Designation: E 1053 – 97 (Reapproved 2002)

Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces[1]

This standard is issued under the fixed designation E 1053; 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 laboratory test method is used to evaluate the virucidal efficacy of liquid, aerosol, or trigger spray antimicrobial solutions on inanimate nonporous environmental surfaces. This test method may be employed with most viruses and is designed for cell culture host systems.

1.2 This test method should be performed only by those trained in microbiological or virological techniques.

1.3 *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 the laboratory safety recommendations.[2]

1.4 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.

2. Referenced Documents

2.1 *ASTM Standards:*

E 1052 Test Method for Efficacy of Virucidal Agents Against Viruses in Suspensions[3]

E 1153 Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces[3]

E 1482 Test Method for Neutralization of Virucidal Agents in Virucidal Efficacy Evaluations[3]

2.2 *Federal Standards:*

Title 40, Code of Federal Regulations (CFR), Environmental Protection Agency, Subchapter E, Pesticide Programs; Part 160, Good Laboratory Practice Standards[4]

Title 21, Code of Federal Regulations (CFR), Food and Drug Administration, Part 58, Laboratory Practice for Nonclinical Laboratory Studies[4]

3. Summary of Test Method

3.1 The virus suspension is dried on an inanimate, nonporous surface. The antimicrobial is added over the dried film as a use dilution solution or sprayed from an aerosol can or trigger spray container following the recommended directions. After exposure at the appropriate temperature (usually 22 ± 2°C) for the recommended time, the virus-antimicrobial mixture is assayed in a host system appropriate for the test virus. The virus titer of an untreated surface is determined by the median infective dose (ID_{50}) method of virus titration. Cytotoxicity to the host system of the antimicrobial at the tested concentration is determined by an LD_{50} method. The virus-antimicrobial mixture is assayed in numerous units of the host system at a dilution just beyond the cytotoxicity range of the antimicrobial. The extent of virus inactivation by the antimicrobial is determined. Results are recorded as $\log_{10}$-virus inactivated.

3.2 This test method is designed to be performed by a trained virologist who is responsible for choosing the appropriate host system for the test virus and applying the techniques necessary for propagation and maintenance of host and test virus. For a reference text, refer to Schmidt and Emmons.[5]

4. Significance and Use

4.1 This test method may be used to determine the effectiveness of liquid and aerosol antimicrobial products against designated prototype viruses.

4.2 The effective antimicrobial concentration should be determined utilizing cell cultures as the host system for specific viruses.

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

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[2] CDC-NIH, *Biosafety in Microbiological and Biomedical Laboratories*, 3rd ed., U.S. Department of Health and Human Services, Washington, DC, May 1993.

[3] *Annual Book of ASTM Standards*, Vol 11.05.

[4] Available from U.S. Government Printing Office, Superintendent of Documents, Washington, DC 20402.

[5] *Diagnostic Procedures for Viral and Rickettsial Infections*, N. J. Schmidt and R. W. Emmons, eds., 6th ed., American Public Health Association, Washington, DC, 1989.

4.3 This test method is applicable for testing of liquid and pressurized antimicrobial products against viruses on inanimate nonporous environmental surfaces.

5. Materials and Reagents

5.1 *Cell Culture Technique:* [5]

5.1.1 *Cell Culture System*, appropriate for test virus.

5.1.2 *Growth Media/Maintenance Media*, Medium 199, Eagle's minimal essential medium (EMEM) or equivalent, supplemented with appropriate concentration of serum (inactivated and mycoplasma-free), antibiotics, and other growth factors as needed.[6]

5.1.3 *Diluent*, the media listed in 5.1.2, phosphate buffered saline, trypticase soy broth supplemented with serum or other similar buffered solutions.

5.1.4 *Plastic Cell Culture Ware*. [7]

5.1.5 *Incubator*, capable of maintaining 37 ± 1°C or other temperature appropriate for replication of the specific test virus.

5.1.6 *Refrigerator*, 4 ± 2°C.

5.1.7 *Test Tubes*, screw-capped.

