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Standard Test Method for Evaluation of Hygienic Handwash and Handrub Formulations for Virus-Eliminating Activity Using the Entire Hand[1]

This standard is issued under the fixed designation E2011; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

INTRODUCTION

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

1. Scope

1.1 This test method is designed to evaluate handwash or handrub agents for their ability to reduce or eliminate viable viruses from the skin of human hands.

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

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

1.3 *This standard may involve hazardous materials, operations and equipment. This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use. The user should consult a reference for laboratory safety recommendations.* (**3-5**)

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E1482 Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization

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

2.2 *AOAC Standard:*

AOAC 960.9 Official Methods of Analysis (2007)[4]

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

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

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

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

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

[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

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

3.1.5 *virus-eliminating (inactivating/removing) agent, n*—any agent that rids hands of viruses by either inactivating 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 uses adult subjects who have provided a written informed consent and whose hands have been determined to be free from any apparent damage at the time of their participation in the study.

4.1.1 Since both hands, including nail beds, of the test subject are exposed to high-titer suspensions of virus, each subject shall be carefully examined for any skin irritations, micro-breaches, or breaks in the hand skin and around the nails using a magnifying glass under well-lighted conditions. Those with any breaches, breaks, or other apparent skin damages shall not participate in the test.

4.1.2 While no fewer than six subjects are recommended for each virus-test substance combination to be evaluated, the number required may vary depending on the intended use of the data and the target regulatory agency.

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

4.3 A prepared suspension of the selected test virus is grown and diluted or concentrated to produce a titer with a minimum of 10^7 infective units/mL. The contaminating virus is applied to the hands and the hands are treated with the test substance according to the manufacturer's directions or with a set test regimen.

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

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

4.5.1 This test method is designed to be performed by a person trained and experienced in working with human pathogenic viruses and their host cells. Such an individual will also be responsible for choosing the appropriate host system for the test virus, and applying the techniques necessary for propagation and maintenance for host system and test virus. For a reference text, see Ref (**6**).

5. Significance and Use

5.1 This test method is designed to evaluate the virus-eliminating activity of hygienic handwash and handrub agents from experimentally-contaminated hands. Such formulations may be further assessed in a clinical trial for their effectiveness in the field. This test method incorporates whole-hand exposure and reflects actual use conditions such as friction during hand decontamination, and also enables alternative product forms such as alcohol- or non-alcohol-based liquids, gels, and foams to be tested according to label directions. It is meant to extend, if required, the results of testing with Test Method E1838, which gives precise reductions in viral infectivity on a limited area of the hands. It may also serve as an alternative test method when product form is not amenable to testing by Test Method E1838.

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

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

6. Equipment and Apparatus

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

6.2 *Incubator*—an incubator at 35 ± 2°C or other appropriate temperature for growing host cells and for incubating virus-infected cultures. If an open system is used for cell culture, a CO_2 incubator will be required.

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

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

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

6.6 *Freezers*—a freezer at –20 ± 2°C for the storage of 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 ± 2°C is necessary for storage of prepared cell culture media and reagents.

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

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

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

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

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

6.10.3 Tap water temperature regulator and temperature monitor to monitor and regulate water temperature at 40 ± 2°C.

6.11 *Liquid Nitrogen Storage for Cells*—an appropriate liquid nitrogen container and liquid nitrogen for cryopreservation of cell line stocks.

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

6.13 *Serological Pipettes*—sterile reusable or single-use pipettes of 10.0-, 5.0-, and 1.0-mL capacity or other suitable capacity.

6.14 *Cell Culture Flasks*—plastic cell culture flasks of 25 cm^2 or 75 cm^2 or other suitable capacity for culturing cells and for preparing virus pools.

NOTE 3—Each plastic flask for growing cell monolayers can be reused by reseeding with new cell cultures up to 10 times before being discarded.

6.15 *Plastic and Glass Vials, Medication (Medicant)*—sterile screw-capped vials will be required for storage of samples.

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

6.17 *Sterile Glass Beads*—3.5 mm in diameter.

6.18 *Glass or Plastic Funnel*—27 cm in diameter.

6.19 *Glass or Plastic Beaker*—200 mL in capacity.

7. Materials and Reagents

7.1 *Cell Culture Media and Supplements*—Culture media and the types and ratios of supplements will vary depending on the cell line. For example, 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 4). Antibiotics may be required in the medium to suppress bacterial contamination.

7.2 *Soil Load:*

7.2.1 Bovine serum, at a final concentration of 5 % in the virus inoculum (see Note 4), if required for the test.

NOTE 4—Serum is considered unsuitable for use as a soil load with rotaviruses because of its rotavirus-inhibitory and trypsin-neutralizing activity.

7.2.2 A tripartite soil load, as an alternative to serum, is prepared from the following stock solutions in phosphate buffer (pH 7.2 to 7.4).

