



Standard Test Method to Assess the Activity of Microbicides against Viruses in Suspension¹

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

1.1 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

2. Referenced Documents

2.1 ASTM Standards:³

E1053 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

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

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.

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

Using the Fingerpads of Adults

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

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

2.2 Federal Standards:⁴

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

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

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

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

5. Significance and Use

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

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

6.1 Cell Culture Technique.⁵

6.1.1 *Cell Culture System* appropriate for test virus.

6.1.2 *Growth Media/Maintenance Media*, 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 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*. See Note 2.

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

6.1.5 *Incubator*, with a 5 to 7 % CO₂ 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.

6.1.7 *Test Tubes*, screw-capped.

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

6.1.9 *Microtitration Kit*. See Note 3.

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

6.2 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.

7. Test Viruses

7.1 To demonstrate the spectrum of virucidal activity of the test substance, it should be tested against viruses with varying

levels of resistance to microbicides. Appendix X1 lists suggested viruses and their host cells.

8. Virus Stock

8.1 Use an appropriate host to prepare virus suspensions. The host system for titrating virus infectivity may be different from that used for preparing the virus pool.

9. Operating Technique

9.1 The test must include the parameters given in Table 1.

9.2 Please refer to Test Method E2197 for details on cytotoxicity and other controls.

9.3 Thoroughly mix virus suspension and then add one part to nine parts of the test substance 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 chosen, immediately neutralize the microbicidal activity by serial ten-fold dilutions into a neutralization solution appropriate for the test substance.

NOTE 4—Perform the virus control (one part of virus + nine parts EBSS) and cytotoxicity control (one part EBSS + nine parts test substance) concurrently with the virucidal test described above. If dilution alone is insufficient to reduce cytotoxicity, gel filtration as described in Test Method E1482 may be used.

9.4 *Virus Recovery*—Inoculate at least four cell culture monolayers per dilution of the virus-test substance mixture with 0.1 mL volumes of each test and control dilution separately onto monolayers of appropriate host cell cultures. Other volumes may be used depending on the type of cell culture vessel employed; however, no less than four separate monolayers of the host cells must be inoculated for each dilution tested. Incubate the cultures at the appropriate temperature and observe for evidence of virus replication (e.g., cytopathic effects, hemagglutination, plaque assay) and/or cytotoxicity

9.5 *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.

9.6 To validate the neutralization, add equal volumes of the neutralized test substance, the neutralizer alone and a control fluid (for example, EBSS) 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 as well as the neutralized test substance for the neutralization to be considered valid. In case of incomplete neutralization, try another neutralizer or use the gel filtration method (Test Method E1482) to reduce cytotoxicity.

TABLE 1 Parameters

Parameter	Summary	Replicates
Cell culture	medium alone	4/group
Virus control	1 part virus + 9 parts medium	4/dilution
Virucidal test	1 part virus + 9 parts test substance	4/dilution
Cytotoxicity control	1 part medium + 9 parts test substance	4/dilution
Neutralization control	neutralized test substance + virus	4/dilution

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.

9.6.1 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 microbicidal activity.

10. Soil Load and Hard Water

10.1 If a soil load is required, add to the virus suspension bovine serum at a final concentration of 5 % or the tripartite mixture as described in Test Method E2197.

10.2 If tests are to be performed in water of a specific hardness, follow the methods listed in Test Method E2197.

11. Calculation of Results

11.1 Use an appropriate method to calculate the control and test samples to determine the level of virus inactivation achieved in relation to the dilution at which cytotoxicity was observed.

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

12. Precision and Bias

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

13. Keywords

13.1 cell cultures; microbicide; suspension test; virucidal test; virucide; viruses in suspension

APPENDIX

(Nonmandatory Information)

X1. VIRUSES

X1.1 Representative enveloped and non-enveloped to assess the virucidal activity of microbicides in suspension. The ATCC numbers of the viruses and their host cells are given in parenthesis, where available.

X1.1.1 *Adenovirus*, Type 2 (VR-846) or Type 5 (VR-5). Cell line options: Human Lung Carcinoma (A549) [CCL-185], HEp-2. [CCL-23], Vero [CCL-81].

X1.1.2 *Canine Parvovirus*, Cornell-780916–80 strain [VR-2017]. Cell line option: A–72 [CRL-1542].

X1.1.3 *Cytomegalovirus*, strain AD-169, [VR-538]. Cell line options: Human diploid lung (MRC-5 [CCL-171] or WI-38 [CCL-75]).

X1.1.4 *Feline calicivirus*, strain F-9 [VR-782]. Cell line option: CRFK [CCL-94].

X1.1.5 *Hepatitis A Virus*, HM-175 strain [VR-2093]. Cell line options: FRhK-4 [CRL-1688].

X1.1.6 *Herpes simplex virus*, Type 1, strain F (1) [VR-733]. Cell line options: VERO [CCL-81], HEp-2 [CCL-23].

X1.1.7 *Influenza A*, A/Hong Kong/8/68 [VR-544], A/PR/8/34 [VR-95]. Cell line options: Madin-Darby Canine kidney (MDCK) [CCL-34]; Rhesus monkey kidney (LLC-MK2) [CCL-7].

X1.1.8 *Murine Norovirus*, Cell line: RAW 264.7 [TIB-71].

X1.1.9 *Respiratory syncytial virus*, Long strain [VR-26]. Cell line options: HEp-2 [CCL-23], MRC-5 [CCL-171].

X1.1.10 *Rhinovirus*, Type 14 [VR-284] or 37 [VR-1147]. Cell line options: MRC-5 [CCL-171], WI-38 [CCL-75], HeLa T⁴⁺.

X1.1.11 *Rotavirus*, Wa strain [VR-2018]. Cell line options: MA-104 [CRL-2378.1] or African green monkey kidney (CV-1) [CCL-70].

X1.1.12 *Vaccinia*, WR strain, [VR-119]. Cell line options: VERO [CCL-81], HEp-2 [CCL-23].

NOTE X1.1—Rhinoviruses grow optimally at $33 \pm 1^\circ\text{C}$.

NOTE X1.2—Fetal bovine serum may be inhibitory to rotavirus and it may also neutralize the trypsin often needed for rotavirus and influenza-virus growth.

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