Designation: E1053 – 11

Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces[1]

This standard is issued under the fixed designation E1053; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method is used to evaluate the virucidal efficacy of liquid, aerosol, or trigger-spray microbicides intended for use on inanimate, nonporous environmental surfaces. This test method may be employed with most viruses, which can be grown in cultured cells.[2] However, other host systems (for example, embryonic eggs) may be used with proper justification and documentation.

1.2 This test method should be performed only by those trained in microbiological and virological techniques in facilities designed and equipped for work with infectious agents at the appropriate biosafety level.

1.3 It is the responsibility of the investigator to determine whether Good Laboratory Practice regulations (GLPs) are required and to follow them where appropriate (40 CFR, Part 160 for EPA submissions and 21 CFR, Part 58 for FDA submissions). Refer to the appropriate regulatory agency for performance standards of virucidal efficacy.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* The user should consult a reference for laboratory safety recommendations.[2]

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D1129 Terminology Relating to Water

E1153 Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces

E1482 Test Method for Neutralization of Virucidal Agents in Virucidal Efficacy Evaluations

E2197 Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals

2.2 *Federal Standards:*[4]

Title 21, Code of Federal Regulations (CFR), Food and Drug Administration, Part 58, Laboratory Practice for Nonclinical Laboratory Studies

Title 40, Code of Federal Regulations (CFR), Environmental Protection Agency, Subchapter E, Pesticide Programs; Part 160, Good Laboratory Practice Standards

3. Terminology

3.1 *Definitions*—For definitions of general terms used in this test method, refer to Terminology D1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *hard water, n*—water with a standard hardness as calcium carbonate.

3.2.2 *neutralization, n*—a process which results in quenching the microbicidal activity of a test substance. This may be achieved through dilution of the test substance to reduce the microbicidal activity, or through the use of chemical agents, called neutralizers, to eliminate microbicidal activity.

3.2.3 *soil load, n*—a solution of one or more organic and/or inorganic substances added to the suspension of the test organism to simulate the presence of body secretions, excretions or other extraneous substances.

3.2.4 *test substance or test formulation, n*—a formulation which incorporates microbicidal ingredients.

4. Summary of Test Method

4.1 The virus suspension is dried on an inanimate, nonporous surface. The test substance is added over the dried film at

[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

Current edition approved Jan. 1, 2011. Published March 2011. Originally approved in 1985. Last previous edition approved in 2002 as E1053 – 97 (2002). DOI: 10.1520/E1053-11.

[2] Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health, Biosafety in Microbiological and Biomedical Laboratories, U.S. Department of Health and Human Services, Washington, DC, December 2009, 422 pp.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[4] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, http://www.access.gpo.gov.

its use-dilution or sprayed from an aerosol can or trigger-sprayer following the manufacturer's directions. Control carriers receive an equivalent volume of a buffer harmless to the test virus and its host cells. After exposure at the appropriate temperature (usually 22 ± 2°C) for the recommended time, the eluates from control and test carriers are assayed for infectivity.

4.2 This test method is designed to be performed by a person trained in culturing and assaying infectious viruses who is responsible for choosing the appropriate host system for the test virus and applying the techniques necessary for propagation and maintenance of the host system and test virus. For a reference text, refer to Lennette et al.[5]

5. Significance and Use

5.1 This test method may be used to determine the effectiveness of liquid, aerosols/foams, and trigger-spray products against designated prototype viruses.

5.2 The number of lots of the test substance and the number of replicates in each test will depend on the requirements of the target regulatory agency.

5.3 Certain regulatory agencies may require additional testing using other carrier tests for product registration purposes.

6. Materials and Reagents

6.1 *Host System and Assay of Infectious Virus*—See Note 2.

6.1.1 *Cell Cultures,* appropriate for test virus.

6.1.2 *Growth and Maintenance Media,* any growth and maintenance media suitable for work with the virus and its host cells.

NOTE 1—Materials and reagents for cell culture may be purchased from biological supply houses.

6.1.3 *Diluent,* Earle's Balanced Salt Solution (EBSS) or other appropriate media.

