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# Standard Test Method for Determining the Virus-Eliminating Effectiveness of Hygienic Handwash and Handrub Agents Using the Fingerpads of Adults<sup>1</sup>

This standard is issued under the fixed designation E1838; 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 ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

## INTRODUCTION

Hands play an important role in the spread of many viruses. Thus, proper and regular hand hygiene is crucial in preventing such spread, particularly in health-care settings, day-care centers, and food-handling establishments. Many viruses that are known to spread through contaminated hands can remain infectious for several hours on human hands, and also may be more resistant than the bacteria commonly used to evaluate the microbicidal activity of handwash and handrub agents (1, 2, 3, 4).<sup>2</sup> Contaminated hands also can readily transfer infectious virus to other surfaces (1, 2, 3). Hand antisepsis has been shown to interrupt the spread of viral infections (5, 6, 7, 8, 9). This test method is to assess the virus-eliminating potential of handwash and handrub agents *in vivo*.

## 1. Scope

1.1 Human skin is not known to carry viruses as a part of its resident microbiota, with the notable exception of papilloma viruses (10). Hands transiently contaminated with viruses can, however, act as vehicles for the spread of many types of viral infections. Hand hygiene is meant to reduce the load of viruses and other transient microorganisms on hands, thereby reducing the risk of disease transmission. Such reductions in the virus load may be due to a combination of virus inactivation and mechanical removal of infectious virus from the skin.

1.2 This test method is designed to determine the comparative virus-eliminating effectiveness of microbicidal or non-microbicidal formulations. This test method is not meant for use with surgical hand scrubs or preoperative skin preps.

NOTE 1—The test method should be performed by persons with training in virology in facilities designed and equipped for work with infectious agents at biosafety level 2 (II).

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

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the*

<sup>1</sup> 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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<sup>2</sup> The boldface numbers in parentheses refer to the list of references at the end of this standard.

*responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

1.5 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

## 2. Referenced Documents

### 2.1 ASTM Standards:<sup>3</sup>

E2011 Test Method for Evaluation of Hygienic Handwash and Handrub Formulations for Virus-Eliminating Activity Using the Entire Hand

E2276 Test Method for Determining the Bacteria-Eliminating Effectiveness of Hygienic Handwash and Handrub Agents Using the Fingerpads of Adults

E2613 Test Method for Determining Fungus-Eliminating Effectiveness of Hygienic Handwash and Handrub Agents Using Fingerpads of Adults

## 3. Terminology

### 3.1 Definitions of Terms Specific to This Standard:

3.1.1 *Health-care personnel (HCP), n*—persons who are directly related to provision of health care services. It includes

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all paid and unpaid persons working in health-care settings, such as physicians, nurses, nursing assistants, therapists, technicians, emergency medical service personnel, dental personnel, pharmacists, laboratory personnel, autopsy personnel, students, trainees, and contractual staff, etc., who have the potential to get themselves exposed to patients and infectious materials.

3.1.2 *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, nonirritating, fast-acting, and efficient in eliminating transient microorganisms from intact skin.

3.1.3 *nonmedicated soap, n*—a soap or detergent that is mild to the skin and does not contain any microbicidal 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.

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

#### 4. Summary of Test Method

4.1 This test method is conducted on a group of adult subjects who have provided informed consent and the skin of whose hands has been determined to be free from any apparent damage. The subjects are to refrain from using any products containing antimicrobial agents for at least one week prior to the test. A known volume of the test virus suspension is placed on a demarcated area on each fingerpad and the inoculum allowed to dry. The contaminated area then is exposed to test or control agent or a vehicle (for example, standard hard water), and rubbed with a randomly chosen fingerpad from the opposite hand for the desired contact time. Virus remaining on the fingerpads is then eluted and the eluates titrated for infectious virus along with the required controls. The infectious units from the two thumbpads or the pair of the fingerpads that were involved in a single treatment will be averaged. Percent or  $\log_{10}$  reductions, or both, in the levels of infectious virus after treatment with the test or control agents are then determined. If two different formulations are being compared in the same test, one of them may be designated as a reference. If desired, one also may use tap water in parallel with the hard water control to determine the influence of water hardness on the test product's virus-eliminating activity.

