recovery of viable spores, use of phosphate buffered saline with 0.1% Tween 80 as a diluent, titer of final spore suspension and carrier specifications should be followed. In addition, the Agency encourages applicants to add a soil load to the spore inoculum due to the association of spores with organic soil at the use site.

This document is not binding on either EPA or any outside parties, and the EPA may depart from it where circumstances warrant and without prior notice. Registrants and applicants may propose and submit alternative practices (e.g., modifications to existing test methodologies) to the Agency for assessment and the Agency will evaluate them for appropriateness on a case-by-case basis.

Note: Standard Operating Procedures (SOPs) referenced in this document may be found on the Microbiology Laboratory Antimicrobial Testing Methods and Procedures website. The SOPs relevant to the guidance document are:

1. Standard Operating Procedure MB-28; Production of Spores of *Clostridium difficile* for Use in the Efficacy Evaluation of Antimicrobial Agents.

2. Standard Operating Procedure MB-31; Quantitative Disk Carrier Test Method (QCT-2) Modified for Testing Antimicrobial Products Against Spores of *Clostridium difficile* (ATCC 43598) on Inanimate, Hard Non-porous Surfaces.

In late 2014, the Agency will initiate a collaborative study with stakeholders to evaluate and if necessary, update the test methods and SOPs as final guidance.

Eligible Products; Only products registered with hospital claims are suitable for the *C. difficile* claim.

Method: For liquid products, the following quantitative test method should be used to generate the efficacy data:

1. ASTM E2197: Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporocidal Activities of Chemicals. Modifications to the method to accommodate testing with *C. difficile* are outlined in this guidance document and Standard Operating Procedure MB-31.

ASTM E2197 is the recommended method. If an applicant plans to use a different method, the protocol should be submitted to the Agency for review prior to conducting the efficacy evaluation.

For towelette and spray formulations, the Agency will accept testing of the liquid expressed directly from towelettes or collected directly from spray containers using the quantitative method specified above and conditions specified in this document. The process used for the collection of the liquid must accompany the study data as well as verification of the formulation chemistry from a relevant sample of the expressed liquid.

For fogger or foam formulations, applicants should consult with the Agency before developing registration data. EPA may require submission of protocols for review for foggers and foams.

NOTE: Updates to standard methods for efficacy occur periodically, thus the Agency encourages the use of the most current version of the published standard.

Acceptable Test Strain: Use *Clostridium difficile* ATCC strain 43598. This strain is clinically relevant and produces toxin B only. *C. difficile* is an obligate anaerobe; testing should ensure anaerobic incubation and spore recovery

conditions (e.g., reduced recovery media). The applicant is responsible for documenting the maintenance of an anaerobic environment during culture incubation.

Spore Production: Spores of ATCC strain 43598 should be produced using the following method:

1. ASTM Standard E2839; Test Method for Production of *Clostridium difficile* Spores for Use in Efficacy Evaluation of Antimicrobial Agents. Refer to Standard Operating Procedure MB-28. Harvest spores at 7-10 days incubation. Following the spore purification steps, the final spore suspension should be 2×10^8 to 8×10^8 spores/mL with $\geq 95\%$ purity (spores).

The acid resistance of the final spore suspension should be assessed against 2.5 M hydrochloric acid. The spores are considered acid resistant if a log reduction of 0-2 is exhibited following 10 minutes of exposure to 2.5 M HCl. The source, identity, spore production protocol, HCl data, and spore titer must accompany each submission.

The final spore suspension should be stored in phosphate-buffered saline containing 0.1 % Tween 80 in small aliquots (e.g. 200 to 400 μ L) at $-20 \pm 1^\circ\text{C}$. Use within one year.

Recovery Media: Use Brain Heart Infusion Agar with Yeast Extract, Horse Blood and Taurocholate (BHIY-HT) for recovery of spores; use the media in a reduced state (free of oxygen). If the medium is prepared in-house and must be reduced, the applicant should document the means of reduction (e.g., exposure time to anaerobic conditions). Commercially prepared BHIY-HT plates should be placed under anaerobic conditions within one hour after the seal is broken on the package.

