

DRAFT GUIDANCE DOCUMENT ON THE CONDUCT OF QUANTITATIVE METHODS FOR EVALUATING THE BACTERICIDAL, FUNGICIDAL, MYCOBACTERICIDAL AND VIRUCIDAL ACTIVITIES OF MICROBICIDES USED ON HARD, NON-POROUS SURFACES

INTRODUCTION

1. Microbicides are routinely used on hard surfaces to interrupt the spread of pathogens in a wide range of sites including healthcare, food production, restaurants, farms veterinary clinics, homes, schools, and other commercial and institutional settings. Over the years, a number of laboratory test methods have been developed to assess the effectiveness of hard surface microbicides. These methods have ranged from testing microorganisms in suspension to drying them on hard carriers and exposing them to the microbicide. Regulatory authorities throughout the world have relied upon data generated using these methods to approve applications/licenses for sale and distribution of these products in commerce. To date, there is no single method for this purpose that can be used and accepted in all OECD member countries.

2. To investigate the feasibility of harmonizing microbicide test methods, in April 2002, the U.S. Environmental Protection Agency (EPA) sponsored an OECD Workshop that included regulatory, academic, and industry experts from Europe, North America, Asia and Australia for the purpose of setting the foundation for moving towards international harmonization for microbicide test methods. The three-day workshop focused on four potential areas for harmonization: test methods, performance standards, labeling, and exchange of information and international coordination (1). Subsequently, the two areas chosen for further work on harmonization were hard surface disinfectants and treated materials. A Steering Committee was assembled to provide oversight for future work and, through funding from the European Commission, a call for tender was issued to initiate a critical review of existing methods to test hard surface microbicides and to provide recommendations to the Steering Committee on desirable attributes of such a method. The contract was awarded to the Centre for Research on Environmental Microbiology (CREM) at the University of Ottawa, Ottawa, Canada. With decades of experience between them in the area of microbicide test methods development, principal investigators, Drs. Syed Sattar, Martin Hamilton and Ms. Susan Springthorpe were tasked with evaluating the existing methods for testing hard surface microbicides and presenting a set of recommendations for developing a harmonized method. The final report from CREM contained ten recommendations to be considered in developing a harmonized test method (2).

3. Following the review and acceptance of the recommendations from CREM, the Task Force on Biocides (TFB) made the decision to continue working on a harmonized method and a Validation Management Group (VMG) was formed to oversee the design and conduct of a multi-laboratory ring-trial to validate the new methods.

4. Under the auspices of the TFB, several face-to-face meetings and conference calls were convened to proceed with selecting a statistician for data analysis, designing the ring-trial, selecting laboratories to conduct the tests, executing the ring-trial, and reviewing the preliminary statistical report. Several pre-validation studies were conducted prior to commencing with the full ring trial, which ran from February 2008 thru July 2008. Twenty seven laboratories, from eight OECD member countries (Australia, Canada, Czech Republic, France, Germany, Italy, UK and USA) participated in the ring-trial. For bacteria, viruses

and mycobacterium, data was submitted by six or more laboratories. Only five laboratories generated fungicidal data.

5. In September 2008, a meeting of the VMG was held to discuss the preliminary report with the statistician. It was determined that additional studies would be needed to augment the validation data from the first trial and to clarify issues presented as a result of the initial trial. This led to refinement of the test methods and Phase 2 testing was carried out between May 2009 and September 2009.

6. Much was learned from Phase 1 (the initial ring-trial). While all the analysts who participated in Phase 1 testing were trained in standard microbiological techniques and had performed testing using other microbicidal methods, they did not receive specific training for the four methods used in the ring-trials. In retrospect, the design of the ring-trial may have been overly ambitious, given the number of test substances and microorganisms that were challenged. While the outcome of Phase 1 was very positive, there was a high degree of variability across the test laboratories. Several laboratories did not follow the study design or were unable to achieve the target number of microorganisms for the water control. The VMG solicited feedback from the laboratory participants and as a result, additional refinements were made to the methods, which guided the design for Phase 2 testing.