6.1.4 *Plastic Cell Culture Ware.*

NOTE 2—Plastic cell culture ware may be purchased from most laboratory supply houses.

6.1.5 *Incubator,* capable of maintaining 36 ± 1°C or other temperature appropriate for replication of the specific test virus; an incubator with 5 to 7 % CO_2 will be needed if an open system is being used for cell culture and virus assay.

6.1.6 *Refrigerator,* 4 ± 2°C.

6.1.7 *Test Tubes,* screw-capped.

6.1.8 *Pipettes,* serological, 10, 1, and 0.5 mL.

6.1.9 *Micropipettors and Tips,* (96-well assay only).

6.1.10 *96-Well Dilution Plates,* (96-well assay only).

6.1.11 *Microtitration Kit.*

NOTE 3—Microtitration kits may be purchased from most laboratory supply houses.

6.1.12 *Petri Plates,* glass, 100-mm diameter, 1-cm deep.

7. Test Viruses

7.1 Appendix X1 lists viruses and their respective host cells as examples for use in this test method.

7.2 To demonstrate that the test substance has broad virucidal activity, it should be shown to be effective against at least one non-enveloped virus.

8. Virus Stock

8.1 The titer of the test virus suspension must be sufficiently high so that at least 10^4 infective units/carrier can be recovered from the inoculated carriers after the inoculum has dried. The host system employed for virus propagation need not be the same as that used for virus recovery and the infectivity assay.

9. Operating Technique

9.1 *Test Substance Diluent*—For test substances requiring dilution in water to obtain a use-dilution, water with a standardized and specified level of hardness, such as 400 ppm as $CaCO_3$, shall be used as the diluent.[6]

9.2 *Cytotoxicity Control*—The objective of this control is to (*1*) determine the dilution of the test substance post-neutralization at which it causes no apparent degeneration (cytotoxicity) of the cell line to be used for measuring virus infectivity and (*2*) assess if the neutralizer in any way reduces or enhances such cytotoxicity. Make an initial 1:2 dilution of the use-dilution of the test substance in the neutralizer and three further ten-fold dilutions of the neutralized test substance in the diluent. Remove the culture medium from the monolayers of the host cell line(s) and put into each test monolayer separately the same volume of inoculum as used in virus titration; control monolayers receive an equivalent amount of diluent (without any neutralizer) only. Another set of monolayers should be exposed to the neutralizer alone. Hold the cultures for the same period of time as used in virus titration and examine them under an inverted microscope for any cytotoxicity. In case of cytotoxicity, a different neutralizer or gel filtration (see Test Method E1482) of the neutralized virus-test substance mixture may be needed.

NOTE 4—If gel filtration is used in the virucidal activity test runs, the neutralized and gel-filtered test substance should be evaluated for the cytotoxicity control.

9.3 *Test Substance Neutralization Control*—To determine the dilution at which neutralization of the test substance has occurred, prepare and inoculate an additional set of cytotoxicity controls with the neutralizer added to the test substance. To validate the neutralization, add equal volumes of the neutralized test substance, the neutralizer alone and a control fluid (for example, PBS) a relatively low number (for example, 1000 to 5000) infective units of the test virus and hold the mixtures for 10 to 20 min at room temperature. Titrate the mixtures for infectious virus. Comparable levels of infective units must be recovered from the control, the neutralizer alone, and the neutralized test substance for the neutralization to be successful. In case of incomplete neutralization, either another neutralizer may be needed or the gel filtration method (Test Method E1482) may be used.

9.4 *Plate Recovery Control*—Vortex the virus suspension thoroughly and place 0.2-mL on the inside bottom surface of

[5] Schmidt, N. J., , Lennette, D. A., Lennette, E. T., and Lennette, E. H. eds., Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections, Seventh Edition, American Public Health Association, Washington, DC, 1995.

[6] AOAC International, Official Methods of Analysis of the AOAC, Arlington, VA, 1990.

each glass petri dish. Allow the virus inoculum to dry under ambient conditions in a laminar flow hood or other suitable chamber with the petri dish cover removed. The drying time of this control should be consistent with that for the test runs. A recovery of at least 10^4 infective units/control dish should be achieved for the test to be considered valid. Pools of certain types of viruses may require concentration by ultracentrifugation to obtain titers high enough to give a minimum of 10^4 infective units/dish after the inoculum has been dried. However, any such concentrated virus must be vortexed well to reduce the presence of viral aggregates.

