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Via Electronic Mail

Subject: HCPA and CBC Comments on Evaluating the Efficacy of Antimicrobial Test Substances on Porous Surfaces in Non-Residential Settings; Interim Guidance and Methods; Docket No. EPA-HQ-OPP-2022-0337

The Household & Commercial Products Association[1] (HCPA) and Center for Biocide Chemistries (CBC) thank the U.S. Environmental Protection Agency (EPA) for the opportunity to provide comments on the *Interim Guidance and Methods for Evaluating the Efficacy of Antimicrobial Test Substances on Porous Surfaces in Non-Residential Settings*.

HCPA and CBC acknowledge the importance of EPA guidance and test methods. They provide the regulated community with essential direction on evaluating products to ensure consistent and measurable evaluation of their benefits to public health. We recognize that the guidance and methods for efficacy claims on porous surfaces are still under development and appreciate the opportunity for stakeholder engagement. Our comments identify critical issues and areas of ambiguity that should be considered and addressed by EPA prior to finalizing the guidance and methods.

Beyond the specific comments outlined further below, we note that the interim guidance and methods seem to be based on ASTM International's (ASTM) *New Test Method for Quantitatively Evaluating the Efficacy of Antimicrobial Test Substances on Hard, Non-Porous Surfaces Against Bacteria*.[2] The ASTM quantitative method (QM) has innate issues that the ASTM work group is actively working to resolve. The QM issues of carrier manufacturer, inert nature of the carrier, mucin preparation and consistency, reproducibility, and bias concerns must still be addressed. These issues are also expected to

[1] The Household & Commercial Products Association (HCPA) is the premier trade association representing companies that manufacture and sell $180 billion annually of trusted and familiar products used for cleaning, protecting, maintaining, and disinfecting homes and commercial environments. HCPA member companies employ 200,000 people in the U.S. whose work helps consumers and workers to create cleaner, healthier and more productive lives.

[2] New Test Method for Quantitatively Evaluating the Efficacy of Antimicrobial Test Substances on Hard, Non-Porous Surfaces Against Bacteria (ASTM WK78068).

negatively impact the reliability and use of the interim methods for porous surface efficacy. Given that ASTM's QM has yet to go through a multi-laboratory collaborative, additional method changes could directly impact the porous surfaces interim methods. Until then, and until our comments are addressed, we strongly encourage the Agency to continue accepting alternative protocols to support porous surface efficacy claims.

Guidance Comments – Document 0002

HCPA and CBC appreciate EPA's diligent efforts in developing guidance for evaluating efficacy of antimicrobial products on porous surfaces. We offer the following specific comments:

The Categorization of Surface Types

The interim guidance currently states: "based on the porous materials selected in the recommended efficacy test methods, this guidance is intended to be representative of clinical and/or institutional environments (non-residential) and to address efficacy of products against public health pathogens when used on soft, porous materials in these settings."

HCPA and CBC request additional clarity from the Agency on the criteria used to delineate and define porous versus non-porous and soft versus hard surfaces as it relates to the allowable surface claims under this guidance. It is currently unclear what master label claims will be accepted with the submission of the prescribed efficacy data.

The Selection of Test Carriers

The interim guidance requires viral and bacterial efficacy testing for three carrier types: vinyl seating fabric ("vinyl face, polyester backing"), non-polyvinyl chloride (non-PVC) fabric ("polyutherane face made with polycarbonate and polyester resins, polyester backing"), and privacy curtain fabric (54 percent polyester, 46 percent fire resistant polyester). The vinyl seating fabric and non-PVC fabric, however, do not reflect the definition of "porous" surfaces.

Vinyl, for example, is generally considered to be non-porous or poorly porous in nature by EPA and registrants and does not represent surfaces most likely to harbor infectious bacteria in clinical and institutional settings.[3] In fact, porous surfaces that are more permeable to potentially infectious fluids are often discouraged in clinical furnishings. According to the Centers for Disease Control and Prevention Guidelines for Environmental Infection Control in Health-Care Facilities,[4] "recovering worn, upholstered furniture (especially the seat cushion) with covers that are easily cleaned (e.g., vinyl), or replacing the item is prudent; minimizing the use of upholstered furniture and furnishings in any patient-care areas where immunosuppressed patients are located (e.g., HSCT units) reduces the likelihood of disease."

[3] Noskin, G. A., et al. (2000). Persistent contamination of fabric-covered furniture by vacomycin-resistant enterococci: Implications for upholstery selection in hospitals. *American Journal of Infection Control*, 28(4), 311-313: https://doi.org/10.1067/mic.2000.108129.

[4] Guidelines for Environmental Infection Control in Health-Care Facilities.

Vinyl fabric is more common in these use sites, and behaves like hard, non-porous surfaces as is demonstrated in EPA's preliminary data.[5] For this reason, EPA has historically accepted the standard hard, non-porous surface test methods to support claims on vinyl surfaces. According to a survey conducted on January 18, 2023, on the number of registrations in the last five years (including primary registrations) that include public health claims for use on vinyl surfaces, out of the 164 total new antimicrobial products approved by EPA, the Agency approved 100 percent of the products with vinyl as a non-porous surface.[6]

Defining the vinyl[7] and non-polyvinyl chloride[8] test carriers in these methods as representative porous surfaces could lead to greater confusion seeing that both carrier types are defined as "inherent fluid barriers" and contain an "advanced soil resistant and stain resistant top coat" on the manufacturer website.

Additionally, the three material types selected as representative of porous surfaces are contradictory to the current OCSPP 810.2000[9] Section E.5 which states: "examples of soft porous surfaces include fabrics (e.g., cotton, polyester, etc.)." It is currently unclear what "porous surfaces" will be allowable in a claim given the representative materials chosen are not all porous and two of the surfaces currently are contradictory to what EPA has historically defined as porous representatives (e.g., cotton and polyester). *We request that the Agency revise the representative surface types chosen to support soft, porous surface claims by minimally removing vinyl and non-polyvinyl chloride as required surface types to avoid registrant and end-user confusion. Specifically, HCPA and CBC also request that EPA confirm whether testing against the three representative carrier materials will support claims against cotton and polyester and ask that the rationale and justification for the Agency's movement away from the use of these two representative materials be provided.*

Surface Compatibility Testing

The interim guidance currently states: "for all porous surfaces tested, the applicant should document compatibility of the product with the porous material per the proposed label prior to use. Data and observations pertaining to physical degradation, pitting, fraying, cracking, delamination, bleaching of dyes, etc., may indicate incompatibility of the product with the porous surface. These data and observations should be submitted in the final report to the Agency."

There is currently no standardized method specified by EPA for determining surface compatibility and registrants would not be able to appropriately satisfy this requirement. Surface compatibility testing is a form of product stewardship performed by registrants to assess the end-user experience over the lifetime of a product's use. This testing does not address the public health concern being tested with the efficacy methods (i.e., reduction of pathogens) and should be excluded as a requirement for efficacy.

Other EPA efficacy methods do not include a requirement for surface compatibility testing. Consistency across methods is critical to ensure a level playing field and avoid confusion. Additionally, the guidance suggests that surface testing would be done within the same protocol/report as the efficacy testing;

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[5] Demonstration Studies on the Use of a Quantitative Test Method for Evaluating the Performance of Disinfectants on Porous Materials.
[6] Pesticide Product Label System (PPLS).
[7] CF Stinson designMix : Catalog : - Hopsack HOP24 Fjord.
[8] CF Stinson designMix : Catalog : - Kid KID17 Blue Sky.
[9] Product Performance Test Guidelines OCSPP 810.2000 General Considerations for Testing Public Health Antimicrobial Pesticides, Guidance for Efficacy Testing.

however, most laboratories do not have the chemistry expertise to conduct this type of testing alongside the efficacy testing.

HCPA and CBC request that EPA remove the requirement to submit surface compatibility testing data.