#### 5. Significance and Use

5.1 This *in vivo* procedure is designed to test the ability of hygienic handwash and handrub agents to reduce levels of selected infectious viruses from experimentally contaminated fingerpads of adults. Since the two thumbpads and all eight fingerpads can be contaminated with virus and used in a given test, it allows for the incorporation of a wet inoculum input control, dried virus recovery control, and up to three replicates

to assess the virus-eliminating efficiency of a test or control agent, or a vehicle material. No more than 100  $\mu\text{L}$  of the virus suspension are required to complete one test.

5.2 This test method is designed to be performed by a trained individual, who is responsible for choosing the appropriate host system for the test virus and applying the techniques necessary for propagation and maintenance of host and test virus. For a reference text, refer to Lennette et al (12).

5.3 Whereas the method described here relates to testing with viruses of human origin, it can be readily adapted to work with animal pathogenic viruses as well as bacteriophages. Standard methods for working with bacteria (Test Method E2276) and fungi (Test Method E2613) are also available.

5.4 Infectious microorganisms left on hands after washing can be reduced further by drying the washed hands with paper, cloth, or warm air (13). A step for the drying of fingerpads after exposure to the control or test product, therefore, has not been included to avoid virus removal by the drying process itself.

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

5.6 The level of viable virus in the dried inocula the control fingerpads should not be less than  $10^4$  infectious units which would permit the detection of up to a 4  $\log_{10}$  reduction in the infectivity titer of the virus by the test product under the conditions of this test method.

#### 6. Equipment and Apparatus

6.1 *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 (11).

6.2 *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  $\text{CO}_2$  incubator will be required.

6.3 *Positive Displacement Pipette*—A pipette and pipette tips that accurately can dispense 10- $\mu\text{L}$  volumes.

6.4 *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.

6.5 *Filter Sterilization System*—A membrane or cartridge filtration system (0.22- $\mu\text{m}$  pore diameter) is required for sterilizing heat-sensitive media and solutions.

6.6 *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  $-70^\circ\text{C}$  or lower is required to store viruses

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

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

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

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

6.9.2 *Tap Water Temperature Regulator and Temperature Monitor*, to monitor and regulate water temperature at  $40 \pm 2^\circ\text{C}$ .

6.10 *Liquid Nitrogen Storage for Cells*—A proper liquid nitrogen container and liquid nitrogen for cryopreservation of the stocks of cell lines.

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

## 7. Materials and Reagents

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

7.2 *Cell Culture Flasks*—Plastic flasks of 25 or 75-cm<sup>2</sup> capacity for culturing cells and for preparing virus pools.

NOTE 2—Each flask for growing cell monolayers can be reused ten or more times before being discarded.

NOTE 3—Plastic cell culture ware may be purchased from most laboratory supply houses.

7.3 *Cell Culture Plates, 6-well*—2.0 mL per well eluent (see 7.8) shall be added. This is used for virus elution from each thumbpad and fingerpad.

NOTE 4—Alternatively, small dishes (for example, 35mm diameter) may be used for virus elution.

7.4 *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 (EMEM) with 5 to 10 % fetal bovine serum (virus- and mycoplasma-tested) is used for growing a wide variety of cells (see Note 5).

NOTE 5—Materials and reagents for cell culture may be purchased from biological supply houses.

### 7.5 Soil Load:

7.5.1 *Bovine Serum*, at a final concentration of 5 % in the virus inoculum (see Note 6).

7.5.2 A Yeast extract/BSA/Mucin tripartite soil load, as an alternative to serum. Add 0.5 g of yeast extract to 10 mL of phosphate buffer. Add 0.5 g of bovine serum albumin (BSA) to 10 mL of phosphate buffer. Add 0.04 g of bovine mucin to 10 mL of phosphate buffer. Prepare the stock solutions separately and sterilize by passage through a 0.22 μm pore diameter membrane filter, aliquot and store at either  $4 \pm 2^\circ\text{C}$  or  $-20 \pm 2^\circ\text{C}$ . To obtain a 500-μL inoculum of the test inoculum, add to 340 μL of the microbial suspension 25 μL BSA, 100 μL mucin and 35 μL of yeast extract stock solutions. This mixture contains approximately 2 g of total protein/L, which is approximately equivalent to the protein content of a 5 % solution of fetal bovine serum.