NOTE 5—The volume of virus inoculum per carrier may be increased depending on the titer of the virus. This volume must be consistent between the plate recovery control and test substance runs. It should be noted, however, that an increased volume will prolong the drying of the inoculum and may lead to increased losses in virus infectivity.

9.4.1 After drying, overlay each dried control film with 2.0 mL of PBS or another buffered solution harmless to the virus and its host cells. Let stand for the same contact time as used for the test carriers and then add an equal amount of neutralizer (2.0 mL). Scrape the inside bottom surface with a sterile cell scraper to resuspend the viral film. This suspension may be considered the 10^{-1} dilution of the virus. Prepare serial 10-fold dilutions using diluent and inoculate an amount appropriate to the test format to no less than four replicate cell monolayers/dilution.

9.5 *Test for Virucidal Activity*—For each lot of the test substance, treat a dried film carrier with 2.0 mL of the use-dilution of a liquid product or the amount of product released during recommended use of the aerosol or trigger spray. Hold for the required contact time. Upon completion of the contact time, immediately add an equal volume of neutralizer (2.0 mL) to the carrier and mix well. Scrape the film to resuspend the virus/test substance/neutralizer mixture. This suspension may be considered the 10^{-1} dilution of the virus. Prepare serial 10-fold dilutions using diluent and inoculate an amount appropriate to the test format to no less than four replicate cell monolayers/dilution starting from 10^{-2}.

10. Soil Load (refer to Test Method E2197)

10.1 The soil load to be incorporated in the suspension of the test organism may consist of a mixture of the following stock solutions in phosphate buffer (pH 7.2):

10.2 Add 0.5 g of tryptone or yeast extract to 10 mL of phosphate buffer.

10.3 Add 0.5 g of BSA to 10 mL of phosphate buffer.

10.4 Add 0.04 g of bovine mucin to 10 mL of phosphate buffer.

10.5 Prepare the solutions separately and sterilize by passage through a 0.22-µm pore diameter membrane filter, aliquot, and store at either 4 ± 2°C or –20 ± 2°C; such solutions can be stored in the refrigerated for about three months and frozen for at least one year.

10.6 To obtain 500 µL of the inoculum, add 25 µL of BSA, 100 µL of mucin, and 35 µL of tryptone or yeast extract stock to 340 µL of the virus suspension.

NOTE 6—Other types of soil load such as animal sera may be used depending on the target regulatory agency and recommended use of the test substance. The soil load mixture given above contains a level of protein roughly equal to that in 5 % serum. Preliminary screening of albumin and mucin is recommended to ensure compatibility with test organism(s).

10.7 If the use-dilution of the test substance is to be prepared in water, follow the methods listed in Test Method E1153 for making the hard water to be used. The test report must clearly specify the level of hardness used in testing for virucidal activity.

11. Additional Controls

11.1 *Cell Culture Control*—To ensure that the host cells are not contaminated with bacteria, fungi, or any cytopathogenic viruses other than those used in the test and to confirm the viability of the cells during the incubation period of the assay, at least four host cell monolayers are left untreated in each test and examined first at the end of the incubation period. Any obvious contamination or degeneration in such monolayers would invalidate the virus titration.

11.2 *Virus Stock Titer Control*—An aliquot of the stock virus used in the test is serially diluted in diluent and inoculated onto the host culture. This is to confirm that the host cells are susceptible to the virus and that the viral infection assay is performed appropriately. This control will also confirm that the titer of the stock virus is appropriate for use in the test. A lack of obvious and typical virus-induced cytopathic effects in the lower dilutions would invalidate the test.