Reduced Test Surface Evaluation

We note that the guidance currently requires a product to achieve the method(s)' performance standards on all three of the representative surfaces, regardless of whether the product includes claims for each of these surfaces. It is currently unclear why all three of these very specific material types would need to be tested if some of these material types do not apply to the product use or claim. For example, if a registrant would like to make a stand-alone vinyl seating fabric claim, they should not be required to test the privacy curtain fabric and non-PVC fabric surfaces.

HCPA and CBC request that EPA revise the guidance to allow standalone surface claims to be made against vinyl seating fabric, privacy curtain fabric, non-PVC fabric, or other porous surfaces, where the master label contains product use direction for the specific surface(s) tested only.

Prerequisites for Non-Porous Testing

The porous surface guidance indicates (page 1) that the methods associated with it address only products with both porous disinfectant claims and hard, non-porous surface disinfectant claims but not products that have only porous claims. In OCSPP 810.2100[10], however, EPA allows sporicidal testing and claims on the porous test carriers (porcelain penicylinders or silk/polyester suture loops) without the requirement to test on the hard, non-porous carriers (stainless steel). Similarly, in the Series 810 FAQ[11], EPA further confirms this stance that no prerequisites are needed. Given these porous surface and non-porous surface claims are often unrelated, it is unclear why this prerequisite has been established. For example, a product formulated for use on porous surfaces may not always be intended for use on non-porous surfaces. Alignment among existing guidance is critical to ensure consistency.

HCPA and CBC request that EPA remove the prerequisite requirement for hard non-porous disinfection surface claims to be made prior to the allowance of porous surface disinfection claims.

Guidance Relation to Existing Soft Surface Claims

EPA has not clarified how the porous surfaces interim guidance and methods relate to the existing EPA approved soft surface sanitization claims made following OCSPP 810.2400,[12] which involve very different tests and carrier types than described in the interim guidance. While the existing non-food contact sanitizer method modified for soft surface testing focuses on fabric surfaces (e.g., polyester, cotton), the interim guidance does not. Based on member experiences, polyester and cotton represent stringent porous surfaces, which is supported by the EPA data provided in the docket with the porous guidance and methods.[13]

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[10] *Ibid.*

[11] OCSPP Frequent Questions for the 2018 Series 810 – Product Performance Test Guidelines: Antimicrobial Efficacy Test Guidelines. Page 9.

[12] Product Performance Test Guidelines OCSPP 810.2400: Disinfectants and Sanitizers for Use on Fabrics and Textiles—Efficacy Data Recommendations.

[13] Docket No. EPA-HQ-OPP-2022-0337.

HCPA and CBC request clarification from EPA on how the soft, porous surface guidance and methods work alongside the existing EPA approved soft surface claims.

We also request that EPA allow for registrants to make standalone porous surface disinfection claims for additional microorganisms.

Bridging of Claims

The interim guidance currently states: "to support claims for additional bacteria, testing should be conducted according to the Interim Quantitative Method for Evaluating the Efficacy of Antimicrobial Test Substances on Porous Surfaces Against Bacteria, but with a reduced number of product lots (two) as specified in the OCSPP 810.2000 Test Guideline" and "all viruses for which claims are desired should be tested."

These statements currently indicate that bridging is not allowed by the guidance. This practice is currently contradictory to the bridging allowances outlined in the Residual Efficacy Guidance,[14] Electrostatic Spray Guidance,[15] and disinfectant towelette section of OCSPP 810.2200[16] Section K.2.

HCPA and CBC request that when the registrant has voluntarily tested the required bacterial strains and worst case virus in both hard non-porous disinfection and soft, porous disinfection, EPA revise the guidance to permit the allowance for the bridging of claims against additional bacterial and viral claims.

We also request that EPA lay out these policies in detail in the final guidance by stating that data supporting more stringent application parameters (i.e., contact times and product dilution) will be bridged to less stringent application parameters as is outlined in OCSPP 810.2000 Section E.8. [17]

Use Sites

In the introduction section of the guidance, EPA states that the guidance is intended to be representative of clinical and/or institutional environments (non-residential). However, the Agency fails to consider how upholstered furniture in a residential setting differs from a long-term care facility or school. It is also unclear why upholstered furniture is considered a separate use from textiles/upholstery, when EPA allows soft surface testing for both surface types under OCSPP 810.2400[18]. Details on the criteria used to determine use sites would help registrants to better understand how and if the methods and guidance affect residential uses.

HCPA and CBC are concerned that end-users of these products will not understand the differentiation unless the Agency gives clear examples and areas of separation. *Therefore, we request that EPA consider end-user confusion when providing instructions on how to separate the residential and non-residential use sites.*

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[14] Guidance for Products Adding Residual Efficacy Claims.
[15] Instructions for Adding Electrostatic Spray Application Directions for Use to Antimicrobial Product Registrations.
[16] Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces, Guidance for Efficacy Testing.
[17] Product Performance Test Guidelines OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides, Guidance for Efficacy Testing.
[18] Product Performance Test Guidelines OCSPP 810.2400: Disinfectants and Sanitizers for Use on Fabrics and Textiles—Efficacy Data Recommendations.

We also request that EPA confirm that the selected carriers represent both residential and non-residential use sites and strongly encourage the Agency to consider carriers that represents both types of use sites to avoid test duplication.

Wetness of Test Carriers

The interim guidance currently states: "conduct a wetness test consistent with that outlined in Methods and Guidance for Testing the Efficacy of Antimicrobial Products Against Spores of *Clostridioides difficile* on Hard Non-Porous Surfaces (September 2022) and provide evidence (such as a photo) to demonstrate that the surface remains wet for the duration of the contact time."

Given the only allowable applications are liquids, sprays (including trigger sprays and aerosols), and foams, this referenced method is irrelevant as it was written to substantiate the use of towelette product contact times. EPA has historically allowed these standard product applications (i.e., liquids, sprays, and foams) to be applied to porous and non-porous surfaces "until visibly wet" when wetness can be visually observed and documented accordingly in the efficacy test for hard, non-porous surfaces. The current method for soft surface non-food contact sanitizer claims outlined in OCSPP 810.2400[19] does not require a wetness test to be performed. Therefore, it is unclear why a wetness test is required in the new interim guidance.

HCPA and CBC request that EPA remove the wetness test requirement to be consistent with the guidance on hard non-porous disinfectants and soft surface non-food contact sanitizers; or, alternatively, provide additional guidance on these unique test carriers as the current reference does not provide sufficient detail.

Contact Time

Section II. k. states that the contact time for disinfectants for use on porous surfaces is consistent with the use on hard, non-porous surfaces as described in OCSPP 810.2200 Test Guideline. However, porous surfaces may require a longer contact time than hard, non-porous surfaces. Though OCSPP 810.2400[20] also outlines a 5-minute limit for porous surface laundry sanitization claims and a 10-minute contact time restriction for disinfection claims, EPA has historically allowed use directions to exceed this limitation by 5 and 10 minutes, respectively, for the laundry sanitizer and disinfection applications. Additionally, the guidance does not limit the contact time for carpet sanitizer applications.

HCPA and CBC request that EPA clarify whether longer contact times (i.e., >10 minutes) would be acceptable if the visible wetness is appropriately documented in the efficacy test; or, alternatively, provide the ability to consult the Agency when appropriate to justify the wetness characteristics supported for a particular application.

Eligible Product Types

The interim guidance currently states: "For products using methods of application beyond those listed here including towelettes, fogging, misting, and electrostatic spray, please consult with the Agency." It is

[19] Product Performance Test Guidelines OCSPP 810.2400: Disinfectants and Sanitizers for Use on Fabrics and Textiles—Efficacy Data Recommendations.
[20] *Ibid.*

unclear why application methods such as towelettes are excluded from this method. EPA currently registers soft, porous sanitization claims with these products.[21]

This exclusion is particularly restrictive given the number of towelette products already registered for use on vinyl surfaces as it has historically and consistently been deemed a hard, non-porous surface by EPA. The Centers for Disease Control and Prevention's Guidelines for Environmental Infection Control in Health-Care Facilities[22] recommends that healthcare environmental surfaces (i.e., areas housing immunosuppressed patients) are disinfected by EPA-registered towelettes due to concerns with disinfectant spraying or fogging in patient-care areas. As seen by this example, other federal agencies rely on the availability of certain product types and excluding those without justification would cause significant confusion.

