~~Designation:E1052–96 (Reapproved 2002)~~

~~Standard Test Method for Efficacy of Antimicrobial Agents Against Viruses in Suspension~~Designation: E1052 – 11

Standard Test Method to Assess the Activity of Microbicides against Viruses in Suspension[1]

This standard is issued under the fixed designation E1052; 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

~~1.1This 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.~~

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

~~1.3~~

1.1 This test method is intended to demonstrate the virucidal activity of test substances with viruses in suspension.

1.2 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).

1.3 Refer to the appropriate regulatory agency for performance standards of virucidal efficacy.

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *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]

~~1.4It 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:*~~

~~E1053Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces~~

ASTM Standards:[3]

~~E1153Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces~~ 1053 Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces

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

E1838 Test Method for Determining the Virus-Eliminating Effectiveness of Hygienic Handwash and Handrub Agents Using the Fingerpads of Adults

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

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

~~Current edition approved Oct. 10, 1996. Published December 1996. Originally published as E1052–85. Last previous edition E1052–85 (1990). DOI: 10.1520/E1052-96R02.~~

Current edition approved Oct. 1, 2011. Published October 2011. Originally approved in 1985. Last previous edition published in 2002 as E1052 – 96 (2002), which was withdrawn in July 2011 and reinstated in October 2011. DOI: 10.1520/E1052-11.

[2] CDC-NIH, *Biosafety in Microbiological and Biomedical Laboratories*, ~~Third~~Fifth Edition, U.S. Department of Health and Human Services, Washington, DC, May ~~1993.~~2009.

[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.

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

2.2 ~~*Federal Standards:*~~

~~Title 40,Code of Federal Regulations (CFR), Environmental Protection Agency, Part 160, Good Laboratory Practice Standard~~ Federal Standards:[4]

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

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

3. Terminology

3.1 *Definitions*—For definitions of general terms used in this test method, refer to Terminology E2756.

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*—the process for inactivating or quenching the activity of a microbicide, often achieved through physical (for example, filtration or dilution) or chemical means.

3.2.2.1 *Discussion*—This neutralization 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 substances or test formulation*, *n*—a formulation which incorporates microbicidal ingredients.

4. Summary of Test Method

~~3.1One 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.~~

~~3.2This 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.~~

4.1 One part of the virus suspension is added to nine parts of the test substance, the mixture held at the desired temperature for the required contact time and then assayed for viable virus in an appropriate host system. For control, one part of the virus is added to nine parts of a buffer harmless to the virus and its host cells. Cell culture, cytotoxicity, and virus susceptibility controls must also be included in each test.

4.2 This test method must 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 cell lines and test virus. For a reference text, refer to Schmidt et al.[5]

~~4.Significance and Use~~

~~4.1This test method is to be used to determine the effectiveness of antimicrobial solutions against designated prototype viruses that are in suspension.~~

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

~~4.3This 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. Significance and Use

5.1 This test method is to determine if a test substance can inactivate viruses in suspension.

---

[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.~~

[5] Schmidt, N. J., Lennette, D. A., and Lennette, E. T., and Lennette, E. H.,eds., *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections*, 7th Edition, Am. Pub. Hlth. Assoc., Washington, DC, 1995 .

5.2 Regulatory agencies may require additional testing using *in vitro* (Test Methods E1053, E2197) or *in vivo* (Test Method E1838) carrier tests for product registration purposes.

6. Materials and Reagents

~~5.1~~

6.1 *Cell Culture Technique.*[5]

~~5.1.1~~

6.1.1 *Cell Culture System* appropriate for test virus.

~~5.1.2~~

6.1.2 *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.~~

~~5.1.3~~*, 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. See Note 1.*

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

6.1.3 *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~~*6.1.2, phosphate buffered saline, trypticase soy broth supplemented with serum, Earle's Balanced Salt Solution (EBSS), or other similar buffered solutions.*

6.1.4 *Plastic Cell Culture Ware.*

~~5.1.5~~*See Note 2.*

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

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

~~5.1.6~~*, with a 5 to 7 % CO_2 atmosphere, capable of maintaining 36 ± 1°C or other temperature appropriate for the specific test virus.*

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

~~5.1.7~~

6.1.7 *Test Tubes*, screw-capped.

~~5.1.8~~

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

~~5.1.9*Microtitration Kit.*~~

~~5.2Additional 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~~

~~6.1To 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.~~

~~6.2To 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.~~

~~6.3Suggested 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.~~

6.1.9 *Microtitration Kit.* See Note 3.

~~NOTE1—Rhinovirus-infected cultures require incubation at 33 ± 1°C.~~