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Standard Test Method for Determining the Virus-Eliminating Effectiveness of Liquid Hygienic Handwash and Handrub Agents Using the Fingerpads of Adult Volunteers¹

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INTRODUCTION

Hands play an important role in the spread of many ~~viral infections, viruses,~~ thus proper and regular handwashing is considered crucial in preventing ~~the spread of viral infections, 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 germicidal activity of handwash and handrub agents (1,2, 3).² Contaminated hands also can readily transfer infectious virus to other surfaces (1,2). Hand antiseptics has been shown to interrupt the spread of viral infections (3)(4). Standardized methods to assess the virus-eliminating potential of handwash and handrub agents have not been available and this test method addresses ~~this the~~ gap.

1. Scope

1.1 Human skin does not carry viruses as a part of its resident flora. Hands transiently contaminated with viruses, however, can act as vehicles for the spread of many types of viral infections. Hygienic hand washing is meant to reduce the load of viruses and other transient microorganisms on hands, thereby reducing the risk of disease transmission. Such reduction in the virus load may be due to a combination of virus inactivation and removal of infectious virus from the skin.

1.2 Standard test methods to assess the capacity of hygienic handwash and handrub agents to reduce virus levels on hands are not presently available. This test method, therefore, has been designed to determine the comparative virus-eliminating effectiveness of germicidal or non-germicidal ~~hygienic handwash agents:~~ 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 (45).

1.3 *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.*

2. Referenced Documents

2.1 ASTM Standards:

D 1129 Terminology Relating to Water³

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

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

E 1115 Test Method for Evaluation of Surgical Hand Scrub Formulation⁴

E 1173 Test Method for Evaluation of a Pre-Operative Skin Preparation⁴

E 1174 Test Method for Evaluation of Health Care Personnel Handwash Formulation⁴

E 2011 Test Method for Evaluation of Handwashing Formulations for Virus-Eliminating Activity Using the Entire Hand⁴

3. Terminology

3.1 *Definitions*—For definitions of general terms used in this test method, refer to Terminology D 1129.

3.2 *Definitions of Terms Specific to This Standard:*

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

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

3.2.3 *virus-eliminating (removing) agent*, *soil(organic) load, n*—~~any agent that rids hands~~—a solution of viruses by either killing them on 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 by dislodging them for subsequent wash-off. ~~other extraneous substances.~~

3.2.4 *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.

3.2.5 *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 volunteers who have provided informed consent and the skin of whose hands has been determined to be free from any apparent damage. Panelists are to refrain from any products containing antimicrobial agents for 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 the control (standard hard water) or test agent for the desired contact time. ~~Following a water rinse, time and~~ virus remaining on the fingerpad is eluted and the eluates are titrated for infectious virus along with the required controls. Percent reductions in the amounts of infectious virus after treatment with the control and test agents are then determined. The fingerpad method gives results that are comparable to those obtained using a whole-hand procedure (1,56). If two different formulations are being compared in the same test, one of them may be designated as a reference and used in place of the hard water control. 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 ~~virucidal~~ virus eliminating activity.

5. Significance and Use

5.1 This in vivo procedure is designed to test the ability of hygienic handwash agents to reduce levels of selected infectious viruses from experimentally contaminated fingerpads of adult volunteers. Since the two thumbpads and all eight fingerpads can be used in any given test, it allows for the incorporation of input virus control (two), amount of virus remaining after the inoculum has been allowed to dry (two), virus eliminated after treatment with a control or reference solution (two), and up to four replicates to assess the virus-eliminating efficiency of the product under test. No more than 100 µL of the virus suspension are required to complete one test. The results of testing with this test method may form the basis for confirmatory tests using a suitable whole-hand test protocol.

5.2 This test method is designed to be performed by a trained ~~virologist, individual,~~ 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 Schmidt and Emmons (67).

5.3 Whereas, this test method relates to testing with viruses of human origin, ~~this test method it~~ it can be readily adapted to work with bacteria, fungi, protozoa and bacteriophages.

5.4 Infectious microorganisms left on hands after washing can be reduced further by drying the washed hands with paper, cloth, or warm air (7)(8). A step for the drying of fingerpads after exposure to the control or test solution, 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 amount of virus on each fingerpad after the drying of the inoculum should not be less than 10⁴ infectious units that would permit the detection of up to a 4 log₁₀ reduction in the infectivity titer of the virus by a given 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 (45).