We recommend that the guidance and methods allow for towelette applications.

Three-Part Soil

The interim guidance currently states: "use the three-part soil load identified in the method."

OCSPP 810.2200[23] currently does not require the use of three-part soil when making hard surface disinfection claims. Hard surface disinfection claims have historically been supported with the addition of animal serum as is outlined in OCSPP 810.2000.[24] Additionally, the requirement for the use of three-part soil appears to disallow for two-step application claims which will lead to further misalignment between hard and soft surface claims. See additional concerns outlined on Page 12, Line 57, of HCPA's and CBC's viral method comments. *We request that EPA revise this guidance to make the use of soil optional to allow for two-step application claims.*

Different organic soil load types may impact test results between hard surface and soft surface testing leading to differing claim parameters. Furthermore, given that many viruses and host cells' frozen stocks already include high levels of animal serum for their survival upon thawing and recovery, the compatibility of the soil for all viruses and their host cells has not been confirmed. OCSPP 810.2000[17] currently states: "additional organic material need not be incorporated into those procedures where at least 5% blood serum is already present in the microbial inoculum to be dried on the surface." This puts 810.2000 and the new guidance in conflict. If three-part soil is added to the existing viral stock soil loads, the levels of soil challenge may be well in excess of 5 percent soil.

HCPA and CBC request that EPA accept alternative soil loads (e.g., fetal bovine serum) to permit application claim alignment, avoid excessive addition of soil to the viral stocks, and avoid the conflict in the two guidelines.

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[21] *Ibid.*

[22] Guidelines for Environmental Infection Control in Health-Care Facilities.

[23] Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces, Guidance for Efficacy Testing.

[24] Product Performance Test Guidelines OCSPP 810.2000 General Considerations for Testing Public Health Antimicrobial Pesticides, Guidance for Efficacy Testing.

Repeat Testing

We request clarification from the Agency on whether the repeat testing policy outlined in the Frequent Questions for the 2018 Series 810 – Product Performance Test Guidelines Section (E)(11) and Appendix II[25] will apply to the products outlined in the interim guidance.

Method Validation and Test Replication

The interim guidance currently instructs to evaluate the three representative material types using five carriers against the product and three untreated control carriers for each lot (or batch) of product on three independent test dates. This amount of replication seems excessive as compared to the hard non-porous disinfection efficacy testing requirements. Additionally, the bacterial data provided by the Agency has small error bars that contradict the need for this degree of replication.

According to EPA's Method Validation of U.S. EPA Microbiological Methods of Analysis Guidance,[26] "regardless of the purpose, reliable, and accurate methods are needed to ensure the validity of the data collected. Methods used for these purposes therefore must be validated before they are published as EPA methods." HCPA and CBC are concerned that the amount of replication outlined in the guidance is due to the lack of a multi-laboratory collaborative study being performed to validate these methods. According to the Guidance,[19] "EPA has historically recommended the use of multi laboratory collaborative studies for the validation of methods that are expected to see widespread use or to support regulatory activity." Given these methods are intended for nationwide use and will be used to support regulatory activity, the Guidance recommends that these methods would be assigned as Tier 3 and should be supported with a multi-laboratory study for proper validation.

Additionally, EPA has provided a limited dataset for the prescribed methods and the validation process documentation is presently unclear. The Agency's Policy[27] (FEM-2009-01) states that, "[i]t is EPA's philosophy that all methods of analysis should be validated prior to issuance as an Agency method. This policy directive addresses the validation of microbiological methods of analysis, which should be validated by a process that, at a minimum, follows the guidelines in Method Validation of U.S. Environmental Protection Agency Microbiological Methods of Analysis (FEM Document Number 2009-01, October 7, 2009; REVISION: December 21, 2016). Any EPA organization that proposes to issue a microbiological method of analysis should ensure and document that the method has been validated according to this policy."

HCPA and CBC request that EPA provide the method validation and statistical analysis performed that demonstrates the need for the prescribed replication outlined in the guidance; or, alternatively, consider performing additional multi-laboratory testing to assess the repeatability and reproducibility of the method in accordance with the Agency's Guidance[19] to theoretically reduce the amount of replication prescribed.

[25] OCSPP Frequent Questions for the 2018 Series 810 – Product Performance Test Guidelines: Antimicrobial Efficacy Test Guidelines.
[26] Method Validation of U.S. Environmental Protection Agency (EPA) Microbiological Methods of Analysis.
[27] Ensuring the Validity of Agency Method Validation and Peer Review Guidelines: Microbiological Methods of Analysis.

Cleaning Requirement

The interim guidance currently states: "additional porous surface materials (carriers) may be chosen by the applicant. The additional materials chosen should be able to be cut into one centimeter diameter discs (two millimeter maximum thickness), withstand physical screening, cleaning and sterilization, drying under desiccation, as well as the vortex steps outlined in the method. The inoculated material should provide the necessary recovery level of each test organism to measure acceptable performance for the claim. Applicants are encouraged to consult with EPA prior to initiating testing with additional porous surfaces."

HCPA and CBC request that the Agency delete the word "cleaning" from the paragraph above given the fact that the method specifically states that no cleaning of the carriers is required.

LCL Testing Requirement

The current requirement outlined in the guidance (Table 2) defines that all testing should be conducted at the Lower Certified Limit (LCL):

Claim	Test Method	Test Organisms	Carrier Types	No. of Lots
Base Bacteria	Interim Quantitative Method for Evaluating the Efficacy of Antimicrobial Test Substances on Porous Surfaces Against Bacteria	*Staphylococcus aureus* (ATCC No. 6538) and *Pseudomonas aeruginosa* (ATCC No. 15442)	VF-01, PCF-03, and NVF-01	3 lots per organism at the LCL for each carrier type
Additional Vegetative Bacteria	Interim Quantitative Method for Evaluating the Efficacy of Antimicrobial Test Substances on Porous Surfaces Against Bacteria	All vegetative bacteria claimed on the label	VF-01, PCF-03, and NVF-01	2 lots per organism at the LCL for each carrier type

This requirement to test at the LCL for Additional Vegetative Bacteria is more stringent than the requirement for hard, non-porous surface disinfection defined in OCSPP 810.2200.[28]

We request that EPA revise the requirement to allow for testing at the nominal concentration for Additional Vegetative Bacteria to align with the hard, non-porous disinfection requirements; or, alternatively, provide an explanation for the added stringency.

Claims for Additional Microorganisms

We understand and appreciate the opportunity for registrants to consult with the Agency to include claims for additional microorganisms that are not under the scope of this guidance. In the case of yeasts, EPA has historically included these microorganisms in testing along with the bacterial test methods (except for *Candida auris*) without the requirement of an additional protocol submission and review. Yeasts, however, can easily be tested like bacteria.

To ensure consistency with other methods and improve efficiencies, HCPA and CBC urge EPA to consider allowing yeasts to be tested similar to additional bacteria with the exclusion of Candida auris.

[28] Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces, Guidance for Efficacy Testing.

Replication of Virucidal Testing

HCPA and CBC have concerns with the feasibility of the viral test method as EPA issued a limited efficacy data set[29] for that test method. The current viral method which only includes data on control counts and no disinfectant data, is inconsistent with EPA's requirement for replicability. Registrants will have a difficult time reproducing a method that has not been substantiated by data or validated by a multi-laboratory collaborative effort. Reproducibility is important so that laboratories can replicate the method as intended by EPA to provide the level of certainty that the Agency needs to approve a public health claim. We note that a requirement for multiple test dates is not a typical practice for viral testing and poses additional difficulty by potentially requiring multiple cell line passages or flasks. Testing each viral lot on separate days can be challenging depending on the virus that is being tested.

