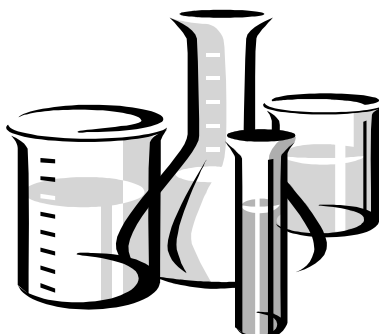




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# Product Performance Test Guidelines

## OCSPP 810.2200: Disinfectants for Use on Hard Surfaces—Efficacy Data Recommendations



## NOTICE

This guideline is one of a series of test guidelines established by the Office of Chemical Safety and Pollution Prevention (OCSPP) (formerly the Office of Prevention, Pesticides and Toxic Substances (OPPTS) prior to April 22, 2010), United States Environmental Protection Agency for use in testing pesticides and chemical substances to develop data for submission to the Agency under the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601, et seq.), the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, et seq.), and section 408 of the Federal Food, Drug, and Cosmetic (FFDCA) (21 U.S.C. 346a), referred to hereinafter as the harmonized test guidelines.

The harmonized test guidelines serve as a compendium of accepted scientific methodologies and protocols that are intended to provide data to inform regulatory decisions under TSCA, FIFRA, and/or FFDCA. This document provides guidance for conducting appropriate tests, and is also used by EPA, the public, and the companies that are subject to data submission requirements under TSCA, FIFRA and/or the FFDCA. At places in this guidance, the Agency uses the word “should.” In this guidance, use of “should” with regard to an action means that the action is recommended rather than mandatory. As a guidance document, these guidelines are not binding on either EPA or any outside parties, and the EPA may depart from the guidelines where circumstances warrant and without prior notice. The methods contained in this guideline are strongly recommended for generating the data that are the subject of the guideline, but EPA recognizes that departures may be appropriate in specific situations. You may propose alternatives to the methods recommended in these guidelines, with your supporting rationale. The Agency will assess such proposals on a case-by-case basis.

For additional information about OCSPP harmonized test guidelines and to access the guidelines electronically, please go to <http://www.epa.gov/ocspp> and select “Test Methods & Guidelines” on the left side navigation menu. You may also access the guidelines in <http://www.regulations.gov> grouped by Series under Docket ID #s: EPA-HQ-OPPT-2009-0150 through EPA-HQ-OPPT-2009-0159, and EPA-HQ-OPPT-2009-0576.

## **OCSPP 810.2200: Disinfectants for use on hard surfaces - efficacy data recommendations**

### **(a) Scope.**

**(1) Applicability.** This guideline describes test methods that EPA believes will generally satisfy testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)(7U.S.C. 136, et seq.) and the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 I/S/C. 346a). It addresses testing to demonstrate the effectiveness of antimicrobial pesticides bearing claims as disinfectants, fungicides, virucides, and tuberculocides.

**(2) Background.** The source materials used in developing this OCSPP test guideline are OPP guidelines 91-2: Products for use on hard surfaces and 91-30: Acceptable methods (Pesticide Assessment Guidelines, Subdivision G, Product Performance. EPA report 540/9-82-026, October 1982).

**(b) Purpose.** This guideline addresses efficacy testing for antimicrobial pesticides intended to be used on hard surfaces, namely disinfectants, fungicides, virucides, and tuberculocides in a variety of product types (water-soluble powders, liquids, sprays, towelettes, etc.).

### **(c) General considerations**

**(1)** This guideline recommends methods for use in tests to be conducted to address the data requirements for pesticide registration. Good Laboratory Practice Standards (GLP) as defined in 40 CFR Part 160 apply to studies to support disinfection on hard, non-porous surfaces. According to 40 CFR §160.17: “EPA may refuse to consider reliable for purposes of supporting an application for a research or marketing permit any data from a study which was not conducted in accordance with this part.” 40 CFR §160.12 (b) requires with any submitted research data “[a] A statement that the study was conducted in accordance with this part; [b] A statement describing in detail all differences between the practices used in the study and those required by this part; or [c] A statement that the person was not a sponsor of the study, did not conduct the study, and does not know whether the study was conducted in accordance with this part.” Note: The Association of Official Analytical Chemicals (AOAC) recommended tests are designed to be conducted as written. For deviations (e.g., cultures grown with shaking instead of static, dilution of culture prior to drying on carriers) proposed to be used in the conduct of these tests, obtain written approval from the Agency and document such deviations in the study reports submitted to the Agency. The Agency may consult with the AOAC prior to accepting modifications to their standardized methods. Refer to OCSPP Test Guideline 810.2000 for general testing recommendations prior to initiating tests.

**(2) Confirmatory testing.** In certain situations an applicant may rely on previously submitted efficacy data to support an application or amendment for registration of a product and submit only confirmatory efficacy data on his own product to demonstrate his ability to produce

an effective formulation. These situations are as listed in paragraphs (C)(3)(i) through (C)(3)(iii) of this guideline:

(i) **Duplicated Product Formulations.** In this situation, the applicant manufactures a formulation which duplicates a product that is already registered with complete supporting efficacy data. The chemical composition, manufacturing procedure, label claims, and directions for use are identical in substance to those of the original registration, and specific references (Master Record ID Numbers [MRID]) to the supporting data developed for the original product are cited by the applicant.

(ii) **Minor Formulation Change in a Registered Product.** In this situation, the change in the formulation is relatively minor, e.g., a change of an inert ingredient. The label claims and directions for use are unchanged from those accepted for the registered formulation, and specific references (MRID) to the supporting data developed for the original formulation are cited by the applicant. If the only change in the formulation is the addition of a fragrance or dye, confirmatory data do not need to be submitted. However, when the product is an aerosol formulation, confirmatory data should be submitted for all formulation changes, including the addition of fragrances and dyes.

(iii) The confirmatory data are to be developed from testing the applicant's own finished product. When the test methodology utilized in deriving the original supporting efficacy data were modified to include additional elements not specified in the recommended method, such as organic soil, hard water, longer or shorter contact time, etc., the confirmatory data should be produced under similarly modified conditions.

**(4) Efficacy claims.** Table 1 provides a quick reference guide to testing for basic claims described in this guideline. Consult the text for detailed testing descriptions.

**Table 1. Testing for basic efficacy claims**

<b>Level of Efficacy</b>	<b>Test Methods</b>		<b>Test Organisms</b>	<b>No. of Batches/Carriers</b>	<b>Evaluation of Success</b>
Limited spectrum disinfectant/hard non-porous surfaces.	Water soluble powders/liquids	AOAC Use-Dilution Method or AOAC Hard Surface Carrier Test (distilled water only)	<i>Staphylococcus aureus</i> (ATCC 6538) or <i>Salmonella enterica</i> (ATCC 10708)	Three batches, one at least 60 days old. 60 carriers against either organism claimed (180 carriers).	59/60 carriers are negative for each batch tested for all methods except AOAC Hard Surface Carrier Test, which is 58/60 carriers are negative for each batch.
	Spray products	AOAC Germicidal Spray Products Test			
	Towelettes	Modified AOAC Germicidal Spray Products Test			
Broad-spectrum disinfectant/hard non-porous	Water soluble powders/liquids	AOAC Use-Dilution Method or	<i>Staphylococcus aureus</i> (ATCC 6538) and	Three batches, one at least 60 days old. 60 carriers against each organism (360	59/60 carriers are negative for each batch tested for all

