US Environmental Protection Agency
Office of Pesticide Programs

Efficacy Testing Standards for Product Data Call-In Responses

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One of the objectives of reregistration is for the Agency to ensure that antimicrobial pesticide products are supported by acceptable efficacy data. As registrants consider the adequacy of their existing product efficacy data or prepare to generate new data in response to a reregistration product Data Call-In (DCI), a thorough understanding of the Agency testing standards is essential. Generally, these testing standards can be found in the Agency's current 810 series (Product Performance testing) Guidelines, published in 2012, which are available on the Office of Pesticide Programs website at http://www.epa.gov/pesticides/science/guidelines.htm. Reregistration product DCIs refer to the 810 Antimicrobial Product Performance testing guidelines for the specific efficacy data requirements. These guidelines identify the efficacy data needed to support reregistration based on the product-specific efficacy claims. All antimicrobial efficacy data needed to support a registration action should be addressed in response to the PDCI either by citing existing data or generating new data. This includes data on the organisms needed to support general efficacy label claims (e.g., disinfectant, sanitizer, etc.), as well as any additional bacterial, viral and fungal organisms on the product label.

Since antimicrobial efficacy testing has evolved in recent years, this supplemental document was developed by the Agency to 1) identify the current efficacy testing criteria that differ from the information in the 2012 Guidelines (for newly generated data), and 2) to summarize the test data standards for registrants intending to cite or submit existing efficacy data in response to a DCI. The information on current testing criteria is intended to inform registrants generating new efficacy data of recent changes to test procedures that, if applicable, should be incorporated into the data development process.

Registrants intending to cite or submit existing (previously generated) data in response to efficacy Data Call-In requirements should ensure that the studies meet the testing standards detailed in the 2012 Guidelines. The test methods used for these studies should be in agreement with the referenced methods as they existed when the guidelines were published (2012). Registrants citing or submitting existing data should also provide documentation which specifies that the studies were performed with active ingredient concentrations at or below the product nominal concentration(s), as defined on the Confidential Statement of Formula (CSF). This documentation should include concentrations, determined by analytical assessment, for each active ingredient and for each product lot tested in the study. Previously developed efficacy studies that are either lacking adequate test material identification, were performed with active ingredient concentrations above the nominal concentration(s), or are inconsistent with the current 810 series Guidelines should not be submitted or cited in support of product reregistration efficacy data requirements.

As indicated above, registrants generating new efficacy data for reregistration should use this supplemental document to identify recent changes to the testing standards, after first consulting the efficacy Guidelines. Registrants should note, since this document includes updated information, it supersedes the current Guidelines in cases where conflicting guidance occurs. As with previously generated studies, new efficacy studies are also expected to include a certificate of analysis which identifies the concentration of each active ingredient in each product lot tested. In addition, all efficacy data submitted in response to a reregistration DCI should be performed with the basic product

formulation, unless otherwise indicated in the specific DCI. Confirmatory efficacy data supporting alternate product formulations should be retained by the registrant.

Registrants generating new data are also expected to use the lower certified limit (LCL) testing approach, which involves testing product lots that are formulated at the lower certified limit(s) of the active ingredient(s) in the product. Efficacy testing at the lower certified limit is deemed necessary for certain label claims in order to demonstrate an antimicrobial product's ability to consistently perform as labeled. The types of efficacy testing that should use the LCL approach are identified in Table 1, which is followed by guidance on LCL testing procedures. The remainder of the document includes sections of the Guidelines with updated testing standards or testing information as described above. Categories or classes of efficacy that have not had significant changes to the guidance since the current Guidelines were published are not addressed in this document.

Table 1. Efficacy Testing Active Ingredient Concentrations

Efficacy Class	Active Ingredient concentration
Basic Disinfection (Hospital, Broad or Limited-Spectrum)	
Required Test Organism(s) (per claim type):	Test at LCL
Additional Bacteria (except CRE):	Test at or below nominal
Carbapenem-resistant *Enterobacteriaceae* (CRE):	Test at LCL
Tuberculocidal Disinfection	
Required Test Organism:	Test at LCL
Fungicidal Disinfection	
Required Test Organism:	Test at or below nominal
Virucidal Disinfection	
Hardest to kill strain (see guidance below):	Test at LCL
Additional viruses (after hardest to kill strain tested at LCL):	Test at or below nominal
Non-Food Contact Sanitizer	
Required Test Organisms:	Test at LCL
Additional Organisms (except CRE):	Test at or below nominal
Carbapenem-resistant *Enterobacteriaceae* (CRE):	Test at LCL
Food Contact Sanitizer	
Required Test Organisms and Additional Microbes:	Test at LCL
Sterilant / Sporicide	
Required Test Organism and Additional Microbes (includes *C. difficile*):	Test at LCL

