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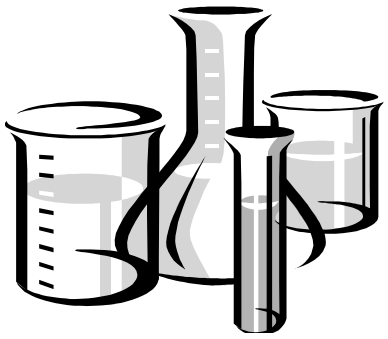
Product Performance Test Guidelines

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 United States Environmental Protection Agency's Office of Chemical Safety and Pollution Prevention (OCSPP) 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 Act (FFDCA) (21 U.S.C. 346a). Prior to April 22, 2010, OCSPP was known as the Office of Prevention, Pesticides and Toxic Substances (OPPTS). To distinguish these guidelines from guidelines issued by other organizations, the numbering convention adopted in 1994 specifically included OPPTS as part of the guideline's number. Any test guideline developed after April 22, 2010 will use the new acronym (OCSPP) in their title.

The OCSPP test guidelines serve as a compendium of accepted standardized 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 the test, and is also used by EPA, the public, and companies that are subject to data submission requirements under TSCA, FIFRA and/or the FFDCA. 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. 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. The procedures 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 recommendations described in these guidelines, and the Agency will assess them for appropriateness on a case-by-case basis.

For additional information about these test guidelines and to access the guidelines electronically, please go to http://www.epa.gov/ocspp and select the topic entitled, "Test Methods and Guidelines." 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.

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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 U.S.C. 346a). It addresses testing to demonstrate the effectiveness of antimicrobial pesticides bearing claims as disinfectants, fungicides, virucides, and tuberculocides. Refer to OCSPP 810.2000: General Considerations for Public Health Uses of Antimicrobial Agents for guidance.

(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) Efficacy claims. This guideline provides guidance for developing data solely for the purpose of supporting the following claims for hard surface disinfectants (limited spectrum disinfectants, general or broad spectrum disinfectants, hospital or healthcare disinfectants) and additional claims (internal toilet and urinal bowl surface disinfectants, and disinfectants with fungicidal, virucidal, and/or tuberculocidal claims). Refer to OCSPP Test Guideline 810.2000 for general testing recommendations prior to testing. See Tables 1 and 2 for a summary of the efficacy claims and recommendations for testing.

(2) Test Methods. Refer to Tables 1 and 2 for information on efficacy test methods recommended to support disinfectant and additional label claims, respectively. The Agency recommends the use of the most recently published version of the standard methods (e.g., AOAC International [AOAC], ASTM). See standard methods for control carrier counts (mean number of viable microbes per carrier) and other method associated information. Agency Standard Operating Procedures for many of the test procedures referenced in the tables are available online at: http://www.epa.gov/pesticides/methods/atmpa2z.htm. Information regarding specifics such as the number of batches to test, performance standards, and control carrier counts is provided in the description for each type of label claim.

(3) **Spray products.** Spray products referenced in Table 1 include aerosol, trigger, and pump dispensers.

(4) **Towelette products.** For products formulated as impregnated wipes or towelettes, refer to Table 1. For evaluation of efficacy, the Agency recommends the use of a modified AOAC Method 961.02 (Germicidal Spray Products as Disinfectants test) or ASTM E2362 (Standard Practice for Evaluation of Pre-saturated or Impregnated Towelettes for Hard Surface Disinfection). 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 sub-culturing 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 10 inoculated carriers (e.g., use 1 towelette for 10 carriers or 6 towelettes for 60 carriers). For each carrier, an unused area of the towelette should be used for wiping. Also, see section (j) of this document on bridging for cases where an applicant formulates a disinfectant towelette using an existing EPA-registered bulk liquid formulation. 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.

(5) **Product batches.** Refer to the claim-specific sections below to identify the type and number of batches to be evaluated for efficacy. Depending on the claim, testing will be conducted on batches (two or three) at or below the lower certified limit(s) (LCL) listed on the confidential statement of formula (CSF) of the product, or at the nominal concentration.

(6) **Soil load.** If a product is intended to be effective in the presence of organic soil (i.e., a one-step disinfectant product), an appropriate organic soil, such as 5 percent blood serum, should be added to the inoculum prior to carrier inoculation.