Level of Efficacy	Test Methods		Test Organisms	No. of Batches/Carriers	Evaluation of Success
surfaces.		AOAC Hard Surface Carrier Test (distilled water only)	<i>Salmonella enterica</i> (ATCC 10708)	carriers).	methods except AOAC Hard Surface Carrier Test, which is 58/60 carriers are negative for each batch.
	Spray products	AOAC Germicidal Spray Products Test			
	Towelettes	Modified Germicidal Spray Test			
Hospital or healthcare disinfectant/hard non-porous surfaces.	Water soluble powders/liquids	AOAC Use-Dilution Method or AOAC Hard Surface Carrier Test (distilled water only)	<i>Staphylococcus aureus</i> (ATCC 6538) and <i>Pseudomonas aeruginosa</i> (ATCC 15442)	Three batches, one at least 60 days old. 60 carriers against each organism (360 carriers).	59/60 carriers are negative for each batch tested for all methods except AOAC Hard Surface Carrier Test, which is 58/60 carriers are negative against <i>Staphylococcus aureus</i> for each batch, and 57/60 carriers are negative against <i>Pseudomonas aeruginosa</i> .
	Spray products	AOAC Germicidal Spray Products Test			
	Towelettes	Modified Germicidal Spray Test			
Fungicidal disinfectant/hard non-porous surfaces.	Water soluble powders/liquids	AOAC Use-Dilution Test modified for fungi or AOAC Fungicidal Test	<i>Trichophyton mentagrophytes</i> (ATCC 9533)	Two batches, ten carriers per batch for the modified AOAC Use Dilution Test, the modified AOAC Germicidal Spray Products Test, and the EPA Towelette Test. Two batches for the AOAC Fungicidal Test.	All fungal spores on all carriers should be killed. For the AOAC Fungicidal Test, all fungal spores should be killed at 10 and 15 minutes to support a 10 minute label claim.
	Spray products	AOAC Germicidal Spray Products Test modified for fungi			
	Towelettes	Modified Germicidal Spray Test			
Virucidal disinfectant/hard non-porous surfaces.	Water soluble powders/liquids	AOAC Use-Dilution Test modified for viruses or ASTM E1053-	Virus claimed on the label or approved surrogate.	Two batches. One surface per batch.	Complete inactivation of the virus. Where cytotoxicity is present, demonstrate a 3 log <sub>10</sub> reduction.
	Spray products	AOAC Germicidal Spray Products Test modified for viruses or ASTM E1053-			
	Towelettes	Modified Germicidal Spray Test			
Tuberculocidal disinfectant/hard non-porous surfaces.	Water soluble powders/liquids	AOAC Tuberculocidal Activity of Disinfectants, Quantitative Tuberculocidal		Two batches, ten carriers per batch.  Two batches, 4 replicates per batch.	10/10 carriers are negative for growth and there is no growth in the additional test media.

Level of Efficacy	Test Methods		Test Organisms	No. of Batches/Carriers	Evaluation of Success
		Activity Test	<i>Mycobacterium bovis</i> BCG	Two batches, ten carriers per batch.	Survival Curve constructed from 4 separate replicates at the 95% confidence level to show probability of one survivor.
	Spray products	AOAC Germicidal Spray Products Test modified for tuberculocidal activity			10/10 carriers are negative for growth and there is no growth in the additional test media.
	Towelettes	Modified Germicidal Spray Test			10/10 carriers are negative for growth and there is no growth in the additional test media.
Additional bacteria/hard non-porous surfaces.	Water soluble powders/liquids	AOAC Use-Dilution Test or AOAC Hard Surface Carrier Test (distilled water only)	Bacteria claimed on the label in addition to the base broad-spectrum claim.	Two batches, ten carriers for each batch.	10/10 carriers are negative for growth of the test organism.
	Spray products	AOAC Germicidal Spray Products Test			

#### (d) Disinfectants

**(1) Limited spectrum products.** This section addresses efficacy testing for disinfectant products with limited efficacy (effective against Gram-negative or Gram-positive bacteria, but not both).

(i) Water-soluble powders and non-volatile liquid products test procedure. The Agency recommends the use of the AOAC International Use-Dilution Methods (Ref. 1) or the AOAC International Hard Surface Carrier Test Methods (distilled water only)(Ref. 2). Sixty carriers for each of three samples, representing three different batches, one of which should be  $\geq 60$  days old, should be tested against *Salmonella enterica* (*S. enterica*)(formerly designated as *Salmonella choleraesuis*)(American Type Culture Collection)(ATCC 10708) for effectiveness against Gram-negative bacteria, or *Staphylococcus aureus* (*S. aureus*)(ATCC 6538) for effectiveness against Gram-positive bacteria. If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(ii) Germicidal spray products (aerosol or pump) and volatile liquid products test procedure. The Agency recommends use of the AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3). Sixty carriers for each of three samples, representing three different

batches, one of which should be  $\geq 60$  days old, should be tested against *S. enterica* (ATCC 10708) for effectiveness against Gram-negative bacteria, or *S. aureus* (ATCC 6538) for effectiveness against Gram-positive bacteria. If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(iii) Single-use towelettes test procedure. The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3) or ASTM E2362 (Ref 4). Sixty carriers for each of three samples, representing three different batches, one of which should be  $\geq 60$  days old, should be tested against *S. enterica* (ATCC 10708) for effectiveness against Gram-negative bacteria, or *S. aureus* (ATCC 6538) for effectiveness against Gram-positive bacteria. If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. Instead of spraying the inoculated surface of the carriers, the product should be tested by wiping the surface of the carriers with the saturated towelette, and then subculturing the carriers after the specified holding time. The towelette should be removed from its container and handled with sterile gloves. One towelette should be used to wipe a minimum of 10 inoculated carriers for a total of 6 towelettes for all 60 carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of carriers. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report.

(iv) Evaluation of limited disinfectant success. For the AOAC International Use-Dilution Methods, the Germicidal Spray Products as Disinfectants test, and single-use towelettes, the product should kill the test microorganisms on 59 out of each set of 60 carriers/slides in  $\leq$  ten minutes. In addition, per the 2009 AOAC revisions for the Use-Dilution Method, the mean log density for *S. aureus* is to be at least 6.0 (corresponding to a geometric mean density of  $1.0 \times 10^6$ ); a mean log density  $< 6.0$  invalidates the test. For the AOAC International Hard Surface Carrier Test Methods, the product should kill the test microorganisms on 58 out of each set of 60 carriers for *S. enterica* or *S. aureus* in  $\leq$  ten minutes. For the Hard Surface Carrier Test, the dried carrier counts should be  $0.5 - 2.0 \times 10^6$  for *Salmonella enterica* and  $1 - 5 \times 10^6$  for *Staphylococcus aureus*.

## **(2) Confirmatory testing for limited spectrum products**

(i) Water-soluble powders and non-volatile liquid products test procedure. The Agency recommends the use of the AOAC International Use-Dilution Methods (Ref.1) or the AOAC International Hard Surface Carrier Test Methods (distilled water only) (Ref. 2). Ten carriers for each of two product samples, representing two different batches of the product, should be tested against either *S. aureus* (ATCC 6538) or *S. enterica* (ATCC 10708)(depending on whether the product is claimed to be effective against Gram-positive or Gram-negative bacteria). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial

inoculum.

(ii) Germicidal spray products (aerosol or pump) and volatile liquid products test procedure. The Agency recommends the AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3). Ten carriers for each of two product samples, representing two different batches of the product, should be tested against either *S. aureus* (ATCC 6538) or *S. enterica* (ATCC 10708)(depending on whether the product is claimed to be effective against Gram-positive or Gram-negative bacteria). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. For pressurized spray products, certification should be furnished specifying that all parts and materials used in manufacturing the container for pressurized spray disinfectants are identical to those specified by the basic manufacturer.