7. One of the key questions to be answered in Phase 2 was whether training of the analysts would reduce the level of variability observed in Phase 1. The results from Phase 2 testing did demonstrate a reduction in variability based on the experience of the analyst. While the intra-laboratory variability was lower in Phase 2, the inter-laboratory variability was on par with Phase 1 and comparable to other standardized methods. These results from Phase 2 led to additional refinements in the text of the test guidelines in order to clarify areas that may have been subject to misinterpretation. The final version of the test guidelines also stress the importance of analyst training and the need for annual proficiency testing on the methods.

8. The report “Validation of Efficacy Methods for Antimicrobials used on Hard Surfaces” (3), along with the four Test Guidelines (for Bacteria, Viruses, Fungi and Mycobacteria) were forwarded for comment to the Working Group of National Coordinators of the Test Guideline Programme.

PURPOSE

9. The purpose of this guidance document is to provide information to regulators and the regulated community on key components that need to be addressed in each Test Guideline in order to meet the specified performance standards and ensure the appropriate data is generated in sufficient quantities to support label claims for public health microbicides.

GUIDING PRINCIPLES AND CONSIDERATIONS

10. The four OECD Test Guidelines use a quantitative method for evaluating the bactericidal, virucidal, fungicidal, and mycobactericidal activities of microbicides to be used on hard, non-porous surfaces.

11. The methods in the four Test Guidelines should be performed by personnel with training in microbiology and aseptic techniques. In order to ensure the validity of test results, each laboratory must establish a proficiency program. Proficiency testing should be conducted on an annual basis. Guidance on the elements to consider when developing a proficiency program can be found in ISO/IEC Standard 17025 (4). The methods should be conducted in facilities that are well equipped to handle infectious microorganisms at the appropriate biosafety level (5) (6).

12. All labware and those parts of equipment coming into direct contact with test organisms, media and reagents must be sterile. All equipment must be maintained and calibrated, as necessary. Maintaining good quality control of all media, reagents, and equipment used in these methods is necessary to ensure the validity of the test results.

13. When conducted as written, data generated using these Test Guidelines will support harmonization efforts for microbicide test methods in OECD member countries. To ensure data meet the criteria of the Council Decision on the Mutual Acceptance of Data please see the OECD website at the link: http://www.oecd.org/department/0,3355,en_2649_34381_1_1_1_1_1,00.html

14. A glossary of the terms common to the four methods is included at the end of this guidance document.

SCOPE OF APPLICATION OF THE GUIDANCE

15. The four Test Guidelines and this guidance document are designed to be used in testing the microbicidal activity of a wide range of formulations used on hard, non-porous surfaces (7). Each of the methods was validated in a multi-laboratory ring-trial against representative microorganism(s): Bacteria – *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus hirae*; Virus – Adenovirus Type 5; Fungus – *Aspergillus niger*; Mycobacterium – *Mycobacterium terrae*. These microorganisms are considered to be the basic representatives for the four test guidelines, however, individual regulatory authorities may require additional microorganisms to be tested to support label claims.

16. The test guidelines were validated for dilutable liquids, powders and ready-to-use formulations but lend themselves for use with other product forms as follows:

- a) Pressurized non-foaming aerosols: the test substance is obtained by spraying the aerosol into a sterile vessel for collection. Using aseptic techniques, 50 µL of the liquid is removed from the vessel and deposited on the inoculated test carrier.
- b) Pump/trigger spray products: the test substance is obtained by spraying the product into a sterile vessel for collection. Using aseptic techniques, 50 µL of the liquid is removed from the vessel and deposited on the inoculated test carrier.
- c) The method may also be suitable for products dispensed as foams or for pre-saturated towelettes/wipes; however, these application types will require further discussion and **may** require a separate validation.

17. No standardized test protocol can fully reflect all possible variations and challenges a microbicide may encounter in the field. Nevertheless, these test methods include the following levels of stringency to better predict the in-use performance of products on hard, non-porous surfaces: (a) the topography of carriers of brushed stainless steel is to assess the ability of the test substance to access target organisms on uneven surfaces, (b) the ratio between the surface area of the disk carrier and the volume of test substance is kept relatively low to better reflect how products are often applied, and (c) the test microbial suspension contains an added soil load to simulate the presence of the residues of body fluids and other substances on pre-cleaned surfaces. However, even the most effective products may fail to work in the field if used inappropriately.

