Series 810 Guidelines FAQ

The following Frequently Asked Questions (FAQs) refer to the revised Product Performance Test Guidelines OCSPP 810.2000, 810.2100, and 810.2200 dated February 2018. The test guidelines may be accessed at the following location: https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-810-product-performance-test-guidelines. Documents pertaining to the revision of the product performance guidelines, including public comment submissions, and the Agency's response to comments are available at www.regulations.gov, in docket EPA-HQ-OPP-2015-0276.

With the exception of confirmatory testing (described under OCSPP guideline 810.2000, section (B)(7)), all studies **initiated** on or after August 28, 2019 should be in compliance with the 2018 revised guidelines for testing. The study initiation date is defined under 40 CFR Part 160.3 as the date the protocol is signed by the study director. Studies that were initiated prior to the implementation date but submitted to the Agency for review after the implementation date may use either the previous version of the guidelines (2012) or the revised (2018) versions, as appropriate. The Agency intends to address confirmatory testing through a separate guidance, which will be made available for public comment prior to finalization. In the interim, applicants should consult with EPA for all confirmatory efficacy questions with the exception of the examples of formulation changes that do not need confirmatory efficacy data, identified in section B(7) of the 810.2000 chapter.

This guidance is not binding on EPA or any outside parties, and EPA may depart from the guidance where circumstances warrant and without prior notice. Registrants and applicants may propose and submit alternative practices (e.g., modifications to the recommended test methodology) to the Agency for assessment. The Agency will evaluate any proposed method modifications for appropriateness on a case-by-case basis. This guidance may be updated in the future.

General Questions:

Question: The "Notice" on the first page of all three test guidelines indicates that the use of the term "should" means an action is recommended, but not mandatory. In many places throughout the 810 documents, "should" is used to describe testing requirements and when testing is required. Could you explain how to interpret the Agency's view of the use of the word "should" in connection with new or detailed testing situations?

Answer: As indicated in the "Notice" preceding all three referenced guidance documents as well as in this and other EPA guidance documents, guidance is not binding on EPA or registrants/applicants. Accordingly, the Agency uses non-mandatory terms in the guidance such as "should." Through these Product Performance Test Guidelines, the Agency seeks to assist registrants' understanding of how the Agency interprets the antimicrobial data requirements in 40 CFR Part 158 Subpart W.

Question: When are submissions expected to be in compliance with the revised 2018 guidelines?

Answer: With the exception of confirmatory testing (described under OCSPP guideline 810.2000, section (B)(7)), all studies **initiated** on or after August 28, 2019 should be in

compliance with the 2018 revised guidelines for testing. The study initiation date is defined under 40 CFR Part 160.3 as the date the protocol is signed by the study director. Studies that were initiated prior to the implementation date but submitted to the Agency for review after the implementation date may use either the previous version of the guidelines (2012) or the revised (2018) versions as appropriate.

The Agency intends to address confirmatory testing through a separate guidance, which will be made available for public comment prior to finalization. In the interim, applicants should consult with EPA for all confirmatory efficacy questions with the exception of the examples of formulation changes that do not need confirmatory efficacy data, identified in section B(7) of the 810.2000 chapter.

Question: What changes can registrants expect for new registration and amendment applications submitted after the August 28, 2019 implementation date of the revised 810.2000, 810.2100 and 810.2200 Guidelines?

Answer: The revised guidelines generally articulate EPA guidance and interpretations that have been in effect for some time. Therefore, EPA does not anticipate any substantial changes to how it processes applications or the ways in which it evaluates and interprets the test data submitted. The Agency recommends compliance with the guidelines, as applicable, to facilitate efficient processing and approval of applications. However, EPA evaluates submissions on a case-by-case basis to determine whether there is adequate support for product registrations or amendments.

Guideline 810.2000

Section: (B)(7) Confirmatory Data

Question: Section 810.2000 (B)(7) in the second paragraph describes the dye and fragrance changes where confirmatory efficacy data are not required to be conducted or submitted. In cases where a combination of dye and fragrance change is proposed, what are the criteria for data submission?

Answer: Where the total change to fragrances and/or dyes are less than or equal to 1.0% (w/w) of the total formulation, confirmatory data are not needed.

Section: (B)(7) Confirmatory Data

Question: Please clarify the submission requirements and examples of when confirmatory testing should be conducted.

Answer: The Agency intends to address confirmatory testing through a separate guidance, which will be made available for public comment prior to finalization. In the interim, applicants should consult with EPA concerning all confirmatory efficacy questions with the exception of the

examples of formulation changes that do not need confirmatory efficacy data under section B(7) of the 810.2000 chapter as follows:

"Specifically, formulation changes do not need confirmatory efficacy data when:
(i) Only the concentration of a fragrance or dye is increased, substituted, or decreased in a formulation, and
(ii) the concentration of fragrances does not exceed 1.0% (w/w) of the total formulation, or
(iii) the total percentage of changes in dyes does not exceed 1.0% (w/w) of the total formulation."

Section: (B)(8) Agency Verification of Efficacy

Question: To make a meaningful determination of efficacy for a product that is registered, how will the Agency conduct testing for evaluation?

Answer: In general, section (B)(8) refers to products that will be tested as part of the revised Antimicrobials Testing Program (ATP). The ATP is currently suspended while the Agency develops a new, risk-based strategy to ensure the effectiveness of public health pesticides used in hospital settings. This approach will be addressed under the Antimicrobial Performance Evaluation Program (APEP) strategy.

Section: (C)(2) Antimicrobial Products with Non-Public Health claims

Question: The current link to the "Crosswalk Table for Non-Public Health Guidelines" accesses a document which states, "EPA's Web Archive: This content is not maintained and may no longer apply." Please provide an updated table.

Answer: The OCSPP 810 Guidelines primarily focus on public health claims. The crosswalk table is specific to testing for non-public health claims and is still accessible via the web archive. The Agency recommends that applicants continue to reference the archived resource and consult with the Agency until a revised table is available.

