



Designation: E1482 – 12

Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization¹

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 reappraisal. A superscript epsilon (ϵ) indicates an editorial change since the last revision or reappraisal.

1. Scope

NOTE 1—The title was formerly Standard Test Method for Neutralization of Virucidal Agents in Virucidal Efficacy Evaluations.

1.1 This practice is intended to be used to reduce the cytotoxic level of the virus-test product mixture prior to assaying for viral infectivity. It is used in conjunction with evaluations of the virucidal efficacy of disinfectant solutions, wipes, trigger sprays, or pressurized disinfectant spray products intended for use on inanimate, nonporous environmental surfaces. This practice may also be used in the evaluation of hygienic handwashes/handrubs, or for other special applications. The practice may be employed with all viruses and host systems.

1.2 This practice should be performed only by persons trained in virology techniques.

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

1.4 This practice will aid in the reduction, but not necessarily elimination, of test product toxicity while preserving the titer of the input virus.

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

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. Referenced Documents

2.1 ASTM Standards:²

E1052 Test Method to Assess the Activity of Microbicides against Viruses in Suspension

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

3. Summary of Test Methods

3.1 After the exposure of a virus to a test product (or handwash/rub product), the virus-product suspension is added to a column of Sephadex³ LH-60, Sephadex³ LH-20, or Sephacryl³ S-1000 Superfine. The column (encased within a sterile centrifuge tube in order to capture the filtrate) is placed in a centrifuge and centrifuged to separate the virus from the test product by gel filtration. Alternatively, samples may be hand-plunged using a syringe plunger. The filtrate (the column flow-through which contains the virus) is assayed in the appropriate host system. The untreated virus control suspension is gel-column filtered, using the same methods/techniques, 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 test product control under the same conditions as those which were used in the test. Results for the virus inactivation and test product cytotoxicity of gel-column filtrates are recorded in the same manner as described in Test Methods E1052 and E1053. The gel-column filtration procedures described in this practice are a modification of the method of Blackwell and Chen.⁴

NOTE 2—A limitation of utilizing columns in virological assays is that

² 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.

³ Sephadex is a registered trademark of Amersham Biosciences. The sole source of supply of the apparatus known to the committee at this time is Amersham Biosciences. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,¹ which you may attend.

⁴ 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.