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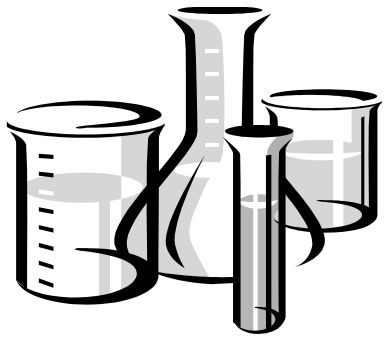
Product Performance Test Guidelines

OCSPP 810.2200:

Disinfectants for Use on Environmental Surfaces

Guidance for Efficacy Testing



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), referred to hereinafter as the harmonized test guidelines. 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 its 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 tests, and are also used by EPA, the public, and the companies that are subject to data submission requirements under TSCA, FIFRA and/or FFDCA. As a guidance document, these guidelines are not binding on either EPA or any outside parties, and the EPA may depart from these 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 these guidelines electronically, see EPA's *Test Guidelines for Pesticides and Toxic Substances* site at https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances. You may also access the guidelines at http://www.regulations.gov and searching by Docket ID #: EPA-HQ-OPP-2015-0276.

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(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) (7 U.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 disinfectant products bearing bactericidal, fungicidal, virucidal, and/or tuberculocidal claims for use on hard surfaces.

(2) Background. This document provides an update to the 2012 OCSPP guideline 810.2200 - "Disinfectants for Use on Hard Surfaces Efficacy Data Recommendations."

(B) Purpose

This guideline addresses efficacy testing for antimicrobial pesticides intended to be used as disinfectants on hard, non-porous surfaces in a variety of product formulations (water-soluble powders, liquids, sprays, towelettes, etc.). Disinfectant products should meet base bactericidal efficacy standards (Table 1) and may bear additional bactericidal, fungicidal, virucidal, and/or tuberculocidal claims supported by data that the agency determines are acceptable.

(C) General Considerations

(1) Test Methods. This document provides guidance concerning test conditions (e.g., the number of test batches and carriers) and success criteria for use with referenced standard methods. For information on test method examples and updates, refer to OCSPP guideline 810.2000, Section B(3) Guideline Updates.

(2) Spray Products. Spray products referenced in this document include aerosol, pressurized spray, trigger, and pump dispensers.

(3) Soil Load, Water Hardness, Lower Certified Limit (LCL), and Neutralization Confirmation. Refer to OCSPP guideline 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides.

(4) Confirmatory Data. Refer to OCSPP guideline 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides for information on confirmatory testing. Specific information related to confirmatory testing is provided in the description for each efficacy claim.

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

(6) Repeat Testing. Refer to OCSPP guideline 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides for guidance on repeat testing of products.

(7) Claims. Tables 1 and 2 below provide a summary of disinfectant claims and testing methods addressed in this guideline.

Table 1. Summary of Testing for Base 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 (UDM) (ref. 1)	*Staphylococcus aureus* (ATCC 6538) or *Salmonella enterica* (ATCC 10708)	For each organism: 3 batches at the LCL; 60 carriers per batch. Note: For UDM, for *Staphylococcus aureus* (ATCC 6538), each batch should be tested on a different day.
	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 (ref. 1)	*Staphylococcus aureus* (ATCC 6538) and *Salmonella enterica* (ATCC 10708) or *Pseudomonas aeruginosa* (ATCC 15442)	For each organism: 3 batches at the LCL; 60 carriers per batch. Note: For UDM, for *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 15442), each batch should be tested on a different day.
	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)		
Hospital or healthcare disinfectant/hard non-porous surfaces	Water soluble powders/liquids	AOAC Use-Dilution Method (ref. 1)	*Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 15442)	For each organism: 3 batches at the LCL; 60 carriers per batch. For UDM, each batch should be tested on a different day.
	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)		

* Table 1 does not include confirmatory testing. For guidance on conducting confirmatory testing, see the respective section for each claim.

** Foaming, fogging, gas, and vapor applications are not included in this category. Applicants should consult with the agency prior to testing to determine the appropriate methodology for product performance testing.

