



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 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.

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. 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.

Current edition approved Oct. 1, 2012. Published November 2012. Originally approved in 1992. Last previous edition approved in 2004 as E1482 – 04. DOI: 10.1520/E1482-12.

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 they are unable to effectively neutralize all actives. Prior to testing, ensure

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 HEP-2 Cell Culture and Herpes simplex Virus," *Journal of the AOAC*, Vol 53, 1970, pp. 1229–1236.

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.

4. Significance and Use

4.1 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.

4.2 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.

4.3 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.

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

5. Reagents and Materials

5.1 Reagents:

5.1.1 *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.⁵ 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.

5.1.2 *Phosphate Buffered Saline (PBS)*.⁶

5.1.3 *Sterile Distilled or Deionized Water*.

5.1.4 *1% Albumin Solution (in PBS)*.

5.2 *Sephadex Gel Filtration Column Assembly*:

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

5.2.2 *Syringe*, 5-cc or 10-cc, disposable.

5.2.3 *Glass wool*, sterilized.

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

5.3 *Labware*:

5.3.1 *Pipettes*, serological, 10-, 5-, and 2-mL.

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

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

5.3.4 *Test Tubes*, 18 by 150 mm.

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

5.4 *Equipment*:

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

5.4.2 *Refrigerator*, 2 to 8°C

5.4.3 *Autoclave*.

6. Procedure

6.1 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. Sterilize slurry by autoclaving. (Example parameters for autoclaving are 121°C at 15 psi (pounds of pressure per square inch) for at least 15 min. Autoclave parameters vary depending on autoclave model, altitude, and so forth.) Allow slurry to cool to room temperature. Store at 2 to 8°C for longer term storage if desired.

6.1.1 Alternatively, Sephadex may be prepared by first preparing a 1% albumin, antibiotics (optional), and PBS solution. This solution is filter sterilized. Sephadex is then added to this filter sterilized solution at the desired concentration of 5 to 22%.

6.2 Select the syringe size to be used depending on the size of the column desired. A 5-cc (mL) syringe is used for 3 to 5-cc (mL) columns (≤ 1.0 mL of sample to be added); a 10-cc (mL) syringe is used for 6 to 8-cc (mL) columns (1.0 to 5.0 mL of sample to be added).

NOTE 4—If sample added is at the higher volume range, the bed size may need to be adjusted so that it is at the maximum allowable height in order to ensure removal of cytotoxic properties.

6.3 Remove the cap from the syringe tip, remove the plunger from the syringe, and place the syringe in an 18 by 150-mm test tube or in another suitable tube holder which can capture the column flow-through during column preparation procedures.

6.4 Place a small wad of glass wool in the syringe to cover the internal tip opening. The wad should have a diameter approximately the same size as the internal syringe diameter, and it should be sufficiently thick to hold the swollen Sephadex beads while allowing water to pass readily. Cover assembly with aluminum foil and autoclave to sterilize.

NOTE 5—Sterilized column assembly without Sephadex slurry can be prepared and stored prior to testing. Additionally, sterile, individually-wrapped syringes and sterile glass wool may be utilized and handled under aseptic techniques to eliminate the need for autoclaving column assemblies.

⁵ *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.

⁶ Dulbecco, R., and Vogt, M., "Plaque Formation and Isolation of Pure Lines with Poliomyelitis Virus," *Journal of Experimental Medicine*, Vol 99, 1954, p. 167.

6.5 Just prior to testing, swirl the Sephadex slurry and pipet Sephadex into the syringe barrel. Allow the excess water to drain, and repeat until the desired bed size of Sephadex has formed. If the column is not used immediately, cover with laboratory film or aluminum foil and store at 2–8°C for a short period of time to prevent the column from drying out.

6.6 To use the column, allow the water to flow through, and then equilibrate (optional) with PBS or a 1 % albumin solution by passing 10 to 20 mL through the column.

6.7 Place the column in a sterile 15- or 50-mL conical centrifuge tube. Cover the column with a tube cap, laboratory film or other suitable cover. Place the tube with the prepared column in the centrifuge and centrifuge at approximately 550 to 650 × g for 3 to 4 min to clear the void volume.

6.8 Remove the column, discard the void volume, and/or place the column in a new tube.

6.9 Gently pipet the appropriate volume of the virus-test product mixture (depending on the column size) onto the Sephadex, place the column in the centrifuge, and centrifuge again for 3 to 4 min at exactly the same r/min as in the previous step. Alternatively, samples may be hand plunged utilizing a syringe plunger to push the liquid through the column to collect the filtrate. Caution should be taken to avoid over plunging of samples, which will push the Sephadex out into the filtrate.

6.10 Remove the column from the centrifuge; if necessary, collect the filtrate (column flow-through), and titrate for infectivity.

6.11 The virus and test product control samples are handled in the same manner as previously described.

6.12 *Optional Control*—To determine the reduction of cytotoxicity of a specific test substance when utilizing gel-

filtration columns, a control whereas the chemically neutralized test product is run through a column is compared with the chemically neutralized test product which is not passed through a column. Compare the toxicity induced cytopathic effects on the host cells to calculate the reduction in toxicity with the use of Sephadex columns.

NOTE 6— It is up to the end user to decide whether the use of gel-filtration columns is appropriate in order to obtain the assay's necessary log reduction.

7. Spray Products

7.1 Prior to applying the virus-product mixture to a Sephadex column (when not using chemical neutralizers), the volume of the mixture may be adjusted (that is, up to 2 mL) with an appropriate aqueous medium such as water, PBS, tissue culture medium, or neutralizer solution in order to obtain enough sample to allow for titration. For example, utilize this practice when alcohol-based spray products which evaporate during the contact period are being tested.

8. Chemical Neutralizers

NOTE 7—Chemical neutralization is recommended for difficult to neutralize actives.

8.1 When utilized, the chemical neutralizer is added at the end of the contact time, and the virus-test product-neutralizer mixture is then immediately added to the Sephadex column.

9. Precision and Bias

9.1 A precision and bias statement cannot be made for this practice at this time.

10. Keywords

10.1 cytotoxicity; disinfectant; gel filtration; neutralization; tissue culture; virucidal; virucidal neutralization method

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