(iii) Single-use towelettes test procedure. The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3) or ASTM E2362 (Ref 4). Ten carriers for each of three samples, representing three different batches, one of which should be  $\geq 60$  days old, should be tested against *S. enterica* (ATCC 10708) for effectiveness against Gram-negative bacteria, or *S. aureus* (ATCC 6538) for effectiveness against Gram-positive bacteria. If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. Instead of spraying the inoculated surface of the carriers, the product should be tested by wiping the surface of the carriers with the saturated towelette, and then subculturing the carriers after the specified holding time. The towelette should be removed from its container and handled with sterile gloves. One towelette should be used to wipe a minimum of 10 inoculated carriers for a total of 1 towelette for all 10 carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of carriers. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report.

(iv) Evaluation of confirmatory limited disinfectant success. The product should kill all the test microorganisms on all carriers in  $\leq 10$  minutes. In addition, per the 2009 AOAC revisions for the Use-Dilution Method, the mean log density for *S. aureus* is to be at least 6.0 (corresponding to a geometric mean density of  $1.0 \times 10^6$ ); a mean log density  $< 6.0$  invalidates the test. For the Hard Surface Carrier Test, the dried carrier counts should be  $0.5 - 2.0 \times 10^6$  for *Salmonella enterica* and  $1 - 5 \times 10^6$  for *Staphylococcus aureus*.

**(3) General or broad spectrum efficacy products.** When a disinfectant is represented in labeling as having efficacy against both Gram-negative and Gram-positive bacteria, the product is considered a general or broad spectrum disinfectant.

(i) Water-soluble powders and non-volatile liquid products test procedure. The Agency recommends the use of the AOAC International Use-Dilution Methods (Ref. 1) or the AOAC



International Hard Surface Carrier Test Methods (distilled water only)(Ref. 2). Sixty carriers for each of three samples, representing three different batches, one of which should be  $\geq 60$  days old, should be tested against both *S. enterica* (ATCC 10708) and *S. aureus* (ATCC 6538). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(ii) Germicidal spray products (aerosol or pump) and volatile liquid products test procedure. The Agency recommends the AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3). Sixty carriers for each of three samples, representing three different batches, one of which should be  $\geq 60$  days old, should be tested against both *S. enterica* (ATCC 10708) and *S. aureus* (ATCC 6538). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(iii) Single-use towelettes test procedure. The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3) or ASTM E2362 (Ref 4). Sixty carriers for each of three samples, representing three different batches, one of which should be  $\geq 60$  days old, should be tested against *S. enterica* (ATCC 10708) for effectiveness against Gram-negative bacteria, or *S. aureus* (ATCC 6538) for effectiveness against Gram-positive bacteria. If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. Instead of spraying the inoculated surface of the carriers, the product should be tested by wiping the surface of the carriers with the saturated towelette, and then subculturing the carriers after the specified holding time. The towelette should be removed from its container and handled with sterile gloves. One towelette should be used to wipe a minimum of 10 inoculated carriers for a total of 6 towelettes for all 60 carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of carriers. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report.

(iv) Evaluation of general or broad spectrum disinfectant success. For the AOAC International Use-Dilution Methods, the Germicidal Spray Products as Disinfectants test, and single-use towelettes, the product should kill the test microorganisms on 59 out of each set of 60 carriers/slides in  $\leq 10$  minutes. In addition, per the 2009 AOAC revisions for the Use-Dilution Method, the mean log density for *S. aureus* is to be at least 6.0 (corresponding to a geometric mean density of  $1.0 \times 10^6$ ); a mean log density  $< 6.0$  invalidates the test. For the AOAC International Hard Surface Carrier Test Methods, the product should kill the test microorganisms on 58 out of each set of 60 carriers in  $\leq 10$  minutes. For the Hard Surface Carrier Test, the dried carrier counts should be  $0.5 - 2.0 \times 10^6$  for *Salmonella enterica* and  $1 - 5 \times 10^6$  for *Staphylococcus aureus*.

#### **(4) Confirmatory testing for general or broad spectrum products**

(i) Water-soluble powders and non-volatile liquid products test procedure. The Agency recommends the use of the AOAC International Use-Dilution Methods (Ref. 1) or the AOAC International Hard Surface Carrier Test Methods (distilled water only)(Ref. 2). Ten carriers for each of two product samples, representing two different batches of the product, should be tested against both *S. aureus* (ATCC 6538) and *S. enterica* (ATCC 10708). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(ii) Germicidal spray products (aerosol or pump) and volatile liquid products test procedure. The Agency recommends the AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3). Ten carriers for each of two product samples, representing two different batches of the product, should be tested against both *S. aureus* (ATCC 6538) and *S. enterica* (ATCC 10708). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. For pressurized spray products, certification should be furnished specifying that all parts and materials used in manufacturing the container for pressurized spray disinfectants are identical to those specified by the basic manufacturer.

(iii) Single-use towelettes test procedure. The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3) or ASTM E2362 (Ref 4). Ten carriers for each of three samples, representing three different batches, one of which should be  $\geq 60$  days old, should be tested against both *S. enterica* (ATCC 10708) and *S. aureus* (ATCC 6538). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. Instead of spraying the inoculated surface of the carriers, the product should be tested by wiping the surface of the carriers with the saturated towelette, and then subculturing the carriers after the specified holding time. The towelette should be removed from its container and handled with sterile gloves. One towelette should be used to wipe a minimum of 10 inoculated carriers for a total of 1 towelette for all 10 carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of carriers. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report.

(iv) Evaluation of confirmatory general or broad spectrum disinfectant success. The product should kill all the test microorganisms on all carriers in  $\leq 10$  minutes. In addition, per the 2009 AOAC revisions for the Use-Dilution Method, the mean log density for *S. aureus* is to be at least 6.0 (corresponding to a geometric mean density of  $1.0 \times 10^6$ ); a mean log density  $< 6.0$  invalidates the test. For the Hard Surface Carrier Test, the dried carrier counts should be  $0.5 - 2.0 \times 10^6$  for *Salmonella enterica* and  $1 - 5 \times 10^6$  for *Staphylococcus aureus*.

**(5) Hospital or healthcare disinfectants.** This section addresses efficacy testing for products recommended for use in hospitals, clinics, dental offices, nursing homes, sickrooms, or

any other healthcare-related facility.

(i) Water-soluble powders and non-volatile liquid product test procedure. The Agency recommends the use of the AOAC International Use-Dilution Methods (Ref. 1) or the AOAC International Hard Surface Carrier Test Methods (distilled water only)(Ref. 2). Sixty carriers for each of three samples, representing three different batches, one of which should be  $\geq 60$  days old, should be tested against *S. aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (*P. aeruginosa*)(ATCC 15442). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(ii) Germicidal spray products (aerosol or pump) and volatile liquid products test procedure. The Agency recommends the use of the AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3). Sixty carriers for each of three samples, representing three different batches, one of which should be  $\geq 60$  days old, should be tested against: *S. aureus* (ATCC 6538), and *P. aeruginosa* (ATCC 15442). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(iii) Single-use towelettes test procedure. The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3) or ASTM E2362 (Ref. 4). Sixty carriers for each of three samples, representing three different batches, one of which should be  $\geq 60$  days old, should be tested against *S. aureus* (ATCC 6538), and *P. aeruginosa* (ATCC 15442). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. Instead of spraying the inoculated surface of the carriers, the product should be tested by wiping the surface of the carriers with the saturated towelette, and then subculturing the carriers after the specified holding time. The towelette should be removed from its container and handled with sterile gloves. One towelette should be used to wipe a minimum of 10 inoculated carriers for a total of 6 towelettes for all 60 carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of carriers. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report.