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

5.1.9 *Microtitration Kit*. [8]

5.1.10 *Petri Plates*, glass, 60-mm diameter, 1 cm deep.

5.2 Additional or equivalent materials and reagents specific to the host recovery system may be necessary. The trained microbiologist or virologist is responsible to choose accordingly as needed.

6. Test Viruses

6.1 To determine virucidal efficacy, a prototype strain from a particular virus family must be tested. Because new strains of viruses are being discovered continuously and methods of isolation and growth are being improved, the following prototypes and the cell cultures in which to grow and test them are suggested. Other strains within a family may be substituted as testing prototypes for specific marketing claims.

6.2 To demonstrate the range of antiviral activity of an antimicrobial, the formulation should be tested against viruses representing a range of resistances to germicides. A possible group of viruses includes a poliovirus (representative of those viruses most resistant to chemical germicides), a herpes virus (representative of those most easily inactivated), and an adenovirus (representative of intermediate resistance to germicides). The following is a list of suggested virus strains that typically are assayed, as well as cell cultures that support their growth.

6.3 Typical test virus strains and cell cultures.

6.3.1 *Poliovirus*, Type 1, Chat strain, ATCC VR-192.

Cell line options: Monkey Kidney Cells (VERO): Human Epidermoid.

Carcinoma, Larynx (HEp-2): African Green Monkey Kidney (CV-1).

6.3.2 *Hepatitis A Virus*, HM-175 strain, ATCC VR-2093.

Cell line options: Fetal Kidney, Rhesus Monkey, Continuous (FRhK-4).

6.3.3 *Herpes Simplex*, Type 1, Strain F (1), ATCC VR-733.

Cell line options: VERO, HEp-2.

6.3.4 *Cytomegalovirus*, strain AD-169, ATCC VR-538.

Cell line options: Human Diploid Lung (MRC-5 or WI-38).

6.3.5 *Adenovirus*, Type 2, Adenoid 6 strain, ATCC VR-2.

Cell line options: Human Lung Carcinoma (A549), HEp-2.

6.3.6 *Influenza A_2*, Hong Kong Strain, ATCC VR-544.

Cell line options: Canine Kidney (MDCK); Rhesus Monkey Cells, Continuous (LLC-MK2).

6.3.7 *Respiratory Syncytial Virus*, Long strain, ATCC VR-26.

Cell line options: HEp-2, MRC-5.

6.3.8 *Vaccinia*, WR strain, ATCC VR-119.

Cell line options: VERO, HEp-2.

6.3.9 *Rhinovirus*, Type 37, Strain 151-1, ATCC VR-1147.

Cell line options: MRC-5, WI-38.

NOTE 1—Rhinovirus-infected cultures require incubation at 33 ± 1°C.

6.3.10 *Rotavirus*, Wa strain, ATCC VR-2018.

Cell line options: Rhesus Monkey Kidney, Continuous (MA-104) or

African Green Monkey Kidney, Continuous (CV-1).

NOTE 2—Some lots of fetal calf serum may be inhibitory to rotavirus.

6.4 *Other Viral Groups*—Virucidal efficacy against certain types of viruses such as *Human Immunodeficiency Virus* must be substantiated in a laboratory having Biosafety Level 3 Facilities.[2]

7. Virus Stock

7.1 The titer of the test virus suspension must be sufficiently high so that at least 10^4 infective units may be recovered from the dried films as described as follows. Utilize an appropriate host to prepare virus suspensions with minimum infectivity titers of about 10^7 to 10^8 infective units/mL. The host system employed for the virus pool need not be the same system used for virus recovery following virus challenge of the antimicrobial. The virus titer of the stock virus is determined by the $CCID_{50}$, plaque assay or other quantifiable measure of infectivity.