NOTE 6—Bovine serum is unsuitable for use as an organic load when working with rotaviruses because of its rotavirus inhibitory and trypsin-neutralizing activity.

7.6 *Standard Hard Water*—Standard hard water prepared in accordance with AOAC 960.09 E and F (14) at a hardness of

200 ppm as calcium carbonate is used for dilution of test substance, as the control solution to determine the baseline level of virus elimination, and to rinse the fingerpads after exposure to the test product. The standard hard water and tap water (if used) must first be tested to ensure that they do not have any virucidal activity against the test virus(es).

NOTE 7—The quality and disinfectant (for example, chlorine) residual in tap water can vary from site to site and at different times at the same site. The use of standard hard water, therefore, is recommended here to avoid variations in results due to differences in tap water quality.

7.7 *Test product*—Two separate manufacturer's lots of the test product may be tested. For handwash products that are used with water, prepare a 25 % solution by adding 1 part product to 3 parts standard hard water. This dilution is necessary because water is used when the product is applied.

7.8 *Eluent for Virus Recovery from Fingerpads*—Minimum Essential Medium (MEM) + 2 % Fetal Bovine Serum (FBS), or Earle's balanced salt solution (EBSS) with a pH of 7.2 – 7.4, or equivalent.

7.9 *Diluent for Virus Titration*—Same as the Eluent for Virus Recovery from Fingerpads.

7.10 *Plastic Vials*—Sterile screw-capped 2.0-mL vials with an inside diameter of about 8 mm are required for demarcation of the fingerpads and to hold various test solutions.

7.11 *Miscellaneous Laboratory Ware*—Automatic pipettes, pipette tips, plastic vials for storing cell and virus stocks, dilution tubes, cluster plates, or flasks for virus titration.

## 8. Test Viruses and Cell Cultures

8.1 See Appendix X1 for recommended viruses and their host cells.

## 9. Subjects

9.1 Recruit a sufficient number of healthy human subjects who have no clinical evidence of dermatoses, open wounds, or other skin disorders (see 4.1). The number of subjects required for a trial is dependent on the number of treatments within a study.

9.2 It is the responsibility of the user of this test method to arrange the necessary clearance for the use of adult subjects for testing and to obtain informed and written consent from those selected for the study before starting the tests.

## 10. Procedure

10.1 The subject will wash his/her hands with a nonmedicated soap for at least 10 s, rinse, and then dry them thoroughly with a clean paper or cloth towel.

NOTE 8—This procedure reduces variability in the test results by removing accumulated oil and dirt from the hands.

10.2 Place about 5 mL of 70 % (v/v) ethanol in the palm of one of the washed hands and instruct the subject to rub it well over the entire surface of both hands until the alcohol and water have evaporated completely.

10.3 Press a thumbpad or fingerpad over the mouth of an empty plastic vial (see 7.9) to demarcate the area to receive the test virus inoculum.

10.4 Using a positive displacement pipette, deposit 10  $\mu\text{L}$  of the virus suspension, with or without a soil load, at the center of each demarcated area of both thumbpads and all eight fingerpads.

10.5 Use thumbpads to determine the level of infectious virus placed in each demarcated area (Wet Inoculum Input Control). Once thumbpads have been contaminated, do not allow the inocula on them to dry and immediately elute them in accordance with 10.10.

10.6 Allow the inoculum on all fingerpads to become visibly dry under ambient conditions. This will generally take 15 to 30 min.

10.7 To determine the level of virus remaining viable after this drying period, elute the virus simultaneously from the two little fingerpads in accordance with 10.11. This is the Dried Virus Recovery Control.

NOTE 9—Using the little fingerpads for the Dried Virus Recovery Control serves as a worst-case scenario

10.8 Randomly select one remaining fingerpad, from one hand, for test product treatment, wherein 20  $\mu\text{L}$  of test product is added to the viral contaminated area, followed by rubbing with a randomly chosen fingerpad (other than the little finger) from the opposite hand for the duration of the contact time. Care should be taken to cover all dried virus inocula with test product during the treatment, and to minimize spillover of the test product.