(iv) Evaluation of hospital or healthcare disinfectant success. For the AOAC International Use-Dilution Methods, the Germicidal Spray Products as Disinfectants test, and single-use towelettes, the product should kill the test microorganisms on 59 out of each set of 60 carriers in  $\leq 10$  minutes. In addition, per the 2009 AOAC revisions for the Use-Dilution Method, the mean log density for *S. aureus* and *P. aeruginosa* is to be at least 6.0 (corresponding to a geometric mean density of  $1.0 \times 10^6$ ); a mean log density  $< 6.0$  invalidates the test. For the AOAC International Hard Surface Carrier Test Methods, the product should kill the test microorganisms on 58 out of each set of 60 carriers for *S. aureus*, and 57 out of each set of 60 carriers for *P. aeruginosa* within ten minutes or less. For the Hard Surface Carrier Test, the dried carrier counts

should be  $1 - 5 \times 10^6$  for both *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

#### **(6) Confirmatory testing for products with hospital or healthcare disinfectant claim**

(i) Water-soluble powders and non-volatile liquid products test procedure. The Agency recommends the use of the AOAC International Use-Dilution Methods (Ref. 1) or the AOAC International Hard Surface Carrier Test Methods (distilled water only) (Ref. 2). Ten carriers for each of two product samples, representing two different batches of the product, should be tested against *S. aureus* (ATCC 6538) *P. aeruginosa* (ATCC 15442). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(ii) Germicidal spray products (aerosol or pump) and volatile liquid products test procedure. The Agency recommends the AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3). Ten carriers for each of two product samples, representing two different batches of the product, should be tested against *S. aureus* (ATCC 6538) and *P. aeruginosa* (ATCC 15442). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. For pressurized spray products, certification should be furnished specifying that all parts and materials used in manufacturing the container for pressurized spray disinfectants are identical to those specified by the basic manufacturer.

(iii) Single-use towelettes test procedure. The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3) or ASTM E2362 (Ref. 4). Ten carriers for each of two product samples, representing two different batches of the product, should be tested against *S. aureus* (ATCC 6538) and *P. aeruginosa* (ATCC 15442). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. Instead of spraying the inoculated surface of the carriers, the product should be tested by wiping the surface of the carriers with the saturated towelette, and then subculturing the carriers after the specified holding time. The towelette should be removed from its container and handled with sterile gloves. One towelette should be used to wipe a minimum of 10 inoculated carriers for a total of 1 towelette for all 10 carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of carriers. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report.

(iv) Evaluation of confirmatory hospital or healthcare disinfectant success. The product should kill all the test microorganisms on all carriers in  $\leq$  ten minutes. In addition, per the 2009 AOAC revisions for the Use-Dilution Method, the mean log density for *S. aureus* and *P. aeruginosa* is to be at least 6.0 (corresponding to a geometric mean density of  $1.0 \times 10^6$ ); a mean log density  $< 6.0$  invalidates the test. For the Hard Surface Carrier Test, the dried carrier counts should be  $1 - 5 \times 10^6$  for both *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**(7) Bridging for disinfectant towelettes.** In some cases, disinfectant towelette formulations are identical to registered liquid formulations. In order to bridge efficacy data from the EPA registered bulk liquid disinfectant used to saturate a towelette or other related product form, the studies in paragraphs (d)(7)(i) and (d)(7)(ii) of this guideline should be conducted and submitted to EPA for review.

(i) Chemical Testing - Comparison of Expressed Liquid from the Towelette(s) to the EPA Registered Liquid Disinfectant Formulation to which it is being bridged: All active ingredients in the expressed liquid should be within the certified limits of the Confidential Statement of Formula of the liquid formula being referenced/bridged. The disinfectant towelettes package should be filled according to the manufacturing specifications. Excess liquid in the bulk towelette containers cannot be poured off for use in the chemical testing for bridging of the efficacy data. The liquid used in the chemical testing should only be that expressed from the towelettes. Three batches (one of which is 60 days old) should be tested. Analytical data for the active ingredients in the expressed liquid should be submitted for review.

(ii) Efficacy Testing - Efficacy testing should be conducted under the same testing conditions (e.g. soil load, contact time, temperature) as used for the bulk liquid testing. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report. For limited disinfectants, broad-spectrum disinfectants, and hospital disinfectants, to bridge bacterial disinfection claims:

(A) Test Procedure. The Agency recommends the use of the AOAC Germicidal Spray Products as Disinfectants test modified for towelettes, using the test organisms specified for limited, broad-spectrum or hospital disinfectant testing. Sixty carriers for each organism should be tested against three different batches of the product (one of which should be  $\geq 60$  days old). Instead of spraying the inoculated surface of the carrier, the product should be tested by wiping the surface of the carrier with the saturated towelette, and then subculturing the carriers after the specified holding time. One towelette should be used to treat 10 carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch.

(B) Evaluation of bactericidal towelette success. The product should kill the test organism on 59 out of 60 carriers. This testing is intended to support bridging of all vegetative bacteria listed on the EPA registered liquid disinfectant used to saturate the towelette to the EPA registered towelette product.

**(8) Disinfectants for Internal Toilet and Urinal Bowl Surfaces Above and Below the Water Line.** This section addresses efficacy testing for products bearing label claims as disinfectants (limited, broad-spectrum, or hospital) for internal toilet and urinal bowl surfaces. Regarding water-soluble powders and non-volatile liquid products test procedure, the Agency recommends the use of the AOAC International Use-Dilution Methods (Ref. 1) modified to include a 5% organic soil challenge added to the bacterial inoculum. Sixty carriers for each of

three samples, representing three different batches, one of which is  $\geq 60$  days old, should be tested against *Salmonella enterica* (ATCC 10708) or *Staphylococcus aureus* (ATCC 6538), for limited disinfectant products; *S. enterica* and *S. aureus*, for broad-spectrum disinfectant products; and *S. aureus* and *Pseudomonas aeruginosa* (ATCC 15442), for hospital disinfectant products. The contained bowl water (-96 fl oz, which represents traditional high volume toilets) should be used to calculate the appropriate use dilution for testing. The contained bowl water for low volume toilets should be measured and used to calculate the appropriate use dilution for testing.

(i) Evaluation of disinfectant success for internal toilet bowl and urinal bowl surfaces. For the AOAC International Use-Dilution Methods and the Germicidal Spray Products as Disinfectants test, the product should kill the test microorganisms on 59 out of each set of 60 carriers/slides within ten minutes or less. In addition, per the 2009 AOAC revisions for the Use-Dilution Method, the mean log density for *S. aureus* and *P. aeruginosa* is to be at least 6.0 (corresponding to a geometric mean density of  $1.0 \times 10^6$ ); a mean log density  $< 6.0$  invalidates the test.

**(9) Additional microorganisms.** This section addresses efficacy testing for broad-spectrum or hospital disinfectants which bear label claims against bacteria other than *Salmonella enterica* (ATCC 10708), *Staphylococcus aureus* (ATCC 6538) or *Pseudomonas aeruginosa* (ATCC 15442).

(i) Water-soluble powders and non-volatile liquid products test procedure. The Agency recommends the use of the AOAC International Use-Dilution Methods (Ref. 1) or the AOAC International Hard Surface Carrier Test Methods (distilled water only)(Ref. 2). Ten carriers should be tested against each specific bacterium for each of two samples representing two different batches. If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(ii) Germicidal spray products (aerosol or pump) and volatile liquid products test procedure. The Agency recommends the use of the AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3). Ten carriers should be tested against each specific bacterium for each of two samples representing two different batches. If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(iii) Single-use towelettes test procedure. The Agency recommends the use of a modified AOAC modified Germicidal Spray Products as Disinfectants test (Ref.3) or ASTM E2362 (Ref. 4). Ten carriers should be tested against each specific bacterium for each of two samples representing two different batches. If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. Instead of spraying the inoculated surface of the carrier, the product should be tested by wiping the surface of the carrier with the saturated towelette, and then subculturing the carriers after the specified holding time. The towelette should be removed from its container and handled with sterile gloves. One towelette should be

used to wipe a minimum of 10 inoculated carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of slides. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report.