Section: (D) Definitions – Biofilm

Question: For the purposes of the 810 test guidelines, OCSPP 810.2000 section D contains a definition of biofilm that does not distinguish between biofilm products that may include public health claims and others that do not. The "Response to Comments" document states that all label claims against biofilm will be considered public health claims unless the label expressly states the use is non-public health. Did the Agency intend to impose a new definition for biofilm or new labeling requirements for this claim? Is the "biofilm" claim limited to public health products?

Answer: Biofilm claims may be made for both public health and non-public health products. Claims against public health organisms must be supported by appropriate efficacy data. Where the term biofilm is used on a label to expressly or impliedly make a public health claim, the claim needs to be supported with efficacy data. The language in the Response to Comments

document was only intended to illustrate this point. EPA did not intend to impose a new definition for biofilm or new labeling requirements.

Section: (E)(1) General Testing considerations - Test substance

Question: 810.2000 section E(1)(d): The Agency recognizes the difficulty of formulating products at the lower certified limit (LCL) for testing and provides acceptable ranges **above** and **below** the LCL based on the product's active ingredient concentration. Will the Agency accept data where the active ingredient concentration is less than the lower bound for the LCL?

Answer: The Agency strongly encourages that testing be conducted as close to the LCL as possible. This ensures that products are subject to an even playing field for efficacy testing and that the test accurately represents the product to be sold. The range around the LCL is to provide a buffer for formulations where it may be difficult to achieve the target concentration of active ingredient (A.I.). In most cases where the concentration of A.I. falls below the lower bound for the LCL, the data will still be acceptable; applicants should consult with the Agency prior to testing to confirm that the lower concentration of A.I. (below the LCL) will not impact product performance evaluation.

Section: (E)(1) General Testing considerations - Test substance

Question: The LCL Testing Guidance (posted by the EPA on December 6, 2013) discusses reactive and unstable chemistries and the difficulty in achieving the allowable test range by direct formulation or by product dilution which is omitted from 810.2000 section E(1). What are the LCL recommendations for reactive and unstable chemistries?

Answer: In the case of products with reactive or unstable chemistries that cannot achieve the allowable test range, registrants should provide a thorough justification detailing all efforts made to obtain acceptable test samples, as well as rationale supporting the chemical basis for product instability. The Agency will address these situations on a case-by-case basis depending on the supporting information provided by the registrant, existing storage stability data for the product, and on how close the attained concentration is to the acceptable LCL range.

Section: (E)(6) General Testing considerations - Dilution of Products for Testing – Hard Water Guidance

Question: How is tap water prepared for efficacy testing? How are adjustments to tap water made if it falls outside of the range?

Answer: A Standard Operating Procedure (SOP) containing specific guidance for how to prepare and adjust tap water for testing is available on the Agency's webpage: Antimicrobial Testing Methods & Procedures Developed by EPA's Microbiology Laboratory under MB-30-01.

Section: (E)(6) General Testing considerations - Dilution of Products for Testing – Hard Water Guidance

Question: What are the allowable ranges around the 200 ppm, 400 ppm and 375 ppm hard water levels cited?

Answer: EPA recommends the AOAC recommended range of -10% and +5% for each type of hard water (e.g., AOAC hard water range = 360-420 ppm calcium carbonate; OECD hard water range = 338-394 ppm calcium carbonate; Tap Water = 180-210 ppm calcium carbonate) as referenced in AOAC 960.09 and the SOP for preparation of hard water on the Agency's webpage: Antimicrobial Testing Methods & Procedures Developed by EPA's Microbiology Laboratory under MB-30-01.

Section: (E)(6) General Testing considerations - Dilution of Products for Testing – Hard Water Guidance

Question: When diluting a concentrated product, what are appropriate label claims (use directions and claims) for using the various types of water?

Answer: This is determined on a case-by-case basis and individual products may have varying water dilution scenarios for efficacy testing. Examples of testing scenarios and corresponding labeling are available in Appendix I.

Section: (E)(8) General Testing considerations - Contact time (Exposure Period)

Question: 810.2000 section (E)(8) indicates that towelette products may use the wetness determination test from 810.2100 section I(1)(a) to determine the maximum contact time. Is this wetness determination test required to be conducted? Is this testing required to be submitted to EPA?

Answer: The Agency recommends conducting wetness determination testing to ensure appropriate selection of contact times for a towelette product (e.g., surface remains wet and does not evaporate prior to completion of contact period). Currently, this test is used for towelette products with claims against *Clostridium difficile* or *Candida auris*.

Section: (E)(9) General Testing considerations - Neutralization Confirmation

Question: Could the neutralization confirmation control be performed after testing?

Answer: In general, we recommend that the neutralization control be run on the same day as the efficacy test. However, we recognize individual methods may have other guidance. For example, AOAC 955.15 specifies the neutralization confirmation must be performed in advance or in conjunction with the Use Dilution test. We also recognize that, for certain types of efficacy studies (e.g., pool field studies, air sanitization, in-tank sanitization, water purification), it is not practical to conduct daily neutralization controls and neutralization testing is typically conducted prior to efficacy testing. Certain situations may also justify repeating neutralization confirmation after the efficacy test. In these cases, a detailed rationale for the neutralization failure should be provided by the laboratory. If the neutralization failure is due to the inoculum level being too high or too low (outside of the acceptable range for the neutralization confirmation control counts), repeat testing of just the neutralization assay is appropriate. For all other failure scenarios, the efficacy test and the neutralization assay are considered invalid and should be

repeated. If there is interference in the neutralization assay caused by contamination, up to two repeat tests may be performed (see Appendix II for information on repeat testing).

Section: (E)10 General Test Considerations - Batch Replication

Question: Batch replication is defined as efficacy testing using a reduced number of product batches (lots) for certain use modifications of a registered product, e.g., the addition of organic soil, a change in hard water concentration or testing temperature, or the use of a porous carrier. Does the performance standard for each of the base organisms tested in the AOAC Use-Dilution Method remain as described in 810.2200(D) when the batch replication policy is employed to reduce the number of test batches?