(7) **Neutralization confirmation.** Testing to verify the effectiveness of the neutralizer should be conducted in advance or concurrently with the efficacy test. The procedure should include conditions relevant to the efficacy test (e.g., growth medium, product dilution and contact time, neutralizer, and microbe).

(8) **Confirmatory data.** Refer to OCSPP 810.2000: General Considerations for Public Health Uses of Antimicrobial Agents, section (b)(7) for situations (i.e., duplicated product formulations and minor formulation changes in a registered product) requiring confirmatory data. Specific information related to confirmatory data is provided in the description for each label claim. 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.

(9) **Additional microorganisms.** For efficacy testing requirements related to broad-

spectrum or hospital disinfectants which bear label claims against bacteria other than *Salmonella enterica* (ATCC10708), *Staphylococcus aureus* (ATCC 6538), or *Pseudomonas aeruginosa* (ATCC 15442), see section (d)(6).

(10) **Contact Time.** All products should meet the performance standard associated with the method and microbe at ≤10 minutes of contact.

Table 1. Summary of Testing for Disinfectant Claims

Claim	Formulation/Test Methods		Test Organisms	No. of Batches/Carriers
Limited spectrum disinfectant/hard non-porous surfaces	Water soluble powders/liquids	AOAC Use-Dilution Method (ref. 1)	*Staphylococcus aureus* (ATCC 6538) or *Salmonella enterica* (ATCC 10708)	Three batches at LCL; 60 carriers against either organism (180 total carriers)
	Spray products	AOAC Germicidal Spray Products as Disinfectants Test (ref. 2)		
	Towelettes	AOAC Germicidal Spray Products as Disinfectants Test modified for towelettes or ASTM E2362 (ref. 3)		
Broad-spectrum disinfectant/hard non-porous surfaces	Water soluble powders/liquids	AOAC Use-Dilution Method	*Staphylococcus aureus* (ATCC 6538) and *Salmonella enterica* (ATCC 10708)	Three batches at LCL; 60 carriers against each organism (360 total carriers)
	Spray products	AOAC Germicidal Spray Products as Disinfectants Test		
	Towelettes	AOAC Germicidal Spray Products as Disinfectants Test modified for towelettes or ASTM E2362		
Hospital or healthcare disinfectant/hard non-porous surfaces	Water soluble powders/liquids	AOAC Use-Dilution Method	*Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 15442)	Three batches at LCL:-60 carriers against each organism (360 total carriers)
	Spray products	AOAC Germicidal Spray Products as Disinfectants Test		
	Towelettes	AOAC Germicidal Spray Products as Disinfectants Test modified for towelettes or ASTM E2362		

Table 2. Summary of Testing for Additional Label Claims

Claim	Formulation/Test Methods		Test Organisms	No. of Batches/Carriers
Fungicidal disinfectant/hard non-porous surfaces	Water soluble powders/liquids	AOAC Use-Dilution Test modified for fungi or AOAC Fungicidal Test (ref. 4)	*Trichophyton mentag*rophytes (ATCC 9533)	Two batches at the LCL; 10 carriers per batch (20 total carriers) for carrier-based methods. Two batches for the AOAC Fungicidal Test.
	Spray products	AOAC Germicidal Spray Products as Disinfectants Test modified for fungi		
	Towelettes	AOAC Germicidal Spray Products as Disinfectants Test modified for towelettes or ASTM E2362		
Virucidal disinfectant/hard non-porous surfaces	Water soluble powders/liquids	ASTM E1053 (ref. 5)	Virus claimed on the label or approved surrogate	Two batches at the nominal concentration; 10 carriers per batch. For non-surrogates, test one surface per batch; for surrogates, test two surfaces per batch.
	Towelettes	AOAC Germicidal Spray Products as Disinfectants Test modified for towelettes or ASTM E2362		
Tuberculocidal disinfectant/hard non-porous surfaces.	Water soluble powders/liquids	AOAC Tuberculocidal Activity of Disinfectants (ref. 8), Quantitative Tuberculocidal Activity Test (ref. 9)	*Mycobacterium bovis* BCG	Two batches at the LCL; 10 carriers per batch.
	Spray products	AOAC Germicidal Spray Products Test modified for tuberculocidal activity		Two batches at the LCL; 10 carriers per batch
	Towelettes	AOAC Germicidal Spray Products as Disinfectants Test modified for towelettes or ASTM E2362		Two batches at the LCL; 10 carriers per batch
Additional bacteria /hard non-porous surfaces.	Water soluble powders/liquids	AOAC Use-Dilution Test	Additional bacteria claimed on the label	Two batches at the nominal concentration; 10 carriers for each batch.
Internal toilet and urinal bowl surfaces above and below the water line	Water-soluble powders and non-volatile liquid products	AOAC Use-Dilution Test modified to include a 5% organic soil challenge added to the bacterial inoculum.	*S. enterica* (ATCC 10708) or *S. aureus* (ATCC 6538) for limited disinfectant products*; S. enterica* and *S. aureus* for broad-spectrum disinfectant products; *S. aureus* and *P. aeruginosa* (ATCC 15442) for	Test sixty carriers against each of three batches of the product at the LCL