(iv) Evaluation of disinfectant success for additional microorganisms. The product should kill all the test microorganisms on all carriers in  $\leq$ ten minutes. The minimum carrier count to make the test valid should be  $1 \times 10^4$ .

**(e) Disinfectants with fungicidal claims.** This section addresses efficacy testing for broad-spectrum or hospital disinfectant products which bear label claims of efficacy against pathogenic fungi.

### **(1) Water soluble powders and non-volatile liquid products**

(i) Test procedures. The Agency recommends the use of the AOAC International Fungicidal Activity of Disinfectants test (Ref. 5). The test should be conducted at 5, 10, and 15 minute exposure times. Two samples representing two different batches of the product should be evaluated for efficacy against *Trichophyton mentagrophytes* (*T. mentagrophytes*)(ATCC 9533). The inoculum employed should provide a concentration of  $\geq 5 \times 10^6$  conidia/mL. If the product is intended to be represented as fungicidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5% blood serum, should be included with the fungal inoculum. The Agency also recommends the use of the AOAC International Use-Dilution Methods (Ref. 1). This test may be modified to conform to appropriate elements (e.g., media, growth conditions, etc.) in the AOAC International Fungicidal Activity of Disinfectants test. Ten carriers for each of two samples representing two different batches of the product should be evaluated against *T. mentagrophytes* (ATCC 9533). The inoculum employed should provide a concentration of  $1 \times 10^4 - 1 \times 10^5$  conidia per carrier. If the product is intended to be represented as fungicidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the fungal inoculum.

(ii) Evaluation of fungicidal success. For the AOAC International Fungicidal Activity of Disinfectants test, all fungal spores at 10 and 15 minutes should be killed to support a 10 minute exposure time. For the AOAC International Use-Dilution Methods, all fungal spores on all 10 carriers should be killed in  $\leq$ ten minutes.

**(2) Germicidal spray products (aerosol or pump) and volatile liquid products—(i)** Test procedures. The Agency recommends the use of the AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3). This test may be modified to conform to appropriate elements (e.g., media, growth conditions, etc.) in the AOAC International Fungicidal Activity of Disinfectants test. Ten carriers for each of two samples representing two different batches of the product should be evaluated against *T. mentagrophytes* (ATCC 9533). The inoculum employed should be modified to provide a concentration of  $1 \times 10^4 - 1 \times 10^5$  conidia per carrier. If the

product is intended to be represented as fungicidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the fungal inoculum.

(ii) Evaluation of fungicidal success. All fungal spores on all 10 carriers should be killed in  $\leq$ ten minutes.

### **(3) Single-Use Towelettes**

(i) Test Procedure. The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3) or ASTM 2362 (Ref. 4). Ten carriers for each of two samples representing two different batches of the product should be evaluated against *T. mentagrophytes* (ATCC 9533). The inoculum employed should be modified to provide a concentration of  $1 \times 10^4 - 1 \times 10^5$  conidia per carrier. If the product is intended to be represented as fungicidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the fungal inoculum. Instead of spraying the inoculated surface of the carrier, the product should be tested by wiping the surface of the carrier with the saturated towelette, and then subculturing the carriers after the specified holding time. The towelette should be removed from its container and handled with sterile gloves. One towelette should be used to wipe a minimum of 10 inoculated carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of slides. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report.

(ii) Evaluation of fungicidal towelette success. All fungal spores on all 10 carriers should be killed in  $\leq$ ten minutes.

**(4) Bridging for disinfectant towelettes.** In some cases, disinfectant towelette formulations are identical to registered liquid formulations. In order to bridge efficacy data from the EPA registered bulk liquid disinfectant used to saturate a towelette or other related product form, the studies in paragraphs (e)(4)(i) and (e)(4)(ii) of this guideline should be conducted and submitted to EPA for review:

(i) Chemical Testing - Comparison of Expressed Liquid from the Towelette(s) to the EPA Registered Liquid Disinfectant Formulation to which it is being bridged: All active ingredients in the expressed liquid should be within the certified limits of the Confidential Statement of Formula of the liquid formula being referenced/bridged. The disinfectant towelettes package should be filled according to the manufacturing specifications. Excess liquid in the bulk towelette containers cannot be poured off for use in the chemical testing for bridging of the efficacy data. The liquid used in the chemical testing should only be that expressed from the towelettes. Three batches (one of which is  $\geq 60$  days old) should be tested. Analytical data for the active ingredients in the expressed liquid should be submitted for review.



(ii) Efficacy Testing - Efficacy testing should be conducted under the same testing conditions (e.g., soil load, contact time, temperature) as used for the bulk liquid testing. This testing allows bridging of data from the registered bulk liquid used to saturate the towel for each type of organism in this paragraph. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report. For fungicidal test procedure, the Agency recommends the use of the AOAC International Germicidal Spray Products as Disinfectants (Ref. 3) modified for fungicidal towelette testing. The test should be modified to conform to appropriate elements (e.g. media, growth conditions) in the AOAC International Fungicidal Activity of Disinfectants test. Ten carriers for each of two samples, representing two batches of the product should be evaluated against *T. mentagrophytes* (ATCC 9533) for the label recommended contact time. The inoculum employed should be at a count to achieve  $1 \times 10^4 - 1 \times 10^5$  conidia per carrier. Instead of spraying the inoculated surface of the carrier, the product should be tested by wiping the surface of the carrier with the saturated towelette, and then subculturing the carriers after the specified holding time. One towelette should be used to wipe a minimum of 10 inoculated carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch.

(A) Evaluation of Fungicidal towelette success. The product should kill the test organism on all 10 carriers in  $\leq$ ten minutes.

(B) Bridging. This testing is intended to support bridging of all fungal test organisms from the EPA registered bulk liquid disinfectant used to saturate the towelette to the EPA registered towelette product.

**(f) Disinfectants with virucidal claims.** This section addresses efficacy testing for broad-spectrum or hospital disinfectant products that bear label claims of effectiveness against viruses. Virucidal products are intended for use on dry inanimate surfaces; therefore, virological data are usually developed by carrier methods. Each specific virus listed on the label should be tested, unless there is an acceptable surrogate for the virus. For label claims against Hepatitis B virus, Hepatitis C virus, and Norovirus, the Duck Hepatitis B virus, Bovine Viral Diarrhea virus, and Feline Calicivirus, respectively, are currently considered acceptable surrogates for testing. Additional guidance and protocols for surrogate virus testing can be found at <http://www.epa.gov/oppad001/regpolicy.htm>. To simulate in-use conditions, the specific virus to be treated (or surrogate as noted in this paragraph) should be inoculated onto hard surfaces (e.g., Petri dishes, glass carriers, or other appropriate test surface), allowed to dry, and then treated with the product according to the directions for use on the product label.

**(1) Water soluble powders and non-volatile liquid products test procedures.** The Agency recommends the use of either the AOAC International Use-Dilution Methods (Ref 1) modified for virucidal testing or the ASTM E1053 Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces (Ref. 6). One surface for each of two samples, representing two different batches of disinfectant, should be tested against a recoverable virus end point titer of  $\geq 10^4$  viable viral particles from the test surface for a specified exposure period ( $\leq 10$  minutes) at room temperature. If the product is intended to be represented as virucidal in

the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the viral inoculum. When viral suspensions are grown in the presence of at least 5% serum, addition of serum to the inoculum is not expected as part of a study to support a one-step label claim.