Table 2. Summary of Testing for Additional Disinfectant Claims*

Claim	Formulation/Test Methods		Test Organisms	No. of Batches/ Carriers
Additional bacteria /hard non-porous surfaces	Water soluble powders/liquids	AOAC Use-Dilution Method (ref. 1)	Additional bacteria claimed on the label	2 batches at the nominal concentration; 10 carriers per batch.
	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)		

Claim	Formulation/Test Methods		Test Organisms	No. of Batches/ Carriers
Internal toilet and urinal bowl surfaces (refer to section (F)(1) for guidance on above vs. below the waterline claims)	Water-soluble powders/liquid	AOAC Use-Dilution Test to include a 5% organic soil challenge added to the bacterial inoculum.	Limited spectrum: *S. enterica* (ATCC 10708) or *S. aureus* (ATCC 6538); Broad spectrum: *S. enterica* or *Pseudomonas aeruginosa* (ATCC 15442) and *S. aureus*; Hospital/Healthcare: *S. aureus* and *P. aeruginosa*	For each organism: 3 batches at the LCL, 60 carriers per batch.
	Spray products ** (for above waterline and dry flush only)	AOAC Germicidal Spray Products as Disinfectants Test (ref. 2)		
Virucidal disinfectant/hard non-porous surfaces	Water soluble powders/liquids	ASTM E1053 (ref. 5) modified for the formulation type	Virus claimed on the label or EPA approved surrogate.	2 batches at the LCL for hardest to kill strain. For all additional viruses, two batches at the nominal concentration. Non-surrogates: 1 surface per batch Surrogates: 2 surfaces per batch.
	Spray products**			
	Towelettes			
Fungicidal disinfectant/hard non-porous surfaces	Water soluble powders/liquids	AOAC Use-Dilution Method modified for fungi or AOAC Fungicidal Activity of Disinfectants (ref. 4)	*Trichophyton interdigitale* (ATCC 9533) (formerly *Trichophyton mentagrophytes*)	2 batches at the LCL; 10 carriers per batch for carrier-based methods. 2 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 (ref. 3)		
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), (ATCC 35743)	2 batches at the LCL; 10 carriers per batch
	Spray products**	AOAC Germicidal Spray Products Test modified for tuberculocidal activity		
	Towelettes	AOAC Germicidal Spray Products as Disinfectants Test modified for towelettes or ASTM E2362		

* Table 2 does not include confirmatory testing. For guidance on conducting confirmatory testing, see the respective section for each claim.

** Foaming, fogging, gas, and vapor applications are not included in this category. Applicants should consult with the agency prior to testing to determine the appropriate methodology for product performance testing.

(D) Base Disinfectant Claims

(1) Limited Spectrum. This section addresses efficacy testing for disinfectant products with limited efficacy (effective against Gram-negative or Gram-positive bacteria, but not both). Limited disinfectants may not have virucidal, fungicidal, or tuberculocidal disinfection claims.

(i) Test Method. For products formulated as liquid and water soluble powders, use the AOAC Use-Dilution Method (ref. 1). For spray products, use the AOAC Germicidal Spray Products as Disinfectants Test (ref. 2). For towelettes, use the AOAC Germicidal Spray Products as Disinfectants Test modified for towelettes or ASTM E2362 (ref. 3) and follow recommendations from section (J) below.

(ii) Test Organism. 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.

(iii) Batches/Number of Carriers. Test three batches of the product at the LCL of the active ingredient(s) (A.I.s). For each batch, test using sixty carriers.

(iv) Control Carrier Counts.

(a) For the AOAC Use-Dilution Methods (ref. 1), the control carrier counts for *S. enterica* should be 1.0×10^5 to 1.0×10^6 CFU/carrier. The control carrier counts for *S. aureus* should be 1.0×10^6 to 1.0×10^7 CFU/carrier.

(b) For the AOAC Germicidal Spray Products as Disinfectants test and towelette methods, the control carrier counts for *S. enterica* should be 1.0×10^4 to 3.2×10^5 CFU/carrier. The counts for *S. aureus* should be 1.0×10^5 to 3.2×10^6 CFU/carrier.

(v) Evaluation of Success.

