UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

August 11, 2023

Agency Response to Public Comments on Interim Guidance for Products Including or Adding Efficacy Claims for Use on Porous Materials in Non-Residential Settings

From December 21, 2022, through February 19, 2023, EPA solicited comments from the public on the clarity of proposed test methods and associated Guidance for Products Including or Adding Efficacy Claims for Use on Porous Materials in Non-Residential Settings.

EPA received comments from five entities and sincerely appreciates the valuable feedback from these organizations and individuals. The attached table is a compilation of the comments received, grouped by topic area, and the Agency's response to those comments. EPA also received unsolicited comments on Memo Document 0003, which are not addressed in this document. A key at the end of this document correlates the comment numbers to the source of the comments that were posted on regulations.gov for docket EPA-HQ-OPP-2022-0337.

The primary areas of comment included the following:

- Carriers and carrier types
- Comments on the guidance and label language
- Edits to the bacterial method
- Edits to the virus method
- Comments on the claim and use sites.

After considering the public comments received, the Agency revised and finalized the guidance document and associated test methods. The revised guidance document and the associated test methods can be found in EPA-HQ-OPP-2022-0337.

Comments Received

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Interpretation of a Porous Surface	Strongly recommend that EPA revisit this guidance document and review its interpretation of a porous surface, which is not in line with industry's position, the test carrier material manufacturer's position or European disinfectant test methodology and will result in significant confusion and inadvertent non-compliance.	1	The term "porous surface" is being replaced with "soft surface textiles"; this nomenclature is defined as a soft, porous surface textile or soft, non-porous surface textiles limited to the surfaces/use sites presented in the guidance document. *Soft surface textile* better represents the clinical and institutional (non-residential) environments for which the guidance is intended.
	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.	2	
	A clear definition of a "porous surface" should be established as a basis for this method to provide clarity for registrants and product users, and to allow the selection of more appropriate test materials aligned with the definition.	161	
Carriers and Carrier Types	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.	13	The carriers indicated in the guidance and methods (vinyl, non-PVC, and privacy curtain) are representative of non-residential use sites. The guidance document is intended to address only the addition of efficacy claims to soft surface textiles in non-residential settings.
	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.	3	*Soft surface textile* is more representative of the clinical and institutional environments (non-residential) and represents the current carrier types outlined in the methods/guidance documents. The guidance document is intended to address only the addition of efficacy claims to soft surface textiles in clinical and institutional (non-residential) settings. To provide clarity, the guidance document was

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	CBC requests that EPA reconsider vinyl as a test material in the method for efficacy on soft, porous surfaces and clarify in the guidance that vinyl is a non-porous material. EPA's data also shows the performance of the vinyl carrier is predicated by the hard, nonporous stainless steel carrier. In the event EPA believes vinyl should continue to be called out in the method, EPA should repropose the method, specifying in detail the reasons for selecting this material, along with clarity about the level of porosity EPA believes this material reflects what should be used in efficacy testing.	150	revised to specify the use sites that are within and those that are outside the scope of the guidance document.
	The combination of three main issues associated with the carriers; 1) lack of permeability, 2) fraying, and 3) distortion after heat sterilization leads Clorox to believe that only one material type can be accommodated for the efficacy test method to support porous claims within the guidance. As this guidance is for porous material product claims, the use of non-permeable carrier materials (VF-01 and NVF-01) in an efficacy evaluation is irrelevant.	155	
	Clorox recommends that PCF-03 cut into 1 cm2 squares be the single representative material used for the supporting method.	166	The methods were revised to include the use of 1 cm^2 squares in addition to the 1 cm diameter circles for all carrier types.
	Clorox recommends the Agency broaden the acceptance of alternate material sources and vendors for privacy curtain fabrics, similar to PCF-03.	156	The carrier types outlined in the method and guidance should be used to for efficacy testing. To provide flexibility, the use of specific color per material was removed.
Claims	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.	4	The carrier types outlined in the method and guidance should be used for efficacy testing and, as described in the guidance, will support the addition of a disinfection claim to products intended for use in clinical and institutional (non-residential) environments. Cotton and polyester are not representative of the surfaces for which this guidance is intended.

