Designation: E 1052 – 96 (Reapproved 2002)

Standard Test Method for Efficacy of Antimicrobial Agents Against Viruses in Suspension[1]

This standard is issued under the fixed designation E 1052; 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 a suspension test used to evaluate the effectiveness of antimicrobial solutions against specific viruses. 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 andCFR, 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 1053 Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces[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 Evaluation[3]

2.2 *Federal Standards:*[4]

Title 40, Code of Federal Regulations (CFR), Environmental Protection Agency, Part 160, Good Laboratory Practice Standard

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

3. Summary of Test Method

3.1 One part of the virus suspension is added to nine parts of the appropriately diluted antimicrobial. The virus is exposed to the virucide for the length of time that is representative of actual use conditions or the label directions of the product (for example, from 15 sec for a handsoap to 10 min or longer for a antimicrobial solution). The tests also should be performed at the temperature most representative of actual use conditions (usually 22 ± 2°C). The virus-antimicrobial mixture is assayed in a host system appropriate for the test virus. The virus titer of the stock virus is determined by the median cell culture infective dose ($CCID_{50}$), plaque assay or other quantifiable measure of infectivity. Cytotoxicity to the host system (from the antimicrobial) at the tested concentration also is determined. The virus-antimicrobial mixture is assayed in numerous units of the host system at a dilution just beyond the cytotoxic range of the antimicrobial. At least three replicate determinations are performed on controls and experimentals to confirm virus inactivation by a batch of antimicrobial. Results are recorded as the median value of $\log_{10}$-virus inactivation.

3.2 This test method is designed to be performed by a trained microbiologist or 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 is to be used to determine the effectiveness of antimicrobial solutions against designated prototype viruses that are in suspension.

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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*, Third Edition, 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, Rickettsial and Chlamydial Infections*, N. J. Schmidt and W. W. Emmons, Eds, Sixth Edition, Amer. Pub. Hlth. Assoc., Washington, DC 1989.

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

4.3 This suspension test is for special applications of virucides, such as inactivation of viruses in contaminated liquid wastes, and as a first stage in determining virucidal potential of liquid chemical germicides, liquid hand soaps, OTC topicals or other skin products. Regulatory agencies may require additional tests to demonstrate overall virucidal activity.

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 the specific test virus.

5.1.6 *Refrigerator*, 4 ± 2°C or other appropriate temperature.

5.1.7 *Test Tubes*, screw-capped.

5.1.8 *Pipettes*, serological, 10, 1, 0.5 mL or calibrated pipettors, or both.

5.1.9 *Microtitration Kit.*[8]

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 the virucidal efficacy, a prototype strain from a particular virus family must be tested. Because new strains of viruses are continuously being discovered 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 claim purposes.

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 are typically assayed, as well as cell cultures that support their growth.

6.3 Suggested test virus strains and cell cultures.

6.3.1 *Poliovirus*, Type 1, Chat strain, American Type Culture Collection (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 claims for certain types of viruses, such as *Human Immunodeficiency Virus* must be substantiated in a laboratory having Biosafety Level 3 Facilities.

7. Virus Stock

7.1 Utilize an appropriate host to prepare high titer virus suspensions with minimum infectivity titers of at least 10^6 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 parameters given in Table 1.

8.2 Thoroughly mix virus suspension. Add one part of virus suspension to nine parts of the appropriately diluted germicide in a sterile medication tube held at the appropriate exposure temperature (usually 22 ± 2°C). Consider this the 10^{-1} dilution of the virus. Following the exposure for the time indicated by

TABLE 1 Parameters

Parameter	Summary	Replicates
Cell culture	medium alone	3/group
Virus control	1 part virus + 9 parts medium	3/dilution
Virucidal test	1 part virus + 9 parts germicide	3/dilution
Cytotoxicity control	1 part medium + 9 parts germicide	3/dilution
Neutralization control	neutralized germicide + virus	3/dilution

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

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

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

the claim, immediately neutralize the antimicrobial by serial ten-fold dilutions in media or other diluent.

NOTE 3—The first dilution may be performed in 50–100 % serum to enhance neutralization. Perform the virus control (one part of virus + nine parts medium) and cytotoxicity control (one part medium + nine parts germicide) concurrently with the virucidal test described above. If excessive cytotoxicity cannot be eliminated by dilution of the virus/germicide mixture, follow Test Method E 1482.

8.3 *Virus Recovery*—Inoculate replicate cell culture monolayers per dilution. (Three replicates per dilution are typically assayed). Inoculate 0.1 mL volumes of each test and control dilution onto the appropriate cell cultures. Other volumes may be used; however, approximately half the volume of each dilution must be assayed. Incubate the cultures at the appropriate temperature and observe for the evidence of virus-specific or cytotoxic effects using the appropriate methods (CPE, hemagglutination, plaque assay).

8.4 *Germicide Neutralization Control*—To determine the dilution at which neutralization of the germicide has occurred, prepare and inoculate an additional set of cytotoxicity controls. Following inoculation of cell cultures, add 0.1 mL (or volume inoculated previously) of the diluted stock virus at approximately 100–1000 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.

9. Organic Soil or Hard Water

9.1 To simulate an organic soil load, if required in testing, add calf or other serum to virus suspensions. The serum should be tested for absence of antiviral inhibitors, see 6.3.10. If serum cannot be used, the test should include another type of material with a protein content at least equal to that of bovine serum.

9.2 If tests are to be performed in water of a specific hardness, follow the methods listed in Test Method E 1153.

10. Calculation of Results

10.1 Use an appropriate method[4] to calculate the control virus titer, inactivated virus titer and cytotoxicity.

10.2 Report the titer of the stock virus, degree of cytotoxicity, the degree of virus inactivation, and the dilution at which neutralization occurred.

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 cell cultures; disinfectant; germicide; handsoaps; suspension test; topical antimicrobial; virucidal test; virus; viruses in suspension