10.8.1 To simulate the post-treatment water rinse when testing handwash agents, expose each treated fingerpad to 1.0 mL of standard hard water in a vial for 5-10 seconds; then elute virus following 10.10.

10.8.2 When testing handrubs, skip Step 10.8.1 and proceed directly to 10.10.

10.9 The other two remaining fingerpads from one hand may be used for treatment by the 2<sup>nd</sup> lot of test product, a reference (control) product, or a vehicle (standard hard water) control, or both.

10.10 For virus elution, press each thumbpad or fingerpad into one well of a 6-well plate or a 35mm-dish, which contains 2.0 mL eluent (= neutralizer), and rubbing continuously for 1 minute. After the rubbing, lift the finger and scrape the pad against the inside rim of the well or dish to recover as much of the fluid as possible into the vessel. Use a separate well or dish for each finger. Recovery values from the 2 thumbpads or the pair of the fingerpads that are involved in the same treatment will be averaged.

10.11 Subjects must be advised to avoid touching anything or anyone (including themselves) with their hands prior to decontamination.

10.12 Immediately following recovery, for all viruses except hepatitis A virus (HAV), decontaminate the thumb-/fingerpads by pressing them for 2 to 3 min over tissue paper or paper towel soaked in 70 % (v/v) ethanol; for HAV, use a 1:10 dilution of domestic bleach (about 5000 ppm available chlorine) in tap water.

10.13 Instruct the subjects to further decontaminate their hands (10.12) by washing them thoroughly with soap and water and drying them well before leaving the test area.

10.14 Titrate the eluates and controls for infectious virus using a minimum of three monolayers for each dilution tested. If titrations cannot be carried out within 3 to 4 h of collection, store samples overnight at  $6 \pm 2^\circ\text{C}$ . Longer storage should be at  $-70^\circ\text{C}$  or lower.

#### 10.15 Controls:

10.15.1 *Cell Viability Control*—To ensure that the host cells are not contaminated with bacteria, fungi or any cytopathogenic viruses other than those used in the test, leave at least two host cell monolayers untreated in each test to be examined first at the end of the incubation period. Any obvious contamination or degeneration in such monolayers would invalidate the virus titration.

10.15.2 *Virus Stock Titer Control*—Perform serial ten-fold dilutions on an aliquot of the stock virus for titration following 10.14. A lack of obvious and typical virus-induced cytopathic effects in such monolayers would also invalidate the test. This control will also confirm the titer of the stock virus and its suitability in testing.

10.15.3 *Cytotoxicity Control*—This control is to (1) determine the dilution of the test product at which it causes no apparent degeneration (cytotoxicity) of the host cells to be used for measuring virus infectivity and (2) assess if the neutralizer in any way reduces or enhances such cytotoxicity. Make an initial 1:20 dilution and one further ten-fold dilution of the use-dilution of the test product in EBSS with and without the neutralizer. Remove the culture medium from the monolayers of the host cell monolayers and put into each test monolayer separately the same volume of inoculum as used in virus titration; control monolayers receive an equivalent volume of EBSS (without any neutralizer) only. Hold the cultures for 30 to 60 min at room temperature and examine them under an inverted microscope for any cell degeneration or destruction. In case of cytotoxicity, a different neutralizer or alternative approaches to the removal/reduction of cytotoxicity may be needed. If no cytotoxicity is observed at either one of the dilutions, the test product and the neutralizer should be subjected to the following interference test.