Answer: Yes. The performance standard in the Evaluation of Success Section 810.2200(D) for the base test organisms (i.e., *Salmonella enterica*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*) remains applicable for AOAC Use-Dilution Method studies performed with a reduced number of test batches as addressed by the Batch Replication Policy outlined in 810.2000(E)(10). The performance standard of up to three positive carriers out of 60 for *S. aureus* and up to 6 positive carriers out of 60 for *P. aeruginosa* remains appropriate when two test batches are tested on independent test dates. Likewise, at this time, the performance standard for *S. enterica* remains at one positive carrier out of 60 and with no need to test each of the two test batches on separate test dates.

Section: (E)(11) Repeat Testing

Question: How many times can you repeat test for contamination in a test system?

Answer: Contamination found in the test system may invalidate the assay in certain situations and applicants have the option to conduct repeat tests up to two times to address contamination. If multiple contamination events occur, the laboratory may consider a quality plan to rectify the issue (e.g., performing a facility/equipment cleaning, replacing the stock tube, purchasing a new lyophilized stock, etc.) as appropriate. Separate laboratory personnel (e.g., study director, technical staff, QA auditor) or testing at a different laboratory are not needed for repeat testing.

Section: (E)(11) Repeat Testing

Question: How many times can repeat testing be conducted due to contamination on the carrier control?

Answer: Applicants have the option to conduct repeat tests up to two times to achieve a valid control. See Appendix II for guidance regarding contaminated carrier control counts and when repeat testing should be conducted.

Section: (E)(11) Repeat Testing

Question: Can a repeat test be conducted for a 60 carrier Qualitative test using *S. aureus, S. enterica* or *P. aeruginosa*, where the total number of positive carriers for growth (including contamination) exceed the performance standards?

Answer: If the presence of a contaminated carrier results in the performance standard being exceeded, the test would be invalidated and applicants have the option to conduct repeat tests up to two times to address contamination. However, if the number of positive carriers due to growth of the target organism alone exceeds the performance standard, then the test would be considered to have failed regardless of additional contaminated carriers and repeat testing should not be conducted. See Appendix III for example scenarios.

Section: (E)(11) Repeat Testing

Question: If a carrier population control is greater than the acceptance criteria, can a test be repeated, and if so, how many times?

Answer: Yes, if the carrier population control is greater than the acceptance criteria and the product fails to achieve the performance standard, applicants have the option to conduct repeat testing up to two times to address a population control failure. However, if the test substance meets the performance standard with a carrier population control count greater than the acceptance criteria, testing does not need to be repeated.

Section: (E)(11) Repeat Testing

Question: How many times can testing be repeated?

Answer: EPA understands that different issues may arise on each subsequent test date. In cases of control failures, applicants have the option to conduct up to two repeat tests to address each type of control failure (e.g., failure to neutralize, soil sterility failure, purity control failure, carrier control failure) to achieve a valid test. Where repeat testing occurs within the same study, invalid data due to these control failures should be reported in appendices of the final report.

Section: (E)(11) Repeat Testing

Question: Is a repeat test necessary for a 60 carrier Qualitative test using *S. aureus, S. enterica* or *P. aeruginosa*, where the total number of positive carriers for growth (including contamination) fall within the performance standards?

Answer: In the case that only one carrier is contaminated and the total number of positive carriers for growth fall within the performance standards, repeat testing is not needed. However, if multiple carriers are found to be contaminated, applicants should conduct repeat testing even when the total number of positive carriers fall within the performance standards.

Applicants have the option to conduct up to two repeat tests to address contamination issues. Refer to Appendix III for example repeat testing scenarios.

Section: (E)(11) Repeat Testing

Question: If you have a 10-carrier qualitative test for *S. aureus* and *P. aeruginosa* and the formulation fails with 1 or more positive tubes out of 10, will EPA allow repeat testing using 60 carriers?

Answer: No, the test has failed for the performance standard and applicants should not proceed with repeat testing using 60 carriers. In these cases, the applicant should report the failure(s) and has the option to conduct a different test by changing the test conditions (e.g., removing soil load or using an increased contact time and/or a higher concentration to support the new efficacy label claim for the product).

Section: (E)(11) Repeat Testing

Question: If you have a 10-carrier qualitative test for confirmatory testing or for an additional organism (e.g., MRSA) and it fails 1/10, can testing be repeated on that failing lot with 60 carriers?

Answer: No, the test has failed for the performance standard and applicants should not proceed with repeat testing using 60 carriers. In these cases, the applicant should report the failure(s) and has the option to conduct a different test by changing the test conditions (e.g., removing soil load or using an increased contact time and/or a higher concentration to support the new efficacy label claim for the product).

Section: (E)(11) Repeat Testing

Question: For a quantitative assay, is it recommended that a repeat test be conducted when any level of contamination occurs which interferes with the ability to interpret results, deeming the test inconclusive?

Answer: For quantitative assays, the following are the Agency's recommended criteria for repeat testing:

- A test in which a sporadic, isolated contaminant(s) is observed on a plate that does not interfere with the reading of results is a valid test. Repeat testing is not needed.
- A test in which systemic contamination is present (e.g., contamination within an entire dilution series) is invalid and applicants have the option to repeat the test up to two times to address contamination.
- A test in which contaminants inhibit the analyst's ability to accurately read a plate(s) is invalid and applicants have the option to repeat the test up to two times to address contamination.

Appendix II and Appendix III provide more information regarding repeat testing for quantitative tests.

Guideline 810.2100

Section: (E) Sporicide Claim

Question: Could a product have a disinfectant label claim against the vegetative form of *Clostridium perfringens*?

Answer: Consistent with other spore forming organisms (e.g., *Clostridium difficile*, *Bacillus anthracis*), testing should be conducted against the spore form of the organism. Stand-alone claims against the vegetative form could be potentially misleading to consumers as these are common spore-formers.