**(2) Germicidal spray products (aerosol or pump) and volatile liquid products test procedure.** The Agency recommends the use of a AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3) modified for virucidal testing or the ASTM E1053 Virucidal Test Method (Ref. 6). One surface for each of two samples, representing two different batches of disinfectant, should be tested against a recoverable virus titer of at least  $10^4$  viable viral particles from the test surface for the exposure period specified on the label. If the product is intended to be represented as virucidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the viral inoculum. When viral suspensions are grown in the presence of at least 5% serum, addition of serum to the inoculum is not expected as part of a study to support a one-step label claim.

**(3) Single-use towelettes test procedure.** The Agency recommends the use of the modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3) or ASTM E1053 (Ref. 6). One surface for each of two samples, representing two different batches of disinfectant, should be tested against a recoverable virus end point titer of  $\geq 10^4$  viable viral particles from the test surface for a specified exposure period ( $\leq 10$  minutes) at room temperature. If the product is intended to be represented as virucidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the viral inoculum. When viral suspensions are grown in the presence of at least 5% serum, addition of serum to the inoculum is not expected as part of a study to support a one-step label claim. Instead of spraying the inoculated surface of the carrier, the product should be tested by wiping the surface of the carrier with the saturated towelette, and then subculturing the carriers after the specified holding time. The towelette should be removed from its container and handled with sterile gloves. One towelette should be used to wipe a minimum of 10 inoculated carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of slides. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report.

**(4) Evaluation of virucidal success.** Following treatment of the test virus with the disinfectant product, the presence of remaining viable virus should then be assayed using an appropriate virological technique (e.g., cytopathogenic effect, fluorescent antibody, plaque count, or animal response). The protocol for the viral assay should provide the information identified in paragraphs (f)(4)(i) through (f)(4)(ix) of this guideline.

(i) The virus recovery (titer) should include a minimum of four determinations per each dilution in the assay system (e.g., tissue culture, embryonated egg, animal infection, etc.).

(ii) Cytotoxicity controls. The effect of the disinfectant on the viral assay system should

include a minimum of four determinations per each dilution.

(iii) The activity of the disinfectant against the test virus should include a minimum of four determinations per dilution in the assay system.

(iv) Neutralization controls. Neutralization controls should be performed (Ref. 7) and should include a minimum of four determinations per each dilution.

(v) Any special methods which are used to increase the virus titer and to detoxify the residual disinfectant should be described.

(vi) The ID<sub>50</sub> values calculated for each assay should be provided.

(vii) The test results should be reported as the reduction of the virus titer by the activity of the disinfectant (ID<sub>50</sub> of the virus control less the ID<sub>50</sub> of the test system) expressed as the logarithm to the base 10 and calculated by a statistical method (e.g., Reed and Munch, Most Probable Number, Spearman-Kärber).

(viii) The product should demonstrate complete inactivation of the virus at all dilutions. If cytotoxicity is present, the virus control titer should be increased to demonstrate a  $\geq 3 \log_{10}$  reduction in viral titer beyond the cytotoxic level. Table 1 provides an example of a typical laboratory report of a single test with one virus, assayed in a tissue culture system.

(ix) A laboratory report of a single test with one virus (recovered from a treated surface) involving a tissue culture assay system should include the details of the methods employed and the information included in Tables 2-1, 2-2 and 2-3:

**Table 2-1: Test Results**

Dilution of Virus	Virus - Disinfectant*	Virus - Control*	Cytotoxic - Control
10 <sup>-1</sup>	T T T T	+ + + +	T T T T
10 <sup>-2</sup>	T T T T	+ + + +	T T T T
10 <sup>-3</sup>	T 0 0 0	+ + + +	T 0 0 0
10 <sup>-4</sup>	0 0 0 0	+ + + +	0 0 0 0
10 <sup>-5</sup>	0 0 0 0	+ + + +	0 0 0 0
10 <sup>-6</sup>	0 0 0 0	+ + + 0	0 0 0 0
10 <sup>-7</sup>	0 0 0 0	+ 0 0 0	0 0 0 0
10 <sup>-8</sup>	0 0 0 0	0 0 0 0	0 0 0 0

Note: T = toxic; + = virus recovered; 0 = no virus recovered

**Table 2-2: Calculation of the Tissue Culture Infective Dose 50 (TCID<sub>50</sub>)**

Values				Accumulated Values			
Virus Dilution Inoculated	No. Infected / No. Inoculated	No. Infected	No. not Infected	No. Infected	No. not Infected	No. Infected / No. Inoculated	% Infected
10 <sup>-1</sup>	4/4	4	0	24	0	24/24	100
10 <sup>-2</sup>	4/4	4	0	20	0	20/20	100
10 <sup>-3</sup>	4/4	4	0	16	0	16/16	100
10 <sup>-4</sup>	4/4	4	0	12	0	12/12	100
10 <sup>-5</sup>	4/4	4	0	8	0	8/8	100
10 <sup>-6</sup>	3/4	3	1	4	1	4/5	80
10 <sup>-7</sup>	1/4	1	3	1	4	1/5	20
10 <sup>-8</sup>	0/4	0	4	0	8	0/8	0

$$\text{TCID}_{50} = 10^{6.5}$$

**Table 2-3: Calculations of the Tissue Culture Lethal Dose 50 (TCLD<sub>50</sub>)**

Values				Accumulated Values			
Virus Dilution Inoculated	No. Toxic / No. Inoculated	No. Toxic	No. not Toxic	No. Toxic	No. not Toxic	No. Toxic / No. Inoculated	% Toxic
10 <sup>-1</sup>	4/4	4	0	9	0	9/9	100
10 <sup>-2</sup>	4/4	4	0	5	0	5/5	100
10 <sup>-3</sup>	1/4	1	3	1	3	1/4	25
10 <sup>-4</sup>	0/4	0	4	0	7	0/7	0
10 <sup>-5</sup>	0/4	0	4	0	11	0/11	0
10 <sup>-6</sup>	0/4	0	4	0	15	0/15	0
10 <sup>-7</sup>	0/4	0	4	0	19	0/19	0
10 <sup>-8</sup>	0/4	0	4	0	23	0/23	0

$$\text{TCLD}_{50} = 10^{2.7} \text{ Therefore: Virus inactivation} = \text{TCID}_{50} - \text{TCLD}_{50} = 10^{3.8} \log 10$$

**(5) Bridging for disinfectant towelettes.** In some cases, disinfectant towelette formulations are identical to registered liquid formulations. In order to bridge efficacy data from the EPA registered bulk liquid disinfectant used to saturate a towelette or other related product form, the studies in paragraphs (f)(5)(i) and (f)(5)(ii) of this guideline should be conducted and

submitted to EPA for review.

(i) Chemical Testing—Comparison of Expressed Liquid from the Towelette(s) to the EPA Registered Liquid Disinfectant Formulation to which it is being bridged: All active ingredients in the expressed liquid should be within the certified limits of the Confidential Statement of Formula of the liquid formula being referenced/bridged. The disinfectant towelettes package should be filled according to the manufacturing specifications. Excess liquid in the bulk towelette containers cannot be poured off for use in the chemical testing for bridging of the efficacy data. The liquid used in the chemical testing should only be that expressed from the towelettes. Three batches (one of which is 60 days old) should be tested. Analytical data for the active ingredients in the expressed liquid should be submitted for review.

(ii) Efficacy Testing: Efficacy testing should be conducted under the same testing conditions (e.g. soil load, contact time, temperature) as used for the bulk liquid testing. This testing allows bridging of data from the registered bulk liquid used to saturate the towel for each type of virus in paragraphs (f)(5)(ii)(A)(1) through (f)(5)(ii)(A)(3) of this guideline. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report.

