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# Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization[1,2]

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 (ε) indicates an editorial change since the last revision or reapproval.

## 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.

NOTE 1—Gel filtration columns may impact virus titer and their use should be taken into consideration when selected for use.

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 ~~entrifugation.~~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 *Units*—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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.7 *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.*

[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] The title was formerly Standard Test Method for Neutralization of Virucidal Agents in Virucidal Efficacy Evaluations.

## 2. Referenced Documents

2.1 *ASTM Standards:*[3]

E1052 Practice to Assess the Activity of Microbicides against Viruses in Suspension

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

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

## 3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this guide, refer to Terminology E2756.

## 4. Summary of Test ~~Methods~~Practice

4.1 After the exposure of a virus to a test ~~product (or handwash/rub product),~~ product, the virus-product suspension is added to a column of Sephadex[4] LH-60, Sephadex[4] LH-20, or ~~Sephacryl~~other ~~S-1000 Superfine.~~ appropriate gel filtration medium. 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.[5]

NOTE 2—A limitation of utilizing columns in virological assays is that they are unable to effectively neutralize all actives. Prior to testing, ensure the effectiveness of gel-filtration columns with the intended product chemistry. In addition, chemical neutralization is recommended to ensure/aid neutralization of certain difficult to neutralize product active(s) in addition to the use of Sephadex columns.

## 5. Significance and Use

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

5.2 The purpose of the practice is to reduce the concentration of the cytotoxic properties of the test product and neutralizers in order to permit the evaluation of viral infectivity at dilutions that would otherwise be toxic to the host cells.

5.3 The practice is applicable to the testing of liquid, pre-saturated towelettes, and pressurized disinfectant products, as well as handwash/rub products.

NOTE 3—When testing ~~handwash/rub~~ products, the ability of the solution to pass through the column must be verified prior to testing. Certain products with high viscosities are unable to pass through columns. If the product is determined to be too viscous, alternative neutralization methods should be employed.

5.4 This practice is compatible with organic soil loads, hard water, disinfectants containing organic solvents, and chemical neutralizers.

[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] 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.

[5] Blackwell, H. H., and Chen, J. H. S., "Effects of Various Germicidal Chemicals on H.EP.2 Cell Culture and *Herpes simplex* Virus," *Journal of the AOAC*, Vol 53, 1970, pp. 1229–1236.

## 6. Reagents and Materials

6.1 *Reagents:*

6.1.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.[6] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

6.1.2 *Phosphate Buffered Saline* (PBS).[7]

6.1.3 *Sterile Distilled or Deionized Water.*

6.1.4 *1% Albumin Solution (in PBS).*

6.2 *Sephadex Gel Filtration Column Assembly:*

6.2.1 *Sephadex LH-60 or LH-20,* compatible with organic solvents. ~~(Sephacryl S-1000 Superfine may be substituted.)~~

6.2.2 *Syringe,* ~~5-cc or 10-cc,~~5 cc or 10 cc, disposable.

6.2.3 *Glass wool,* sterilized.

6.2.4 *Centrifuge tube,* ~~15- and/or 50-mL,~~15 and/or 50 mL, conical, sterile, and disposable.

6.3 *Labware:*

6.3.1 *Pipettes,* serological, ~~10-, 5-, and 2-mL.~~10, 5, and 2 mL.

6.3.2 *Erlenmeyer Flask,* sterile, ~~250-mL~~250 mL or other suitable sterilizable container.

6.3.3 *Test Tube Rack or Holder,* for ~~15- and 50-mL~~15 and 50 mL tubes.

6.3.4 *Test Tubes,* 18 by 150 mm.

6.3.5 *Laboratory Film*, or other sealing film. (Aluminum foil may also be used to cover the syringe/glass-wool/tube assembly and then autoclaved).

6.4 *Equipment:*

6.4.1 *Centrifuge,* clinical, with rotor and shields capable of holding ~~15- and/or 50-mL~~15 and/or 50 mL centrifuge tubes, and running at a r/min that generates 550 to 650 × g.

6.4.2 *Refrigerator,* 2 to ~~8°C~~8 °C

6.4.3 *Autoclave.*

## 7. Procedure

7.1 Suspend the Sephadex in a large excess of sterile distilled or deionized water in an Erlenmeyer flask or other suitable sterilizable container. Use an amount of Sephadex sufficient for the number of columns to be prepared (approximately 0.5 g of Sephadex per column) or prepare a larger volume slurry to give a final suggested concentration of 5 to 22 % Sephadex g/v. The

[6] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For Suggestions on the testing of reagents not listed by the American Chemical Society, see *Annual Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

[7] Dulbecco, R., and Vogt, M., "Plaque Formation and Isolation of Pure Lines with Poliomyelitis Virus," *Journal of Experimental Medicine*, Vol 99, 1954, p. 167.