10.15.4 *Control for Neutralization Effectiveness and Interference with Viral Infectivity*—Levels of the test product which show no obvious cytotoxicity could still reduce or enhance the ability of the challenge virus to infect or replicate in host cells, thus interfering with the estimation of its virucidal activity. An interference control must, therefore, be included to rule out such a possibility. Remove the culture medium from the host cells and inoculate each one of the test monolayers with the same volume of inoculum as used in virus titration with a 1:20 dilution of the test product in EBSS with and without neutralizer. Controls receive EBSS alone (without the neutralizer). Hold the monolayers at room temperature for 30 to 60 min and inoculate each with a low number of infective units of the challenge virus. Incubate the monolayers for virus adsorption, place maintenance medium in the cultures, incubate them for the time required for virus replication and examine them for cytopathology or foci of virus infection. Any significant

difference in virus infectivity titer is indicative of the test product's or the neutralizer's ability to affect the virus susceptibility of the host cells. In such a case, a different neutralizer or alternative approaches to the removal of the residues of the test product in the samples to be titrated for virus infectivity may be needed.

## 11. Repetitions and Statistical Evaluations

11.1 This test method should be performed at least once with no less than three subjects.

11.2 This test method is designed to include the two thumbpads to determine the level of viable virus placed on the fingerpads (Wet Inoculum Input Control), two little fingerpads to assess the level of viable virus remaining after the drying of the inoculum (Dried Virus Recovery Control), two fingerpads to determine the extent of virus elimination after treatment with the test product (including the post-treatment rinse with the standard hard water, if the test product is a handwash), and four fingerpads to assess the level of virus eliminated after treat-

ments by the 2<sup>nd</sup> lot of the test product, or a reference (control) agent, or the vehicle (for example, standard hard water) alone.

11.3 The difference in the level of infectious virus in the Wet Inoculum Input Control and the Dried Virus Recovery Control represents the loss in virus infectivity due to the drying of the inoculum on the fingerpads. The amount of infectious virus remaining after the drying of the inoculum shall be used as the baseline to determine the extent of virus elimination after treatment with the test or the control product.

## 12. Precision and Bias

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

## 13. Keywords

13.1 adenovirus; cell culture; cytotoxicity; eluent; fingerpads; hepatitis A virus; hygienic handwashing; hygienic handrub agent; infection control; influenza virus; *in vivo* testing; microbicial soap; rhinovirus; rotavirus; skin microbiota; soil load; standard hard water; virus; virus elution

## APPENDIX

### (Nonmandatory Information)

#### X1. TEST VIRUSES AND CELL CULTURES

X1.1 Viruses and their host cells recommended for use in this method; ATCC numbers, where available, are in parenthesis.

X1.2 The selection of the following test viruses is based on their (1) relative safety to subjects as well as experimenters, (2) ability to grow to titers sufficiently high for testing, (3) property to produce cytopathic effects or plaques, or both, in cell cultures, (4) potential to spread through contaminated hands, and (5) relative resistance to agents used in hand hygiene. Other strains or types of viruses may be substituted provided they meet the preceding criteria.

NOTE X1.1—There is insufficient information on whether the passage history, culture conditions, and strain differences of viruses can influence the efficiency of their elimination by hand hygiene agents. Caution must be exercised, however, when substituting viruses as this may lead to variations in results from one laboratory to another.

X1.2.1 *Human Adenovirus*—Type 2 (VR-846) or Type 5 (VR-5): Cell line options: Human Lung Carcinoma (A549) (CCL-185), Hep-2 (CCL-23), and Vero (CCL-81).

X1.2.2 *Hepatitis A Virus Strain HM-175 (VR-1402)*:—Recommended cell line FRhK-4 (CRL-1688).

X1.2.3 *Human Rotavirus Wa (VR-2018)*—Recommended cell line: CV-1 (CCL-70) or MA-104 (CRL-2378). Prior to rotavirus inoculation, cell monolayers must be washed at least three times with EBSS to remove the serum from the growth medium. All diluents, maintenance media, and agar overlays also must be free from serum. Most rotaviruses also require the presence of trypsin in the medium for growth and plaques formation.

X1.2.4 *Human Rhinovirus Type 37 (VR-1147) or Rhinovirus 14 (VR-284)*—Recommended cell line: MRC-5 (ATCC CCL-171), WI-38 (CCL-75) or HeLa T4+ cells.

X1.2.5 *Murine Norovirus Type 1*—A surrogate for human noroviruses; recommended cell line: RAW 264.7 (TIB-71)

X1.2.6 *Feline calicivirus*—A surrogate for human noroviruses; recommended cell line: CrFK (CCL-94).

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