Note that the LCL testing approach applies to all batches tested in a study, and that aged product batches are not needed when testing is performed under this approach. Since achieving test samples exactly at the lower certified limit can be challenging, an active ingredient concentration range above the lower certified limit which may be used for efficacy testing has been established. Individual

active ingredient concentrations within this range (above the LCL) are considered representative of the lower certified limit for efficacy testing purposes. The established test concentration range above the LCL is determined as follows:

- For products with a nominal concentration less than or equal to 1.0%, the tested value for that active ingredient may be up to 2.0% above the lower certified limit stated on the CSF.

- For products with a nominal concentration above 1.0% and less than or equal to 20.0%, the tested value for that active ingredient may be up to 1.0% above the lower certified limit stated on the CSF.

- For products with a nominal concentration above 20.0% and less than or equal to 100.0%, the tested value for that active ingredient may be up to 0.6% above the lower certified limit stated on the CSF.

Using this approach, a product with a nominal concentration (active ingredient) of 7.00% and a lower certified limit of 6.65% (based on 40 CFR 158.350), would have a testing range of 6.65% to 6.71%. In this example the nominal concentration is greater than 1.0% and less than 20%, therefore the appropriate testing range would be up to 1.0% above the LCL of 6.65% (or 6.65% to 6.71%). Products with multiple active ingredients should use this approach to determine the usable testing range for each active in the product.

Registrants are expected to develop formulated product samples for efficacy testing within this range. In cases where efforts to formulate product within this range have failed, product samples may be diluted from a higher active ingredient concentration to achieve the target range. If product dilution is performed, a diluent that is not expected to increase product efficacy should be employed. Water is the preferred diluent in these situations. Products likely to be reactive or less stable in the presence of water may be diluted with a primary solvent already present in the product formulation. The use of emulsifiers or surfactants as diluents should be avoided even if they are already present in the subject formulation, as these may alter product efficacy. Registrants should consult with the Agency prior to testing if an appropriate diluent cannot be found. Efficacy studies developed using test samples diluted with material considered likely to increase sample efficacy may be rejected by the Agency. When dilution or any other alteration is performed to achieve the acceptable LCL range, the efficacy report should specify exactly how the product (test material) was modified prior to testing.

As indicated in Table 1, only the hardest or most difficult to kill virus on a product label should be tested at the lower certified limit(s). The most difficult to kill virus can be determined from the viral disinfection hierarchy groups below. Using this approach, group 1 viruses are the hardest to kill (or the most resistant viruses to biocidal chemicals). Group 2 viruses are less difficult to kill than group 1 viruses, and group 3 viruses are the least difficult to kill using biocidal chemicals. Therefore, testing a group 1 virus (if on the product label) at the LCL allows all other viruses on the label, (whether group 1, 2, or group 3) to be tested at or below the nominal concentration.

1. **Small non-enveloped 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 most resistant representative virus (or acceptable surrogate) such as Feline calicivirus for testing.

2. **Large non-enveloped viruses**. 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 most resistant representative virus (or acceptable surrogate) such as Adenovirus for testing.

3. Enveloped 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 most resistant representative virus (or acceptable surrogate) for testing.

Disinfection Claims

Table 2. Disinfection: Basic Claims

Claim	Product Form / Test Methods		Organism(s)	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 each against either organism claimed (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 each against both organisms (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 each against both organisms (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 Notes:

- **Carrier control counts for Reference 1:** The mean log density for *S. aureus* and *P. aeruginosa* should be at least 6.0 (geometric mean density of 1.0×10^6) and not above 7.0 (geometric mean density of 1.0×10^7). The mean log density for *S. enterica* should be at least 5.0 (geometric mean density of 1.0×10^5) and not above 6.0 (geometric mean density of 1.0×10^6).
- **Performance measures for Reference 1:** For the AOAC Use-Dilution Methods 955.15 and 964.02 (against *S. aureus* and *P. aeruginosa*), conduct three independent tests on different test days against the test organism(s). 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. Use-dilution testing of *S. aureus* and/or *P. aeruginosa* should follow the additional criteria specified in the UDM Performance Standard Revision Document on the US EPA, Antimicrobials Division web page

(http://www.epa.gov/oppad001/regpolicy.htm). For the AOAC Use-Dilution Method 955.14 (against *S. enterica*), the performance standard is 0-1 positive carriers out of sixty.

