



Standard Test Method for Determining the Virus-Eliminating Effectiveness of Hygienic Handwash and Handrub Agents Using the Fingerpads of Adults¹

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 (ϵ) 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**).² 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**). Standardized methods to assess the virus-eliminating potential of handwash and handrub agents have not been available and this test method addresses the gap.

1. Scope

1.1 Human skin is not known to carry viruses as a part of its resident microbiota. 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 (**6**).

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 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 Antimicrobial Agents.

Current edition approved April 1, 2010. Published May 2010. Originally approved in 1996. Last previous edition approved in 2002 as E1838 – 02. DOI: 10.1520/E1838-10.

² The boldface numbers in parentheses refer to the list of references at the end of 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 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:³

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 Adult Subjects

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 *hygienic (health-care personnel) handwash agents, n*—agents generally used for handwashing by personnel in

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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.

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

3.1.3 *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.

3.1.4 *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.

3.1.5 *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 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 the control (standard hard water) or test agent for the desired contact time and virus remaining on the fingerpad is eluted and the eluates titrated for infectious virus along with the required controls. Percent and/or $\log_{10}$ reductions in the levels 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 (2, 7), another ASTM standard (Test Method E2011). 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 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 used in any given test, it allows for the incorporation of input virus control (two), virus remaining viable 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 substance 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 further tests using a suitable whole-hand test protocol (for example, Test Method E2011).

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 (8).

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 (9). A step for the drying of fingerpads after exposure to the control or test substance, 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 on each fingerpad after the drying of the inoculum 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 substance 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 (6).

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.

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 subjects 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—Plastic 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.

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

7.3 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 4—Materials and reagents for cell culture may be purchased from biological supply houses.

7.4 Soil Load:

7.4.1 Bovine Serum, at a final concentration of 5 % in the virus inoculum (see [Note 5](#)).

7.4.2 A tripartite soil load, as an alternative to serum. Add 0.5 g of tryptone or 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 $0.22\ \mu\text{m}$ 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 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 5—Bovine serum is 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* ([10](#)) to a standard 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).

7.6 Test Substance—At least two separate manufacturer's lots of the substance shall 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.

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 (pH [7.2-7.4](#)).

7.9 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.

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

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 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 subjects 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. It is recommended that virus from the two thumbpads be eluted simultaneously. The simultaneous exposure and elution of the two fingerpads to be used for the control or test substance is also recommended.

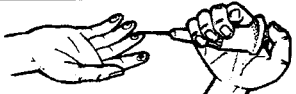
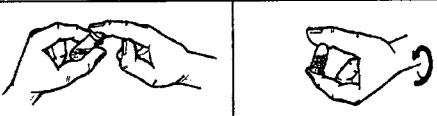
10.2 The subject will wash his/her hands with a nonmedicated soap for at least 10 s, rinse, and then dry them thoroughly with a clean paper or cloth towel. This procedure reduces variability in the test results by removing accumulated oil and dirt from the hands. Place about 5 mL of 70 % (v/v) ethanol 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 (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 (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 (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 thumbpads have been contaminated, do not allow the inocula on them to dry and immediately elute them in accordance with [10.11](#).

10.6 Allow the inoculum on all fingerpads to become visibly dry under ambient conditions (Step 4). This will generally take 15 to 30 min.

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.	

NOTE—In Step 6, scraping of fingerpad surface is not required when testing handrubs.

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

10.7 To determine the amount of virus remaining viable after this drying period, elute the virus simultaneously from two randomly selected fingerpads in accordance with 10.11 (see Step 8 below).

10.8 Expose the dried inoculum on the required number of randomly selected fingerpads, 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 30 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 without any inversions.

10.9 After the desired contact time, scrape the fingerpad on the inside rim of the vial to recover as much of the fluid as possible (Step 6). For handrubs, do not scrape the fingerpad; the fluid remaining on the skin should be allowed to remain in place and recovered during the elution process.

10.10 To simulate the post-treatment water rinse when testing handwash agents expose the treated fingerpads to 1 mL of standard hard water for 5-10 seconds (Step 7). When testing handrubs, skip Step 7 and proceed directly to Step 8.

10.11 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 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 (Step 8).

10.12 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 (Step 9).

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 4 to 10°C. Longer storage should be at -70°C or lower.