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Guidance Document	HCPA and CBC request that EPA remove the requirement to submit surface compatibility testing data.	5	The guidance was revised to remove the provision regarding surface compatibility testing data.
	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.	6	The carrier types outlined in the method and guidance should be used for efficacy testing and, as described in the guidance, will support the addition of a disinfection claim to products intended for use in clinical and institutional (non-residential) environments. The guidance document is intended to address only broad label language regarding use surfaces. The guidance document does not address the addition of disinfection claims on specific surfaces. The Agency is concerned that the inclusion of specific surface material claims on product labels could lead to product misuse and/or confusion by requiring the end-user to discern a specific surface type. The guidance document is also intended to address only the addition of disinfectant claims for soft surface textiles on products that have also met the performance standard for hard, non-porous surface claims.
	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.	7	The guidance document is not intended to address the addition of stand-alone soft surface textile claims (e.g., products with only soft surface textile claims). Data for hard, non-porous surface claims should be submitted before soft surface textile claims are added pursuant to the process described in the guidance document.
	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.	8	Guidelines to test fabric carriers (for EPA-approved soft surface claims) are not applicable to the soft surface textile guidance. The soft surface textile guidance addresses claims for non-launderable, non-residential

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			pliable surfaces; refer to Table 1 (Summary of Testing to Support Efficacy Claims for Use on Soft Surface Textiles) in the Guidance Document for information on other soft surface claims.
	We also request that EPA allow for registrants to make standalone porous surface disinfection claims for additional microorganisms.	9	This guidance document is not intended to address stand-alone soft surface textile claims (e.g., products with only soft surface textile claims) for additional microorganisms.
	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.	10	Due to the surface complexity of soft surface textiles compared to hard, non-porous surfaces, this guidance is only intended to address claims added to a product where the additional bacteria and viruses have been assessed on all three soft surface textile types for at least two lots of product at the nominal concentration.
	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.	11	Due to the surface complexity of soft surface textiles compared to hard, non-porous surfaces, this guidance is only intended to address claims added to a product where the additional bacteria and viruses have been assessed on all three soft surface textile types for at least two lots of product at the nominal concentration. This guidance is not intended to address bridging of data from hard, non-porous surfaces to soft surface textiles.
	We recommend that the guidance and methods allow for towelette applications.	16	Towelette applications are outside the scope of the guidance document. For products using methods of application beyond those listed in the guidance, including towelettes, please consult with the Agency.
	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 II25 will apply to the products outlined in the interim guidance	18	For retesting questions, please contact AD_efficacy@epa.gov.

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	HCPA and CBC request that the Agency delete the word "cleaning" from the Section II.d. given the fact that the method specifically states that no cleaning of the carriers is required.	20	The revised guidance reflects this change.
	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.	21	The revised guidance reflects this change to allow for testing at the nominal concentration for additional vegetative 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.	22	The guidance is intended to address claims of efficacy against vegetative bacteria and viruses. If fungal claims are desired, registrant are encouraged to submit a protocol for review along with preliminary data.
	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.	23	As with prior guidance, test each lot on separate test days; however, multiple bacteria/viruses and/or surface types may be tested on the same day.
	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, nonporous disinfection claims, which requires only a single carrier for non-surrogate viruses or two carriers for surrogate viruses.	24	The Guidance for Products Adding Residual Efficacy Claims (https://www.epa.gov/pesticide-registration/guidance-products-adding-residual-efficacy-claims) also requires assessing five carriers per lot for both EPA's Test Method for Evaluating the Efficacy of Antimicrobial Surface Coatings (EPA MLB SOP MB-40) and EPA's Method for the Evaluation of Antimicrobial Activity of Hard, Non-porous Copper-Containing Surface Products (EPA MLB SOP MB-41) for testing performed with both bacteria and viruses. In addition, five carriers accommodates the potential for an increase in carrier to carrier variability observed with soft surface textile carriers over hard, non-porous carriers.

