Designation: E1052 – 20

Standard Practice 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

1.1 This practice 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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* The user should consult a reference for the laboratory safety recommendations.[2]

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E1053 Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces

E1482 Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization

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

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.

[1] This practice 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.

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

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

4. Summary of Practice

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 practice 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 practice is to determine if a test substance can inactivate viruses in suspension.

5.2 Regulatory agencies may require additional testing using *in vitro* (Practice E1053, Test Method 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 % 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.

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. A virus endpoint titer of at least 10^4 infective units should be recovered for the test to be considered valid. Pools of certain types of viruses may require concentration by ultra-centrifugation to obtain titers high enough to give a minimum of 10^4 infective units. However, any such concentrated virus must be vortexed well to reduce the presence of viral aggregates.

NOTE 4—If the virus control has a virus endpoint titer of $>10^6$ infective units the virus culture may be standardized by dilution to target a virus endpoint titer of 10^4 to 10^6 infective units.

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. In order to reduce cytotoxicity, gel filtration columns may be used prior to performing or following serial ten-fold dilutions for individual dilutions.

NOTE 5—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. If gel filtration columns are used in the test they will also be used in the virus control and cytotoxicity control.

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 .