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Standard Test Method for Evaluation of Hygienic Handwash and Handrub Formulations for Virus-Eliminating Activity Using the Entire Hand¹

This standard is issued under the fixed designation E2011; 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.

INTRODUCTION

Mechanical removal and/or in situ inactivation of viruses by hygienic handwash and handrub agents can be assessed using artificially-contaminated hands of adults. This test method uses the entire surface of both hands in contrast to only the fingerpads in the procedure described in Test Method E1838. However, the reported results from these two methods are comparable. (1, 2)²

1. Scope

1.1 This test method is designed to evaluate handwash or handrub agents for their ability to reduce viral contamination from hands.

NOTE 1—A knowledge of virological techniques is required for this test method.

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

1.3 *This standard may involve hazardous materials, operations and equipment. 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. The user should consult a reference for laboratory safety recommendations. (3-5)*

2. Referenced Documents

2.1 *ASTM Standards:*³

E1482 Test Method for Neutralization of Virucidal Agents in Virucidal Efficacy Evaluations

¹ This test method 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 boldface numbers in parentheses refer to a list of references at the end of this test method.

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.

E1838 Test Method for Determining the Virus-Eliminating Effectiveness of Hygienic Handwash and Handrub Agents Using the Fingerpads of Adults

2.2 *AOAC Standard:*

AOAC 960.9 Official Methods of Analysis (1990)⁴

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *hygienic (health-care personnel) handwash agents, n*—agents generally used for handwashing by personnel in hospitals, other health-care facilities, day-care centers, nursing homes, and food-handling establishments; should be safe for repeated use, non-irritating, fast-acting, and efficient in eliminating transient microorganisms from intact skin.

3.1.2 *hygienic handrub agents (that is, hand sanitizers), n*—agents generally used for hand hygiene by personnel in hospitals, other health-care facilities, day-care centers, nursing homes, and food-handling establishments that do not require rinsing; should be safe for repeated use, non-irritating, fast-acting, and efficient in eliminating transient microorganisms from intact skin.

3.1.3 *non-medicated soap, n*—a soap or detergent that is mild to the skin and does not contain any germicidal chemicals.

3.1.4 *soil (organic) load, n*—a solution of one or more organic and/or inorganic substances added to the suspension of the test organism to simulate the presence of body secretions, excretions or other extraneous substances.

3.1.5 *virus-eliminating (killing/removing) agent, n*—any agent that rids hands of viruses by either killing them on the skin or by dislodging them for subsequent wash-off.

⁴ Available from AOAC International, 481 North Frederick Ave., Suite 500, Gaithersburg, Maryland 20877-2417, http://www.aoc.org.

3.1.6 *virus inactivating agent, n*—any agent that renders a virus noninfectious.

4. Summary of Test Method

4.1 This test method uses adult subjects who have provided a written informed consent and whose hands have been determined to be free from any apparent damage at the time of their participation in the study. While no fewer than six subjects are recommended for each virus-test substance combination to be evaluated, the number required may vary depending on the intended use of the data and the target regulatory agency.

4.2 All subjects should refrain from using any antimicrobials starting at least one week prior to the experimental contamination of their hands.

4.3 A prepared suspension of the selected test virus is grown and diluted or concentrated to produce a titer with a minimum of 10^8 infective units/mL. The contaminating virus is applied to the hands and the hands are treated with the test formulation according to the manufacturer's directions or with a set test regimen.

4.4 The virus titer recovered after treatment with the test substance is compared to a control. For the control, the test virus is applied to the hands and recovered after the subject has treated the hands with standard hard water (200 ppm as calcium carbonate) or vehicle, or both, instead of the test substance.

4.5 The virus on experimentally-contaminated hands is exposed to the test substance for the length of time that is representative of actual use conditions of the product, for example, from 10 to 20 s for a handsoap and 20 to 30 s for a handrub. The virus to be recovered after exposure to the test substance is assayed in a cell culture system appropriate to the test virus. The virus titer of the stock, test samples, and controls is determined by a suitable infectivity assay. Cytotoxicity of the host cell culture system caused by the test substance or vehicle at the tested concentration is also determined. The virus-test substance mixture is assayed in numerous units of the host system at a dilution just beyond the cytotoxicity range of the formulation tested. At least three replicate determinations are performed on controls (untreated) and test samples (treated) to confirm the extent of virus elimination by the number of lots required by the target regulatory agency. Results are recorded and $\log_{10}$ and/or percent reduction in virus infectivity are calculated.

4.5.1 This test method is designed to be performed by a trained microbiologist or virologist who is responsible for choosing the appropriate host system for the test virus, and applying the techniques necessary for propagation and maintenance for host system and test virus. For a reference text, see Ref (6).

5. Significance and Use

5.1 This test method is designed to evaluate the virus-eliminating activity of hygienic handwash and handrub agents from experimentally-contaminated hands. Such formulations may be further assessed in a clinical trial for their effectiveness

in the field. This test method incorporates whole-hand exposure and reflects actual use conditions such as friction during hand decontamination, and also enables alternate product forms such as alcohol- or non-alcohol-based liquids, gels, and foams to be tested according to label directions. It is meant to extend, if required, the results of testing with Test Method E1838, which gives precise reductions in virus infectivity on a limited area of the hands. It may also serve as an alternative test method when product form is not amenable to testing by Test Method E1838.

5.2 This test method is not meant for use with surgical hand scrubs or preoperative skin preparations.

NOTE 2—Application of viruses on the entire surface of both hands entails a greater risk to the subjects than using fingerpads only. Therefore, greater care is needed to ensure that the hands of the participants are free from any apparent damage. Also, virus preparations must be thoroughly screened for, or documented to be free from, extraneous or adventitious pathogens before use in such tests.

6. Equipment and Apparatus

6.1 *Laminar Flow Cabinet*—a Class II biological safety cabinet. The procedures for the proper maintenance and use of such cabinets are given in Ref (3, 4).

6.2 *Incubator*—an incubator at $36 \pm 1^\circ\text{C}$ for growing host cells and for incubating virus-infected cultures. If an open system is used for cell culture, a CO_2 incubator will be required.

6.3 *Positive Displacement Pipette*—a pipette and pipette tips that can accurately dispense 10 to 20 μL volumes.

6.4 *Sterilizer*—any steam sterilizer suitable for processing cell culture media and reagents. The steam supplied to the sterilizer must be free from additives toxic to cell cultures.

6.5 *Filter Sterilization System*—a membrane or cartridge filtration system (0.22 μm pore diameter) is required for sterilization of heat-sensitive media and solutions.

6.6 *Freezers*—a freezer at $-20 \pm 2^\circ\text{C}$ for the storage of fetal bovine serum and other additives for cell culture media. A second freezer at -70°C or lower is required to store viruses.

6.7 *Refrigerator*—a refrigerator at $4 \pm 2^\circ\text{C}$ is necessary for storage of prepared cell culture media and reagents.

6.8 *Timer*—any stopwatch that can be read in minutes and seconds.

6.9 *Magnetic Stirrer and Magnets*—magnetic stirrer and magnets must be large enough to hold a 5-L beaker or Erlenmeyer flask for preparing cell culture media or other solutions.

6.10 *Handwashing Sink*—a sink of sufficient size to permit subjects to wash hands without touching hands to sink surface.

6.10.1 Water faucet(s) are to be located above the sink at a height that permits the hands to be held higher than the elbow during the washing procedures. Faucets with electronic sensors or those that are wrist-, elbow-, knee-, or foot-operated are preferred to avoid recontamination of the washed hands.

6.10.2 Mild, proven non-antimicrobial soap, preferably liquid.