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	HCPA and CBC request that EPA update the table to read “all additional viruses claimed on porous surfaces” and “Hardest to kill 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.	26	The terms "all additional viruses" and "hardest to kill virus" will remain in the guidance document, consistent with EPA's Series 810 Product Performance Test Guidelines 810.2200: Disinfectants for Use on Environmental Surfaces, Guide for Efficacy Testing.
	HCPA and CBC request that EPA confirm the acceptance criteria is a mean of the tested carriers.	165	Each lot of the product should achieve a minimum mean 4.0-log reduction in ≤ 10 minutes ± 5 seconds for qualifying bacteria when compared to the controls to support soft surface textile disinfectant claims pursuant to this guidance for each of the three representative soft surface textiles. Each lot of the product should achieve a minimum mean 3.0-log reduction in ≤ 10 minutes ± 5 seconds for qualifying viruses when compared to the controls to support soft surface textile disinfectant claims pursuant to this guidance for each of the three representative soft surface textiles.
	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.	27	This guidance does not address claims against specific viruses (e.g., SARS-CoV-2). Two lots of product at the LCL should be tested for the hardest to kill virus. For additional information on selecting the most difficult to kill virus, see EPA’s Emerging Viral Pathogens Guidance (https://www.epa.gov/pesticide-registration/emerging-viral-pathogen-guidance-and-status-antimicrobial-pesticides). Two lots of product at the nominal concentration should be tested for all other viruses claimed on the label for each carrier type
	HCPA and CBC request that the Agency expand eligibility for inclusion in EPA’s “Common Pathogen” lists.	28	For specific requests, please contact the Antimicrobials Division Ombudsman (pesticidequestions@epa.gov).

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	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. REMOVE 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. 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.	29	The language was changed from "routinely contact skin" to "currently in contact with skin" to not confuse the end user and does not restrict chemistries on surface that contact skin.
	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. Given the inconsistency, HCPA and CBC request the removal of the sample language.	30	The guidance is intended to address only disinfectant claims for soft surface textiles, separate from a sanitization claim. To add claims for spot disinfection of launderable clothing using a modified version of the methods identified in the guidance, please submit a protocol for review.
	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.	31	This guidance is intended to address the addition of additional bacteria and viruses to the label after certain prerequisites are met, see Sections II and III.
	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.	33	EPA is no longer prioritizing Public Health Emergency requests for new products that address surface transmission of SARS-CoV-2 and will no longer expedite new product registrations, emerging viral pathogen claims, and SARS-CoV-2 claims for products intended to kill SARS-CoV-2 on surfaces.

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	The interim guidance accompanying the methods includes a requirement for surface compatibility testing, a novel requirement in efficacy guidelines, and from the Antimicrobials Division under its Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) authority. Specifically, the interim guidelines require that 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." CBC notes that this testing requirement is not relevant to the efficacy of antimicrobial pesticides on porous surfaces and should be outside the scope of this interim guidance and the Antimicrobials Division data requirements. EPA should limit efficacy methods to efficacy testing and delete this requirement as inappropriate for an efficacy protocol.	151	The revised guidance reflects this change.
	Clorox recommends that the Agency eliminate the need for submission of surface compatibility information associated with any GLP efficacy data.	157	
Interim while changes to guidance are made	We note that due to the high volume of significant technical comments on the method, including the concern regarding the use of vinyl as a test surface, we encourage EPA to make further changes to the method before encouraging registrants to use the method in seeking approval of new claims.	153	Interim guidance is posted for comment and use; registrants may obtain claims using the interim guidance and methods throughout the comment and revision processes.
	While EPA considers comments and changes to update its methods, we encourage EPA to continue to approve claims based on alternative protocols for efficacy on porous, soft surfaces on a case-by-case basis.	154	
	Based upon the experience and test results from Clorox, Ecolab, and other laboratories working with the proposed method thus far, the EWG respectfully requests the Interim Guidance and methods be further refined prior to use by registrants to obtain claims.	162	

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Label	Section IV of the interim guidance accompanying the methods provides labeling and additional information for products for use on surfaces of certain porous materials. As part of the sample directions for use, EPA suggests language "Do not use on surfaces that routinely contact skin (i.e., clothing, sheets, towels). CBC notes that a warning regarding skin contact is dependent on separate toxicological data and should not be part of the efficacy guidance. We note that some of the soft surfaces used in this guidance, such as vinyl fabrics for chairs, could routinely contact skin. Therefore, we suggest removing example IV. c. ii. from the sample directions for use.	152	Section IV.c.ii has been removed.
	Clorox encourages the Agency to continue to accept variations in label language as it contributes to clarity with unique product usage and directions for use.	158	Using label language that is the same as or similar to the example label language in the guidance will help EPA to more efficiently review labels. However, EPA will review all label language on a case-by-case basis.
PRIA Submissions	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.	32	AD is not expediting reviews of applications to add soft surface textile disinfectant claims. PRIA codes were updated.
Wetness Test	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.	14	The wetness testing provision was removed from the guidance document.
	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.	15	If a contact time greater than 10 minutes (i.e., different from the range identified in the guideline) is needed (e.g., for a unique use-site or organism claim), consultation with the agency prior to testing is recommended and a modification to the standard approach may be needed.