(a) For the AOAC Use-Dilution Method, *Staphylococcus aureus* (ATCC 6538) only, conduct three independent tests (i.e., three batches tested on three different test days). The performance standard for *S. aureus* is no more than three positive carriers out of 60 per test. The performance standard for *S. enterica* is no more than one positive carrier out of 60 per test.

(b) For the AOAC Germicidal Spray Products as Disinfectants test and towelette methods, the product should kill all of the test microorganisms on 59 out of each set of 60 carriers.

(vi) Confirmatory Testing. 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 of the test microorganisms on all carriers.

(2) General or Broad Spectrum. For disinfectants with label claims against both Gram-negative and Gram-positive bacteria, the product is considered a general or broad spectrum disinfectant.

(i) Test Method. For products formulated as liquid and water soluble powders, use the AOAC Use-Dilution Method (ref. 1). For spray products, use the AOAC Germicidal Spray Products as Disinfectants Test (ref. 2). For towelettes, use the AOAC Germicidal Spray Products as Disinfectants Test modified for towelettes or ASTM E2362 (ref. 3) and follow recommendations from section (J) below.

(ii) Test Organism. To demonstrate efficacy, testing should be conducted against both *S. enterica* (ATCC 10708) and *S. aureus* (ATCC 6538). Alternatively, *P. aeruginosa* (ATCC 15442) may be tested instead of *S. enterica*.

(iii) Batches/Number of Carriers. For each organism, test 60 carriers against each of three batches of the product at the LCL of the A.I.(s).

(iv) Control Carrier Counts.

(a) For the AOAC Use-Dilution Methods, control carrier counts for *S. enterica* should be 1.0×10^5 to 1.0×10^6 CFU/carrier. Where *P. aeruginosa* is used instead of *S. enterica*, the counts should be 1.0×10^6 to 1.0×10^7 CFU/carrier. For *S. aureus*, the counts should be 1.0×10^6 to 1.0×10^7 CFU/carrier.

(b) For the AOAC Germicidal Spray Products as Disinfectants test and towelette methods, the control carrier counts for *S. enterica* should be 1.0×10^4 to 3.2×10^5 CFU/carrier. Where *P. aeruginosa* is used instead of *S. enterica*, the counts for *P. aeruginosa* should be 1.0×10^5 to 3.2×10^6 CFU/carrier. The counts for *S. aureus* should be 1.0×10^5 to 3.2×10^6 CFU/carrier.

(v) Evaluation of Success.

(a) For the AOAC Use-Dilution Methods, *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 15442) only, conduct three independent tests (i.e., three batches tested at the LCL of the A.I.(s) on three different test days). The performance standard for *S. aureus* is no more than three positive carriers out of 60 per test. The performance standard for *S. enterica* is no more than one positive carrier out of 60 per test. The performance standard for *P. aeruginosa* is no more than six positive carriers out of 60 per test.

(b) For the AOAC Germicidal Spray Products as Disinfectants test and towelette methods, the product should kill all of the test microorganisms on 59 out of each set of 60 carriers/slides.

(vi) Confirmatory Testing. 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 at the LCL of the A.I.(s) should be tested against *S. aureus* (ATCC 6538) and *S. enterica* (ATCC 10708) or *P. aeruginosa* (ATCC 15442). For all methods, the product should kill all of the test microorganisms on all carriers.

(3) Hospital or Healthcare. 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) Test Method. For products formulated as liquid and water soluble powders, use the AOAC Use-Dilution Method (ref. 1). For spray products, use the AOAC Germicidal Spray Products as Disinfectants Test (ref. 2). For towelettes, use the AOAC Germicidal Spray Products as Disinfectants Test modified for towelettes or ASTM E2362 (ref. 3) and follow recommendations from section (J) below.

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

(iii) Batches/Number of Carriers. For each organism, test 60 carriers against each of three batches of the product at the LCL of the A.I.(s).

(iv) Control Carrier Counts.

(a) For the AOAC Use-Dilution Methods, the control carrier counts for *S. aureus* and *P. aeruginosa* should be 1.0×10^6 to 1.0×10^7 CFU/carrier.

