

**Guidance on Test Methods for
Determining the Efficacy of
Antimicrobial Products for Inactivating
Bacillus anthracis Spores on Inanimate
Surfaces
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Preface

Following the “anthrax attacks” in late 2001, the EPA Office of Pesticide Programs (EPA/OPP) has played a critical role in regulating the use of antimicrobial chemicals for decontaminating facilities and in conducting research on the sporicidal efficacy of available chemicals.

Because no antimicrobial products were registered at that time specifically for inactivation of *Bacillus anthracis* spores, EPA had to issue crisis exemptions under Section 18 of FIFRA to permit the use of these products to treat contaminated facilities and their contents. Altogether, EPA received 63 requests for crisis exemptions, of which it approved 28 and denied 35.

Rather than have to issue crisis exemptions in the future if another bioterrorism attack occurs, EPA/OPP plans to allow for the registration of products that have claims to inactivate *Bacillus anthracis* spores (hereafter referred to as “anthrax-related products”) so that they will be readily available to trained, qualified persons for decontaminating buildings and their contents.

Toward this end, EPA is proposing guidance on the product performance (i.e., efficacy) data needed to support the registration of anthrax-related products. This proposal is set forth in this document and in a draft insertion to the document entitled “OPPTS 810.2100A. Products for Use on Hard Surfaces—Basic Efficacy Data Recommendations.”

On July 17-19, 2007, the FIFRA Scientific Advisory Panel (SAP) will meet publicly to consider the scientific basis for the efficacy test methods and associated criteria described in the cited document.

Following is background information on the proposed efficacy test methods that would support the registration of anthrax-related products. Details are available in the cited references, which are being provided to the SAP.

1. Introduction

EPA regulations require that product performance (efficacy) studies be submitted to support registration of an antimicrobial product for which public health claims are made such as “disinfect” or “sterilize” (40 CFR Part 158.640). In addition, any claim of inactivation of a specific microorganism should be supported by valid data that demonstrate the efficacy of the product against that particular microorganism. At the July 17-19 SAP meeting, the EPA will present draft guidance concerning what efficacy testing should be conducted to support the registration of an anthrax-related product.

EPA’s draft guidance describes three basic options for a registrant to follow in pursuing such a registration:

1. For any liquid, gas or vapor “sterilant/sporicide” product, conduct the full AOAC Official Method 966.04 on silk suture loops and porcelain penicylinders, which represent porous and nonporous surfaces, respectively. In addition, conduct “confirmatory testing” using AOAC 966.04, but instead of *B. subtilis* or *C. sporogenes*, use virulent *B. anthracis* spores on carriers made of porcelain and silk. If both tests are passed (by showing no growth on any carrier), then the product may be registered as a “sterilant/sporicide” with a claim that the product inactivates *B. anthracis* spores on inanimate surfaces. [Note: Any gas or vapor product would also have to undergo a “simulated use test” (see section 4. below).]
2. For any liquid, gas or vapor “sporicidal decontaminant” product, conduct the *Bacillus* component of AOAC Official Method 966.04 on silk suture loops and porcelain penicylinders using virulent *B. anthracis* spores (instead of *B. subtilis* or *C. sporogenes*). The test would be conducted without the *Clostridium* requirement. If the product passes this test, it could be registered as a “sporicidal decontaminant” with a claim that the product inactivates *B. anthracis* spores on inanimate surfaces. [Note: Any gas or vapor product would also have to undergo a “simulated use test” (see section 4. below).]
3. For any liquid, gas or vapor “sporicidal decontaminant” product, conduct a well developed, quantitative sporicidal test method acceptable to EPA using virulent *B. anthracis* spores on porous and/or non-porous surfaces (with coupon materials acceptable to EPA). If the product achieves at least a 6 log reduction of target spores on nonporous and/or porous surfaces, it could registered as a “sporicidal decontaminant” with a claim that the product inactivates *B. anthracis* spores on inanimate surfaces. [Note: Any gas or vapor product would also have to undergo a “simulated use test” (see section 4. below).]

An issue that applies to all three testing options is the possible use of surrogate *Bacillus* spores in place of virulent *Bacillus anthracis* spores. Also, the issue of whether various coupon materials may be used for quantitative sporicidal tests applies to the third testing option.