Section: (E) Sporicide Claim

Question: For surface-specific sporicide claims under 810.2100(E), what are the carrier types and how many test carriers should be tested?

Answer: For surface-specific sporicide claims:

1. To add a **hard, non-porous surface** sporicidal claim to a liquid product, perform testing on the base strains using **stainless steel penicylinders** (60 carriers/lot/strain, 3 lots). Testing on suture loops or porcelain penicylinders is not necessary.
2. To add a **hard, non-porous and porous surface** sporicidal claim to a liquid product, perform testing on the base strains using the **porcelain penicylinders** (60 carriers/lot/strain, 3 lots). Testing on suture loops or stainless steel penicylinders is not necessary.
3. To add a **soft, porous surface** sporicidal claim to a liquid product, perform testing on the base strains using **suture loops** (60 carriers/lot/strain, 3 lots). Testing on stainless steel or porcelain penicylinders is not necessary.

Carrier types for testing should be tailored to the label claims and EPA may recommend alternate surface types. Similar to disinfection testing, for example, sporicidal testing using stainless steel penicylinders alone generally would support general hard, nonporous surface claims and use sites on labels. Note, verification testing should be conducted in all of the scenarios described above as detailed in section (E)(vi) of Guideline 810.2100.

Section: (I) Hospital or Healthcare Disinfectant with Sporicidal Activity against Clostridium difficile Claim

Question: Is verification testing needed for *Clostridium difficile*?

Answer: No verification testing is needed at this time.

Guideline 810.2200

Section: (G)(5) and (6)(vii) Virucidal Claims

Question: 810.2200 section (G)(5) and (6)(vii) indicate that the log survivors and log reductions should be reported per assayed volume and per carrier surface. Reporting per the carrier surface is new. How should this calculation be performed?

Answer: Please see Appendix IV for sample calculations. Note that most probable number (MPN) software is no longer necessary for any viral protocols.

Section: (J) Towelette Products and (K)(2) Bridging for Towelettes – Efficacy Testing

Question: What application procedure should be used for bacterial, fungal, tuberculocidal, and viral testing for towelette disinfection claims?

Answer: For testing bacterial, mycobacterial and fungal disinfection claims for towelettes, EPA recommends the use of AOAC 961.02 or ASTM E2362. In these methods, one wipe is used to treat 10 carriers. Typically, each carrier is wiped back and forth 3 times for a total of 6 passes before moving to the next carrier. A pass is defined as moving from one side of the carrier to the other with a single motion. For virucidal claims, EPA recommends use of ASTM E1053. When testing with EPA recommended viral surrogates, please reference the protocols outlined in 810.2000. Due to the large carrier used for viral testing, one towelette is used to wipe one test carrier. Typically, this testing is also performed with 6 passes across the carrier surface. Due to the large carrier size and the small folded size of the wipe, consider whether to treat the carrier in two parallel sections using the same wipe, or whether the wiping technique used in E2896 should be employed for viral testing.

Section: (K)(1) Bridging for EPA-Registered Liquids and Disinfectant Towelettes: Chemical Analysis

Question: 810.2200 section (K)(1) indicates that mechanically expressed liquid from towelettes should be used for chemical analysis when bridging a disinfectant towelette to an EPA-registered bulk liquid formulation. What does “mechanically expressed” mean?

Answer: “Mechanically expressed” includes any physical means of extracting liquid from a towelette product (other than pouring off excess liquid from the bulk towelette container). “Mechanically expressing” includes but is not limited to:

- Squeezing using a gloved hand
- Centrifuging the liquid from the towelette
- Compressing the towelette using a plunger inside of a syringe

For alternative methods of extraction, applicants should consult with the Agency prior to testing and provide a justification for why the mechanical methods of extracting or expressing would not be appropriate.

APPENDICES

Appendix I – Label Use-Directions for Dilutable Products

Where the label use-directions require the user to dilute a concentrated product, these are label examples based on varying types[1] of water used in efficacy testing for a given product:

Scenario 1 (mixed use of hard water): A registered product has existing hard water label claims prior to the implementation of the 2018 guidelines. For example, the organisms on the label were previously tested and approved at 300 ppm AOAC hard water. New data is submitted for existing or new microorganisms using 400 ppm AOAC hard water. The following are acceptable label directions:

- Label: Dilute with [tap] water AND/OR
- Label: Can be diluted with hard water AND/OR
- Label: Use up to 300 ppm hard water

Sample Label Language: *"For use as a Daily Disinfectant: Dilute 6 fl. oz in water [up to 300 ppm hardness]. Pre-clean heavily soiled areas. Apply Use Solution by coarse trigger sprayer to hard, non-porous surfaces. Spray 6-8 inches from the surface, making sure to wet surfaces thoroughly. All surfaces must remain wet for the required time indicated in the directions for use."*

Scenario 2 (mixed use of hard water): A registered product has existing hard water label claims prior to the implementation of the 2018 guidelines. The organisms on the label were tested at a level of water hardness that is less than the revised hard water recommendations (e.g., 300 ppm AOAC hard water). Testing is conducted for new microorganisms using 200 ppm hard tap water. The following are acceptable label directions:

- Label: Dilute with [tap] water AND/OR
- Label: Can be diluted with hard water AND/OR
- Label: Dilute with up to 200 ppm hard water

Sample Label Language:
"For use as a Daily Disinfectant: Dilute 6 fl. oz in water [up to 200 ppm hardness] ..."

Scenario 3 (mix of different types of hard water, all following the revised 2018 guidelines): A registered product has hard water label claims prior to the implementation of the 2018 guidelines. The organisms on the label were tested using any combination of the recommended hard water types specified in the revised 2018 guidelines (e.g., *S. aureus* disinfection using 200 ppm tap water, *C. auris* disinfection using 375 ppm OECD synthetic hard water). The following are acceptable label directions:

- Label: Dilute with [tap] water AND/OR

[1]Hard water is defined as:

- Regular (un-softened) tap water with a minimum of 200 ppm calcium carbonate
- AOAC Synthetic hard water of 400 ppm calcium carbonate
- OECD hard water formula of 375 ppm hard water

(OCSPP 810.2000 (E)(6))

Softened water is defined as water with levels of calcium carbonate below the respective concentration indicated above for each type of water.