			hospital disinfectants	

(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). Refer to Table 1 for test methods. Conduct the following:

(i) **Test organism.** To demonstrate efficacy, testing should be conducted 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.

(ii) **Batches/number of carriers.** Test three batches of the product at the LCL. For each batch, test using sixty carriers.

(iii) **Control carrier counts for AOAC Use-Dilution Methods (ref. 1).** For the AOAC Use-Dilution Methods, the mean log density for *S. enterica* is to be at least 5.0 (corresponding to a geometric mean density of 1.0×10^5) and not above 6.0 (corresponding to a geometric mean density of 1.0×10^6). A mean log density <5.0 or >6.0 invalidates the test. The mean log density for *S. aureus* is to be at least 6.0 (corresponding to a geometric mean density of 1.0×10^6) and not above 7.0 (corresponding to a geometric mean density of 1.0×10^7). A mean log density <6.0 or >7.0 invalidates the test.

(iv) **Control carrier counts for spray products and single-use towelettes.** The mean log density for *S. enterica* is to be at least 4.0 (corresponding to a geometric mean density of 1.0×10^4) and not above 5.5 (corresponding to a geometric mean density of 3.2×10^5). A mean log density <4.0 or >5.5 invalidates the test. The mean log density for *S. aureus* is to be at least 5.0 (corresponding to a geometric mean density of 1.0×10^5) and not above 6.5 (corresponding to a geometric mean density of 3.2×10^6). A mean log density <5.0 or >6.5 invalidates the test.

(v) **Performance measure.**

(A) For the AOAC Use-Dilution Methods, conduct three independent tests (i.e., three batches tested once on three different test days) against the test microbe at the LCL. The performance standard for *S. aureus* is 0-3 positive carriers out of sixty. The performance standard for *S. enterica* is 0-1 positive carriers out of sixty.

Contamination of one carrier (culture tube) is allowed per 60-carrier set; occurrence of more than one contaminated carrier invalidates the test results.

(B) For the AOAC Germicidal Spray Products as Disinfectants test and towelette methods, the product should kill the test microorganisms on 59 out of each set of 60 carriers/slides. Contamination of only one carrier (culture tube) is allowed per 60-carrier set; occurrence of more than one contaminated carrier invalidates the test results.

(vi) **Confirmatory testing for limited spectrum products.** For confirmatory testing, follow the above testing guidance in sections (c) and (d)(1), with the exception that ten carriers for each of two different batches of product should be tested against *S. aureus* (ATCC 6538) or *S. enterica* (ATCC 10708). For all methods, the product should kill all the test microorganisms on all carriers. Control carrier counts specifications should be met. For a valid test, no contamination of any carrier is allowed.

(2) **General or broad spectrum efficacy products**. For disinfectants with label claims against both Gram-negative and Gram-positive bacteria, the product is considered a general or broad spectrum disinfectant. Refer to Table 1 for test methods. Conduct the following:

(i) **Test organism**. To demonstrate efficacy, testing should be conducted against both *S. enterica* (ATCC 10708) and *S. aureus* (ATCC 6538.

(ii) **Batches/number of carriers.** For each organism, test sixty carriers against each of three batches of the product at the LCL.