Carriers: Use disks (1 cm in diameter) made from 0.7 mm thick sheets of brushed and magnetized stainless steel – carriers are single use. Refer to Standard Operating Procedure MB-31 for the exact manufacturing specifications for the carriers. Use a positive displacement pipette with a 10ul tip to deliver the spores to the carrier. Inoculated carriers should be stored in a dessicator and used within 24 hours of drying.

Soil Load: Applicants are strongly encouraged to incorporate a soil load in the spore suspension prior to carrier inoculation as *C. difficile* is often associated with organic matter at the use site. The use of a 3-part soil load identified in

MB-31 and incorporated into the test inoculum is recommended. The final volume can be reduced accordingly, e.g., 12.5 µl of 5% bovine serum albumin, 17.5 µl of 5% yeast extract and 50 µl of 0.4% mucin to 170 µl of the spore suspension to obtain a total of 250 µl spore suspension.

Diluent: Phosphate-buffered saline (PBS) with 0.1% Tween 80 (PBS-T).

Filters: For the filtration step in ASTM Standard E2197, use a hydrophilic polyethersulfone (PES) membrane with 0.22 µm pore diameter.

Neutralizer Confirmation: Testing should be conducted to determine the appropriate neutralizer for the product; the data must be submitted with the product efficacy data.

Contact Time: The contact time for testing should not exceed 10 minutes.

Control Carriers: Mean control carrier counts should be $>10^6$ to $<10^7$ spores/carrier.

Number of Batches and Test Carriers per Batch: Three batches of product should be tested on three different test days at or below the lower certified limit(s) listed on the statement of formula of the product. Use 10 carriers for the treatment and 3 carriers for the control per test.

Incubation Conditions: BHIY-HT plates used for recovery of controls should be incubated anaerobically using either an anaerobic chamber or jar at $36\pm 1^\circ\text{C}$ for 48 ± 4 hours. BHIY-HT plates used for recovery of treated spores should be incubated anaerobically using either an anaerobic chamber or gas jar at $36\pm 1^\circ\text{C}$ for 72 ± 4 hours; if there are zero or few colonies after 72 ± 4 hours, incubate an additional 48 ± 4 hours.

Product Performance: A minimum 6 log reduction in viable spores is required.

Label Claim: Kills and/or inactivates spores of *Clostridium difficile* on hard, non-porous surfaces. The product should achieve a mean log reduction of ≥ 6 logs based on recoverable spores.

Special Label Instructions for Cleaning Prior to Disinfection against *Clostridium difficile* spores: All products bearing *Clostridium difficile* sporicide claims are to include these specific cleaning directions:

- **Personal Protection:** Wear appropriate barrier protection such as gloves, gowns, masks or eye covering.
- **Cleaning Procedure:** Fecal matter/waste must be thoroughly cleaned from surfaces/objects before disinfection by application with a clean cloth, mop, and/or sponge saturated with the sporicidal product. Cleaning is to include vigorous wiping and/or scrubbing, until all visible soil is removed. Special attention is needed for high-touch surfaces. Surfaces in patient rooms are to be cleaned in an appropriate manner, such as from right to left or left to right, on horizontal surfaces, and top to bottom, on vertical surfaces, to minimize spreading of the spores. Restrooms are to be cleaned last. Do not reuse soiled cloths.
- **Infectious Materials Disposal:** Materials used in the cleaning process that may contain feces/wastes are to be disposed of immediately in accordance with local regulations for infectious materials disposal.

Data Submission: Information on the test system must be submitted including but not limited to the test design, spore production method, ATCC strain, spore titer, efficacy test method, neutralization study design and outcome, individual plate counts for treated and control carriers, calculations including log reduction values. Any deviations to standard methods should be noted and supplied to the Agency. For the purpose of product registration, all studies are to be conducted following the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Good Laboratory Practice Standards, 40 CFR Part 160, including media quality assessments and spore production.