- Label: Can be diluted with hard water AND/OR
- Label: Dilute with up to 200 ppm hard water

Sample Label Language:
"For use as a Daily Disinfectant: Dilute 6 fl. oz in water [up to 200 ppm hardness] …"

Scenario 4 (level of water hardness exceeds the revised 2018 guidelines): A registered product has hard water label claims prior to the implementation of the 2018 guidelines. The microorganisms on the label were tested using 500 ppm AOAC hard water (or higher). The following are acceptable label directions:
- Label: Dilute with [tap] water AND/OR
- Label: Can be diluted with hard water AND/OR
- Label: Dilute with up to 500 ppm hard water

Sample Label Language:
"For use as a Daily Disinfectant: Dilute 6 fl. oz in water [up to 500 ppm hardness] …"

Scenario 5 (mix of hard water consistent with the revised 2018 guidelines and soft water): A registered product has hard water label claims prior to the implementation of the 2018 guidelines. Organism X was tested in 400 ppm AOAC hard water and organism Y was tested using sterile deionized water. The following are acceptable label directions:
- Label: Dilute using [tap] water for organisms X. Dilute using sterile deionized water for organisms Y [and X].
- Label: Can be diluted with hard water for organisms X. Dilute using sterile deionized for organisms Y [and X].

Sample Label Language:
"For use as a Daily Disinfectant: Dilute 6 fl. oz in deionized water …*
**deionized water for organism Y and tap water for organism X"*

Scenario 6 (mix of hard water consistent with the revised 2018 guidelines and previous testing at below 200 ppm): A registered product with new data against organism X using tap water with 200 ppm hardness (post implementation of the 2018 guidelines) and old data against organism Y using tap water with 100 ppm hardness (prior to the implementation of the 2018 guidelines). The following are acceptable label directions:
- Label: Dilute using [tap] water for organism X. For organism Y, dilute using water softened to less than or equal to 100 ppm.

Sample Label Language: *"For use as a Daily Disinfectant: Dilute 6 fl. oz in water with a minimum water hardness of 100 ppm…*
**water softened to less than or equal to 100 ppm hardness for organism Y and tap water for organism X"*

Scenario 7 (soft water or deionized water): New or registered product tested with soft water.
- Consult with Agency prior to testing.

Appendix II – Revised Repeat Testing Policy

Revised language for OCSPP Guideline 810.2000, section (E)(11): Repeat Testing.

Since the posting of the 2018 guidelines in February 2018, the Agency received additional comments and concerns from industry regarding approaches for repeat testing. As a result, the Agency has adjusted the repeat testing guidance to address stakeholder concerns. The new guidance is as follows:

"Repeat Testing. The Agency defines repeat testing as retesting a product lot under the same conditions as the original test (e.g., same number of carriers, temperature, contact time, soil load, dilution rate, etc). Repeat testing is appropriate only in certain cases.

The following table provides a general retesting strategy for evaluation of antimicrobial product efficacy.

- Failed tests are defined as test outcomes in which the product did not meet the performance standard for the efficacy claim. Performance standards are outlined in the OCSPP 810.2000 series in the "Evaluation of Success" sections for each efficacy claim.
- Contaminants are defined as microorganisms which are not the test organism that are present in the study. Methods such as gram staining, colony morphology, and biochemical assays should be used to identify the contaminant and presence and/or absence of the test organism. Results should be provided to EPA.

Outcome	**Passed/Failed Test**	**Lot Retest?**	**# of Repeats per Lot**
1. Mean control carrier count level above acceptable range	Failed	Yes	2
2. Mean control carrier count level above acceptable range	Passed	Not necessary	N/A
3. Mean control carrier count level below acceptable range	Failed	No	N/A
4. Mean control carrier count level below acceptable range	Passed	Yes	2
5. Presence of contamination	See *Contamination in Subculture Media* and *Contamination in Control Carrier Counts or Neutralization Assays (for qualitative tests) or Quantitative Efficacy Tests* below.		
6. Neutralization verification assay failure	Passed or Failed	Yes	2
7. Documented control failure (including sterility control and test system control)	Passed or Failed	Yes	2
8. Documented operator error or test system failure which results in an invalid study as deemed by the Study Director	Passed or Failed	Yes	2
9. Verified product contamination for EPA registered products	N/A	No, notify EPA immediately	N/A

Contamination in Subculture Media

- In the case where a contaminant and the test organism are both identified to be present in the subculture tube, the outcome is considered a positive carrier.

- For a 60-carrier qualitative test
    - One contaminated carrier (subculture medium tube) may occur per 60-carrier test, without a retest, if the total number of positive carriers including the contaminant meets the performance standard.
    - A test where the total number of positive carriers, including the contaminant, exceeds the performance standard by one positive carrier is invalid and may be repeated up to two times using another 60-carrier test.
    - A test with more than one contaminated carrier is invalid and may be repeated up to two times using another 60-carrier test.
- For a 10-carrier qualitative test
    - A test with one or more contaminated carrier(s) is invalid and may be repeated up to two times using another 10-carrier test.

Contamination in Control Carrier Counts or Neutralization Assays (for qualitative tests) or Quantitative Efficacy Tests

- A test in which a sporadic, isolated contaminant(s) is observed on a plate that does not interfere with the reading of results is a valid test. Repeat testing is not needed.
- A test in which systemic contamination is present (e.g., contamination within an entire dilution series) is invalid and may be repeated up to two times to achieve a valid test.
- A test in which contaminants inhibit the analyst's ability to accurately read a plate(s) is invalid and may be repeated up to two times to achieve a valid test.