(iii) **Control carrier counts for AOAC Use-Dilution Methods.** For the AOAC Use-Dilution Methods, the mean log density for *S. enterica* is to be at least 5.0 (corresponding to a geometric mean density of 1.0×10^5) and not above 6.0 (corresponding to a geometric mean density of 1.0×10^6). A mean log density <5.0 or >6.0 invalidates the test. The mean log density for *S. aureus* is to be at least 6.0 (corresponding to a geometric mean density of 1.0×10^6) and not above 7.0 (corresponding to a geometric mean density of 1.0×10^7). A mean log density <6.0 or >7.0 invalidates the test.

(iv) **Control carrier counts for spray products and towelettes.** The mean log density for *S. enterica* is to be at least 4.0 (corresponding to a geometric mean density of 1.0×10^4) and not above 5.5 (corresponding to a geometric mean density of 3.2×10^5). A mean log density <4.0 or >5.5 invalidates the test. The mean log density for *S. aureus* is to be at least 5.0 (corresponding to a geometric mean density of 1.0×10^5) and not above

6.5 (corresponding to a geometric mean density of 3.2 x 10^6). A mean log density <5.0 or >6.5 invalidates the test.

(v) **Performance measure.**

(A) For the AOAC Use-Dilution Methods, conduct three independent tests (i.e., three batches tested at the LCL on three different test days) against the test microbe. The performance standard for *S. aureus* is 0-3 positive carriers out of sixty. The performance standard for *S. enterica* is 0-1 positive carriers out of sixty. Contamination of only one carrier (culture tube) is allowed per 60-carrier set; occurrence of more than one contaminated carrier invalidates the test results.

(B) For the AOAC Germicidal Spray Products as Disinfectants test and towelette methods, the product should kill the test microorganisms on 59 out of each set of 60 carriers/slides. Contamination of only one carrier (culture tube) is allowed per 60-carrier set; occurrence of more than one contaminated carrier invalidates the test results.

(vi) **Confirmatory testing for general or broad spectrum products.** For confirmatory testing, follow the above testing guidance in sections (c) and (d) (2), with the exception that ten carriers for each of two different batches of product (within the CSF) should be tested against *S. aureus* (ATCC 6538) and *S. enterica* (ATCC 10708). For all methods, the product should kill all the test microorganisms on all carriers. Control carrier counts specifications should be met. For a valid test, no contamination of any carrier is allowed.

(3) 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. Refer to Table 1 for test methods. Conduct the following:

(i) **Test organism.** To demonstrate efficacy, testing should be conducted against *S. aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 15442).

(ii) **Batches/number of carriers.** For each organism, test sixty carriers against each of three batches of the product at the LCL.

(iii) **Control carrier counts for AOAC Use-Dilution Methods.** For the Use-Dilution Methods, 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 x 10^6) and not above 7.0 (corresponding to a geometric mean density of 1.0 x 10^7). A mean log density <6.0 or >7.0 invalidates the test.

(iv) **Control carrier counts for spray products and towelettes.** The mean log density for both *S. aureus* and *P. aeruginosa* is to be at least 5.0 (corresponding to a geometric mean density of 1.0×10^5) and not above 6.5 (corresponding to a geometric mean density of 3.2×10^6). A mean log density <5.0 or >6.5 invalidates the test.

(v) **Performance measure.**

(A) For the AOAC Use-Dilution Methods, conduct three independent tests (i.e., three batches at the LCL tested on three different test days) against the test microbe. The performance standard for *S. aureus* is 0-3 positive carriers out of sixty. The performance standard for *P. aeruginosa* is 0-6 positive carriers out of sixty. Contamination of only one carrier (culture tube) is allowed per 60-carrier set; occurrence of more than one contaminated carrier invalidates the test results.

(B) For the AOAC Germicidal Spray Products as Disinfectants test and towelette methods, the product should kill the test microorganisms on 59 out of each set of 60 carriers/slides. Contamination of only one carrier (culture tube) is allowed per 60-carrier set; occurrence of more than one contaminated carrier invalidates the test results.

(vi) **Confirmatory testing for products with hospital or healthcare disinfectant claim.** For confirmatory testing, follow the above testing guidance in sections (c) and (d) (3), with the exception that ten carriers for each of two different batches of product (within the CSF) should be tested against *S. aureus* (ATCC 6538) and *P. aeruginosa* (ATCC 15442). For all methods, the product should kill all the test microorganisms on all carriers. Control carrier counts specifications should be met. For a valid test, no contamination of any carrier is allowed.

