



Standard Test Method for Evaluation of Handwashing Formulations for Virus- Eliminating Activity Using the Entire Hand¹

This standard is issued under the fixed designation E 2011; 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

Physical removal, inactivation *in situ*, elimination and a combination of these actions to reduce the spread of viral infections with effective handwashing has become an achievable goal. Artificial contamination of hands with selected test viruses provides a usable model. This guide is closely related to another ASTM test of handwash agents which restricts contamination and sampling to a smaller area of the hand, Test Method E 1836, Determining the Virus Eliminating Effectiveness of Liquid Hygienic Handwash Agents Using the Fingerpads of Adult Volunteers. This test method tests a larger area of the hands than the fingerpad method; however, reported results are comparable. (1)²

1. Scope

1.1 This test method is designed to evaluate antimicrobial agents in formulations for utility and effectiveness for virus-eliminating activity using human subjects.³

1.2 *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. (2, 3)*

2. Referenced Documents

2.1 ASTM Standards:⁴

2.1.1 Use the most current editions of the standards referenced herein.

E 1052 Test Method for Efficacy of Virucidal Agents Against Viruses in Suspension

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

E 1482 Test Method for Neutralization of Virucidal Agents

in Virucidal Efficacy Evaluations

E 1836 Test Method for Determining the Virus-Eliminating Effectiveness of Liquid Hygienic Handwash Agents Using the Fingerpads of Adult Volunteers

2.2 AOAC Standard:⁵

AOAC 960.9

3. Summary of Test Method

3.1 This test method is conducted on subjects selected from a group of adult volunteers who have provided a written informed consent and whose hands have been determined to be free from any apparent damage. All subjects should have refrained from the use of any antimicrobials for at least one week prior to initiation of the test and be supplied with selected products free from antimicrobials for use during this week. At least 12 to 15 subjects are selected from this group for the test. The number of required subjects may vary with the virus tested.

3.2 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 washed with the test formulation according to the manufacturer's directions or with a set test regimen.

3.3 The virus titer recovered after washing with the test product is compared to a control titer of virus. For the control, a titer of virus is applied to the hands and recovered from subjects washing with standard hard water (200 ppm as calcium carbonate) or vehicle, or both, instead of the formulation.

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

Current edition approved March 10, 1999. Published September 1999.

² The boldface numbers in parentheses refer to a list of references at the end of this test method.

³ A knowledge of virological techniques is required for this test method.

⁴ 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.

⁵ Available from Association of Organic and Analytical Chemists International, Gaithersburg, MD.

3.4 The virus is exposed to the virucide 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. The virus to be recovered after exposure to the test germicide 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 the a suitable infectivity assay. Cytotoxicity of the host cell culture system caused by the test germicide at the tested concentration is also determined. The virus-germicide mixture is assayed in numerous units of the host system at a dilution just beyond the cytotoxicity range of the germicide. At least three replicate determinations are performed on controls (untreated) and test samples (treated) to confirm virus elimination by a sample of the test germicide. Results are recorded as the median value of $\log_{10}$ - reduction in virus infectivity.

3.4.1 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 and test virus. For a reference text, see Ref. (4).

4. Significance and Use

4.1 This test method should be used to evaluate the virus-eliminating effectiveness of these formulations after handwashing. Effective formulations can be further evaluated in a clinical trial on human subjects. Published data have shown (1) that results of in vitro tests do not accurately reflect what occurs when this class of products is used in the health care facility. This test method involves the incorporation of whole hand exposure and friction from washing, reflecting actual use conditions in human subjects. It is meant to confirm the results of testing with Test Method E 1836. This method gives precise reductions on a limited area of the finger, the fingerpads.

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

5. Equipment and Apparatus

5.1 *Laminar Flow Cabinet*—A Class II biological safety cabinet is required for virus work. The procedures for the proper maintenance and use of such cabinets are given in Ref. (5).

5.2 *Incubator*—An incubator at $36 \pm 1^\circ\text{C}$ is needed 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. Work with rhinoviruses will require an incubator at $33 \pm 1^\circ\text{C}$.

5.3 *Positive Displacement Pipette*—A pipette and pipette tips that can accurately dispense 10 to 20 μL volumes is required.

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

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

5.6 *Freezers*—A freezer at $-20 \pm 2^\circ\text{C}$ is required 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.

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

5.8 *Timer*—Any stop watch that can be read in minutes and seconds is acceptable.

5.9 *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.

5.10 *Handwashing Sink*—A sink of sufficient size to permit subjects to wash hands without touching hands to sink surface is required.

5.10.1 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.

5.10.2 Bland, proven non-antimicrobial soap, preferably liquid, is needed.

5.10.3 Tap water temperature regulator and temperature monitor to monitor and regulate water temperature at $40 \pm 2^\circ\text{C}$ is needed.

5.11 *Liquid Nitrogen Storage for Cells*—An appropriate liquid nitrogen container and liquid nitrogen for cryopreservation of the stocks of cell lines are needed.

5.12 *Inverted Microscope*—An inverted microscope with 10X eye pieces and 5X, 10X and 40X objectives.

5.13 *Serological Pipettes*—Sterile reusable or single-use pipettes of 10.0, 5.0 and 1.0 mL capacity are needed.

5.14 *Cell Culture Flasks*⁶—Plastic cell culture flasks of 25 cm^2 or 75 cm^2 capacity for culturing cells and for preparing virus pools are needed.

NOTE 1—Each flask for growing cell monolayers can be reused by reseeding with new cell cultures 10 or more times before being discarded.

5.15 *Plastic and Glass Vials, Medication (Medicant)*—Sterile screw-capped vials will be required or post test sampling of hands.

5.16 *Miscellaneous Labware*—Required are: automatic pipettes, pipette tips, plastic vials for storing cell and virus stocks, dilution tubes, cluster plates or flasks for virus titration.

5.17 *Sterile Glass beads*—Beads that are 3.5 mm in diameter are needed.

6. Materials and Reagents

6.1 *Cell Culture Media and Supplements*—Culture media and the types and ratios of supplements will vary depending on the cell line. Eagle's minimal essential medium (EMEN) with 5 to 10 % fetal bovine serum (virus- and mycoplasma-tested) is used for growing a wide variety of cells (see Note 2). Antibiotics may be required in the medium to suppress bacterial growth.

⁶ Plastic cell culture ware and other related supplies may be purchased from most laboratory supply houses.