(b) For the AOAC Germicidal Spray Products as Disinfectants test and towelette methods, the control carrier counts for both *S. aureus* and *P. aeruginosa* should be 1.0×10^5 to 3.2×10^6 CFU/carrier.

(v) Evaluation of Success.

(a) For the AOAC Use-Dilution Methods, conduct three independent tests (i.e., three batches at the LCL of the A.I.(s), tested on three different test days) against the test microbe. The performance standard for *S. aureus* is no more than three positive carriers out of 60 per test. The performance standard for *P. aeruginosa* is no more than six positive carriers out of 60 per test.

(b) For the AOAC Germicidal Spray Products as Disinfectants test and towelette methods, the product should kill all of the test microorganisms on 59 out of each set of 60 carriers/slides.

(vi) Confirmatory Testing. 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 at the LCL of the A.I.(s) should be tested against

S. aureus (ATCC 6538) and *P. aeruginosa* (ATCC 15442). For all methods, the product should kill all of the test microorganisms on all carriers.

(E) Additional Bacteria

Applicants may request label claims for additional bacteria other than the test microorganism(s) identified under the base disinfectant claims, provided that the target microbe is likely to be present in or on the recommended use areas and surfaces. These claims are subject to agency approval. 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 the recommendations in Table 1, conduct additional testing as described below:

(1) Test Method. For products formulated as liquid and water soluble powders, use the AOAC Use-Dilution Method (ref. 1). For spray products, use the AOAC Germicidal Spray Products as Disinfectants Test (ref. 2). For towelettes, use the AOAC Germicidal Spray Products as Disinfectants Test modified for towelettes or ASTM E2362 (ref. 3) and follow recommendations from section (J) below.

(2) Test Organism. Testing should be conducted against each specific bacterium to be claimed on the label. For limited disinfectants, select additional bacteria from the same Gram type of the required strain supporting the disinfection claim (e.g., where *S. aureus* supports the Limited Disinfection claim, select only additional Gram positive bacteria).

(3) Batches/Number of Carriers. Test ten carriers against each of two batches of the product at or below the nominal concentration.

(4) Control Carrier Counts. The control carrier counts should be 1×10^4 to 1×10^5 CFU/carrier.

(5) Evaluation of Success. The product should kill all of the test microorganisms on all ten carriers (i.e. no positive carriers).

(F) Internal Toilet Bowl and Urinal Surfaces

This section addresses efficacy testing for products recommended for use on internal toilet bowl and urinal surfaces.

(1) Test Method. Regarding the test procedure for water-soluble powders and liquid products, the agency recommends the use of the AOAC Use-Dilution Methods modified to include a 5% organic soil challenge added to the bacterial inoculum.

(i) For products designed for use in water flush toilets and urinals for the entire internal surface of the bowl (including below the waterline), the contained bowl water (96 fl. oz. of water, representing a traditional high volume toilet) should be used to calculate the appropriate use-dilution for testing. Refer to OCSPP guideline 810.2000 for guidance on Dilution of Products for Testing.

(ii) For products designed for use in dry/vacuum flush toilets, dry/waterless urinals, and internal toilet bowl surfaces above the waterline only, products may be tested as ready-to-use. Spray products may be tested using the AOAC Germicidal Spray Products as Disinfectants Test modified to include a 5% organic soil challenge added to the bacterial inoculum.

(2) Test Organisms. Test against *S. enterica* (ATCC 10708) or *S. aureus* (ATCC 6538) for limited disinfectant products; *S. enterica* and/or *P. aeruginosa* (ATCC 15442) and *S. aureus* for broad-spectrum disinfectant products; and *S. aureus* and *P. aeruginosa* (ATCC 15442) for hospital disinfectant products.

(3) Batches/Number of Carriers. Test 60 carriers against each of three batches of the product at the LCL of the A.I.(s).

(4) Control Carrier Counts. Refer to the control carrier counts specified for the desired disinfection claim (limited, broad-spectrum, or hospital/healthcare disinfectant).

(5) Evaluation of Success. Refer to the performance standards specified for the desired disinfection claim (limited, broad-spectrum, and hospital/healthcare).