³ Annual Book of ASTM Standards, Vol 11.04¹.

⁴ Annual Book of ASTM Standards, Vol 11.05.

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 CO_2 incubator will be required. Work with rhinoviruses will require an incubator at $33 \pm 1^\circ\text{C}$.

6.3 *Positive Displacement Pipette*—A pipette and pipette tips that accurately can dispense 10- μL 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- μm 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°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 *Magnetic Stirrer and Magnets*—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 panelists to wash hands without touching hands to sink surface.

6.10.1 *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.

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

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

6.12 *Inverted Microscope*—An inverted microscope with 10 \times eye pieces and 5 \times , 10 \times , and 40 \times 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*⁵—Plastic cell culture flasks of 25 or 75-cm² 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.

7.3 *Cell Culture Media and Supplements*⁶—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 3).

7.4 *Organic Soil Load*:

7.4.1 *Fetal Bovine Serum*, at a final concentration of 5 % in the virus inoculum (see Note 3).

7.4.2 *Peptone* (a pancreatic digest of casein);

7.4.2 A tripartite soil load, as an alternative to serum. ~~Dissolve 7.6~~ Add 0.5 g of ~~peptone powder in 1 L~~ tryptone to 10 mL of physiological saline (0.85 % NaCl). ~~Sterilize 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 autoclaving or 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 tryptone stock solutions. This peptone solution should contain 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 3—Fetal bovine serum is considered unsuitable for use as an organic load when working with rotaviruses because of its rotavirus inhibitory and trypsin-neutralizing activity.

7.5 *Standard Hard Water*—The quality and disinfectant (for example, chlorine) residual in tap water can vary from site to site and also 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. Water prepared in accordance with AOAC 960.09 *E* and *F* (89) to a standard hardness of 200 ppm as calcium carbonate is used for dilution of test products, 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).

7.6 *Test Agents*—At least two samples of the product shall be tested.

7.7 *Diluent for Virus Titration*—Earle's balanced salt solution (EBSS) with a pH of 7.2-7.4.

7.8 *Eluent for Virus Recovery from Fingerpads*—EBSS containing 1 % tryptone. (pH 7.2-7.4).

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

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

⁵ Plastic cell culture ware may be purchased from most laboratory supply houses.

⁶ Materials and reagents for cell culture may be purchased from biological supply houses.

8. Test Viruses and Cell Cultures

8.1 The selection of the following test viruses is based on their (a) relative safety to the volunteers as well as experimenters, (b) ability to grow to titers sufficiently high for testing, (c) property to produce cytopathic effects or plaques, or both, in cell cultures, (d) potential to spread through contaminated hands, and (e) relative resistance to agents used in hygienic handwashing. Other strains or types of viruses may be substituted provided they meet the preceding criteria.

NOTE 4—There is insufficient information on whether the passage history, culture conditions, and strain differences of viruses can influence the efficiency of their elimination by hygienic handwash agents. Caution must be exercised, however, when substituting viruses as this may lead to variations in results from one laboratory to another.

~~8.2 Poliovirus Human Adenovirus Type 1 (Chat), American Type Culture Collection 4 (ATCC VR-192) or Poliovirus Type 1 Lse 2ab (ATCC VR-59), VR-4; Recommended cell line: CV-1 line derived from the kidney of the African green monkey lines for making virus pools and infectivity titrations are 293 (CRL-1573) and Vero (ATCC CCL-7081) cells, respectively.~~

~~8.3 Human Rotavirus Wa Hepatitis A Virus Strain HM-175 (ATCC VR-2018402);— Recommended cell line: CV-1 line FRhK-4 (ATCC CCL-70).~~

~~8.3.1 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. CRL-1688).~~

~~8.4 Human Rhinovirus Type 37 Rotavirus Wa (ATCC VR-1147) or Rhinovirus 14 (ATCC VR-284)— Recommended VR-2018)— Recommended cell line: MRC-5 CV-1 (ATCC CCL-1710) or WI-38 (ATCC CCL-75), MA-104 (CRL-2378).~~

~~8.4.1 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.~~

~~8.5 Influenzavirus Human Rhinovirus Type A (H1N1), Hong Kong 37 (ATCC VR-51147) or Rhinovirus 14 (ATCC VR-284)— Recommended cell line: MDCK cells MRC-5 (ATCC CCL-171), WI-38 (ATCC CCL-75) or HeLa T⁴⁺ cells. (Incubation of infected cells at 33°C is required for optimal virus replication).~~

9. Panelists

9.1 Recruit a sufficient number of healthy adult human volunteers who have no clinical evidence of dermatoses, open wounds, or other skin disorders (see 4.1). The number of volunteers 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 panelists/volunteers for testing and to obtain informed and written consent from those selected for the study before starting the tests.