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Use sites	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.	12	The labeling guidance provided in guidance document's introduction and section IV.d gives clear examples of product application areas.
Soil Load	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.	17	The revised methods clarify that 3-part soil is appropriate for use in these methods.
Method Validation	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 to theoretically reduce the amount of replication prescribed.	19	EPA received numerous requests from stakeholders to issue guidance and associated methods to address claims for products with antimicrobial efficacy against bacteria and viruses on soft surface textiles. The proposed method, the Quantitative Method for Evaluating the Efficacy of Antimicrobial Test Substances on Hard, Non-Porous Surfaces, is currently in use for registrants seeking claims against *Candida auris* and *Clostridioides difficile*. Using an existing method for a novel purpose was selected to expedite the issuance of the guidance and associated methods in response to the persistent requests from stakeholders. Pursuit of multi-laboratory testing may have delayed release of the guidance and associated methods substantially.
	EWG suggests that additional method feasibility testing followed by validation take place before EPA allows these methods to be used by registrants to obtain a soft, porous surface claim.	160	
	HCPA and CBC further request that once a collaborative is completed that the replication be revisited to possibly further reduce this requirement of five treated carriers	25	
	The EWG companies would then propose a collaboration with the EPA MLB lab and other interested parties to conduct additional joint testing with the method to determine appropriate improvements before its use by registrants to support soft, porous surface claims.	163	

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	FEM-2009-01 updated December 21, 2016, EPA states: "It 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." The 2016 Method Validation Guidelines clearly recommend multi-laboratory collaboration to demonstrate each method's suitability, reliability, reproducibility and performance. The interim porous efficacy methods appear to have been tested minimally and only within a single laboratory for bacteria, and the viral method evaluation appears to be limited to untreated controls.	164	
Virus Method	Line 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.	37	The revised method reflects this change.
	Line 13-14. 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."	38	If a deviation to the method is required, provide justification for the deviation (e.g., including data) in advance of testing to the Antimicrobials Division (AD_Efficacy@epa.gov) and document all deviations in the study report.
	Line 15-17. 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.	39	Chemical neutralizers are the stated means of neutralization for this method. If a column is required to reduce cytotoxicity (i.e., the entire neutralizer volume passed through the column), consult with the Antimicrobials Division (AD_Efficacy@epa.gov) in advance of testing.

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	Line 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).	40	The medium used to grow the virus is recommended to be used as the neutralizer; however, the method states that other neutralizers may be used if needed.
	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.	41	The revised methods clarify that 3-part soil is appropriate for use in these methods. While animal serum is often required to propagate virus, it may not be required to freeze the virus; preparation of frozen virus stocks is not specified in the method. Virus stocks may be washed to remove animal serum prior to freezing or immediately after thawing (prior to testing). The revised method states MEM instead of CGM.
	Line 57. OCSPP 810.2200 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).	42	The revised methods clarify that 3-part soil is appropriate for use in these methods.
	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.	43	The revised method includes an example of a vial that may be used.
	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.	44	The revised method reflects this change.

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	Line 123-124. The manufacturer websites 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.	45	The revised method reflects this change.
	Line 123-124. 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 patterns and colors examples to allow for the use of alternative colors and patterns based on availability.	46	The revised method reflects this change.
	Line 123-124. 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	47	The carrier types outlined in the method should be used for efficacy testing. To provide flexibility, the use of a specific color per material was removed. The guidance document does not address the addition of disinfection claims on specific surfaces. The Agency is concerned that the inclusion of specific surface material claims on product labels could lead to product misuse and/or confusion by requiring the end-user to discern a specific surface type. The carriers selected represent the materials/spaces listed in the guidance and as such should be compatible with them.
	Line 123-124. 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.	48	
	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.	49	The revised method reflects this change.
	Line 131. 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.	50	The revised method reflects this change.