(6) Confirmatory Testing. For confirmatory testing, follow the above testing guidance in sections (C) and (F)(1)-(5), with the exception that ten carriers for each of two different batches of product should be tested against *S. aureus* (ATCC 6538), *S. enterica* (ATCC 10708) and/or *P. aeruginosa* (ATCC 15442), as described above. For all methods, the product should kill all of the test microorganisms on all carriers.

(G) Virucidal Claims

Virucidal claims may be added to a broad spectrum or hospital disinfectant product (liquid, spray, towelette) for use on hard, non-porous surfaces, subject to agency approval. To simulate in-use conditions, the specific virus to be treated (or EPA acceptable surrogate) should be inoculated onto hard, non-porous 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) Test Method. Use ASTM 1053 Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces (ref. 5), modified for the formulation type.

(2) Test Organism. Each specific virus listed on the label should be tested, unless there is an EPA acceptable surrogate for the virus. Refer to OCSPP guideline 810.2000, section (E)(3) for list of acceptable surrogates. The host cell line should exhibit cytopathic effects (CPE) in the presence of the selected virus. The study report should identify the host cell line by source and strain.

(3) Number of Batches. Test two batches of product at the LCL of the A.I.(s) for the hardest to kill strain on the label.

(4) Carriers/Surfaces. Test one carrier/surface per batch; for EPA accepted surrogates, test two carriers/surfaces per batch.

(5) Viral Concentration. Test each batch against a minimum recoverable virus end point titer of $\geq 10^{4.80}$ (6.3×10^4) viable viral particles per test carrier/surface for a specified exposure period. To achieve this range, the virus culture may be standardized by concentration or dilution. The method used to standardize the culture should be documented in the raw data and reported.

(6) Evaluation of 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 below.

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

(ii) For cytotoxicity control, the effect of the disinfectant with addition of the neutralizer on the viral assay system should include a minimum of four determinations (e.g., tissue culture, embryonated egg) per each dilution. For approved surrogates of animal infection, the viral assay system should include a minimum of two determinations per each dilution. While cytotoxicity may be determined in advance, this control should be performed on the day of testing for all lots.

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

(iv) Neutralization controls should be performed (Ref. 6) and should include a minimum of four determinations (e.g., tissue culture, embryonated egg) per each dilution. For approved surrogates of animal infection, the viral assay system should include a minimum of two determinations per each dilution. While the neutralizer control may be determined in advance, this control should be performed on the day of testing for all lots.

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

(vi) The 50% infectious dose (ID_{50}) values (for all carriers) for each assay should be calculated by using an appropriate statistical method (e.g., Reed and Munch, Most Probable Number, Spearman-Karber).

(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). The log reduction calculation should take into consideration the level of cytotoxicity and neutralization. Results should be reported per assayed volume and per carrier/surface (e.g., 50% tissue culture infectious dose ($TCID_{50}$)/0.1mL and $TCID_{50}$/carrier).

(viii) The product should demonstrate a ≥3 log_{10} reduction on each surface in the presence or absence of cytotoxicity.

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

(x) A laboratory report of a single test with one virus involving a tissue culture assay system should include detailed results obtained from each test and control assay; see examples of information in Tables 3-5:

Table 3. Example of Virucidal Test Results for Treated Carriers

Dilution of Virus	Virus - Disinfectant* (treated carriers)	Virus - Control* (control carriers)	Cytotoxicity – Control (disinfectant and neutralizer without virus)	Neutralization Control (neutralized disinfectant inoculated with low dose virus)
10^{-1}	T T T T	+ + + +	T T T T	T T T T
10^{-2}	0 0 0 0	+ + + +	0 0 0 0	+ + + +
10^{-3}	0 0 0 0	+ + + +	0 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 4. Sample Tissue Culture Infective Dose 50 ($TCID_{50}$) Data–Control Counts (Reed and Munch)

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
Log $TCID_{50}$/0.1mL	6.5						
Log $TCID_{50}$/carrier (0.1 mL challenge)	7.2						

Table 5. Sample Tissue Culture Lethal Dose 50 ($TCLD_{50}$) Data–Treated Carriers (Reed and Munch)