Repeat Testing Criteria:

1. All product lots should be tested at the appropriate concentration for the claim (i.e., LCL or nominal concentration of the A.I.s).
2. Testing should be performed under the original testing conditions.
3. A valid rationale to support retesting should be provided in the report.
4. In case of contamination, run all identification tests necessary to rule out the test system organism. Results should be included in the final study report.
5. Report all passing and failing data.

For any exceptional circumstances that fall outside of the scope of this guidance, applicants are encouraged to consult with the Agency prior to conducting any additional repeat testing.

In cases where repeat testing is not appropriate, and the test conditions are identical to the label conditions, the applicant should report the failure(s) and may choose to conduct a different test by changing the test conditions (e.g., removing soil load or using an increased contact time and/or a higher concentration to support the new efficacy label claim for the product)."

Appendix III – Repeat Testing Scenarios

Example Repeat Testing Scenarios

Test Parameters	Example outcome	Example Performance Standard/ (# positive/ total # carriers)	Conclusion
AOAC UDM S. aureus 60 carriers	4 total positive tubes; 2 positives test organism and 2 contaminants	3/60	Invalid / Retest
	3 total positive tubes; 1 positive test organism and 2 contaminants	3/60	Invalid / Retest
	3 total positive tubes; 2 positive test organism and 1 contaminant	3/60	Valid / Pass / No retesting necessary
	5 total positive tubes; 4 positive test organism and 1 contaminant	3/60	Valid / Fail / No retesting
AOAC Germicidal Spray Test/ Towelette S. aureus 60 carriers	2 total positive tubes; 1 positives test organism and 1 contaminant	1/60	Invalid/ Retest
	1 total positive tube which is a contaminant	1/60	Valid / Pass / No retesting
	3 total positive tubes; 2 positive test organism and 1 contaminant	1/60	Valid / Fail / No retesting
Quantitative Test Methods (e.g. NFCS, QCT, FCS, control counts for qualitative tests)	Test and or control contamination	Any isolated contamination which interferes with the recording or interpretation of results OR systemic contamination in a test results in an invalid test.	Invalid/ Repeat Testing

Appendix IV – Virucidal Calculations

Sample calculation of $TCID_{50}$ using the Spearman-Karber method

The $TCID_{50}$ (50% Tissue Culture Infectious Dose) refers to the dilution where 50% of the inoculated cell cultures exhibit cytopathic effects due to infection by the test virus.

Example 1- No cytotoxicity

Conditions:

- *200 µL of virus is dried onto the carrier.*
- *2 mL of media (plate recovery control) or test substance is added to the carrier.*
- *The mixture is immediately passed through a Sephadex or Sephacryl column after the contact time, with no additional neutralizer volume, and the full 2 mL of filtrate is collected. This is considered the 10^{-1} dilution.*
- *The 10^{-1} dilution is tenfold serially diluted in a dilution medium.*
- *100 µL of 10^{-1} to 10^{-7} dilutions is inoculated into the cell culture well.*
- *The limit of detection for $TCID_{50}$/0.1 mL is $10^{0.50.}$*

Results:
Plate Recovery Control

Dilution	Plate Recovery Control	Percent Infection
Cell Control	0 0 0 0	-
10^{-1}	+ + + +	100
10^{-2}	+ + + +	100
10^{-3}	+ + + +	100
10^{-4}	+ + + +	100
10^{-5}	+ + + 0	75
10^{-6}	0 0 + +	50
10^{-7}	0 0 0 0	0
$TCID_{50}$/100 µL	$10^{5.75}$	-
$TCID_{50}$/carrier	$10^{6.05}$	-

(+) = Positive for the presence of test virus
(0) = No test virus recovered and no cytotoxicity present

Calculations:

Using the Spearman Karber method as follows:

Negative Log of $TCID_{50}$/100 µl = -log of 1st dilution assayed – [((Σ of % mortality at each dilution/100) – 0.5) x (log of dilution)]

Negative Log of $TCID_{50}$/100 µl = -1 - [(((100 + 100 + 100 + 100 + 75 + 50)/100) – 0.5) X (1)]
= -1 – [((5.25) – (0.5)) x 1]
= -1 – 4.75Log of $TCID_{50}$/100 µl = 5.75
Therefore, the $TCID_{50}$/100 µl = $10^{5.75}$

To calculate the $TCID_{50}$/carrier for the plate recovery control, multiply by 2 [note 2 is used since 200 µl of virus is applied to the carrier surface]

$TCID_{50}$/carrier = (Antilog of 5.75) X 2 = 1,124,682.65

The Log_{10} of 1,124,682.65 = 6.05 so the $TCID_{50}$/carrier = $10^{6.05}$

To calculate the $TCID_{50}$/carrier for the treated carrier,

Dilution	Virus + Test Substance Lot 1	Percent Infection
Cell Control	0 0 0 0	-
10^{-1}	+ + + +	100
10^{-2}	0 0 + +	50
10^{-3}	0 0 0 0	0
10^{-4}	0 0 0 0	0
10^{-5}	0 0 0 0	0
10^{-6}	0 0 0 0	0
10^{-7}	0 0 0 0	0
$TCID_{50}$/100 µL	$10^{2.00}$	-
$TCID_{50}$/carrier	$10^{2.30}$	-
Log_{10} Reduction	3.75	-

(+) = Positive for the presence of test virus
(0) = No test virus recovered and no cytotoxicity present

Negative log of $TCID_{50}$/100 µl = -1 - [(((100 + 50 + 0 + 0 + 0)/100) – 0.5) X (1)]
= -1 – [((1.5) – (0.5)) x 1]
= -1 – 1.5
= -2
Log of $TCID_{50}$/100 µl = 2
$TCID_{50}$/100 µl = $10^{2.0}$

To calculate the $TCID_{50}$/carrier, multiply by 2 [note 2 is used since 200 µl of virus is applied to the carrier surface]