We suggest that EPA revise the guidance to allow for testing product lots on the same day to align with historically acceptable practices for hard, non-porous disinfection claims and to ensure the same cell line passages are being used. We also request that the Agency revise the carrier replication requirements from "five treated carriers" to "three treated carriers" per lot for surrogate and non-surrogate viruses to reduce the cost and extensive laboratory staffing burden necessary to meet this requirement. Moving to three treated carriers will still be more rigorous than the current viral method replication for hard, non-porous disinfection claims, which requires only a single carrier for non-surrogate viruses or two carriers for surrogate viruses.[30] *HCPA and CBC further request that once a collaborative is completed that the replication be revisited to possibly further reduce this requirement.*

Section III of the guidance currently states that each lot of the product should achieve a minimum mean 3.0-log reduction for qualifying viruses. *HCPA and CBC request that EPA confirm the acceptance criteria is a mean of the tested carriers.*

The current requirement outlined in the guidance (Table 3) specifies that "all viruses claimed on the label" are tested at the nominal concentration.

Claim	Test Method	Test Organisms	Carrier Types	No. of Lots
Base Bacteria	Interim Quantitative Method for Evaluating the Efficacy of Antimicrobial Test Substances on Porous Surfaces Against Bacteria	*Staphylococcus aureus* (ATCC No. 6538) and *Pseudomonas aeruginosa* (ATCC No. 15442)	VF-01, PCF-03, and NVF-01	3 lots per organism at the LCL for each carrier type
Virucidal	Interim Quantitative Method for Evaluating the Efficacy of Antimicrobial Test Substances on Porous Surfaces Against Viruses	Hardest to kill virus	VF-01, PCF-03, and NVF-01	2 lots at the LCL for each carrier type
		All viruses claimed on the label	VF-01, PCF-03, and NVF-01	2 lots at the nominal concentration for each carrier type

"Hardest to kill virus" and "all viruses claimed on the label" are contradictory. *HCPA and CBC request that EPA update the table to read "all additional viruses claimed on porous surfaces" and "Hardest to kill*

[29] Memorandum – Demonstration Studies on the Use of a Quantitative Test Method for Evaluating the Performance of Disinfectants on Porous Materials.

[30] Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces, Guidance for Efficacy Testing.

virus claimed on porous surfaces" to clarify the list of viruses being tested and exclude the hardest to kill virus which is required to be tested at the LCL concentration.

We also request that EPA address claims against SARS-CoV-2, explicitly confirming that two batches at the nominal concentration are acceptable where the virus is not the "hardest to kill" virus for porous surface claims.

Labelling and Additional Information

We appreciate EPA making products eligible for inclusion on List N if they meet the criteria in the Emerging Viral Pathogens (EVP) guidance or are supported by appropriate testing for a qualifying virus.

However, HCPA and CBC request that the Agency expand eligibility for inclusion in EPA's "Common Pathogen" lists.[31]

Sample Directions for Use

The sample directions for use provided in the guidance should be revised to be more consistent with other EPA guidance/regulation and methods. The wording used for the instructions may be interpreted as contradictory. Therefore, HCPA suggests the following modifications:

c. Sample directions for use:
   i. Apply in a limited area (spot treatment), monitor treated area for wetness for duration of the contact time, and allow to dry.
   ~~ii. Apply to surfaces only. Do not use on surfaces that routinely contact skin (i.e., clothing, sheets, towels).~~
   ~~iii. Only for use on non-launderable surfaces or those that may be laundered on an infrequent (non-routine) basis.~~

The language restricting use of chemistries on surfaces that contact skin (IV. c. iii.) is not applicable to all products. Instead, it is dictated by the precautionary and hazard language present on the master label and supported by acute toxicity data.[32] *While we understand that this is sample language and alternative language would be acceptable, we request that EPA remove this from the sample directions for use to avoid confusion.*

Additionally, the language restricting use of chemistries on surfaces that are non-launderable or infrequently laundered (IV.c.ii) does not align with currently accepted porous surface sanitization claims.[33] *Given the inconsistency, HCPA and CBC request the removal of the sample language.*

Preparing an Application for Registration

HCPA and CBC note that additional bacterial and virucidal claims are not addressed throughout Section V of the guidance and request that EPA address these claims to avoid confusion.

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[31] Antimicrobial Products Registered with EPA for Claims Against Common Pathogens.
[32] EPA Label Review Manual.
[33] Product Performance Test Guidelines OCSPP 810.2300: Sanitizers for Use on Hard Surfaces—Efficacy Data Recommendations.

We also note that the Pesticide Registration Improvement Act (PRIA) codes referenced in the guidance will need to be updated to reflect the new PRIA 5 codes and fees. Section 5 also references the content that should be included in the cover letter to EPA, implying that there is an expedited process for these types of PRIA submissions. It is our understanding that the expedited process for review of these types of claims no longer exists as it was implemented only during the COVID-19 pandemic. *Please clarify whether this information is not necessary because an expedited process does not exist.*

Microbiology Laboratory Branch Memorandum – Document 0003

Study Overview

HCPA and CBC request that EPA confirm the Active Ingredient (AI) levels for the citric acid, hydrogen peroxide, quaternary ammonium compound that the laboratory applied to the porous materials to measure the log reduction (LR).

Antimicrobial Treatments

The test conditions refer to ready-to-use (RTU) treatments against *Pseudomonas aeruginosa* and *Staphylococcus aureus*; HCPA wishes to know which items were diluted in hard water under the test conditions.

Additionally, given the fact that the only results reported were for the Human Coronavirus, HCPA and CBC request that EPA provide any additional viral method validation work performed, accompanied by the raw data for all preliminary bacterial and viral efficacy testing performed.

Comments on the Interim Quantitative Method for Evaluating the Efficacy of Antimicrobial Test Substances on Porous Surfaces Against Viruses – Document 0004

LOCATION	COMMENT
Lines 13-14	The method currently specifies that each carrier receives 10 µL of microbial inoculum with a three-part organic and inorganic soil load. Three-part soil contains mucin, yeast extract, and bovine serum albumin, all of which are organic soil load components. HCPA and CBC request that EPA remove "and inorganic" from the method.
	We also request that EPA allow flexibility in modifying the growth and drying conditions for alternative organisms by adding the following statement: "appropriate modifications to the method may be required when testing organisms not specified herein."
Lines 15-17	The method currently specifies that liquid neutralization must be performed. HCPA and CBC request that EPA expand this section to allow for the use of columns and chemical neutralizers to aid in neutralization as a mitigation step to prevent cytotoxicity as in ASTM E1053.[34]

[34] ASTM E1053-20 Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces.

Lines 48-51	EPA's recommended neutralizer for the test system is the same medium used to grow the virus (e.g., Complete Growth Media (CGM)), however, this is not always effective. When trying to grow the virus to high titers, different media may be used that are not necessary when simply growing the strains or cells. Therefore, we request that EPA revise the method to add the allowance for alternative neutralization approaches (e.g., the use of sterile medium).
Line 57	This section does not address situations where animal serum (e.g., fetal bovine serum) is present in the viral suspension as the viral growth medium. The stock viruses often require animal serum to grow to high titers and successfully thaw for use in testing. This already puts anywhere from one percent to 20 percent animal serum in the viral inoculum. The required three-part soil on top of this would present an additional challenge and unnecessary redundancy in organic load. Dilution of the stock to thin out the animal serum present may not be possible. HCPA and CBC request that EPA revise this section to allow for the animal serum in the viral suspension to be considered in calculating the soil load as in standard industry practice for hard surface disinfection claims. OCSPP 810.2200[35] currently does not require the use of three-part soil when making viral hard surface disinfection claims. Additionally, different organic soil load types can impact test results between hard surface and soft surface testing. We are concerned with the compatibility of mixing organic soil types and feel that the effects on the viruses and/or host cells are unclear. Given the limited data set provided, HCPA and CBC request that EPA revise this section to make the use of three-part soil an optional step and permit alternative soil load allowances (e.g., fetal bovine serum).
Line 97	HCPA and CBC request that EPA supply the vendor and catalog number of a suggested vial with lids used as an example in this section. Making this an example would allow for alternative vials with lids to be used to provide vendor flexibility.
Line 111	15 mL conical centrifuge tubes are listed as the only option for this section. Often, however, alternate conical tube volumes or tube types are used (e.g., 50 mL conical tubes). We request that EPA list 15 mL conical centrifuge tubes as an example to allow for alternate volumes or tube types.
Lines 123-124	The manufacturer websites[36,37,38] carrier component percentages differ from those listed currently in the method. HCPA and CBC request that EPA clarify whether these exact carrier component percentages must be followed when sourcing carriers. If so, please revise the method to align to the current carrier manufacturer percentages. The method currently specifies patterns and colors for carrier materials (e.g., Mambo, Hopsack, and Blue Sky). We are concerned that these specified materials may not always be available. Please revise this section to make these exact