- **Carrier control counts for Reference 2, Ref. 2 modified for towelettes, and Reference 3:** The mean log density for *S. enterica* should be at least 4.0 (geometric mean density of 1.0×10^4) and not above 5.5 (geometric mean density of 3.2×10^5). The mean log density for *S. aureus* and *P. aeruginosa* should be at least 5.0 (geometric mean density of 1.0×10^5) and not above 6.5 (geometric mean density of 3.2×10^6).
- **Performance measures for Reference 2, Ref. 2 modified for towelettes, and Reference 3:** For the AOAC Germicidal Spray Products as Disinfectants test and towelette methods, the product should kill the test micro-organisms on 59 out of each set of 60 carriers/slides in ≤ ten minutes.

Table 3. Disinfection: Fungicidal and Virucidal Claims

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, ten 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, one surface per batch (or two surfaces per batch for surrogate testing).
	Spray products	ASTM E1053		
	Towelettes	AOAC Germicidal Spray Products as Disinfectants Test modified for towelettes or ASTM E2362		

Table 3 Notes:

- **Fungal concentrations for AOAC Fungicidal Activity of Disinfectants test:** The inoculum employed with the AOAC Fungicidal Activity of Disinfectants test should provide a concentration of ≥ 5×10^6 conidia/mL.
- **Carrier control counts for Use-Dilution and Germicidal Spray fungicidal testing:** For the AOAC Use-Dilution method and the Germicidal Spray Products as Disinfectants test modified for fungicidal activity or for towelettes, the inoculum employed should provide a concentration of 1×10^4 to 1×10^5 conidia per carrier.
- **Fungicidal performance measures:** For the AOAC Fungicidal Activity of Disinfectants test, all fungal spores at 10 and 15 minutes should be killed (no positive carriers) to support a 10 minute exposure time. For the Use-Dilution and Germicidal Spray fungicidal testing, all fungal spores on all 10 carriers per batch should be killed (no positive carriers) in ≤ ten minutes.
- When performing fungicidal testing with the modified methods in Table 2, the methods should be altered to conform to the applicable elements (e.g., media, growth conditions) in the AOAC Fungicidal Activity of Disinfectants test (reference 4).
- **Viral concentrations for all virucidal test methods in Table 3:** Test each batch against a recoverable virus end point titer of ≥ 10^4 viable viral particles from the test surface for a specified exposure period (≤10 minutes) at room temperature. See current 810.2200 Guidelines for specific performance measures and reporting details for viral testing.

Table 4. Disinfection: Tuberculocidal and Additional Bacteria Claims

Tuberculocidal disinfectant/ hard non-porous surfaces	Water soluble powders/liquids	AOAC Tuberculocidal Activity of Disinfectants (ref. 6), Quantitative Tuberculocidal Activity Test (ref. 7)	*Mycobacterium bovis* (BCG)	Two batches at LCL, ten carriers per batch (4 replicates per batch, ref. 7).
	Spray products	AOAC Germicidal Spray Products Test modified for tuberculocidal activity		Two batches at LCL, ten carriers per batch.
	Towelettes	AOAC Germicidal Spray Products as Disinfectants Test modified for towelettes or ASTM E2362		Two batches at LCL, ten carriers per batch.
Additional bacteria disinfectant/ hard non-porous surfaces.	Water soluble powders/liquids	AOAC Use-Dilution Test (AOAC Int. 955.14 modified for bacterium added)	Additional bacteria claimed on the label	Two batches, ten carriers for each batch.
	Spray or towelettes products	AOAC Germicidal Spray Products Test/modified for towelettes		

Table 4 Notes:

- **Carrier control counts for AOAC Tuberculocidal Activity of Disinfectants test:** The mean log density for *M. bovis* should 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 1.0×10^6).
- **Carrier control counts for tuberculocidal spray/towelette methods:** For the AOAC Germicidal Spray Products as Disinfectants test modified for tuberculocidal activity or for towelettes, the mean log density of *M. bovis* should be at least 4.0 (corresponding to a geometric mean density of $\geq 1.0 \times 10^4$).
- **Performance measures for all tuberculocidal methods in Table 4:** All *M. bovis* 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.
- **Carrier control counts for additional bacteria:** The mean log density for additional bacteria should be at least 4.0 (corresponding to a geometric mean density of $\geq 1.0 \times 10^4$).
- **Performance measures for additional bacteria:** All bacteria (test organism) on all ten carriers for both batches should be killed in ≤ ten minutes.
- When performing tuberculocidal testing with modified methods in Table 4, methods should be altered to conform to the applicable elements (e.g., media, growth conditions) in the AOAC Tuberculocidal Activity of Disinfectants test (reference 6).
- Products formulated solely with quaternary ammonium compounds as the active ingredient(s) should be supported with validation testing to confirm tuberculocidal label claims. For this validation testing, one additional product sample should be tested in a separate laboratory, or in the same laboratory using different study director, technical staff and quality assurance unit, with the same test procedure and conditions as used in the primary laboratory testing.
- For tuberculocidal testing, the Quantitative Tuberculocidal Activity Test should only be used for glutaraldehyde-based formulations.