STUDY DESIGN

18. This method uses disks (1 cm in diameter) of brushed stainless steel as a default carrier to represent hard, non-porous environmental surfaces. Each disk receives 10 µL of the test organism in a soil load. The inoculum is dried and exposed to 50 µL of the use-dilution of the test substance; control carriers receive an equivalent volume of a fluid harmless to the test organism (water control). The contact time and temperature may vary as required. A neutralizer, validated before performing the actual microbicide testing is added at the end of the contact time and the disks then eluted. Most or all of the eluate volume from each disk is assayed for the presence of viable organisms. Log₁₀ reductions in the viability of the test organism are calculated in relation to the viability count on the control carriers.

Discussion of the control carriers

19. Control carriers are used to determine the count of viable organisms on the carriers after the inoculum has been dried. They are prepared and treated in the same manner as the test carriers with the exception that the control carriers receive 50 µL phosphate-buffered saline (PBS) instead of the test substance. The count obtained from the control carriers is used to calculate the log₁₀ reduction following treatment with the test substance. At least four control carriers are used in each test. The mean viability count on the dried control carriers must be between 0.5 log₁₀ and 1.5 log₁₀ higher than the defined performance standard. The upper limit of 1.5 logs is set to exclude the influence of too high an inoculum on the results and to enable a fair comparison of the test substances. The basis to which these numbers (0.5 log₁₀ – log₁₀) are added will vary depending upon the log₁₀ reduction required to meet the performance standard as defined.

Number of test carriers

20. Three test carriers for one concentration/dilution of a test substance and four control carriers are recommended for these methods per test run (repetition). Individual regulatory authorities may require a higher number of test carriers.

Soil load

21. All microbial test suspensions for inoculating the carriers are to contain the three-component soil load specified in the methods. The total protein content of the mixture is roughly equal to that in 5% bovine serum and will satisfy label claims as a one-step cleaner/disinfectant. The mixture can be adjusted to 0.5% to support label claims that specify a pre-cleaning step prior to use of the disinfectant. Individual regulatory authorities may require other types and levels of soil depending on the label claims.

Diluent for test substances

22. If a diluent is not specified on the label of products sold as concentrates, it is assumed that tap water will be used to dilute the product to its in-use level. Given the wide range of tap water hardness geographically, the diluent for such products will be water with a standard hardness of 180 ppm as CaCO₃. A 375 ppm (as CaCO₃) water hardness would support a hard water label claim. Individual regulatory authorities may accept other levels of water hardness.

Contact time

23. The selected contact time for the test and control carriers will determine the label use directions, and should reflect the realities of field applications based on the labeled use sites. Products to be used on high-touch surfaces should be tested with a contact time of no longer than five minutes.

Products for surfaces other than those stated above may be tested with longer contact times in line with the existing laws, rules, guidelines or recommendations for the use of those products, and may require prior approval from target regulatory authorities.

Concentration of the test substance

24. The test substance should be prepared and tested according to the label directions for use. Additional concentrations may be tested to determine the limits of the product's activity.

Testing additional microorganisms

25. The recommended test organisms for use in regulated testing are specified in the individual Test Guidelines. Prior to initiating the tests, applicants should check with their regulating authority to determine whether additional test organisms and test parameters will be required to meet relevant regulatory requirements. The strain numbers given are for the American Type Culture Collection (ATCC). Equivalent strains from other established culture collections such as the National Collection of Type Cultures (NCTC) might be acceptable alternatives. If other test organisms are employed, the growth and recovery media, incubation requirements and any other test parameters should be detailed as necessary (8).

Number of test runs and test days

26. Due to the observed levels of variability in the ring-trials, applicants should have their products tested in multiple laboratories and/or on multiple test days to achieve a higher confidence level for their recommendations of use. Table 1 outlines the number of test runs and test days, which varies depending upon the number of laboratories chosen to conduct the test. Check with your regulatory authority to determine whether the test runs require different batches and if any batches need to be aged prior to use in the test.

Table 1

Number of Laboratories	Number of Runs		Number of Test Days/Run	
1	1 run		3 separate days	
2	1 st Lab	1 run	1 st Lab	1 day
	2 nd Lab	1 run	2 nd Lab	2 separate days
3	1 run		1 test day	

Performance standards

27. The target performance standards (log reduction) for each of the four test guidelines are outlined as follows:

- Disinfectant – Bacteria 5 log₁₀ reduction
- Disinfectant – Virus 3 log₁₀ reduction

- Disinfectant – Fungi 4 log₁₀ reduction
- Disinfectant – Mycobacteria 5 log₁₀ reduction

Table 2 provides the mean log reduction, for each test run, (based on a 30% standard deviation observed in the ring-trial) that is required to achieve the target performance standards.

