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Designation: E1482 – 04 E1482 – 12

Standard Test Method Practice for Neutralization of Virucidal Agents in Virucidal Efficacy EvaluationsUse of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization[1]

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

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.*

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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

ASTM E1482-12
https://standards.iteh.ai/catalog/standards/sist/07945a18-b477-4b4c-a996-a30ae48a81bb/astm-e1482-12

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[3] 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.[4]

[1] This test method practice is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agentsand is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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

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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.

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) (see Dulbecco and Vogt[6]).
5.1.3 *Sterile Distilled or Deionized Water.*

5.2 *Sephadex Gel Filtration:*
5.2.1 *Sephadex LH-60-120,* 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 20 mL, conical, sterile, and disposable.
5.2.5 *Centrifuge,* clinical, with rotor and shields capable of holding 15 and 50-mL centrifuge tubes, and running at a r/min that generates 600 × g.
5.2.6 *Refrigerator,* 4°C.

5.3 *Labware:*
5.3.1 *Pipettes,* serological, 10, 5, and 1 mL, in 0.1-mL graduations.
5.3.2 *Erlenmeyer Flask,* sterile, 250 mL.
5.3.3 *Test Tube Rack or Holder,* for 15 and 50-mL tubes.
5.3.4 *Test Tube,* 18 by 150 mm.
5.3.5 *Laboratory Film*,[7] or other sealing film.

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6. Procedure

6.1 Suspend the Sephadex in a large excess of sterile distilled or deionized water in an Erlenmeyer flask. Use an amount of Sephadex sufficient for the number of columns to be prepared (approximately 0.5 g of Sephadex per column). Close the flask with a plastic film or closure and allow the Sephadex to swell overnight at 4°C. Sterilize by autoclaving at 121°C and 15 lb of pressure for 15 min. Allow to cool to room temperature.

6.2 Select the syringe size to be used depending on the size of the column desired. A 5-cc syringe is used for 3-cc columns (0.6 mL of sample to be added); a 10-cc syringe is used for 6-cc columns (1.2 mL of sample to be added). 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 a suitable tube holder above a sink or liquid receptacle.

6.3 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.

6.4 Swirl the Sephadex slurry and pipet Sephadex into the syringe. Allow the excess water to drain, and repeat until the desired bed size of Sephadex has formed. If the column is not used immediately, seal or plug the syringe tip, add a layer of distilled water above the column, cover with parafilm, and store at 4°C.

6.5 To use the column, allow the water to flow through, and then equilibrate with PBS by passing 10 mL of PBS through the column.

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

6.6 Cover the column with laboratory film or another film, and place the column in a sterile 15 or 50-mL conical centrifuge tube. Place the tube with the column in the centrifuge and centrifuge at approximately 600 × g for 3 min to clear the void volume. Record the r/min used for this step.

6.7 Remove the column, discard the void volume, and place the column in a new tube.

6.8 Gently pipet 0.6 or 1.2 mL of the virus-disinfectant mixture (depending on the column size) onto the Sephadex, place the column in the centrifuge, and centrifuge again for 3 min at exactly the same r/min as in the previous step.

6.9 Remove the column from the centrifuge, collect the filtrate (column flow-through), and titrate for infectivity.

6.10 The virus and disinfectant control samples are handled in the same manner.

7. Spray Products

7.1 Prior to applying the virus-product mixture to a Sephadex column, the volume of the mixture is adjusted to 2.0 mL with an appropriate aqueous medium such as water, PBS, tissue culture medium, or neutralizer solution.

8. Chemical Neutralizers

8.1 When utilized, the chemical neutralizer is added after the contact time, and the virus-disinfectant-neutralizer mixture is applied to the Sephadex column.

9. Precision and Bias

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

10. Keywords

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

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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.*