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Standard Test Method for Neutralization of Virucidal Agents in Virucidal Efficacy Evaluations[1]

This standard is issued under the fixed designation E 1482; 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 laboratory test method is intended for 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 problems, 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:*

E 1052 Test Method for Efficacy of Virucidal Agents Intended for Special Applications[2]

E 1053 Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces[2]

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 LH60-120. 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 E 1052 and E 1053. The gel filtration procedures described in this test method are a modification of the method of Blackwell and Chen.[3]

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.[4] Other grades may be

[1] This test method is under the jurisdiction of ASTM Committee E-35 on Pesticides and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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[2] *Annual Book of ASTM Standards*, Vol 11.05.

[3] 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.

[4] "Reagent Chemicals, American Chemical Society Specifications," Am. Chemical Soc., Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see "Analar Standards for Laboratory U.K. Chemicals," BDH Ltd., Poole, Dorset, and the "United States Pharmacopeia."

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