(4) Disinfectants for internal toilet and urinal bowl surfaces above and below the water line. To add claims to existing limited, broad-spectrum, or hospital products for internal toilet and urinal bowl surfaces, conduct the following:

(i) **Test method.** Regarding water-soluble powders and non-volatile liquid products test procedure, the Agency recommends the use of the AOAC Use-Dilution Methods modified to include a 5% organic soil challenge added to the bacterial inoculum.

(A) The contained bowl water (96 fl oz of 200-400 ppm hard water, 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 using 200-400 ppm hard water for testing.

(ii) **Test organisms.** Test against *S. enterica* (ATCC 10708) or *S. aureus* (ATCC 6538), for limited disinfectant products; *S. enterica* and *S. aureus*, for broad-spectrum disinfectant products; and *S. aureus* and *P. aeruginosa* (ATCC 15442), for hospital disinfectant products. Follow the testing recommendations (i.e., control carrier counts, and performance measure) specified for limited, broad-spectrum, and hospital disinfectant claims.

(iii) **Batches/number of carriers.** Test sixty carriers against each of three batches of the product at the LCL.

(iv) **Performance Measure.** Meet the performance measures specified for each claim (e.g., hospital claim) and specified microbe(s).

(5) Additional microorganisms. Label claims for additional microbes other than the designated test microorganism(s) is permitted, provided that the target microbe is likely to be present in or on the recommended use areas and surfaces. This section addresses efficacy testing for limited, broad-spectrum or hospital disinfectants which bear label claims against bacteria other than *S. enterica* (ATCC 10708), *S. aureus* (ATCC 6538) or *P. aeruginosa* (ATCC 15442). Following recommendations for test methods, per the product formulation as provided in Table 1, conduct additional testing as described below:

(i) **Test organism**. To demonstrate efficacy, testing should be conducted against each specific bacterium to be claimed on the label.

(ii) **Batches/number of carriers**. Test ten carriers against each of two batches of the product at the nominal concentration.

(iii) **Control carrier counts for all methods.** The minimum carrier count for a valid test should be a mean of 1×10^4 CFU/carrier.

(iv) **Performance measure.** The product should kill all the test microorganisms on all ten carriers. For a valid test, no contamination of any carrier is allowed.

(e) Fungicidal claims. A fungicidal claim may be added to an existing broad-spectrum or hospital disinfectant product. Refer to Table 2 for test methods. Conduct the following:

(1) Test organism. To demonstrate efficacy, testing should be conducted against *Trichophyton mentagrophytes* (ATCC 9533).

(2) Batches/number of carriers.

(i) **For AOAC Fungicidal Activity of Disinfectants Test**. Two batches of product at the LCL should be evaluated for efficacy at 5, 10, and 15 minute exposure times. The method is a suspension test.

(ii) **For AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Test, and towelettes.** Tests may be modified to conform to appropriate elements (e.g., media, growth conditions) in the AOAC Fungicidal Activity of Disinfectants test. Test ten carriers against each of two batches of product at the LCL.

(3) Titer of conidial suspension/control counts.

(i) **AOAC Fungicidal Activity of Disinfectants test**. The inoculum employed should provide a concentration of $\geq 5 \times 10^6$ conidia/mL.

(ii) **AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Test, and single-use towelettes**. The inoculum employed should provide a concentration of $1 \times 10^4 - 1 \times 10^5$ conidia per carrier.

(4) Performance measure.

(i) **AOAC International Fungicidal Activity of Disinfectants test**. All fungal spores at 10 and 15 minutes should be killed (no positive carriers).

(ii) **AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Test, and towelettes**. All fungal spores on all 10 carriers should be killed (no positive carriers).

(f) Virucidal claims. Virucidal claims may be added to existing broad-spectrum or hospital disinfectant products (liquids, sprays, towelettes). Virucidal products are intended for use on dry inanimate surfaces; therefore, data are developed using carrier-based methods. To simulate in-use conditions, the specific virus to be treated (or surrogate) 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. Refer to Table 2 for test methods. Conduct the following:

(1) Test organism. Each specific virus listed on the label should be tested, unless there is an acceptable surrogate for the virus. For example, 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 considered acceptable surrogates for testing. Additional guidance and protocols for surrogate virus testing can be found at http://www.epa.gov/oppad001/regpolicy.htm.