Lastly, EPA’s draft guidance proposes to require “simulated use testing” to

support registration of gas and vapor sterilants/sporicides and sporicidal decontaminants. This testing would demonstrate a product's ability to be applied in large, enclosed spaces and to achieve the same concentration, temperature, and relative humidity that was necessary to achieve 100% kill (i.e., no growth on any of the 720 carriers) in the qualitative laboratory test, or a 6 log reduction in the quantitative test.

2. Available Sterilant/Sporicides Efficacy Test Methods

2.1 AOAC Official Method 966.04--Sporicidal Activity of Disinfectants Test (Method I)

The original AOAC Official Method 966.04 (Method I) was developed in the 1960s and adopted by EPA and the FDA as a validated product performance test that demonstrates that a product is a "sterilant" or "sporicide" (Reference 1). The AOAC procedure is qualitative and determines the ability of an antimicrobial product to inactivate two spore types (*Bacillus subtilis* and *Clostridium sporogenes*) on porcelain and silk carriers. A sterilant or sporicide is a substance that destroys all microorganisms on inanimate surfaces, including vegetative and spore forms of bacteria and fungi, as well as viruses. All of EPA's registered sterilants/sporicides were tested with this method and were shown to pass the requirement of no growth on any carrier.

Until or unless the AOAC Official Method 966.04 is formally replaced by another method, EPA and FDA will continue to require it for making regulatory decisions about sterilants. However, EPA, FDA and other agencies have been working collaboratively to make improvements to this test and to compare the performance of relatively new quantitative tests to the qualitative AOAC Method 966.04.

2.2. Modifications to the AOAC Official Method 966.04 (Method II)

EPA led a multi-laboratory collaborative study, facilitated by AOAC International, to officially modify and improve the AOAC Official Method 966.04 (Reference 3). These modifications/improvements included:

1. Replacement of the current sporulation medium (soil extract nutrient broth) with a synthetic, standardized sporulation medium (nutrient agar amended with manganese sulfate).
2. Replacement of the porcelain carriers currently being used with stainless steel penicylinders. (This change is not currently recommended for use until the *Clostridium* component is collaboratively modified for stainless steel.)
3. Addition of a carrier count procedure for enumerating spores.
4. Establishment of a target spore titer per carrier as a minimum of 1×10^5 and a maximum of approximately 1×10^6 spores/carrier.

5. Addition of a neutralization confirmation protocol.
6. Editorial changes to the procedure.

The collaborative study, initiated in June 2005, involved five laboratories. These laboratories had existing microbiology programs, had conducted the AOAC method 966.04 in the past, and were suitable testing labs based on an EPA quality assurance readiness-review process. The OPP Microbiology Laboratory was the lead laboratory; others included the U.S. Food and Drug Administration (FDA) in Denver, CO; FDA in Winchester, MA; MICROBIOTEST, Inc. in Sterling, VA; and Advanced Sterilization Microbiology Lab in Irvine, CA.

The OPP laboratory provided the following: The study design and the necessary protocols; test forms; data sheets; media preparation sheets for test chemicals, media, and reagents; test chemicals; garden soil; and porcelain penicylinders. Using a step-wise comparative process, modifications involving the new sporulation medium and the new carrier type were evaluated against the current method using three parameters:

1. Spore counts on carriers
2. HCl resistance
3. Efficacy against common sporicidal agents

The study design required that three carrier-medium combinations be tested against one chemical (a high and low treatment) on the same day (six 30-carrier tests). An acceptable randomization method was decided by the lead laboratory before experimentation was initiated. This involved a randomized order of testing of chemicals and carrier-medium combinations for each participating laboratory.

The data evaluation was qualitative (i.e., how many positives versus negatives), which was acceptable to the AOAC. The data were collected and summarized by August 12, 2005. Statistical analysis was performed by Dr. Martin Hamilton (Montana State University).

The final, validated Method II has been published in the Journal of AOAC International (Reference 3).

The most significant concerns associated with method 966.04 are the qualitative nature of the method, the use of raw garden soil extract as a source of minerals for spore production, the lack of a standardized procedure for enumeration of spores, and the lack of a target spore load. An official AOAC collaborative study was conducted to modify and improve selected aspects of the method. The proposed modifications were limited to liquid formulations, *B. subtilis*, and the hard surface carrier (porcelain penicylinder) components of the method.