[35] Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces, Guidance for Efficacy Testing.
[36] CF Stinson designMix : Catalog : - Kid KID17 Blue Sky.
[37] CF Stinson designMix : Catalog : - Hopsack HOP24 Fjord.
[38] CF Stinson designMix : Catalog : - Mambo MAM34 Nights.

	patterns and colors examples to allow for the use of alternative colors and patterns based on availability. We are also concerned that the dyes or components present in the fabric carriers selected for testing may not be inert (e.g., dyes, flame retardant components, soil resistant coatings, stain resistant coatings). We request that EPA provide data generated to address compatibility of these fabrics and clarify that the Agency will accept retest arguments as a result of observed incompatibility. We also recommend that EPA revise this section to state: "If carrier incompatibility is observed, consult the Agency to discuss the use of alternative carriers" to allow for flexibility.[39] [40]
Line 131	As seen in the photos in the method, 1 cm round carriers may not always be exactly round and, therefore, not exactly 1 cm in diameter across all areas of the carrier. HCPA and CBC request that EPA revise this section to state that the diameter should be approximately 1 cm in diameter to account for potential variability in the roundness of the carrier punch. Additionally, please also revise this section to allow for square 1 cm X 1 cm carriers to be utilized if a carrier punch is not available.
Line 133	The method currently states to visually screen the carriers to ensure "consistent surface characteristics." HCPA and CBC request that EPA provide photos or detailed, written examples to clarify what characteristics are acceptable and unacceptable (e.g., fraying, ripping, discoloration, backing separation) regarding the screening of both sides of the carriers.
Line 135	The method currently states that pre-cleaning is not considered necessary, but it could be necessary in some instances. We request that EPA clarify that cleaning of the carriers would be allowable if deemed necessary.
Line 137	Carrier sterility is often assessed concurrently with an efficacy study. HCPA and CBC request that EPA revise this section to address how to test the carrier sterility and to allow for the carrier sterility control to be performed "prior to or concurrently with efficacy testing."
Line 138	The phrase "minor distortion" is vague regarding the carrier preparation, leaving each reader to define this for themselves. HCPA and CBC request that EPA consider providing photos or detailed, written examples regarding what is considered acceptable and unacceptable distortion (e.g., cupping, doming).
Line 140	Tracking photos to their carrier number and inclusion of photos into raw data can all be problematic in a Good Laboratory Practice (GLP) efficacy study. Given the large number of carriers that would need to be tracked due to the replication required in this method, we are concerned that this would create an undue burden on laboratories that is not requested for in any other disinfection efficacy method. Visual confirmation of carrier acceptability can be documented in writing by the technician. HCPA and CBC request that EPA revise this section to allow for written affirmation of acceptable carrier condition.

[39] McDonnell G. Alternative AOAC sporicidal test carrier for evaluating peracetic acid-based sterilants (modification of AOAC official method 966.04). J AOAC Int. 2003 Mar-Apr;86(2):407-11. PMID: 12723925.

[40] McDonnell G, Amato R, Malchesky PS, Harrington S, Muzic DS, Marchant RE. Use of Dacron as an alternative carrier for evaluating oxidizing sterilants in the AOAC sporicidal test. J AOAC Int. 2000 Mar-Apr;83(2):269-75. PMID: 10772163.

Line 141	HCPA and CBC recommend that EPA revise this section to allow for carriers to be kept for up to six months after sterilization as in recent revisions made to ASTM *New Test Method for Quantitatively Evaluating the Efficacy of Antimicrobial Test Substances on Hard, Non-Porous Surfaces Against Bacteria*).[5] Based on preliminary physical observations, re-sterilized carriers indicate that an additional run through an autoclave may be possible without significant additional distortion of the carriers. We request that EPA consider including an option to re-sterilize carriers if desired.
Line 142	HCPA and CBC request that EPA clarify how often the carrier cytotoxicity checks must be performed (e.g., carrier cytotoxicity checks should be performed once per cell line, prior to or concurrent with testing). Additionally, please clarify how many wells should be plated for this control (e.g., plate a minimum of two wells) and confirm that serial dilutions are not required.
Line 146	Observing cells for cytotoxicity daily is not a necessary practice. HCPA and CBC request that EPA revise this statement to allow for daily monitoring as an option.
Line 151	We request that EPA revise the concentration instructions for the virus stock stating "~100,000 x g for 4 hours at 4°C" as an example rather than the only way to concentrate virus stocks. Not all viruses may tolerate or need this duration, but others may need longer. The duration is also dependent upon the equipment available.
Line 166	HPCA and CBC request that EPA allow for the use of glass petri dishes.
Line 178	The recent revisions made to ASTM's *New Test Method for Quantitatively Evaluating the Efficacy of Antimicrobial Test Substances on Hard, Non-Porous Surfaces Against Bacteria*[41] indicates that the pipet tip should be perpendicular to the carrier surface during inoculation. Consistency across methods is key in reducing registrant confusion. HCPA and CBC request that EPA add instructions to keep the pipet tip perpendicular to the carrier surface during inoculation.
Line 179	As line 135 indicates that carriers are not required to be pre-cleaned, we suggest the deletion of the word "clean" from this sentence.
Line 188	According to the Memorandum[42], EPA was only able to perform testing of this method against human coronavirus. Based on this data, it is unclear whether other viruses will survive drying via desiccation for 45-60 minutes; more sensitive viruses may not survive these conditions. Therefore, we request that EPA allow for alternate drying conditions to be used (e.g., drying via biosafety cabinet, <45 minutes) by adding the following statement: "appropriate modifications to the method may be required when testing organisms not specified herein. These modifications are acceptable without consulting EPA." We also recommend that EPA conduct additional feasibility testing to confirm the compatibility of this test method with alternative viruses. Furthermore, some of these carriers are prone to static cling and therefore flip or attach themselves to the petri dish lid during desiccation. HCPA and CBC request

[41] New Test Method for Quantitatively Evaluating the Efficacy of Antimicrobial Test Substances on Hard, Non-Porous Surfaces Against Bacteria (ASTM WK78068).