11.3 *Control for Interference with Virus Infectivity (optional)*—Levels of the test substance which show no obvious cytotoxicity could still reduce or enhance the ability of the challenge virus to infect or replicate in host cells, thus interfering with the estimation of its virucidal activity. An interference control should, therefore, be included to rule out such a possibility. Remove the culture medium from the host cells and inoculate each one of the test monolayers with the same volume of inoculum as used in virus titration and a 1:10 dilution of the test substance in the neutralizer. Controls receive PBS alone (without the neutralizer). Another set of monolayers should be exposed to the neutralizer alone. Hold the monolayers at room temperature for 30 to 60 min and inoculate each with a low number (for example, 20 to 50) of infective units of the challenge virus. Incubate the monolayers for virus adsorption, place maintenance medium in the cultures, incubate them for the time required for virus replication and examine them for cytopathology or foci of virus infection. Any significant difference in virus infectivity compared to the control is indicative of the test substance's or the neutralizer's ability to affect the virus susceptibility of the host cells. In such a case, a different neutralizer or alternative approaches to the removal of the residual test product in the samples to be titrated for virus infectivity may be needed.

12. Calculation

12.1 When a most probable number (MPN) assay for virus titration is employed, use the method of Reed and Muench or Spearman-Kärber to calculate reductions in virus infectivity.

12.2 Report the titer of the stock virus, titer of recovered virus (plate recovery control), degree of cytotoxicity (cytotoxicity control), the degree of virus inactivation (results of virucide test), and the dilution at which neutralization and absence of viral interference occurred (neutralization/viral interference control).

13. Precision and Bias

13.1 A precision and bias statement cannot be made for this test method at this time.

14. Keywords

14.1 carrier test; cell or other tissue cultures; disinfectant; infection control; microbicide; surface test; virucidal test; virus; viruses on dried environmental surfaces

APPENDIX

(Nonmandatory Information)

X1. RECOMMENDED TEST VIRUS STRAINS AND THEIR HOST CELLS

X1.1 ATCC numbers are given, when available, in parentheses.

X1.1.1 *Adenovirus,* Type 4 (VR-4) or Type 5 (VR-5). Cell line options for making virus pools and infectivity titrations are 293 (CRL-1573) and Vero (CCL-81) cells, respectively.

X1.1.2 *Canine parvovirus,* Cornell-780916-80 strain, (VR-2017). Cell line: A-72 (CRL-1542).

X1.1.3 *Cytomegalovirus,* strain AD-169 (VR-538). Cell line: MRC-5 (ATCC CCL-171) orWI-38 (CCL-75).

X1.1.4 *Feline calicivirus,* strain F9 (VR-782). Cell line: CRFK (CCL-94).

X1.1.5 *Hepatitis A Virus,* HM-175 strain (VR-2093). Cell line: Rhesus monkey kidney - FRhK-4 (CRL-1688).

X1.1.6 *Herpes Simplex Virus,* Type 1, strain F (1) (VR-733). Cell line: Vero (CCL-81), HEp-2 (CLL-23), primary rabbit kidney.

X1.1.7 *Influenza A,* Hong Kong Strain (VR-544); PR-8 (VR-95). Cell line: Canine kidney (MDCK); established (e.g., LLC-MK2) or primary monkey kidney cells.

NOTE X1.1—Animal serum may be inhibitory to influenza viruses and also to trypsin used for influenza virus culture.

X1.1.8 *Murine Norovirus,* Cell line: RAW 264.7 (TIB-71).

X1.1.9 *Respiratory Syncytial Virus,* Long strain (VR-26). Cell line: HEp-2, MRC-5 (CCL-171), HeLa (CCL-2).

X1.1.10 *Rhinovirus,* type 37, strain 151-1 (VR-1147); Rhinovirus type 14 (VR-284). Cell line: MRC-5 (CCL-171), WI-38 (CCL-75), H1-HeLa (CRL-1958).

X1.1.11 *Rotavirus,* Wa strain, (VR-2018). Cell line: Rhesus monkey kidney (MA-104) (CRL-2378) or African green monkey kidney - CV-1 (CCL-70).

NOTE X1.2—Animal serum may be inhibitory to rotaviruses and also to trypsin used for rotavirus culture.

X1.1.12 *Vaccinia Virus,* WR strain (VR-119). Cell line: Vero (CCL-81), HEp-2 (CLL23).