10.15 Controls:

10.15.1 *Cell Culture Control*—To ensure that the host cells are not contaminated with bacteria, fungi or any cytopathogenic viruses other than those used in the test. At least two host cell monolayers are left untreated in each test and 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 Susceptibility Control*—At least one host cell monolayer receives the test virus at a high enough level to produce cytopathology to make sure that they remain susceptible to it. A lack of obvious and typical virus-induced cytopathic effects in such a monolayer would also invalidate the test.

10.15.3 *Cytotoxicity Control*—The objective of this control is to (1) determine the dilution of the test substance at which it causes no apparent degeneration (cytotoxicity) of the cell line 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 substance in EBSS with and without the neutralizer, if any. Remove the culture medium from the monolayers of the host cell line(s) and put into each test monolayer separately the same volume of inoculum as used in virus titration; control monolayers receive an equivalent amount 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 cytotoxicity. 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 substance and the neutralizer should be subjected to the following interference test.

10.15.4 *Control for Interference with Virus Infectivity*—Levels of the test substance 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 substance 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 (20 to 50) 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 substance'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 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 (7, 11, 12). 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 adenovirus; cell culture; cytotoxicity; eluent; fingerpads; hepatitis A virus; hygienic handwashing; hygienic handrub agent; infection control; influenza virus; *in vivo* testing;

microbicidal 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 4 (VR-4) or Type 5 (VR-5): Recommended lines for making virus pools and infectivity titrations are 293 (CRL-1573) and Vero (CCL-81) cells, respectively.

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)

REFERENCES

- (1) Sattar, S. A. and Ansari, S. A., “The fingerpad protocol to assess hygienic hand antiseptics against viruses,” *J. Virol. Methods*, Vol 103, 2002, pp. 171–181.
- (2) Ansari, S. A., Sattar, S. A., Springthorpe, V. S., Wells, G. A., and Tostowaryk, W., “Rotavirus Survival on Human Hands and Transfer of Infectious Virus Animate and Nonporous Inanimate Surfaces,” *Journal of Clinical Microbiology*, Vol 26, 1988, pp. 1513–1518.
- (3) Mbithi, J. N., Springthorpe, V. S., Boulet, J., and Sattar, S. A., “Survival of Hepatitis A Virus on Human Hands and Its Transfer on Contact with Animate and Inanimate Surfaces,” *Journal of Clinical Microbiology*, Vol 30, 1992, pp. 757–763.
- (4) Sattar, S.A., Abebe, M., Buetti, A., Jampani, H. & Newman, J. “Determination of the activity of an alcohol-based hand gel against human adeno-, rhino-, and rotaviruses using the fingerpad method,” *Infection Control & Hospital Epidemiology*, Vol. 21, 2000, pp. 516–519.
- (5) Gwaltney, J. M., Moskalski, P. B., and Hendley, J. O., “Interruption of Experimental Rhinovirus Transmission,” *Journal of Infectious Diseases*, Vol 142, 1980, pp. 811–815.
- (6) CDC-NIH, *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed., U.S. Department of Health and Human Services, Washington, DC, 1999.
- (7) Mbithi, J. N., Springthorpe, V. S., and Sattar, S. A., “Comparative In Vivo Efficiency of Hand-Washing Agents Against Hepatitis A (HM-175) and Poliovirus Type 1 (Sabin),” *Applied and Environmental Microbiology*, Vol 59, 1993, pp. 3463–3469.
- (8) Lennette, E.H., Lennette, D. A., and Evelyne T. Lennette, E. T. eds., *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections*, Seventh Edition, American Public Health Association, Washington, DC, 1995.
- (9) Ansari, S. A., Springthorpe, V. S., and Sattar, S. A., “Comparison of Cloth-, Paper- and Warm Air-Drying in Eliminating Viruses and Bacteria from Washed Hands,” *American Journal of Infection Control*, Vol 19, 1991, pp. 243–249.
- (10) AOAC International, *Official Methods of Analysis of the AOAC*, Arlington, VA, 1990.
- (11) Ansari, S. A., Sattar, S. A., Springthorpe, V. S., Wells, G. A., and Tostowaryk, W., “In Vivo Protocol for Testing Efficacy of Hand-Washing Agents Against Viruses and Bacteria: Experiments With Rotavirus and *Escherichia coli*,” *Applied and Environmental Microbiology*, Vol 55, 1989, pp. 3113–3118.
- (12) Steinmann, J., Nehrkorn, R., Meyer, A., and Becker, K. “Two In-Vivo Protocols for Testing Virucidal Efficacy of Handwashing and Hand Disinfection,” *Zentralblatt für Hygiene*, Vol 196, 1995, pp. 425–436.

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