[42] Memorandum – Demonstration Studies on the Use of a Quantitative Test Method for Evaluating the Performance of Disinfectants on Porous Materials.

	that EPA provide instructions on how to address these situations regarding the discard of carriers.
Line 200	Please provide instructions on what to do if the inoculum soaks into the carrier and drying cannot be visualized or confirmed. HCPA and CBC also request that EPA provide pictures of the freshly inoculated carriers before and after drying, and pictures of acceptable and unacceptable dried carriers. Please also include a statement that unacceptable dried carriers are not used in the test.
Line 213	The method currently states that the test substance must completely cover the inoculum spot. The inoculum spot may not be observable on the specified privacy curtain fabric and vinyl seating fabric. HCPA and CBC request that EPA modify this section to state that if the inoculum spot is not visible (and therefore the test substance coverage cannot be assessed), the study should be considered invalid.
Line 234	The volume of neutralizer to use is not specified in this section of the method. Allowing for volumes <10 mL would reduce the number of wells inoculated in the 10^0 dilutions, thus reducing the number of cell culture plates required. HCPA and CBC request that EPA add the neutralizer volume (e.g., >5 mL) and add instructions to cap the vial prior to vortex mixing for added clarification. The carriers used in this method may float in the neutralizer. We request that EPA add instructions on how to ensure that the carrier is effectively submerged in the neutralizer at the end of the contact time by adding the following statement: "Note: As some carriers are prone to floating, ensure each carrier's treated surface comes into contact with the neutralizer by [add instruction]." Additionally, please provide instructions to ensure that the carrier moves during the vortex mixing process.
Line 242	Based on the limited dataset,[43] it is unclear if the 30±5 second vortex is sufficient for all viruses. HCPA and CBC request that EPA revise this section to state "at least 30±5 second vortex" and add an option to use sterile glass beads in the vortex instructions to aid in the removal of the virus if needed.
Line 245	It is currently unclear if dilutions are started 30 minutes after completion of the vortex-mix steps of the neutralization or started 30 minutes after the neutralizer is first applied to the treated carriers. We request that EPA revise this section to state: "initiate dilutions within 30 minutes after completion of the vortex mixing" for clarity.
Line 247	The method currently requires eight wells assayed per dilution. HCPA and CBC request that EPA revise the method to specify a minimum of four wells assayed per dilution to align with ASTM E1053[44] and OCSPP 810.2200.[9]
Line 249	The method states to plate a minimum 80 percent of the volume of the 10^0 vial and of each dilution tube. If dilutions are performed using 0.5 mL of the 10^0 vial and 4.5 mL of the test medium, plating 1 mL in quadruplicate will meet the minimum 80 percent requirement. We request that EPA revise the neutralizer

[43] Memorandum – Demonstration Studies on the Use of a Quantitative Test Method for Evaluating the Performance of Disinfectants on Porous Materials.

[44] ASTM E1053-20 Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces.

	volume (e.g., >5 mL) as in Line 234 and revise this section to allow for plating in quadruplicate, minimally, to align with ASTM E1053[8] and OCSPP 810.2200.[45]
Line 255	The method currently states an upper limit of 5.5 log viral particles/carrier. The viral levels may be a challenge if test material cytotoxicity is a problem. HCPA and CBC request that EPA revise this criterion to allow for levels higher than 5.5 log viral particles/carrier to address high cytotoxicity.
Line 258	The abbreviation for Dulbecco's Phosphate Buffered Saline is defined as DPBS on page 3. HCPA and CBC request that EPA update the entire method to align with the definition provided (DPBS) as opposed to PBS.
Lines 270-271	We request that EPA add a calculation example for the mean $\log_{10}$ density across the treated carriers and the control carriers as well as a calculation example for the TCID50 per carrier.
Line 278	In some cases, the neutralization assay may be performed on the same day as efficacy testing. HCPA and CBC request that EPA allow for the neutralization assay to be performed prior to or concurrent with the efficacy study. Additionally, in instances where the control is performed prior to testing, we request that variations in the media/reagent lots be considered acceptable.
Line 282	Variation in TCID50 assays necessitates that this acceptance criteria is 1.0 log difference instead of 0.5 log difference. HCPA and CBC request that update the acceptance criteria to "1.0 log difference" throughout Attachment I.
Line 299, 303, 306, & 311	The method states to proceed with step 3, however, it does not list a step 3. HCPA and CBC request that EPA clarify whether this should state "proceed with section IV." instead.
Line 304	If the Complete Growth Media (CGM) is used as the neutralizer, Treatment 3 and Treatment 2 are identical. We request that EPA clarify that Treatment 3 is only necessary if the CGM and the neutralizer are different.
Line 320	HCPA and CBC request that EPA clarify that the samples should be titrated in the same manner as in the test. Additionally, please replace "cell" with "cell line" for clarity.
Line 324, 327	The instructions for adsorption are absent in the efficacy test section of the method. HCPA and CBC request that EPA add the adsorption instructions to the appropriate efficacy test section. Additionally, if one parameter requires a media change, please add a note indicating "all test and control parameters of the same dilution should be changed."
Line 327	The method currently requires a DPBS wash. This is not necessary if changing the media; please allow for this wash to be optional.
Line 354	The method currently states that the cytotoxicity control must be performed prior to performing the neutralization assay. HCPA and CBC request that EPA revise this section to state that the cytotoxicity determinations do not need to be performed on the day of efficacy testing as well.

[45] Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces, Guidance for Efficacy Testing.

Line 356	The method is currently unclear as to when the neutralizer effect on the cell line control needs to be performed. Please revise this section to only require this control for non-MEM or RPMI based media.
Line 357, 383	The use of FBS is not appropriate for all viruses; HCPA and CBC request that EPA revise this to state "CGM."
Line 360, 377	Some viruses need rapidly dividing cells at a 50-70 percent confluency to grow. We request that EPA modify the language to read "cells at the appropriate confluency" instead of "80-95% confluent monolayer."
Line 372	The neutralization assays state to use 10 mL of neutralizer, however this section states to use 20 mL of neutralizer. Allowing for volumes <10 mL would reduce the number of wells inoculated in the 10^0 dilutions, thus reducing the number of cell culture plates required. HCPA and CBC request that EPA revise to ">5 mL" to align with the requested revision to Line 234.
Line 379, 405	We request that EPA revise the method to state "10^0" instead of "100" to correct the typographical error.
Line 382	Typically, the washing of the cells is done after the absorption period of the dilution. HCPA and CBC request that EPA revise the instructions to state "wash the cells after adding the dilutions."
Line 389	Changing the media is extremely labor intensive and unnecessary if allowing cytotoxicity up to 10^{-1} dilution. HCPA and CBC request that EPA revise to state "to monitor and change as necessary" without including specific times for flexibility.
Line 413	The method currently states to perform a three-part soil effect on the cell line control within the cytotoxicity determination (Attachment 2). HCPA and CBC request that EPA clarify that if this control is performed prior to the neutralization assay, it does not need to be repeated during efficacy testing. We also request that EPA allow the performance of the three-part soil effect once per cell line type since the cytotoxicity shouldn't change. A certified copy of the results would be included in each study report for the applicable cell line.
Lines 419-420	The method currently states that no cytotoxicity can be observed in this control. Please advise on what to do if cytotoxicity is observed in this control. Separately, please advise whether animal sera could be used as an alternative organic soil load if the three-part soil is toxic to the cell line. HCPA and CBC also request that EPA revise the statement "observe daily for cytotoxicity" to be optional.

Interim Quantitative Method for Evaluating the Efficacy of Antimicrobial Test Substances on Porous Surfaces Against Bacteria – Document 0005

LOCATION	COMMENT
Line 16-17	The method currently specifies that each carrier receives 10 µL of microbial inoculum with a three-part organic and inorganic soil load. Three-part soil contains mucin, yeast extract, and bovine serum albumin, all of which are organic soil load components. We request that EPA remove "and inorganic" from the method.

Line 24	HCPA and CBC request that EPA allow flexibility in modifying the growth and drying conditions for alternative organisms by adding the following statement: "appropriate modifications to the method may be required when testing organisms not specified herein."
Line 48	Please revise this section to demonstrate that dextrose may be added prior to inoculation on the day inoculated based on recent revisions made to ASTM's *New Test Method for Quantitatively Evaluating the Efficacy of Antimicrobial Test Substances on Hard, Non-Porous Surfaces Against Bacteria*.[46]
Line 71	OCSPP 810.2200[47] currently does not require the use of three-part soil when making bacterial hard surface disinfection claims. Additionally, different organic soil load types can impact test results between hard surface and soft surface testing. HCPA and CBC request that EPA consider the use of three-part soil as an optional step and revise this section to allow for alternative soil load requirements (e.g., fetal bovine serum).
Line 123	We request that EPA supply the vendor and catalog number of a suggested vial with lids used as an example in this section. Making this an example would allow for alternative vials with lids to be used to provide vendor flexibility.
Line 137	15 mL conical centrifuge tubes are listed as the only option for this section. Often, however, alternate conical tube volumes or tube types are used (e.g., 50 mL conical tubes). HCPA and CBC request that EPA list 15 mL conical centrifuge tubes as an example to allow for alternate volumes or tube types.
Line 142	The manufacturer websites[48,49,50] carrier component percentages differ from that listed currently in the method. HCPA and CBC request that EPA clarify whether these exact carrier component percentages must be followed when sourcing carriers. If so, please revise the method to align to the current carrier manufacturer percentages. The method currently specifies patterns and colors for carrier materials (e.g., Mambo, Hopsack, and Blue Sky). We are concerned that these specified materials may not always be available. HCPA and CBC request that EPA revise this section to make these exact patterns and colors examples to allow for the use of alternative colors and patterns based on availability.
Line 152	As seen in the photos in the method, 1 cm round carriers may not always be exactly round and therefore, not exactly 1 cm in diameter across all areas of the carrier. HCPA and CBC request that EPA clarify whether this diameter should instead be approximately 1 cm in diameter to account for potential variability in the roundness of the carrier punch. Additionally, please also revise this section to allow for square 1 cm X 1 cm carriers to be utilized if a carrier punch is not available.