$TCID_{50}$/carrier for the treated carrier = (Antilog of 2.0) X 2 = 200

The log of 200 = 2.30 $Log_{10,}$ so the $TCID_{50}$/carrier = $10^{2.3}$

The log reduction is:

($LogTCID_{50}$/carrier for the plate recovery control) - ($LogTCID_{50}$/carrier for the treated carrier)

6.05-2.3= 3.75

Example 2 – No Cytotoxicity

Conditions:

- *200 µL of virus is dried onto the carrier.*
- *2 mL of media (plate recovery control) or test substance is added to the carrier.*
- *The mixture is immediately passed through a Sephadex or Sephacryl column after the contact time, with no additional neutralizer volume, and the full 2 mL of filtrate is collected. This is considered the 10^{-1} dilution.*
- *The 10^{-1} dilution is tenfold serially diluted in a dilution medium.*
- *250 µL of 10^{-1} to 10^{-7} dilutions is inoculated into the cell culture well.*
- *The limit of detection for $TCID_{50}$/0.25 mL is $10^{0.50.}$*

Results:

Plate Recovery Control

Dilution	Plate Recovery Control	Percent Infection
Cell Control	0 0 0 0	-
10^{-1}	+ + + +	100
10^{-2}	+ + + +	100
10^{-3}	+ + + +	100
10^{-4}	+ + + +	100
10^{-5}	+ + + 0	75
10^{-6}	0 0 + +	50
10^{-7}	0 0 0 0	0
$TCID_{50}$/250 µL	$10^{5.75}$	-
$TCID_{50}$/carrier	$10^{5.65}$	-

(+) = Positive for the presence of test virus
(0) = No test virus recovered and no cytotoxicity present

Calculations:

Using the Spearman Karber equation as follows:

Negative log of $TCID_{50}$/250 µl = -log of 1^{st} dilution assayed – [((Σ of % mortality at each dilution/100) – 0.5) x (log of dilution)]

Negative log of $TCID_{50}$/250 µl = -1 - [(((100 + 100 + 100 + 100 + 75 + 50)/100) – 0.5) X (1)]
= -1 – [((5.25) – (0.5)) x 1]
= -1 – 4.75
= -5.75
Log of $TCID_{50}$/250 µl = 5.75
$TCID_{50}$/250 µl = $10^{5.75}$

To calculate the $TCID_{50}$/carrier for the plate recovery control, multiply by 0.8 [note 0.8 is used since 200 µl of virus is applied to the carrier surface (200 µl /250 µl)]

$TCID_{50}$/carrier = (Antilog of 5.75) X 0.8 = 449,873

The log of 449,873 = 5.65 $Log_{10,}$ so the $TCID_{50}$/carrier = $10^{5.65}$

The calculations for the treated carriers and the log reduction would be performed consistent with what is presented in the first example but with the adjustment for the 250 µl inoculation volume.

Example 3 - With Cytotoxicity

Conditions:

- *200 µL of virus is dried onto the carrier.*
- *2 mL of media (plate recovery control) or test substance is added to the carrier.*
- *The mixture is immediately passed through a Sephadex or Sephacryl column after the contact time, with no additional neutralizer volume, and the full 2 mL of filtrate is collected. This is considered the 10^{-1} dilution*
- *The 10^{-1} dilution is tenfold serially diluted in a dilution medium.*
- *100 µL of 10^{-1} to 10^{-7} dilutions is inoculated into the cell culture well.*
- *The limit of detection for $TCID_{50}$/0.1 mL is $10^{2.5.}$*

Results:

Dilution	Plate Recovery Control	Virus + Test Substance Lot 1	Percent mortality for the treated carrier
Cell Control	0 0 0 0	0 0 0 0	-
10^{-1}	+ + + +	T T T T	100
10^{-2}	+ + + +	T T T T	100
10^{-3}	+ + + +	0 0 0 0	0
10^{-4}	+ + + +	0 0 0 0	0
10^{-5}	+ + + 0	0 0 0 0	0
10^{-6}	+ + 0 0	0 0 0 0	0
10^{-7}	0 0 0 0	0 0 0 0	0
$TCID_{50}$/100 µL	$10^{5.75}$	$\leq 10^{2.5}$	
$TCID_{50}$/carrier	$10^{6.05}$	$\leq 10^{2.80}$	
Log_{10} Reduction	N/A	≥ 3.25	

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present
(T) = Cytotoxicity

The plate recovery control is the same as calculated in example 1.

Calculations:

Using the Spearman Karber equation as follows:

Negative log of $TCID_{50}$/100 µl = -log of 1st dilution assayed – [((Σ of % mortality at each dilution/100) – 0.5) x (log of dilution)]

Negative log of $TCID_{50}$/100 µl = -1 - [(((100 + 100 + 0 + 0 + 0)/100) – 0.5) X (1)]
= -1 – [((2) – (0.5)) x 1]
= -1 – 1.5
= -2.5
Log of $TCID_{50}$/100 µl = 2.5
$TCID_{50}$/100 µl = $\leq 10^{2.5}$

To calculate the $TCID_{50}$/carrier for the treated carrier, multiply by 2 [note 2 is used since 200 µl of virus is applied to the carrier surface]

$TCID_{50}$/treated carrier = (Antilog of 2.5) X 2 = 632.46

The log of 632.46 = 2.8 $Log_{10,}$ so the $TCID_{50}$/carrier = $\leq 10^{2.8}$

The log reduction is:

(Log$TCID_{50}$/carrier for the plate recovery control) - (Log$TCID_{50}$/carrier for the treated carrier)

6.05- $\leq$ 2.8= $\geq$ 3.25

Example 4- Use of a Chemical Neutralizer

Conditions:

- *400 µL of virus is dried onto the carrier*
- *2 mL of media (plate recovery control) or test substance is added to the carrier.*
- *2 ml of chemical neutralizer is added after the contact time and mixed. This 4 ml of mixture is considered the 10^{-1} dilution*
- *The 10^{-1} dilution is tenfold serially diluted in a dilution medium*
- *100 µL of 10^{-1} to 10^{-7} dilutions is inoculated into the cell culture well.*
- *The limit of detection for $TCID_{50}$/0.1 mL is $10^{0.50.}$*