(A) Virucidal Test Procedure. The Agency recommends the use of either the AOAC International Germicidal Spray Products as Disinfectants (Ref. 3) modified for virucidal towelette testing or the ASTM E1053 (Ref. 6) modified for virucidal towelette testing.

(1) To support bridging of all viral claims, the most difficult to inactivate small-sized non-enveloped virus, from the viral strains registered for the bulk liquid, should be selected for testing. Examples of small-sized non-enveloped viral families include members of the Picornaviridae family (e.g., poliovirus, enterovirus, hepatitis A virus, rhinovirus), and Parvoviridae family (e.g., parvovirus).

(2) To support bridging of viral claims for large-sized non-enveloped and enveloped viral strains, the most difficult to inactivate large-sized non-enveloped virus, from the viral strains registered for the bulk liquid, should be selected for testing. Examples of large-sized non-enveloped viral families include members of the Adenoviridae family (e.g., adenovirus), Reoviridae family (e.g., rotavirus), and Papillomaviridae family (e.g., papillomavirus).

(3) To support bridging of viral claims for enveloped viral strains, the most difficult to inactivate enveloped virus, from the viral strains registered for the bulk liquid, should be selected for testing. Examples of enveloped viral families include members of the Coronaviridae family (e.g., coronavirus), Flaviviridae family (e.g., hepatitis C virus), Herpesviridae family (e.g., herpes virus), Poxviridae family (e.g., vaccinia), Hepadnaviridae family (e.g., hepatitis B virus), Orthomyxoviridae family (e.g., Influenza), Paramyxoviridae family (e.g., parainfluenza) and Retroviridae family (e.g., human immunodeficiency virus).

(B) Ten carriers for each of two samples, representing two batches of disinfectant, should be tested against a recoverable dried virus end point titer of  $\geq 10^4$  viral particles from the test

surface for a specified exposure period at room temperature. Instead of spraying the inoculated surface of the carrier, the product should be tested by wiping the surface of the carrier with the saturated towelette, and then subculturing the carriers after the specified holding time. One towelette should be used to wipe a minimum of 10 inoculated carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch.

The protocol for the viral assay should provide the information identified in paragraphs (f)(5)(ii)(B)(1) through (f)(5)(ii)(B)(7) of this guideline:

(1) The virus recovery (titer) should include a minimum of four determinations for each dilution in the assay system (e.g., cell culture, embryonated egg, animal infection).

(2) Cytotoxicity controls. The effect of the test substance on the viral assay system should include a minimum of four determinations for each dilution.

(3) The activity of the test substance against the test virus should include a minimum of four determinations for each dilution in the assay system.

(4) Neutralization controls. Neutralization controls should be performed (Ref. 7) and should include a minimum of four determinations per each dilution.

(5) Any special methods which are used to increase the virus titer and to detoxify the residual test substance should be described.

(6) The LD<sub>50</sub> values calculated for each assay should be provided.

(7) The test results should be reported as the reduction of the virus titer by the activity of the test substance (LD<sub>50</sub> of the virus control less the LD<sub>50</sub> of the test system) expressed as the logarithm to the base 10 and calculated by a statistical method (e.g., Reed and Munch, Most Probable Number, Spearman-Kärber).

(C) Evaluation of virucidal success. The product should demonstrate complete inactivation of the virus at all dilutions. If cytotoxicity is present, a  $\geq 3$ -log reduction in viral titer should be demonstrated beyond the cytotoxic level recovered from the carrier surface.

**(g) Disinfectants with tuberculocidal claims.** This section addresses efficacy testing for broad-spectrum or hospital disinfectant products which bear label claims of effectiveness as tuberculocides. In the Agency's "Data Call-In Notice for Tuberculocidal Effectiveness for All Antimicrobial Pesticides with Tuberculocidal Claims," dated June 13, 1986 (Ref. 8), applicants were given the option of choosing from one of three test methods (AOAC Tuberculocidal Activity of Disinfectants test, a modified AOAC Tuberculocidal Activity of Disinfectants test, or the Quantitative Tuberculocidal Activity Test) for conducting tuberculocidal efficacy tests. In general, the Agency does not believe that the Quantitative Tuberculocidal Activity Test (a

suspension test) is appropriate for disinfectant formulations used on hard surfaces. An exception to this is for glutaraldehyde-based products, which have never been validated in the AOAC Tuberculocidal Activity of Disinfectants test (a carrier based test). Therefore, the Quantitative Tuberculocidal Activity Test should only be used for glutaraldehyde-based products. The Agency strongly recommends all other formulations to use the carrier-based AOAC Tuberculocidal Activity of Disinfectants test.

**(1) Water-soluble powders and non-volatile liquid products test procedures.** The Agency recommends the test procedures in paragraphs (g)(1)(i) through (g)(1)(iv) of this guideline.

(i) AOAC International Tuberculocidal Activity of Disinfectants test. The AOAC International Tuberculocidal Activity of Disinfectants test (Ref. 9) employing a 10 minute contact time and 20°C temperature. Ten carriers for each of two samples representing two different batches of the product should be tested against *Mycobacterium bovis* (BCG)(*M.bovis*). If the product is intended to be represented as tuberculocidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(ii) AOAC International Tuberculocidal Activity of Disinfectants test with modifications. The AOAC International Tuberculocidal Activity of Disinfectants test with modifications to the 10 minute contact time and/or 20°C temperature (Ref. 9). Ten carriers for each of two samples representing two different batches of the product should be tested against *M. bovis* (BCG). If the product is intended to be represented as tuberculocidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(iii) Evaluation of tuberculocide success. For the AOAC International Tuberculocidal Activity of Disinfectants test, all organisms on all carriers should be killed, and there should be no growth in any of the inoculated subculture media.

(iv) Validation testing for Quaternary Ammonium Compounds. Products formulated solely with quaternary ammonium compounds as the active ingredient(s) should be supported with validation testing to confirm their tuberculocidal label claim. One additional product sample should be tested in a different laboratory from the original one, or in the same laboratory using different study director, technical staff and quality assurance unit, using the same test procedure and conditions as used in the first laboratory test.

## **(2) Glutaraldehyde formulations**

(i) Test Procedure. For glutaraldehyde formulations, the Agency recommends the Quantitative Tuberculocidal Activity Test. This test has been published in the Agency's "Data Call-In Notice for Tuberculocidal Effectiveness for All Antimicrobial Pesticides with Tuberculocidal Claims," dated June 13, 1986 (Ref. 8). Two samples, representing two different batches of the product should each be utilized in at least four separate studies (a total of at least

eight studies), against *M. bovis*, so that upper 95 percent confidence limits can be determined for each point on the survival curve. If the product is intended to be represented as tuberculocidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(ii) Evaluation of tuberculocide success. For the Quantitative Tuberculocidal Activity Method, survival curves should be constructed from the average of four separate replicates so that the upper 95% Confidence Limit can be determined for each point on the curve. The minimum time claimed for efficacy is determined by finding the point where the average survival curve intersects the probability of one survivor. If the data show a four-log reduction, but the survivor curve does not intersect the one-survivor line, the minimal time is found by extrapolating the upper 95% confidence limit curve such that the value where it intersects the one survivor line is not 50% greater than when the survivor curve intersects the one survivor line.