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	4	0	4/4	100
10^{-2}	0/4	0	4	0	4	0/4	0
10^{-3}	0/4	0	4	0	8	0/8	0
10^{-4}	0/4	0	4	0	12	0/12	0
10^{-5}	0/4	0	4	0	16	0/16	0
10^{-6}	0/4	0	4	0	20	0/20	0
10^{-7}	0/4	0	4	0	24	0/24	0
10^{-8}	0/4	0	4	0	28	0/28	0
Log $TCID_{50}$/0.1mL	≤ 1.5						
Log $TCID_{50}$/carrier (0.1 mL challenge)	≤ 2.2						
Log_{10} Reduction	≥ 5						

(7) Confirmatory Testing. For confirmatory testing, follow the above testing guidance in sections (C) and (G)(1)-(6) for the hardest to kill virus(es). The groups and the presumed levels of resistance are provided below:

(i) Small Non-Enveloped Viruses (<50 nm) (expected to have a high level of resistance to inactivation). 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 Parvoviridae family (e.g., parvovirus) select the most resistant representative virus (or EPA acceptable surrogate) such as Feline calicivirus (surrogate for human norovirus) for testing.

(ii) Large Non-Enveloped Viruses (>50 nm) (expected to have an intermediate level of resistance to inactivation). For label claims to inactivate large non-enveloped viruses such as 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 EPA acceptable surrogate) such as Adenovirus for testing.

(iii) Enveloped Viruses (expected to have a low level of resistance to inactivation). 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 EPA acceptable surrogate) for testing.

(H) Fungicidal Claims

A fungicidal claim may be added to a broad-spectrum or hospital disinfectant product, subject to agency approval. Fungicidal testing is conducted on fungal conidia (spores).

(1) Test Method. For products formulated as liquid and water soluble powders, use the AOAC Use-Dilution Method (ref. 1) modified for fungi or AOAC Fungicidal Activity of Disinfectants (ref. 4). For spray products, use the AOAC Germicidal Spray Products as Disinfectants Test (ref. 2) modified for fungi. For towelettes, use the AOAC Germicidal Spray Products as Disinfectants Test modified for towelettes or ASTM E2362 (ref. 3) and follow recommendations from section (J) below.

(2) Test Organism. Testing should be conducted against *Trichophyton interdigitale* (ATCC 9533) (formerly *Trichophyton mentagrophytes*). (Additional fungi claims may also be added, subject to agency approval, following the testing guidance in this section.)

(3) Batches/Number of Carriers.

(i) For the AOAC Fungicidal Activity of Disinfectants test, two batches of product at LCL of the A.I.(s) should be evaluated for efficacy. The method is a suspension test.

(ii) For the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Test (with or without modification for 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 of the A.I.(s).

(4) Titer of Conidial Suspension/Control Counts.

(i) For the AOAC Fungicidal Activity of Disinfectants test, the inoculum employed should provide a concentration of 5×10^6 to 5×10^7 conidia/mL.

(ii) For the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Test, and single-use towelettes, the control carrier counts should be 1×10^4 to 1×10^5 conidia per carrier.

(5) Evaluation of Success.

(i) For the AOAC Fungicidal Activity of Disinfectants test, all fungal spores at 10 minutes should be killed (no growth) to support the label claim for a ten-minute contact time.

(ii) For the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Test (with or without modification for towelettes), all fungal spores on all twenty carriers should be killed (no positive carriers).

(6) Confirmatory Testing. For confirmatory testing, follow the above testing guidance in sections (C) and (H)(1)-(5).

(I) Tuberculocidal Claims

Tuberculocidal claims may be added to a broad spectrum or hospital disinfectant product, subject to agency approval.

(1) Glutaraldehyde-based Products

(i) Test Method. For glutaraldehyde products, the agency recommends the Quantitative Tuberculocidal Activity Test (MB-16: Standard Operating Procedure for Quantitative Suspension Test Method for Determining Tuberculocidal Efficacy of Disinfectants Against *Mycobacterium bovis* (BCG)). Four replicates from each of two different batches of the product should be tested, against *Mycobacterium bovis* (BCG) (*M. bovis*) (ATCC 35743).