10. Procedure

~~10.1 Fig. 1 shows the main steps for this test method and the following procedure refers to the relevant panel number(s) in it. method.~~

~~10.2 The volunteer 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. This procedure reduces variability in the test results by removing accumulated oil and dirt from the hands. Place about 3-5 mL of 70 % (v/v) ethanol in the palm of one of the washed hands and instruct the volunteer to rub it well over the entire surface of both hands until the alcohol and water have evaporated completely (Panel (Step 1)).~~

~~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 (Panels 2 and 3). (Step 2).~~

~~10.4 Using a positive displacement pipette, deposit 10 µL of the virus suspension, with or without a soil load, at the center of each demarcated area (Panel 4).~~

~~10.5 For virus elution, place the contaminated area of the thumb/finger over the mouth of a plastic vial (see 7.9) containing 1 mL of the eluent. Invert the vial with the pad still over it, and allow the eluent to remain in contact with the inoculated contaminated area for 5 to 10 s. Invert the vial 20 times with the pad still in place. Repeat the soak and inversion step once more. Finally, turn the vial upright and scrape the pad against the inside rim of the vial to recover as much of the fluid as possible (Panels 7 and 9).~~

~~10.6 It (Step 3).~~

~~10.5 It is recommended that thumbpads be used to determine the amount of infectious virus placed in each demarcated area (Input Control). Once a thumbpad has been contaminated (Panel 5), contaminated, do not allow the inoculum to dry and immediately elute it in accordance with 10.5. Decontaminate 10.10.~~

~~10.6 Allow the thumbpad by pressing it for 2 to 3 min over tissue paper or paper towel soaked in a 1:10 dilution of domestic bleach (about 5000 ppm available chlorine) in tap water.~~

~~10.7 Repeat 10.2-10.6 with the other thumbpad to obtain the second input virus sample (Panel 5).~~

~~10.8 Repeat 10.3 and 10.4 and with the fingerpads but allow the inoculum on all fingerpads to become visibly dry under ambient conditions (Panel 6): (Step 4). This will generally take 20 to 30 min.~~

~~10.7 To determine the amount of virus remaining viable after this drying period, elute the virus from two randomly selected fingerpads in accordance with 10.5.~~



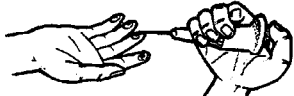




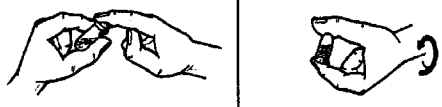

Step #	
(1) The panelist washes hands with non-germicidal soap and tap water and dries them with paper towel. About 3-5 mL of 70%(v/v) ethanol is placed on the hands and they are rubbed together till dry.	
(2) Each digit is pressed against the mouth of an empty cryovial (8 mm inside diam.) to demarcate the target area.	
(3) 10 µL of virus with or without soil load is placed at center of each demarcated area. Inoculum from the two thumbpads is eluted immediately (Step 8 below) to act as 'input' control for virus.	
(4) Inoculum on fingerpads allowed to become visibly dry (20-25 minutes). Two randomly selected fingerpads are eluted immediately (Step 8) at the end of drying ('baseline' control).	
(5) Dried inoculum on at least two randomly selected fingerpads is exposed to 1 mL of test product or control fluid in a cryovial for desired contact time, with specified number of full inversions.	
(6) Skin scraped against inside lip of vial to collect as much fluid as possible. For waterless handwash agents or to determine virus elimination after exposure to the product alone, fingerpads can be eluted (Step 8) without further treatment	
(7) To simulate post-treatment rinsing of hands, fingerpads are exposed to 1 mL of hard water for 5-10 seconds. Virus can be eluted (Step 8) at this stage or after drying of hands. To determine virus removal after the drying of washed hands, they can be dried in air or with paper or cloth towel for specified time and virus recovered from them.	
(8) To elute virus, the digit is placed on the mouth of a cryovial with 1 mL of eluent and subjected to 20 full inversions; skin is scraped against inside lip of vial to collect as much fluid as possible. The eluates and controls are titrated for virus and log ₁₀ reductions calculated.	
(9) The panelist decontaminates the hands by pressing the inoculated areas for 2-3 minutes against a tissue or paper towel soaked in either 70% ethanol or a 1:10 dilution of domestic bleach. The panelist then washes hands thoroughly with soap and water and dries them well before leaving the test area.	

