



Designation: E1482 – 04

# Standard Test Method for Neutralization of Virucidal Agents in Virucidal Efficacy Evaluations<sup>1</sup>

This standard is issued under the fixed designation E1482; 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 ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

## 1. Scope

1.1 This test method covers the use, in conjunction with evaluations of the virucidal efficacy, of disinfectant solutions or pressurized disinfectant spray products intended for use on inanimate nonporous environmental surfaces or for other special applications. The test method may be employed with all viruses and host systems.

1.2 This test method should be performed only by persons trained in microbiology and virology.

1.3 This test method utilizes gel filtration technology. The effectiveness of the test method is dependent on the ratio of gel bed volume to sample size and uniformity in the preparation of columns and centrifugation conditions. The effectiveness of this test method is maximized by investigator practice and experience with gel filtration techniques.

1.4 This test method will reduce, but not necessarily eliminate, disinfectant toxicity while preserving the titer of input virus.

1.5 The values stated in SI units are to be regarded as the standard.

1.6 *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.*

## 2. Referenced Documents

2.1 *ASTM Standards:*<sup>2</sup>

[E1052 Test Method to Assess the Activity of Microbicides against Viruses in Suspension](#)

[E1053 Test Method to Assess Virucidal Activity of Chemi-](#)

[cals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces](#)

## 3. Summary of Test Method

3.1 After the exposure of a virus to a disinfectant, the virus-disinfectant suspension is applied to a column of Sephadex<sup>3</sup> LH60-120, or Sephacryl S-1000 Superfine. The column is placed in a centrifuge and centrifuged to separate the virus from the disinfectant by gel filtration. The filtrate (the column flow-through that contains the virus) is assayed in the appropriate host system. The untreated virus control suspension is similarly gel filtered, and the virus titer of the filtrate is determined by assay of infectivity. The residual cytotoxicity of the disinfectant is determined by gel filtration of the disinfectant control under the same conditions. Results for the virus inactivation and disinfectant cytotoxicity of gel filtrates are recorded in the same manner as described in Test Methods [E1052](#) and [E1053](#). The gel filtration procedures described in this test method are a modification of the method of Blackwell and Chen.<sup>4</sup>

## 4. Significance and Use

4.1 This test method is to be used for the removal of virucidal agents from agent-virus mixtures, or from agent-neutralizer-virus mixtures, after the contact period and before the inoculation of these mixtures into host systems for assay of infectivity.

4.2 The purpose of the test method is to reduce the concentration of agents and neutralizers in order to permit the evaluation of viral infectivity at dilutions that would otherwise be toxic to the host.

4.3 The test method is applicable to the testing of liquid and pressurized disinfectant products.

4.4 This test method is compatible with organic soil loads, hard water, disinfectants containing organic solvents, and chemical neutralizers.

<sup>1</sup> This test method is under the jurisdiction of ASTM Committee [E35](#) on Pesticides and Alternative Control Agents and is the direct responsibility of Subcommittee [E35.15](#) on Antimicrobial Agents.

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<sup>2</sup> For referenced ASTM standards, visit the ASTM website, [www.astm.org](http://www.astm.org), or contact ASTM Customer Service at [service@astm.org](mailto:service@astm.org). For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

<sup>3</sup> Sephadex is a registered trademark of Amersham Biosciences.

<sup>4</sup> Blackwell, H. H., and Chen, J. H. S., "Effects of Various Germicidal Chemicals on H.EP2 Cell Culture and *Herpes simplex Virus*," *Journal of the AOAC*, Vol 53, 1970, pp. 1229-1236.