(2) In limited situations such as towelette bridging and confirmatory testing, the Agency will allow testing of the most resistant virus on the label based on disinfection hierarchy. The categories and the presumed levels of resistance are provided below:

(i) **Small non-enveloped viruses** (most resistant viruses). For label claims to inactivate small non-enveloped viruses such as members of the Picornaviridae family (e.g., poliovirus, enterovirus, hepatitis A virus, rhinovirus), and the Caliciviridae family (e.g., parvovirus) select the most resistant representative virus (or acceptable surrogate) such as Feline calicivirus for testing.

(ii) **Large non-enveloped viruses** (intermediate resistant viruses). For label claims to inactivate large non-enveloped viruses such as members of the members of the Adenoviridae family (e.g., adenovirus), Reoviridae family (e.g., rotavirus), and Papillomaviridae family (e.g., papillomavirus), select the most resistant representative virus (or acceptable surrogate) such as Adenovirus for testing.

(iii) **Enveloped viruses** (least resistant viruses). For label claims to inactivate enveloped viruses such as 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), select the most resistant representative virus (or acceptable surrogate) for testing.

(3) **Soil load**. 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.

(4) **Number of batches**. Test two batches of product at the nominal concentration.

(5) **Surfaces.** For non-surrogates, test one surface per batch; for surrogates, test two surfaces per batch.

(6) **Viral concentration**. Test each batch against a recoverable virus end point titer of $\geq 10^4$ viable viral particles from the test surface for a specified exposure period.

(7) **Performance measure**. 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 below.

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

(ii) Cytotoxicity control. The effect of the disinfectant (with addition of the neutralizer) on the viral assay system should include a minimum of four determinations (i.e., four replications) per each dilution. Note: Level of cytotoxicity may be determined in advance or for one test and is not required to be performed with each test.

(iii) The activity of the disinfectant (treated carriers) against the test virus should include a minimum of four determinations (i.e., four replications) per dilution in the assay system.

(iv) Neutralization control. Neutralization controls should be performed (Ref. 6) and should include a minimum of four determinations (i.e., four replications) per each dilution. The neutralizer control may be determined in advance or for one test and is not required to be performed with each test.

(v) Any special methods (e.g., use of columns) which are used to increase the virus titer and to detoxify the residual disinfectant should be described in the protocol.

(vi) The ID_{50} values (control carrier counts) calculated for each assay should be provided.

(vii) The test results should be reported as the log reduction of the virus (ID_{50} of the virus control carriers less the ID_{50} of the test carriers) as calculated by an appropriate statistical method (e.g., Reed and Munch, Most Probable Number, Spearman-Karber).

(viii) The product should demonstrate complete inactivation of the virus at all dilutions.

(ix) If cytotoxicity is present, the virus control titer should be increased to demonstrate a ≥ 3 $\log_{10}$ reduction in viral titer beyond the cytotoxic level.

(x) A laboratory report of a single test with one virus involving a tissue culture assay system should include the details of the methods employed; see examples of information in Tables 2-1, 2-2 and 2-3:

(A) **Performance measure.** The product should demonstrate complete inactivation of the virus at all dilutions. If cytotoxicity is present, a ≥ 3-log reduction in viral titer should be demonstrated beyond the cytotoxic level recovered from the carrier surface.

Table 2-1: Example of Virucidal Test Results for One Test

Dilution of Virus	Virus - Disinfectant* (treated carriers)	Virus - Control* (control carriers)	Cytotoxicity – Control (disinfectant and neutralizer without virus)
10^{-1}	T T T T	+ + + +	T T T T
10^{-2}	T T T T	+ + + +	T T T T
10^{-3}	T 0 0 0	+ + + +	T 0 0 0
10^{-4}	0 0 0 0	+ + + +	0 0 0 0
10^{-5}	0 0 0 0	+ + + +	0 0 0 0
10^{-6}	0 0 0 0	+ + + 0	0 0 0 0
10^{-7}	0 0 0 0	+ 0 0 0	0 0 0 0
10^{-8}	0 0 0 0	0 0 0 0	0 0 0 0

Note: T = toxic to cell line; + = virus recovered; 0 = no virus recovered
*Recovery of virus from surfaces.