Results:
Plate Recovery Control

Dilution	Plate Recovery Control	Percent mortality
Cell Control	0 0 0 0	-
10^{-1}	+ + + +	100
10^{-2}	+ + + +	100
10^{-3}	+ + + +	100
10^{-4}	+ + + +	100
10^{-5}	+ + + 0	75
10^{-6}	0 0 + +	50
10^{-7}	0 0 0 0	0
$TCID_{50}$/mL	$10^{5.75}$	-
$TCID_{50}$/carrier	$10^{6.35}$	-

(+) = Positive for the presence of test virus
(0) = No test virus recovered and no cytotoxicity present

Calculations:

Using the Spearman Karber equation as follows:

Negative log of $TCID_{50}$/100 µl = -log of 1st dilution assayed – [((Σ of % mortality at each dilution/100) – 0.5) x (log of dilution)]

Negative log of $TCID_{50}$/100 µl = -1 - [(((100 + 100 + 100 + 100 + 75 + 50)/100) – 0.5) X (1)]
= -1 – [((5.25) – (0.5)) x 1]
= -1 – 4.75
= -5.75
log of $TCID_{50}$/100 µl= 5.75
$TCID_{50}$/100 µl = $10^{5.75}$

To calculate the $TCID_{50}$/carrier for the plate recovery control, multiply by 4 [note 4 is used since 400 µl of virus is applied to the carrier surface]

$TCID_{50}$/carrier = (Antilog of 5.75) X 4 = 2,249,365.3

The log of 2,249,365.3= 6.35 $Log_{10,}$ so the $TCID_{50}$/carrier = $10^{6.35}$

To calculate the $TCID_{50}$/carrier for the treated carrier,

Dilution	Virus + Test Substance Lot 1	Percent Infection
Cell Control	0 0 0 0	-
10^{-1}	+ + + +	100
10^{-2}	0 0 + +	50
10^{-3}	0 0 0 0	0
10^{-4}	0 0 0 0	0
10^{-5}	0 0 0 0	0
10^{-6}	0 0 0 0	0
10^{-7}	0 0 0 0	0
$TCID_{50}$/mL	$10^{2.00}$	-
$TCID_{50}$/carrier	$10^{2.60}$	-
Log_{10} Reduction	3.75	-

(+) = Positive for the presence of test virus
(0) = No test virus recovered and no cytotoxicity present

Negative log of $TCID_{50}$/100 µl = -1 - [(((100 + 50 + 0 + 0 + 0/100)) – 0.5) X (1)]
= -1 – [((1.5) – (0.5)) x 1]
= -1 – 1.5
= -2
$TCID_{50}$/100 µl = $10^{2.00}$

To calculate the $TCID_{50}$/carrier for the, multiply by 4 [note 4 is used since 400 µl of virus is applied to the carrier surface]

$TCID_{50}$/carrier for the treated carrier = (Antilog of 2.0) X 4 = 400

The log of 400 = 2.60 $Log_{10,}$ so the $TCID_{50}$/carrier = $10^{2.60}$

The log reduction is:

($LogTCID_{50}$/carrier for the plate recovery control) - ($LogTCID_{50}$/carrier for the treated carrier)

6.35-2.6= 3.75

Example 5 - Surrogate virus

Conditions:

- *200 µL of virus is dried onto the carrier*
- *2 mL of media (plate recovery control) or test substance is added to the carrier.*
- *The mixture is immediately passed through a Sephadex or Sephacryl column after the contact time, with no additional neutralizer volume, and the full 2 mL of filtrate is collected. This is considered the 10^{-1} dilution*
- *The 10^{-1} dilution is tenfold serially diluted in a dilution medium*
- *100 µL of 10^{-2} to 10^{-7} dilutions is inoculated into the cell culture well.*
- *The limit of detection for $TCID_{50}$/0.1 mL is $10^{0.50.}$*

Results:

Dilution	Plate Recovery Control		Virus + Test Substance Lot 1		Virus + Test Substance Lot 2	
	Rep 1	Rep2	Rep 1	Rep2	Rep 1	Rep2
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
	+ + + +	+ + + +	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-2}	+ + + +	+ + + +	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-3}	+ + + +	+ + + +	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-4}	+ + + +	+ + + +	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-5}	+ 0 + +	+ 0 0 +	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-6}	0 + 0 0	+ 0 + 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-7}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$/100 µL	$10^{5.50}$	$10^{5.50}$	$\leq 10^{1.50}$	$\leq 10^{1.50}$	$\leq 10^{1.50}$	$\leq 10^{1.50}$
Average $TCID_{50}$/100 µL	$10^{5.50}$		$\leq 10^{1.50}$		$\leq 10^{1.50}$	
Average $TCID_{50}$/carrier	$10^{5.80}$		$\leq 10^{1.80}$		$\leq 10^{1.80}$	
Log_{10} Reduction	N/A		≥ 4.00		≥ 4.00	

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present

Calculations:

To calculate the $TCID_{50}$/carrier for the plate recovery control,

$TCID_{50}$/100 µL = $10^{5.50}$

(Antilog of 5.50) X 2 [note: 2 is used since 200 µl is applied to the carrier surface] = 632,455.532

The log of 632,455.532 = 5.80 $Log_{10,}$ so the $TCID_{50}$/carrier = $10^{5.80}$

To calculate the $TCID_{50}$/carrier for the treated carrier,

$TCID_{50}$/100 µL = $\leq 10^{1.50}$

(Antilog of 1.5) X 2 = 63.2456

The log of 63.2456 = 1.8 Log_{10} = $TCID_{50}$/carrier= $\leq 10^{1.8}$

Log_{10} reduction per carrier surface (200 µL): 5.80 Log_{10} - ≤ 1.80 Log_{10} = ≥ 4.00 Log_{10} reduction in viral titer