**(3) Germicidal spray products and volatile liquid products—**(i) Test procedure. The Agency recommends the AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3), using the media, test culture, and other elements described in the AOAC International Tuberculocidal Activity of Disinfectants test. Ten carriers for each of two samples representing two different batches of the product should be tested against *M. bovis* (BCG). If the product is intended to be represented as tuberculocidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(ii) Evaluation of tuberculocide success. When using the AOAC International Germicidal Spray Products as Disinfectants test, all organisms on all carriers/slides should be killed, and there should be no growth in any of the inoculated subculture media.

#### **(4) Single-Use Towelettes**

(i) Test Procedure. The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test (Ref.3) or ASTM 2362 (Ref. 4). Ten carriers for each of two samples representing two different batches of the product should be evaluated against *M. bovis* (BCG). If the product is intended to be represented as tuberculocidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. Instead of spraying the inoculated surface of the carrier, the product should be tested by wiping the surface of the carrier with the saturated towelette, and then subculturing the carriers after the specified holding time. The towelette should be removed from its container and handled with sterile gloves. One towelette should be used to wipe a minimum of 10 inoculated carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of slides. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report.



(ii) Evaluation of tuberculocidal towelette success. All organisms on all carriers should be killed, and there should be no growth in any of the inoculated subculture media.

**(5) Bridging for disinfectant towelettes.** In some cases, disinfectant towelette formulations are identical to registered liquid formulations. In order to bridge efficacy data from the EPA registered bulk liquid disinfectant used to saturate a towelette or other related product form, the studies in paragraphs (g)(5)(i) and (g)(5)(ii) of this guideline should be conducted and submitted to EPA for review.

(i) Chemical Testing - Comparison of Expressed Liquid from the Towelette(s) to the EPA Registered Liquid Disinfectant Formulation to which it is being bridged: All active ingredients in the expressed liquid should be within the certified limits of the Confidential Statement of Formula of the liquid formula being referenced/bridged. The disinfectant towelettes package should be filled according to the manufacturing specifications. Excess liquid in the bulk towelette containers cannot be poured off for use in the chemical testing for bridging of the efficacy data. The liquid used in the chemical testing should only be that expressed from the towelettes. Three batches (one of which is 60 days old) should be tested. Analytical data for the active ingredients in the expressed liquid should be submitted for review.

(ii) Efficacy Testing: Efficacy testing should be conducted under the same testing conditions (e.g. soil load, contact time, temperature) as used for the bulk liquid testing. This testing allows bridging of data from the registered bulk liquid used to saturate the towel for each type of organism in paragraph (g)(5)(ii)(A) of this guideline. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report.

(A) Test Procedure. The Agency recommends the use of the modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3) or ASTM 2362 (Ref. 4). The test should be modified to conform to appropriate elements (e.g., media, growth conditions, etc) in the AOAC International Tuberculocidal Activity of Disinfectants test. Ten carriers for each of two samples, representing two batches of the product, should be tested against *M. bovis* BCG. Instead of spraying the inoculated surface of the carrier, the product should be tested by wiping the surface of the carrier with the saturated towelette, and then subculturing the carriers after the specified holding time. One towelette should be used to wipe a minimum of 10 inoculated carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch.

(B) Evaluation of tuberculocidal towelette success. All organisms on all carriers should be killed, and there should be no growth in any of the inoculated subculture media.

(C) This testing is intended to support bridging of all mycobacteria listed on the EPA registered liquid disinfectant used to saturate the towelette to the EPA registered towelette product.

**(h) Data collection and reporting—(1) General.** To assist in the proper review and

evaluation of product performance, complete descriptions of the test employed and the results obtained should be submitted to the Agency. All test reports should include, at the least, the following information:

- (i) Study title;
- (ii) Product Identity;
- (iii) Guideline number/Data Requirement;
- (iv) Identification of the testing laboratory or organization;
- (v) Location where the test was performed;
- (vi) Name(s) of the person(s) responsible for the test;
- (vii) Statement of Confidentiality Claims;
- (viii) Statement of 40 CFR Part 160 Good Laboratory Practice compliance and Quality Assurance Statement;
- (ix) Purpose of the study;
- (x) Date and time of the start and end of the test;
- (xi) Test employed and any modifications (e.g., organic soil, hard water, etc.), when using standard tests (e.g., AOAC, ASTM, etc.) all deviations to the test methods should be reported;
- (xii) Test microorganisms employed, including identification of the specific strain (ATCC or other);
- (xiii) Description of the test substance, including the percent of active ingredient;
- (xiv) Concentration or dilution of the product tested and how prepared;
- (xv) Number of samples, batches and replicates tested;
- (xvi) Manufacture date of each product batch;
- (xvii) Identification of all material or procedural options employed, where such choice is provided for or recommended in the test method selected (e.g., growth media, drying time for inoculated carriers, neutralization confirmation and/or subculture media, secondary subculturing);

- (xviii) Test exposure conditions (e.g., contact time, temperature, and relative humidity);
- (xix) Complete reports of results obtained for each replication;
- (xx) Any control data essential to establish the validity of the test.
- (xxi) Carrier counts;
- (xxii) Statistical treatment of the data;
- (xxiii) Conclusions;
- (xxiv) References;
- (xxv) Appendices, including study protocol and all raw data reports associated with the conduct of the study.

The applicant is encouraged to use the EPA's standard efficacy report format, which may be found at <http://www.epa.gov/oppad001/efficacystudystandards.htm>.

**(2) Data for modifications of recommended methods.** When recommended methods are modified to support specific claims and/or use patterns for a product, the protocol, identifying and describing each modification, should be provided with the study report. The applicant should submit the proposed modification to the Agency for review and evaluation prior to initiation of the test.

**(3) Data for other methods.** When recommended methods, or modifications thereto, are not employed to develop efficacy data (such as actual in-use or many kinds of simulated-use testing), complete testing protocols should be submitted with the test reports. All materials and procedures employed in testing should be described in a manner consistent with original research reports published in technical or scientific journals. Where references to published reports or papers are made, copies or reprints of such references should be provided with the test reports. The applicant should submit the proposed testing protocols for in-use or simulated-use studies (with a proposed label to show the claims to be supported by the protocol) to the Agency for review and evaluation prior to initiation of the test.

(i) References. The references in this paragraph may be consulted for additional background information:

(1) *Official Methods of Analysis of the AOAC International*, Chapter 6, Disinfectants, Use-Dilution Methods (955.14, 955.15, & 964.02), Current edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(2) *Official Methods of Analysis of the AOAC International*, Chapter 6, Disinfectants, Hard Surface Carrier Test Methods, Current edition. AOAC International, Suite 500, 481 North

Frederick Avenue, Gaithersburg, MD 20877-2417.

(3) *Official Methods of Analysis of the AOAC International*, Chapter 6, Disinfectants, Official Method 961.02 Germicidal Spray Products as Disinfectants, Current edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(4) *Annual Book of ASTM Standards*, Standard Practice for Evaluation of Pre-saturated or Impregnated Towelettes for Hard Surface Disinfection, Designation E2362. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428, current edition.

(5) *Official Methods of Analysis of the AOAC International*, Chapter 6, Disinfectants, Official Method 955.17 Fungicidal Activity of Disinfectants. Current edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(6) *Annual Book of ASTM Standards*, Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces, Designation E1053. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428, current edition.

(7) *Annual Book of ASTM Standards*, Standard Test Method for Neutralization of Virucidal Agents in Virucidal Efficacy Evaluations, Designation E1483. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428, current edition.

(8) *Official Methods of Analysis of the AOAC International*, Chapter 6, Disinfectants, Official Method 965.12 Tuberculocidal Activity of Disinfectants. Current edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(9) Environmental Protection Agency, Data Call-in Notice for Tuberculocidal Effectiveness Data for All Antimicrobial Pesticides with Tuberculocidal Claims (Registration Division, Office of Pesticide Programs, June 13, 1986).