Table 2-2: Example Calculation (Spearman-Karber) of the Tissue Culture Infective Dose 50 ($TCID_{50}$) – for the Control Counts

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^{-1}	4/4	4	0	24	0	24/24	100
10^{-2}	4/4	4	0	20	0	20/20	100
10^{-3}	4/4	4	0	16	0	16/16	100
10^{-4}	4/4	4	0	12	0	12/12	100
10^{-5}	4/4	4	0	8	0	8/8	100
10^{-6}	3/4	3	1	4	1	4/5	80
10^{-7}	1/4	1	3	1	4	1/5	20
10^{-8}	0/4	0	4	0	8	0/8	0

$TCID_{50} = 10^{6.5}$ (6.5 logs)

Table 2-3: Example Calculation (Spearman-Karber) of the Tissue Culture Lethal Dose 50 ($TCLD_{50}$) – for the Treated Carriers

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^{-1}	4/4	4	0	9	0	9/9	100
10^{-2}	4/4	4	0	5	0	5/5	100
10^{-3}	1/4	1	3	1	3	1/4	25
10^{-4}	0/4	0	4	0	7	0/7	0
10^{-5}	0/4	0	4	0	11	0/11	0
10^{-6}	0/4	0	4	0	15	0/15	0
10^{-7}	0/4	0	4	0	19	0/19	0
10^{-8}	0/4	0	4	0	23	0/23	0

$TCLD_{50} = 10^{2.7}$ Therefore: Virus inactivation = $TCID_{50}$ - $TCLD_{50} = 10^{3.8}$ $\log^{10}$ (a log reduction of 3.8)

(g) **Tuberculocidal claims**. Tuberculocidal claims may be added to broad-spectrum or hospital disinfectant products. In the Agency's "Data Call-In Notice for Tuberculocidal Effectiveness for All Antimicrobial Pesticides with Tuberculocidal Claims," dated June 13, 1986 (Ref. 7), 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. Based on experience since 1986, the Agency does not recommend the use of the Quantitative Tuberculocidal Activity Test (a suspension test) for testing disinfectant formulations for use on hard surfaces. An exception to this is for glutaraldehyde-based products, which have not 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 for all other liquid formulations, use of the carrier-based AOAC Tuberculocidal Activity of Disinfectants test. Refer to Table 2 for recommendations per formulation type. Conduct the following:

(1) **Test organism**. To demonstrate efficacy, testing should be conducted against *Mycobacterium bovis* (BCG) (*M. bovis*). Test cultures of *M. bovis* (BCG) may be grown using agitation; consult with EPA regarding the methodology for producing these cultures.

(2) **Batches/number of carriers**. Test ten carriers against each of two different batches of product at the LCL.

(3) **Control carrier counts for AOAC Tuberculocidal Activity of Disinfectants test and AOAC Tuberculocidal Activity of Disinfectants test with modifications**. Per the 2012 revisions for the AOAC Tuberculocidal Activity of Disinfectants test, the mean log density is to be at least 4.0 (corresponding to a geometric mean density of 1.0×10^4) and not above 6.0 (corresponding to a

geometric mean density of 1.0 x 10^6). A mean log density < 4.0 or > 6.0 invalidates the test.

(4) **Control carrier counts for AOAC Germicidal Spray Products as Disinfectants test and towelettes**. The log density of *M. bovis* should be ≥ 4.0 (corresponding to a geometric mean density of ≥1.0 x 10^4 CFU/carrier) a mean log density of <4.0 invalidates the test.

(i) Evaluate the product against *M. bovis* BCG for the label recommended contact time.

(5) **Performance Measure.** *Mycobacterium* on all carriers (in primary subculture medium) should be killed (no positive carriers), and there should be no growth in any of the associated subculture media. For a valid test, no contamination of any carrier or secondary subculture medium is allowed.

(6) The spray and towelette tests should be modified to conform to appropriate elements (e.g., media, growth conditions, etc.) as in the AOAC Tuberculocidal Activity of Disinfectants test.

(7) **Media Performance**. The performance of the subculture media should be confirmed in advance or concurrently with testing. The results should be submitted with the product performance data.