The study included the evaluation of a replacement for soil extract nutrient broth and an establishment of a minimum spore titer per carrier, both considered crucial for the improvement and utilization of the method. Additionally, an alternative hard surface material and a neutralization confirmation procedure were evaluated. Based on the results

of this study, it was determined that nutrient agar amended with 5 µg/mL manganese sulfate as a sporulation medium, the spore enumeration procedure, a target carrier count (10⁵ to approximately 10⁶ spores/carrier) and the neutralization confirmation procedure were suitable modifications.

The modifications have been approved by AOAC-International (AOACI), and the manuscript describing the study has been accepted for publication. Additional studies are planned in 2007-2008 to assess the impact of the modifications when applied to suture loops and with gaseous formulations.

3. Options for Supporting Registration of Anthrax-Related Products

3.1 Sterilant/Sporicide plus *B. anthracis* Claim

The AOAC Sporicidal Activity of Disinfectants Test (AOAC Official Method 966.04, Methods I and II) should first be conducted for any liquid, gas or vapor product to demonstrate that it is a sterilant/sporicide on porous and non-porous environmental surfaces. A product must inactivate viable spores on all 720 carriers in the AOAC sporicidal test. Second, “confirmatory testing” needs to be conducted on two samples (representing two batches of product) using virulent *B. anthracis* spores, or a surrogate acceptable to EPA, to show that the product inactivates these spores on all 120 carriers (porcelain penicylinders and silk suture loops). If both tests are passed, then the product may be registered as a “sterilant” or “sporicide” with a claim that the product inactivates *B. anthracis* spores on inanimate surfaces. [Note: Any gas or vapor product would also have to undergo a “simulated use test” as well (see section 4. below).]

3.2 Sporicidal Decontaminant (Qualitative Testing) plus *B. anthracis* Claim

A product may be registered as a “sporicidal decontaminant” if it is successfully tested using the AOAC Official Method 966.04 to demonstrate the product’s effectiveness on porcelain or silk carriers only tested against virulent *B. anthracis* spores, or a surrogate acceptable to EPA. Sixty carriers representing either or both of two types of carriers (porcelain penicylinders and silk suture loops) should be tested on three samples representing three different batches of product, one of which should be at least 60 days old. If one surface type is tested, then there are 60 carriers per sample, or a total of 180 carriers; if two surfaces types are tested, then the total number of carriers is 360. [Note: Any gas or vapor product would also have to undergo a “simulated use test” as well (see section 4. below).]

3.3 Sporicidal Decontaminant (Quantitative Testing) plus *B. anthracis* Claim

A product may be registered as a “sporicidal decontaminant” if it is successfully tested using a well established, quantitative sporicidal test method acceptable to EPA using virulent *B. anthracis* spores (or a surrogate acceptable to EPA) on porous and/or nonporous surfaces. The product should be tested on three samples representing three different batches of product, one of which should be at least 60 days old. The number of carriers will depend upon the method that is used. The product needs to achieve at least a 6 log reduction of virulent *B. anthracis* spores (or a surrogate) on either

nonporous or porous surfaces. [Note: Any gas or vapor product would also have to undergo a “simulated use test” as well (see section 4. below).]

4. Simulated Use Testing for Gas and Vapor Products

Simulated use testing is needed to support registration of gas and vapor sterilants/sporicides and sporicidal decontaminants. This testing would demonstrate a product’s ability to be applied effectively in large, enclosed spaces rather than just in small spaces (such as glove boxes). The purpose of the test would be to:

- Assure that key parameters for efficacy (chemical concentration, temperature, relative humidity and contact time) are accurately monitored and maintained throughout the enclosed space, and
- Establish product generation rate (lbs/hr) and rate/volume (lbs/hr/ft³).

Generally, the test chamber or space should be at least the size of a typical office. Key parameters such as ambient temperature, relative humidity, gas concentration, gas injection rate and/or total mass usage, and contact time should be monitored throughout the test.

Measurements should show that the same concentration, temperature, and relative humidity, can be maintained for the required contact time that were necessary to achieve 100% kill (i.e., no growth on any of the carriers) in the qualitative laboratory test, or a 6 log reduction in the quantitative test is demonstrated in the simulated-use test. In addition, measurements of the fumigant mass injection/generation rate (e.g., pounds/hour), divided by the volume of the simulated use test bed, that was used to arrive at the required generation rate/volume (e.g., pounds per hour/cubic foot) for the fumigation, should be included with the data, and listed on the product label.

5. References are listed in the Charge Memorandum dated 6/7/07)