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	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.	51	The revised method reflects this change.
	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.	52	Pre-cleaning would be a deviation to the methods. If a deviation to the method is required, provide justification for the deviation in advance of testing to the Antimicrobials Division (AD_Efficacy@epa.gov) and document all deviations in the study report.
	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."	53	The revised method reflects this change.
	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).	54	The revised method reflects this change.
	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.	55	The method requests documentation that screened/sterile carriers are acceptable for use (e.g., not distorted, frayed, etc.). This documentation can be on the testing paperwork (e.g., via a check box indicating that carriers were acceptable for use); however, it requested to include a photograph of a representative carrier being used for testing after sterilization in the submission. The method was updated to include allowance for written documentation of carrier acceptability.

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	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). 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.	56	The revised method reflects this change.
	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.	57	The revised method reflects this change.
	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.	58	The revised method reflects this change.
	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.	59	The revised method reflects this change.
	Line 166. HPCA and CBC request that EPA allow for the use of glass petri dishes.	60	The revised method reflects this change.
	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 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.	61	The revised method reflects this change.
	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.	62	The revised method reflects this change.

Comment Category	Specific Comment	Comment #	Agency Response
	Line 188. According to the Memorandum, 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."	63	If a deviation to the method is required, provide justification for the deviation (e.g., including data) in advance of testing to the Antimicrobials Division (AD_Efficacy@epa.gov) and document all deviations in the study report.
	Line 188. We also recommend that EPA conduct additional feasibility testing to confirm the compatibility of this test method with alternative viruses.	64	If compatibility concerns arise while testing different viruses/cell lines, please contact the Antimicrobials Division at AD_Efficacy@epa.gov.
	Line 188. 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 that EPA provide instructions on how to address these situations regarding the discard of carriers.	65	The revised method reflects this change.
	Line 200. Please provide instructions on what to do if the inoculum soaks into the carrier and drying cannot be visualized or confirmed.	66	Language was added to the method to address inoculum soaking into the carrier. For informational purposes, it may be advantageous to visualize how the inoculum and test substance absorbs into the carrier prior to testing. For privacy curtain fabric in particular, the inoculum is anticipated to soak into the carrier. When test substance is applied to the privacy curtain fabric it is anticipated to soak into and through the carrier (e.g., the test substance may be observed to pool around the carrier); this scenario is only anticipated for privacy curtain fabric.
	Line 200. 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.	67	Pictures of inoculated carriers and test substance added to the carriers were added to the method.

Comment Category	Specific Comment	Comment #	Agency Response
	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.	68	The method has been revised to indicate that if inoculum soaks/absorbs into the carrier and is no longer visible, carriers are still acceptable to use. Examples of typical acceptable and unacceptable carriers are also provided.
	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 100 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.	69	The revised method reflects this change.
	Line 234. 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]."	70	The revised method reflects this change.
	Line 234. Additionally, please provide instructions to ensure that the carrier moves during the vortex mixing process.	71	The revised method reflects this change.
	Line 242. Based on the limited dataset, 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.	72	The recovery processing was revised to match that identified in the bacterial method; the bacterial method does not include a provision for the use of glass beads. If a deviation to the method is required, provide justification for the deviation (e.g., including data) in advance of testing to the Antimicrobials Division (AD_Efficacy@epa.gov) and document all deviations in the study report.
	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.	73	The revised method reflects this change.

Comment Category	Specific Comment	Comment #	Agency Response
	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 E105344 and OCSPP 810.2200.	74	The method was revised to reflect plating a minimum of 80% of the total volume per dilution allowing the use of different size well plates.
	Line 249. The method states to plate a minimum 80 percent of the volume of the 100 vial and of each dilution tube. If dilutions are performed using 0.5 mL of the 100 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 volume (e.g., >5 mL) as in Line 234 and revise this section to allow for plating in quadruplicate, minimally, to align with ASTM E10538 and OCSPP 810.2200.45	75	The revised method reflects this change.
	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.	76	If a deviation to the method is required, provide justification for the deviation (e.g., including data) in advance of testing to the Antimicrobials Division (AD_Efficacy@epa.gov) and document all deviations in the study report.
	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.	77	The revised method reflects this change.
	Line 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 $TCID_{50}$ per carrier.	78	The revised method includes an equation to calculate the mean $\log_{10}$ density across treated and control carriers, and a calculation for $TCID_{50}$ 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.	79	The revised method reflects this change. Variation in the media/reagent lots is acceptable; however, differences in performance between batches of media may lead to misleading neutralization results.