[46] New Test Method for Quantitatively Evaluating the Efficacy of Antimicrobial Test Substances on Hard, Non-Porous Surfaces Against Bacteria (ASTM WK78068).
[47] Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces, Guidance for Efficacy Testing.
[48] CF Stinson designMix : Catalog : - Kid KID17 Blue Sky.
[49] CF Stinson designMix : Catalog : - Hopsack HOP24 Fjord.
[50] CF Stinson designMix : Catalog : - Mambo MAM34 Nights.

Line 153	The method currently states to visually screen the carriers to ensure "consistent surface characteristics." We request that EPA provide photos or detailed, written examples to clarify what characteristics are acceptable and unacceptable (e.g., fraying, ripping, discoloration, backing separation) regarding the screening of both sides of the carriers.
Line 155	The method currently states that pre-cleaning is not considered necessary, but it could be necessary in some instances. HCPA and CBC request that EPA clarify that cleaning of the carriers would be allowable if deemed necessary.
Line 157	Carrier sterility is often assessed concurrently with an efficacy study. HCPA and CBC request that EPA revise this section to address how to test the carrier's sterility and to allow for the carrier sterility control to be performed "prior to or concurrently with efficacy testing."
Line 158	The phrase "minor distortion" is vague regarding the carrier preparation, leaving each reader to define this for themselves. We request that EPA consider providing photos or detailed, written examples regarding what is considered acceptable and unacceptable distortion (e.g., cupping, doming).
Line 160	Tracking photos to their carrier number and inclusion of photos into raw data can all be problematic in a Good Laboratory Practice (GLP) efficacy study. Given the large number of carriers that would need to be tracked due to the replication required in this method, HCPA and CBC are concerned that this would create undue burden on laboratories that is not required for any other disinfection efficacy method. Visual confirmation of carrier acceptability can be documented in writing by the technician. HCPA and CBC request that EPA revise this section to allow for written affirmation of acceptable carrier condition.
Line 162	We request that EPA include a footnote to share practical learnings on carrier preparation (e.g., how to use, troubleshoot, and maintain the carrier punch, the allowance to apply weights to the carriers after sterilization to keep them flat, the allowance that carriers can be kept for up to six months after sterilization as in recent revisions made to ASTM's *New Test Method for Quantitatively Evaluating the Efficacy of Antimicrobial Test Substances on Hard, Non-Porous Surfaces Against Bacteria*).[51]
Line 166	HCPA and CBC request that EPA revise this section to demonstrate that dextrose may be added prior to inoculation, on the day inoculated based on recent revisions made to ASTM's *New Test Method for Quantitatively Evaluating the Efficacy of Antimicrobial Test Substances on Hard, Non-Porous Surfaces Against Bacteria*[52].
Lines 174-175	The method currently specifies to confirm that that 24±2 hour culture titer is at 10^8 CFU/mL. We request that EPA revise this section to make the culture titer verification step optional.
Lines 180-184	15 mL conical centrifuge tubes are listed as the only option for this section. Often, however, alternate conical tube volumes or tube types are used (e.g., 50 mL conical tubes). HCPA and CBC request that EPA list 15 mL conical centrifuge tubes as an example to allow for alternate volumes or tube types.

[51] New Test Method for Quantitatively Evaluating the Efficacy of Antimicrobial Test Substances on Hard, Non-Porous Surfaces Against Bacteria (ASTM WK78068).
[52] *Ibid*.

Line 183	This and other sections of the method currently do not describe the option to allow for 10 mL of *Pseudomonas aeruginosa* to be centrifuged. This request for this allowance is based on recent revisions made to ASTM's *New Test Method for Quantitatively Evaluating the Efficacy of Antimicrobial Test Substances on Hard, Non-Porous Surfaces Against Bacteria*[53] and provides helpful flexibility. We, therefore, request that EPA reword this section to include the following information: "If necessary, the culture may be harvested from two 10 mL 24±2 hour broth cultures to centrifuge a maximum of 10 mL *P. aeruginosa*; record the associated information."
Line 209	*Pseudomonas aeruginosa* colonies become too large to read individually after this amount of time in the incubator. HCPA and CBC request that EPA allow for *Pseudomonas aeruginosa* plates (or *Staphylococcus aureus* and any alternative bacteria) to incubate for 48±4 hours to allow for optimal reading.
Line 213	We request that EPA revise this to allow for the use of glass petri dishes.
Line 223	The recent revisions made to ASTM *New Test Method for Quantitatively Evaluating the Efficacy of Antimicrobial Test Substances on Hard, Non-Porous Surfaces Against Bacteria*[54] state that the pipet tip should be perpendicular to the carrier surface during inoculation. Consistency across methods is key in reducing registrant confusion. HCPA and CBC request that EPA add instructions to keep the pipet tip perpendicular to the carrier surface during inoculation.
Line 224	As line 176 indicates that carriers are not required to be pre-cleaned, we suggest that EPA delete the word "clean" from this sentence for consistency.
Line 238	The method currently specifies that the vacuum must remain on during desiccation. The vacuum does not need to remain on if the stopcock is closed. This is addressed in the most recent version of ASTM's *New Test Method for Quantitatively Evaluating the Efficacy of Antimicrobial Test Substances on Hard, Non-Porous Surfaces Against Bacteria*[55]. HCPA and CBC request that EPA include the following language to keep it consistent with the aforementioned method: "by either leaving the vacuum on during the drying period with the desiccator stopcock opened or turning the vacuum off with the stopcock closed." According to the memorandum[56], EPA was only able to perform testing of this method against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Based on this data, it is unclear whether other bacteria will survive drying via desiccation for 45-60 minutes. More sensitive organisms may not survive these conditions, therefore HCPA and CBC request that EPA allow for alternate drying conditions to be used (e.g., drying for <45 minutes, alternative methods like humidity chamber, incubator, or Biosafety cabinet) by adding the following statement: "appropriate modifications to the method may be required when testing organisms not specified herein. These modifications are acceptable without consulting EPA."

[53] *Ibid*.
[54] *Ibid*.
[55] *Ibid*.
[56] Memorandum – Demonstration Studies on the Use of a Quantitative Test Method for Evaluating the Performance of Disinfectants on Porous Materials.