FIG. 1 Procedure for In Vivo Evaluation of the Virus-eliminating Activity of Handwash and Handrub Agent Using the Fingerpad Test

10.9 Use test product 10.10 (see Step 8 below).

10.8 Expose the dried inoculum on four the required number of randomly selected fingerpads, and control or reference solution on two remaining fingerpads. Place by placing 1.0 mL of the in-use dilution of the test product, control, or reference solution in a plastic vial (7.9). Place a virus-contaminated fingerpad over the mouth of the vial and invert it. Allow the contents of the vial to remain in contact with the contaminated area for 10 to 15 s (Step 5) while subjecting the vial to 10 full inversions. For viscous formulations, invert the vial and keep its contents in contact with the contaminated area for 20 sec without any inversions. Scrape the fingerpad on the inside rim of the vial to recover as much of the fluid as possible.

10.10 Measure out 15.0 possible (Step 6).

10.9 To simulate the post-treatment water rinse when testing handwash agents expose the treated fingerpads to 1 mL of standard hard water in a sterile 25-cm² plastic cell culture flask or a similar vessel which has a mouth with an inside diameter for 5-10 seconds (Step 7).

10.10 For virus elution, place the contaminated area of 8 to 10 mm. Place the fingerpad exposed to the test product, control, or reference solution thumb/finger over the mouth of a plastic vial (see 7.9) containing 1 mL of the flask eluent. Invert the flask vial with the fingerpad pad still in place; over it, and subject it allow the eluent to five full inversions over a period of 3 to remain in contact with the inoculated contaminated area for 5 to 10 s. Scrape Invert the fingerpad on vial 20 times with the pad still in place. Repeat the soak and inversion step once more. Finally, turn the vial upright and scrape the pad against the inside rim of the flask vial to remove as much of the fluid as possible (Panels 10 and 11). This elution procedure results (Step 8).

10.11 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 100-fold 1:10 dilution of the test product domestic bleach (about 5000 ppm available chlorine) in the eluent. This level of dilution has been found to immediately arrest the virucidal activity of most hygienic handwash agents while also rendering them nontoxic to cell cultures.

10.11 Elute remaining virus from the fingerpads using the procedure in 10.5. tap water (Step 9).

10.12 Instruct the panelists to further decontaminate their hands (10.611); by washing them thoroughly with soap and water and drying them well before leaving the test area.

10.13 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 4 to 10°C. Longer storage should be at – 70°C.

11. Repetitions and Statistical Evaluations

11.1 This test method should be repeated at least two times with at least two panelists.

11.2 This test method is designed to include the two thumbpads to determine the amount of virus placed on the fingerpads (inoculum control), two fingerpads to assess the amount of virus remaining after the drying of the inoculum (dried virus control), two fingerpads to determine the extent of virus elimination after treatment with standard hard water alone, and four fingerpads to assess the amount of virus eliminated after the combined effect of exposure to the test product and the post-treatment rinse with the standard hard water.

11.3 The difference in the amount of infectious virus in the inoculum control and the dried virus control represents the loss in virus infectivity due to the drying of the inoculum. The amount of infectious virus remaining after the drying of the inoculum must be used as the baseline to determine the extent of virus elimination after treatment with the control solution or the test product.

12. Precision and Bias

12.1 *Precision*—The precision of this test method within two laboratories has been determined and compared with that of a whole-hand protocol (5,9,10)(6,10,11). The efficiency of the test method of virus elution from the fingerpads has been tested with five different viruses and has been found to be about 85 %.

13. Keywords

13.1 cell culture; cytotoxicity; eluent; fingerpads; germicidal soap; hygienic handwashing; infection control; influenzavirus; in vivo testing; organic load; poliovirus; rhinovirus; rotavirus; skin flora; standard hard water; virus; virus elution

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