8. Operating Technique

8.1 The test must include the following parameters.

Parameter	Summary	Replicates
Cell Culture Control	Medium alone	4 per group
Virus Control	Virus surface + 2 mL medium	4 per dilution
Virucidal Test	Virus surface + 2 mL antimicrobial	10 per dilution
Cytotoxicity Control	2 mL antimicrobial + medium	4 per dilution
Neutralization Control	Neutralized antimicrobial + virus	4 per dilution

8.2 *Cytotoxicity Control*—Prior to studying the effects of the antimicrobial on viruses, determine its cytotoxic effect on

[6] Materials and reagents for tissue culture may be purchased from biological supply houses.

[7] Plastic tissue culture ware may be purchased from most laboratory supply houses.

[8] Microtitration kit may be purchased from most laboratory supply houses.

the host system by an LD_{50} determination. Use serial 2-fold dilutions and inoculate 4 units/dilution. At least two replicate determinations are performed as cytotoxicity controls.

8.3 *Germicide Neutralization Control*—If excessive cytotoxicity cannot be eliminated by dilution of the virus/germicide mixture, follow Test Method E 1482. Use serum or other chemicals to neutralize the cytotoxic effect of the germicide. To determine the dilution at which neutralization of the germicide has occurred, prepare and inoculate an additional set of cytotoxicity controls with the neutralizer added to the diluent. Following inoculation of cell cultures, add 0.1 mL (or volume inoculated previously) of the diluted stock virus at approximately 100 to 1000 infectious units to each dilution. Those dilutions that are toxic to the cells or do not exhibit virus replication, or both, are not included in the $\log_{10}$ reduction calculations of the germicidal activity.

8.4 *Virus Control*—After cytotoxicity is known, perform test against viruses. Shake virus suspension thoroughly and place 0.2-mL amounts on bottoms of four 60-mm petri plates. Allow the virus films to dry for 30 ± 5 min at 37 ± 2°C or 60 ± 5 min at 22 ± 2°C. Do not use a humidified CO_2 incubator for drying. A recovery of at least 10^4 infectious units/surface should be achieved. Use two films as the virus controls; add 2.0 mL PBS or other suitable neutralizing diluent over film, let stand for the same contact time as for the experimentals and then scrape surface with sterile rubber policeman to resuspend film. This solution is the 10^{-1} dilution of the virus. Immediately prepare serial 10-fold dilutions and inoculate 0.1-mL amounts into 4 host system units/each dilution.

8.5 *Virucidal Test*—Each of the remaining films is treated with the antimicrobial, 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 container. After the appropriate time contact at 22 ± 2°C (usual room temperature range), add sufficient PBS or neutralizing diluent to each plate to dilute beyond the cytotoxicity of the antimicrobial; then, scrape surface with sterile rubber policeman to resuspend film. Inoculate 0.1 mL of each virus-antimicrobial dilution onto ten tissue culture test tubes (macro), or 0.05 mL onto 10 monolayers in microculture (if used). Allow the virus to adsorb one hour at 37°C and then add 0.9-mL (macro) or 0.10-mL (micro) maintenance media/monolayer. Incubate at 37°C or other appropriate temperature and observe cell sheet for evidence of virus-specific cytopathic effect.

9. Organic Soil or Hard Water

9.1 To simulate an organic soil load, if required in testing, add calf serum or other serum to virus suspensions. The serum should be tested for absence of antiviral inhibitors, see 6.3.10. Pancreatic digest of casein may be used as an alternative to serum; 7.6 g of tryptone/L of physiological saline (0.85 %) contains 2.0 g of total protein, which is approximately equivalent to the protein content of a 5 % solution of serum.

9.2 If tests are to be performed with hard water as a diluent of liquid disinfectants, follow the methods listed in Test Method E 1153.

10. Calculation

10.1 Use the method of Reed and Muench or Spearman-Karber[2] to calculate the control virus titer and cytotoxicity.

10.2 Report the titer of the stock virus (virus control), degree of cytotoxicity (cytotoxicity control), the degree of virus inactivation (results of virucide test), and the dilution at which neutralization occurred (neutralization control).

11. Precision and Bias

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

12. Keywords

12.1 carrier test; cell cultures; disinfectant; fomites; germicide; infection control; surface test; virucidal test; virus; viruses on dried environmental surfaces