	Some of these carriers are prone to static cling and therefore flip or attach themselves to the petri dish lid during desiccation. We request that EPA provide instructions on how to address these situations regarding the discard of carriers.
Lines 248-249	HCPA and CBC request that EPA provide instructions on what to do if the inoculum soaks into the carrier and drying cannot be visualized or confirmed. We also request that EPA provide pictures of the freshly inoculated carriers before and after drying, and pictures of acceptable and unacceptable dried carriers demonstrating that inoculum spreading and rolling are not allowable and should be grounds for invalidating a study.
Line 275	The method currently states that the test substance must completely cover the inoculum spot. The inoculum spot may not be observable on the specified privacy curtain fabric and vinyl seating fabric. HCPA and CBC request that EPA modify this section to state that if the inoculum spot is not visible (and therefore the test substance coverage cannot be assessed) the study should be considered invalid.
Line 282	The viral test method currently allows for the use of up to 20 mL of neutralizer if neutralization is an issue. We request that EPA revise this section to allow for the use of up to 20 mL of neutralizer, if needed to aid in neutralization. The carriers used in this method may float in the neutralizer. HCPA and CBC request that EPA add instructions on how to ensure that the carrier is effectively submerged in the neutralizer at the end of the contact time by adding the following statement: “Note: As some carriers are prone to floating, ensure each carrier’s treated surface comes into contact with the neutralizer by [add instruction].” Additionally, please provide instructions to ensure that the carrier moves during the vortex mixing process.
Lines 288-294	Lines 288-294 describe a series of vortex and settle steps for neutralized cultures. As written, there is no ± time allowance for the five-minute settle periods. Additionally, it is unclear whether this settle period starts immediately following neutralization of each carrier and would need to be tracked per carrier, or whether one would let all carriers sit for five minutes after everything is neutralized. HCPA and CBC request that EPA clarify this section regarding the timing. Please also provide an approximate time window, or including "approximately", for each five-minute settling step. The carriers used in this method may adhere to the vial cap during the vortex mixing procedures. We request that EPA add instructions to ensure carriers are in the neutralizer during the five-minute settle periods and ensure that the carriers are submerged in the neutralizer during vortex mixing.
Line 290-294	Based on the limited dataset,[57] it is unclear if the 30-second vortex is sufficient for all microorganisms. HCPA and CBC request that EPA revise the vortex time to read “at least 30±5 seconds” and add an option to use sterile glass beads to the vortex instructions to aid in the removal of the microorganism if needed.

[57] *Ibid.*

Line 295	It is currently unclear if dilutions are started 30 minutes after completion of the vortex-mix steps of the neutralization or, started 30 minutes after the neutralizer is first applied to the treated carriers. HCPA and CBC request that EPA revise this section to state: “initiate dilutions within 30 minutes after completion of the vortex mixing” for clarity.
Lines 321	We request that EPA confirm that one filtration plate will be performed for the neutralizer sterility and one filtration plate will be performed for the Phosphate Buffered Saline (PBS) Sterility.
Line 324	The statement “Incubate plates at 36±1°C for 48±4 h for control carriers and for a minimum of 72±4 h for treated carriers” implies that one could incubate the treated carrier plates for >76 hours as it describes a "minimum" of 72±4 hours. HCPA and CBC request that EPA remove the words “minimum of” for clarity.
Line 326	The method recommends monitoring agar plates daily to optimize colony counting. This practice is often difficult to implement in GLP testing and is not an optimal practice for GLP laboratories. HCPA and CBC request that EPA modify the language to make an allowance for monitoring the agar plates daily as an optional practice or remove this altogether.
Lines 347	We request that EPA clarify when further confirmatory analyses and isolation streaks on selective media will be required.
Line 362-363	HCPA and CBC request that EPA add a calculation example for the mean $\log_{10}$ density across the treated carriers and the control carriers.
Line 364-368	The corresponding interim guidance document Section II. j.[58] states that "each of the five treated carriers for each material type should have a minimum 4.0-log reduction." The calculations provided in the method do not encompass log reduction calculations for individual carriers as is required by the guidance. HCPA and CBC request that EPA include a calculation description in the method to instruct users how to perform log reduction calculations for individual carriers. Similarly, we encourage EPA to include an example on how greater than (>) or less than (<) symbols are carried throughout the calculations and judged against the performance criteria (see Attachment 1).
Line 419	The method currently states “i.e., Vitek” when discussing the confirmatory identification procedures. HCPA and CBC request that EPA revise this to state “e.g., Vitek” for clarity.
Line 433	In some cases, the neutralization assay may be performed on the same day as efficacy testing. HCPA and CBC request that EPA allow for the neutralization assay to be performed prior to or concurrent with the efficacy study. Additionally, in instances where the control is performed prior to testing, we request that variations in the media/reagent lots be considered acceptable.
Line 436	HCPA and CBC request that EPA revise the acceptance criteria for the neutralization control to be 1 log difference instead of the ≤50 percent difference in colony counts because this requirement may be impractical for certain organisms.

[58] EPA Interim Guidance for Products Including or Adding Efficacy Claims for Use on Porous Materials in Non-Residential Setting. Page 4.

Line 458	The neutralization control testing does not include a treatment plus fabric combination to simulate the test. We are concerned that the fabrics may impede neutralization. It is immaterial what happens with product alone and neutralizer, as that is not how the test is run. Conducting the method in this manner could lead to erroneous neutralization results and an exaggeration of product efficacy due to lack of neutralization. HCPA and CBC request that EPA add an additional control to assess the neutralizer and product and carrier simulation which includes the vortex and settle process utilized in the test before adding organism.
Line 473	We request that EPA add the word “minutes” after “10±1.”
Line 479	HCPA and CBC suggest that EPA change this statement to read “Initiate filtration within 30 minutes following vortex mixing" to ensure consistency in testing.
Line 482	The method recommends counting and recording CFUs daily, up to 72±4 hours (for the neutralization assay). This practice is often difficult to implement in GLP testing and is not an optimal practice for GLP laboratories. HCPA and CBC request that EPA modify the language to make an allowance for monitoring the agar plates daily as an optional practice or remove this altogether. Additionally, we request that the section is revised to state “...48±4 h and count the colonies. Incubate an additional 24±4 h if no or few colonies are present at 48±4 h and recount the colonies.” Please also define what is meant by “few colonies.”

HCPA and CBC thank EPA in advance for its consideration of these comments. Please do not hesitate to contact us if you wish to discuss further.

Sincerely,

Ligia Duarte Botelho
Senior Director, Regulatory Affairs
Household & Commercial Products Association

Anastasia Swearingen
Executive Director
ACC Center for Biocide Chemistries

CC: Anne Overstreet, BEAD Acting Director
Anita Pease, Antimicrobials Division Director
Kristen Willis, Antimicrobials Division Acting Deputy Director

ATTACHMENT 1: CALCULATION WHERE ONE CARRIER IS TOO NUMEROUS TO COUNT AT ALL DILUTIONS

TABLE 4: CARRIER POPULATION CONTROL RESULTS

Test Organism: *Staphylococcus aureus* (ATCC 6538)							
Volume Plated: 0.100 mL							
Carrier #	Dilution Factor				CFU/ carrier	Log_{10}	Geometric Mean (Average Log_{10})
	10^{-1}	10^{-2}	10^{-3}	10^{-4}			
1	T, T	T, T	60, 60*	5, 9	1.2×10^7	7.08	1.1×10^7 (7.04)
2	T, T	T, T	60, 49*	7, 11	1.1×10^7	7.04	
3	T, T	T, T	51, 47*	0, 5	9.8×10^6	6.99	

CFU = Colony Forming Units
T = Too Numerous to Count (>300 colonies)
*Dilution used for calculations

TABLE 5: TEST CARRIER DATA

eus (ATCC 6538)		
Carrier #	Survivors at the 10^0 dilution	
	1.000 mL	0.100 mL
1	144, 178*	15, 9
2	1, 1*	0, 0
3	0, 1*	0, 0
4	202, 208*	29, 23
5	T, T	T, T*

T = Too Numerous to Count (>300 colonies)
*Dilution used for calculations

TABLE 6: TEST RESULTS

Staphylococcus aureus (ATCC 6538)					
Carrier #	CFU/ Carrier	Log_{10}	Average Log_{10}	Geometric Mean	Percent Reduction
1	3.22×10^3	3.51	>2.90	$>7.94 \times 10^2$	<99.9928%
2	2×10^1	1.30			
3	2×10^1	1.30			
4	4.10×10^3	3.61			
5	$>6.00 \times 10^4$	>4.78			

CFU = Colony Forming Units
A value of <1 CFU was used in place of zero for calculation purposes.