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	Line 282. Variation in $TCID_{50}$ 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.	80	In the method, a difference between treatments of 0.5 logs is anticipated to be easily achievable, regardless of the calculation method used ($TCID_{50}$ or MPN).
	Line 299, 303, 306, and 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.	81	The revised method reflects this change.
	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.	82	The revised method reflects this change.
	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.	83	The revised method reflects this change.
	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.	84	Adsorption may be utilized as necessary for virus/cell line systems for which it is required.
	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."	85	The revised method reflects this change.
	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.	86	The revised method reflects this change.
	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.	87	The revised method reflects this change.
	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.	88	The method was revised to indicate that the cell line control should be performed for neutralizers other than CGM.
	Line 357, 383. The use of FBS is not appropriate for all viruses; HCPA and CBC request that EPA revise this to state "CGM."	89	The revised method reflects this change.

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	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."	90	The revised method reflects this change.
	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 100 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.	91	The revised method addresses the use of different volumes of neutralizer.
	Line 379, 405. We request that EPA revise the method to state "100" instead of "10^0" to correct the typographical error.	92	The revised method reflects this change.
	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."	93	The revised method reflects this change.
	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.	94	The revised method provides the requested 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.	95	The impact of three-part soil on the cell line control has been removed from the method. EPA's Microbiology Laboratory Branch has not observed three-part soil be toxic to cell lines (i.e., CRFK, RAW, Vero, LLC MK2, HepG2, MDCK, MRC-5, A549). If three-part soil is observed to be toxic to a cell line, email AD_Efficacy@epa.gov for guidance.
	Line 413. 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.	96	
	Line 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.	97	
	Line 419-420. 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.	98	

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	Line 419-420. HCPA and CBC also request that EPA revise the statement "observe daily for cytotoxicity" to be optional.	99	
Bacteria Method	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.	99	The revised method reflects this change.
	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."	100	If a deviation to the method is required, provide justification for the deviation (e.g., including data) in advance of testing to the Antimicrobials Division (AD_Efficacy@epa.gov) and document all deviations in the study report.
	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.	101	The revised method reflects this change.
	Line 71. OCSPP 810.22004 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).	102	The revised method clarifies that 3-part soil is appropriate for use in this method.
	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.	103	The revised method includes an example of a vial that may be used.
	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.	104	The revised method reflects this change.

Comment Category	Specific Comment	Comment #	Agency Response
	Line 142. The manufacturer websites 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.	105	The revised method reflects this change.
	Line 142. 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.	106	The revised method reflects this change.
	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.	107	The revised method reflects this change.
	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.	108	The revised method reflects this change.
	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.	109	If a deviation to the method is required, provide justification for the deviation in advance of testing to the Antimicrobials Division (AD_Efficacy@epa.gov) and document all deviations in the study report.
	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."	110	The revised method reflects this change.

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	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).	111	The revised method reflects this change.
	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.	112	The method requests documentation that screened/sterile carriers are acceptable for use (e.g., not distorted, frayed, etc.). This documentation can be on the testing paperwork (e.g., via a check box indicating that carriers were acceptable for use); however, it would be helpful to include a photograph of a representative carrier being used for testing after sterilization in the submission. The method was updated to include allowance for written documentation of carrier acceptability.
	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).	113	The revised method reflects this change.
	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.	114	The revised method reflects this change.
	Line 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.	115	The method indicates that the anticipated culture concentration of 10^8 CFU/mL, however, performance of a titer assay is optional.

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	Line 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.	116	The revised method reflects this change.
	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 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."	117	The revised method reflects this change.
	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.	118	Test substance product chemistry impacts colony development; as such, recovery for some product chemistries may be delayed until after 48 hours thereby necessitating up to 72 h of incubation. Monitoring plates daily helps optimize counts.
	Line 213. We request that EPA revise this to allow for the use of glass petri dishes.	119	The revised method reflects this change.
	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 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.	120	The revised method reflects this change.
	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.	121	The revised method reflects this change.

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	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. 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."	122	The revised method reflects this change.
	Line 238. According to the memorandum, 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."	123	If a deviation to the method is required, provide justification for the deviation (e.g., including data) in advance of testing to the Antimicrobials Division (AD_Efficacy@epa.gov) and document all deviations in the study report.
	Line 238. 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.	124	The revised method reflects this change.