Food Contact Surface Sanitizing Claims

Non-halide Products (Sanitizing rinses formulated with quaternary ammonium compounds, chlorinated trisodium, and anionic detergent-acid formulations)

- Three samples, representing three different batches should be tested at or below the lower certified limit(s) listed on the confidential statement of formula of the product against both Escherichia coli (ATCC 11229) and Staphylococcus aureus (ATCC 6538). The organism titer should be at least $\geq 1.0 \times 10^9$ CFU/mL.
- For a valid test, the number control counts should fall between 7.5×10^7 and 1.25×10^8 CFU/mL for both microbes.
- The product must achieve a log reduction of 99.999% in counts for both organisms within 30 seconds when compared to the numbers control. The 30 second test contact time supports a one minute label claim contact time.

Sterilants and Sporicidal Claims *

Testing for Liquid Products (Ready-to-Use, Dilutable Concentrates and Water Soluble Powder Formulations)

- The Agency recommends use of the AOAC International Official Method 966.04 Sporicidal Activity of Disinfectants test (revised 2013). Neutralizer confirmation must be demonstrated using AOAC 966.04, method II, section h using growth media and incubation conditions suitable for each microbe.
- Sixty carriers of each (porcelain penicylinders and silk suture loops) should be tested against spores of both *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584). For a valid test, a mean control count of 1×10^5-1×10^6 spores per carrier required for both microbes on both carrier types. The inoculated carriers must meet the acid resistance requirements.

Testing for *Bacillus anthracis*

- The Agency recommends use of the AOAC International Official Method 966.04 Sporicidal Activity of Disinfectants test (Method II, revised 2013, see Ref. 1) using virulent *B. anthracis* spores (or a surrogate acceptable to the Agency).
- Sixty carriers representing either or both of two types of surfaces (porcelain penicyclinders and/or silk suture loops) should be tested on three samples representing three different batches of product, tested at or below certified limits of the product.
- The inoculum employed must provide a target count of 1×10^5 -1×10^6 spores per carrier.
- Media sterility controls and system controls (check for aseptic technique during carrier transfer process) are recommended per method.

Testing for Sporicidal Decontaminants—Quantitative Testing

- The Agency recommends the use of a well-developed, quantitative sporicidal test method acceptable to the Agency such as AOAC Method 2008.05 or ASTM method E2197-11 using virulent B. anthracis spores (or a surrogate acceptable to EPA) on porous and/or non-porous surfaces acceptable to EPA. The inoculum employed must provide a target count of 1×10^7 spores per carrier.
- The product must be tested on three samples representing three different batches of product, tested at or below certified limits (LCL) of the product.
- The coupon material(s) should be representative of those found at the sites(s) that appear on the product's labeling, and be acceptable to the Agency.

- The product must achieve a mean log reduction of ≥6 logs based on recoverable spores.

Testing for water-soluble powders and non-volatile liquid products for Commercial Sterilants for Aseptic Packaging of Low Acid Food

- The Agency recommends modifications of the AOAC International Official Method 966.04 as described above to demonstrate the sterilant efficacy of commercial sterilants for aseptic packaging of low acid foods.
- Sixty carriers representing one type of surface (stainless steel penicylinders) must be tested against spores on three samples representing three different batches of the product.
- The inoculum employed must provide a count of $1 \times 10^5 - 1 \times 10^6$ spore forming units per carrier.
- Other modifications to the AOAC Method 966.04 to address this use should be submitted to the Agency for review and approval prior to conducting the tests.
- The type of spore former to be used is dependent on the type of chemical sterilant as follows:

    (1) Hydrogen peroxide based sterilants should be tested against *B. subtilis* (ATCC 19659) and *C. sporogenes* (ATCC 3584)

    (2) Peroxyacid based sterilants should be tested against *B. subtilis* (ATCC 19659), *C. sporogenes* (ATCC 3584), and *B. cereus* (ATCC 14579)

    (3) For sterilant classes other than those listed above, consultation with EPA and FDA is recommended prior to generation of product data to identify the organisms for supporting aseptic packaging label claims.

- The product must kill the test spores on all of the carriers without any failures.

* Products with label claims for *Clostridium difficile* should request a time extension equivalent to 4 months after the Agency's final guidance on *C. difficile* testing is published.

References:

(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) *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.

(7) 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).