Table 2

CLAIM	Disinfectant	Disinfectant	Disinfectant	Disinfectant
	Bacteria 99.999%	Viruses 99.9%	Fungi 99.99%	Mycobacteria 99.999%
Performance standard (Log reduction)	5	3	4	5
# LABS				
1	5.8	3.8	4.8	6.3
2	5.4	3.6	4.6	6.2
3	5.1	3.3	4.3	6.1

TEST REPORTS

28. Any submissions requesting label claims must accompany detailed results and raw data from testing as described in the test guideline along with evidence for validation of neutralization.

29. The test report must include the following information:

Test and Control Substances

- A description of the test substance; physical state, color and pH, trade name or identification number (ID) lot/batch number(s) and/or date of manufacture and/or expiration date.
- Chemical nature and relative concentrations of active ingredients.

Details on the Test Method

Test organism

- Source
- Scientific name and strain number
- Growth and recovery media

Test Conditions

- Temperature
- Contact time
- Soil load (type and amount)

Results

- CFU per carrier
- Log₁₀ Reduction
- Neutralization validation
- Copies of the raw data

Conclusion

REFERENCES

- (1) OECD (2002). Report of the Efficacy Workshop on Certain Antimicrobial Biocides, Arlington, VA, U.S.A., OECD Meeting held in April 2002.
- (2) European Commission DG Env. F.2 (2005). Harmonization of Hard Surface Disinfectant Test Methodology in OECD Countries.
- (3) OECD Report on the Validation of Efficacy Methods for Antimicrobials used on Hard Surfaces, November 2009.
- (4) ISO/IEC 17025 General Requirements for the Competence of Testing and Calibration Laboratories, 2nd Edition: http://www.iso.org/iso/Catalogue_detail?csnumber=39883
- (5) Biosafety in Microbiology and Biomedical Laboratories (2007) 5th Ed., U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health.
- (6) Laboratory Biosafety Guidelines 3rd Edition, 2004: <http://www.phac-aspc.gc.ca/ols-bsl/lbg-ldmbl/>
- (7) Springthorpe, V.S. and Sattar, S.A. (2005b). Carrier tests to assess microbicidal activities of chemical disinfectants for use on medical devices and environmental surfaces. J. AOAC International 88: 182-201.
- (8) ASTM International (2006). Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides. Method E-2197-02, Vol. 11.05. ASTM International, West Conshohocken, PA, U.S.A.

GLOSSARY

BSA: Bovine Serum Albumin

Carrier is an inanimate surface to be inoculated with the test organism

CFU: Colony Forming Unit.

Control fluid (PBS) is the fluid placed on the control carriers in place of the test substance

Eluate is recovered eluent that contains the test organism (inactivated or not).

Eluent is any liquid that is harmless to the test organism(s) and that is added to a carrier to recover these from it.

Inoculum: Test organism in soil load.

Neutralization is a process to quench microbicidal or microbistatic activity of a test substance remaining at the end of the contact time. This process may be achieved by dilution of the organism-test substance mixture and/or by adding to it one or more chemical neutralizers.

PBS: Phosphate Buffer Saline

Soil load is a solution of one or more organic and inorganic substances added to the suspension of the test organism to simulate their presence in body secretions, excretions, or other extraneous substances. It presents the test substance with a challenge to overcome the chemical demand from the soil load and the physical shielding of test organism that it may provide.

Stock culture is the frozen, refrigerated or lyophilized form of the test organism.

Test substance is a compound or formulation that is under evaluation for its microbicidal activity.

Test organism is one selected for testing – usually for its susceptibility/resistance characteristics. It also may be referred to as a *surrogate*, *simulant*, *target* or *marker microbe*. Ideally, it should be easy and safe to handle, and readily identifiable.

Test suspension is the suspension of the test organism used to prepare the stock culture or working culture.

Working culture is the suspension of the test organism prepared for use in the test.