(ii) Inoculum Titer. The organism titer should be 1×10^7 - 1×10^8 CFU/ml.

(iii) Evaluation of Success. Each of the four replicates should demonstrate $\geq 1.0 \times 10^4$ CFU ($\geq$4.0 log) kill of the test organism for both batches at the stated contact time.

(2) For Non-Glutaraldehyde Products

(i) Test Method. For liquid formulations, the agency recommends the use of the AOAC Tuberculocidal Activity of Disinfectants (ref. 8). For spray products, use the AOAC Germicidal Spray Products Test (ref. 2) modified for tuberculocidal activity and for towelettes products, use AOAC Germicidal Spray Products as Disinfectants Test (ref. 2) modified for towelettes or ASTM E2362 (ref. 3).

(ii) Test Organism. Testing should be conducted against *M. bovis* (ATCC 35743). Test cultures of *M. bovis* (BCG) may be grown using agitation; (refer to MB-16: Standard Operating Procedure for Quantitative Suspension Test Method for Determining Tuberculocidal Efficacy of Disinfectants Against Mycobacterium bovis (BCG) protocol for agitation procedure).

(iii) Batches/Number of Carriers. Test ten carriers against each of two different batches of product at the LCL of the A.I.(s).

(iv) Control Carrier Counts. The control carrier counts should be 1.0×10^4 to 1.0×10^6 CFU/carrier.

(v) Evaluation of Success. *M. bovis* on all carriers should be killed (no positive carriers), and there should be no growth in any of the associated subculture media.

(vi) 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.

(3) Verification Testing for Quaternary Ammonium Compounds

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

(4) Confirmatory Testing

For confirmatory testing, follow the above testing guidance in sections (C) and (I)(1) or (I)(2).

(J) Towelette Products

To evaluate the efficacy of products formulated as impregnated wipes or towelettes, 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). One towelette should be used to wipe ten inoculated carriers, except for virucidal efficacy testing, for which one towelettes should be used to wipe the inoculated carrier/surface. For each carrier, an unused surface of the towelette should be used for wiping. Also, see section (K) of this document on bridging for cases where an applicant formulates a disinfectant towelette using an existing EPA-registered bulk liquid formulation. The agency recommends using a wiping procedure similar to ASTM 2896-12, accounting for the differences in carrier size used for the assay. 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.

(K) 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 be evaluated for concentration and 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 should not be poured off for use in the chemical testing for data bridging, rather the liquid used in the chemical testing should only be that mechanically 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 described in the study report.

(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 the claim) as used for the bulk liquid testing. The agency recommends using a wiping procedure similar to ASTM 2896-12, accounting for the differences in carrier size used for the assay. 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) Disinfectant (Bactericidal) Claims. Test the product using the methods and performance criteria specified in section (D) for towelettes formulations. 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) Virucidal 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. Test the product using the methods and performance criteria specified in section (G) for towelette products – all other viruses on the label will be supported by this testing. Test one surface for each of two batches of the product at the LCL of the A.I.(s). The product should demonstrate a ≥ 3 $\log_{10}$ reduction on every surface in the presence or absence of cytotoxicity.

(iii) Fungicidal Claims. Test the product using the methods and performance criteria specified in section (H) for towelette formulations. 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.

(iv) Tuberculocidal Claims. Test the product using the methods and performance criteria specified in section (I) for towelette formulations. This testing is intended to support bridging of all remaining strains of *Mycobacterium bovis* listed on the EPA registered liquid disinfectant used to saturate the towelette to the EPA registered towelette product.

(L) Data Collection and Reporting

Refer to OCSPP guideline 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides for guidance. The applicant is encouraged to use the EPA's Standardized Efficacy Study Report and Efficacy Study Summary.

(M) 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, MB-16: Standard Operating Procedure for Quantitative Suspension Test Method for Determining Tuberculocidal Efficacy of Disinfectants Against *Mycobacterium bovis* (BCG) (Biological and Economic Analysis Division, Office of Pesticide Programs).

(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).

(10) Environmental Protection Agency, Biological and Economical Analysis Division, Office of Pesticide Programs. Antimicrobial Testing Methods & Procedures Developed by EPA's Microbiology Laboratory.