Comment Category	Specific Comment	Comment #	Agency Response
	Line 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.	125	Language was added to the method to address inoculum soaking into the carrier. For informational purposes, it may be advantageous to visualize how the inoculum and test substance absorb into the carrier prior to testing. For privacy curtain fabric in particular, the inoculum is anticipated to soak into the carrier. When test substance is applied to the privacy curtain fabric it is anticipated to soak into and through the carrier (e.g., the test substance may be observed to pool around the carrier); this scenario is only anticipated for privacy curtain fabric.
	Line 248-249. 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.	126	Pictures of inoculated carriers and test substance added to the carriers were added to the method.
	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.	127	The method has been revised to indicate that if inoculum soaks/absorbs into the carrier and is no longer visible, carriers are still acceptable to use. Examples of typical acceptable and unacceptable carriers are also provided.
	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.	128	The use of up to 20 mL of neutralizer for viral testing is primarily for cytotoxicity issues. If additional neutralizer volume is required for bacterial testing, provide justification for the deviation (e.g., including data) in advance of testing to the Antimicrobials Division (AD_Efficacy@epa.gov) and document all deviations in the study report.

Comment Category	Specific Comment	Comment #	Agency Response
	Line 282. 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]."	129	The revised method reflects this change.
	Line 282. Additionally, please provide instructions to ensure that the carrier moves during the vortex mixing process.	130	The revised method reflects this change.
	Line 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.	131	The revised method reflects this change.
	Line 288-294. 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.	132	The revised method reflects this change.
	Line 290-294. Based on the limited dataset, 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.	133	The vortex time was revised to include 30±5 seconds. If a deviation to the method is required (e.g., use of glass beads), provide justification for the deviation (e.g., including data) in advance of testing to the Antimicrobials Division (AD_Efficacy@epa.gov) and document all deviations in the study report.
	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.	134	The revised method reflects this change.

Comment Category	Specific Comment	Comment #	Agency Response
	Line 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.	135	The revised method reflects this change.
	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.	136	The revised method reflects this change.
	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.	137	The method states that colonies "may" be counted daily, making clear that this is an optional practice. Monitoring plates daily helps optimize counts.
	Line 347. We request that EPA clarify when further confirmatory analyses and isolation streaks on selective media will be required.	138	The revised method reflects this change.
	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.	139	The revised method includes an equation to calculate the mean $\log_{10}$ density across treated and control carriers.
	Line 364-368. The corresponding interim guidance document Section II. j. 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.	140	The revised method reflects this change.
	Line 364-368. 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).	141	Calculations are provided in the method. The mean log reduction value, exclusive of any greater than or less than symbols, is judged against the performance criteria.
	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.	142	The revised method reflects this change.

Comment Category	Specific Comment	Comment #	Agency Response
	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.	143	The revised method reflects this change. Variation in the media/reagent lots is acceptable; however, differences in performance between batches of media may lead to misleading neutralization results.
	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.	144	The acceptable neutralization criterion under this method is to be within 50% of the titer control. The average challenge per 0.01 mL inoculum in the neutralization assay is 20-200 CFU; use of a log difference is not practical in this situation.
	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.	145	An additional treatment which assesses the combination of carrier, treatment, and neutralizer was added to the method.
	Line 473. We request that EPA add the word “minutes” after “10±1.”	146	The revised method reflects this change.
	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.	147	The revised method reflects this change.
	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.	148	The revised method reflects this change.

Comment Category	Specific Comment	Comment #	Agency Response
	Line 482. 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."	149	The method was revised to clarify the incubation time.
Positive Feedback	Clorox appreciates the Agency's recognition that there may be additional pathways for product uses which are not described in this guidance. We support the Agency's recognition to continue broadening the scope of the interim guidance.	159	Thank you for your review of the documents and positive feedback.

Comment ID	Source	Comment Numbers
EPA-HQ-OPP-2022-0337-0007	Anonymous	1
EPA-HQ-OPP-2022-0337-0008	Household & Commercial Products Association HCPA and American Chemistry Council Center for Biocide Chemistries (CBC)	2-149, 165
EPA-HQ-OPP-2022-0337-00009	The Clorox Company	155-159, 166
EPA-HQ-OPP-2022-0337-0010	American Chemistry Council Center for Biocide Chemistries (CBC)	150-154
EPA-HQ-OPP-2022-0337-0011	Efficacy Working Group (EWG)	160-164

*Comments 34-36 were unsolicited comments related to Memo Document 0003 and were not addressed.