(h) Glutaraldehyde formulations

(1) **Test Procedure.** For glutaraldehyde formulations, the Agency recommends the Quantitative Tuberculocidal Activity Test. This test has been referenced in the Agency's "Data Call-In Notice for Tuberculocidal Effectiveness for All Antimicrobial Pesticides with Tuberculocidal Claims," dated June 13, 1986 **(**Ref. 7**).** 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.

(2) **Performance measure.** 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. The test substance should demonstrate $\geq$1.0 x 10^4 CFU kill of the test organism at the stated contact time (i.e., a $\geq$4 $\log_{10}$ reduction of the test organism).

(3) The organism titer should be $\geq$ 1 x 10^7 CFU/ml.

(i) 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.

(j) Bridging for EPA-registered liquids and disinfectant towelettes. In cases where an applicant formulates a disinfectant towelette using an existing EPA-registered bulk liquid formulation, the following chemistry and efficacy studies should be conducted and submitted to EPA for review.

(1) **Bridging: Chemical Analysis**.

(i) All active ingredients in the expressed liquid should evaluated for concentration and must be within the certified limits of the Confidential Statement of Formula of the liquid formula being referenced (bridged).

(ii) The disinfectant towelettes package should be filled according to the manufacturing specifications. Excess liquid in the bulk towelettes containers is not to be poured off for use in the chemical testing for data bridging, rather the liquid used in the chemical testing should only be that expressed from the towelettes.

(iii) Two batches (expressed liquid) of the towelette should be tested.

(iv) Analytical data for the active ingredients in the expressed liquid should be submitted for review.

(v) The means used to express the liquid should be included in the study protocol.

(2) **Bridging: Efficacy Testing**. Efficacy testing should be conducted under the same testing conditions (e.g. soil load, contact time, temperature) on two or three batches (depending on claim) as used for the bulk liquid testing. 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. All testing requirements for controls, control carrier counts and performance standards should be followed for each test method/microbe combination. For each type of claim, see specific details below:

(i) **Limited, broad-spectrum and hospital disinfectants**. The Agency recommends the use of the AOAC Germicidal Spray Products as Disinfectants test modified for towelettes, using the test organisms (and control count requirements for a valid test) specified for the primary claim. Test sixty carriers against each of **three** batches of the product at the nominal concentration. The product should kill the test microorganisms on 59 out of each set of 60 carriers/slides. This testing is intended to support bridging of **all remaining vegetative** bacteria listed (with the exception of the tuberculocidal claim) on the EPA registered liquid disinfectant used to saturate the towelette to the EPA registered towelette product.

(ii) **Fungicidal claims**. The Agency recommends the use of the AOAC Germicidal Spray Products as Disinfectants test 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. Evaluate the product against *T. mentagrophytes* (ATCC 9533) for the label recommended contact time. Test ten carriers against each of **two** batches of product at the nominal concentration. The product should kill (no positive carriers) the test organism on all 10 carriers. This testing is intended to support bridging of **all remaining fungi** listed on the EPA registered liquid disinfectant used to saturate the towelette to the EPA registered towelette product.

(iii) **Tuberculocidal Claims**. Bridging of data for towelettes is not allowed for tuberculocidal claims.

(iv) **Virus Claims**. Based on the bulk liquid label, claims for small non-enveloped, large non-enveloped and enveloped viruses can be supported by selecting the most resistant virus on the existing label– **all other viruses on the label** will be supported by this testing. Test ten carriers for each of **two** batches of the product at the nominal concentration on one surface type. 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.

(k) Data collection and reporting.

Refer to OCSPP 810.2000: General Considerations for Public Health Uses of

Antimicrobial Agents for guidance. 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.

(l) **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, Official Method 961.02 Germicidal Spray Products as Disinfectants. Current edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(3) Standard Practice for Evaluation of Pre-saturated or Impregnated Towelettes for Hard Surface Disinfection, ASTM Designation E2362. Current edition. ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

(4) *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.

(5) Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces, ASTM Designation E1053., Current edition. ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

(6) Standard Test Method for Neutralization of Virucidal Agents in Virucidal Efficacy Evaluations, ASTM Designation E1483.Current edition. ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

(7) 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).

(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) Ascenzi, J.M., et al., A More Accurate Method for Measurement of Tuberculocidal Activity of Disinfectants. *Applied Environmental Microbiology*,

Vol. 53, No. 9, 1987, pp. 2189-2192. (Suspension-based assay).