7.2.2.1 Add 0.5 g of tryptone or yeast extract to 10 mL of the buffer.

7.2.2.2 Add 0.5 g of bovine serum albumin (BSA) to 10 mL of the buffer.

7.2.2.3 Add 0.04 g of bovine mucin to 10 mL of the buffer.

7.2.2.4 Prepare the stock solutions separately and sterilize by passage through a 0.22-µm pore diameter membrane filter, aliquot and store at either 4 ± 2°C or –20 ± 2°C. Use within a validated shelf-life.

7.2.2.5 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/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.

7.3 *Standard Hard Water*—Water prepared according to AOAC 960.9 to a standard hardness of 200 ppm as calcium carbonate is used for dilution of test substance. This is the control solution to determine the baseline level of virus elimination, and to rinse the hands after exposure to the test substance.

7.4 *Number of Test Substance Lots to be Used*—The number of separate manufactured lots (batches) of each test formulation to be tested will depend on the specific requirements of the target regulatory agency.

7.5 *Diluent for Virus Titration*—Earle's balanced salt solution (EBSS) or other appropriate dilution medium with a pH of 7.2 to 7.4.

7.6 *Eluent for Virus Recovery from Hands*—EBSS or other appropriate dilution medium containing 1 % peptone and 0.1 % Polysorbate 80 at final concentrations.

7.7 *Sterile Disposable Gloves*—Loose-fitting, unlined, powder-free gloves which possess no antiviral or cytotoxic properties, or equivalent. (Plastic bags with low bioburden may be used in place of gloves.)

8. Test Viruses and Cell Cultures

8.1 See Appendix X1 for suggested viruses and host cells.

8.2 Virus stocks as well as host cells used for virus propagation may contain adventitious viruses or other pathogens potentially harmful to human subjects. Therefore, great care should be used in the selection and use of such materials to be applied on human hands.

9. Preparation of Virus Stocks and Determination of Infectivity Titer

9.1 Use appropriate host cells to prepare the virus pool. The virus pool should contain $\geq 10^7$ infective unit/mL.

9.2 Remove growth or maintenance medium and inoculate 0.1 mL of virus (control flasks receive 0.1 mL of EBSS instead) into each flask (for example, 75 cm^2) with a confluent cell monolayer and allow 60 to 120 min for virus adsorption. Place 15 mL of maintenance medium into each inoculated flask and

reincubate until about 75 to 95 % of each infected cell monolayer shows virus-induced cytopathology. Control monolayers must remain free from any apparent degeneration or contamination. Freeze (–20 to –90°C) and thaw (room temperature) the infected flasks three times to disrupt host cells for virus release. Centrifuge the cell suspension at 4°C for 10 to 20 min at approximately 1000 x*g* to sediment the cell debris, collect supernatant, aliquot if necessary, and store it at –6 to –90°C in suitable aliquots.

NOTE 5—Alternative flask size, inoculum volume, and/or medium volume may be used as appropriate.

9.3 A titer of $\geq 10^7$ infective units/mL is required for the testing and ultra-centrifugation of virus pools may be needed to achieve such levels of infectivity for the contamination of hands.

10. Controls

10.1 *Cell 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 assay.

10.2 *Virus Susceptibility Control*—To ensure that the host cells remain susceptible to the test virus, at least three host cell monolayers will receive a level of the test virus sufficient to produce cytopathology. A lack of obvious and typical virus-induced cytopathic effects in such a monolayer would also invalidate the test.

10.3 *Cytotoxicity Control*—This control applies only in tests which utilize handrub agents. Its objective is to determine if residues of the test substance in the neutralized eluates can produce any apparent degeneration (cytotoxicity) of the cell line for measuring viral infectivity.

10.3.1 Place 0.5 mL of EBSS or other appropriate medium as a mock inoculum in the cupped hands of the subject and ask him/her to rub them together in a lathering motion not reaching above the wrists for 90 s. Then place in the cupped hands the same volume of the handrub as required for the test. Elute each hand with 40 mL of an eluent containing an appropriate neutralizer (see 10.5). Inoculate host cell monolayers with the eluate, and incubate at 35 ± 2°C or another appropriate temperature for the number of days suitable for the test virus. At the end of the incubation period, observe the cell monolayer in an inverted microscope for any signs of cytotoxicity. Absence of any apparent degeneration of the cells indicates freedom from cytotoxicity that could interfere with the scoring for viral infectivity. If cytotoxicity is detected, a different neutralizer or alternative approaches (**7**) to the removal/reduction of cytotoxicity may be needed.

10.4 *Control for Interference with Viral Infectivity*—This control also applies to handrub agents only. 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 determination of its virucidal activity. An interference control must, therefore, be included to rule out such a possibility.

10.4.1 This control can be run using the same eluate as described in 10.3. First, expose the cell monolayers to the eluate or cell culture medium with or without neutralizer (controls) for 60 min and then challenge them with a defined number of infective units of the test virus. Complete the rest of the procedure for an infectivity assay and incubate the cultures as needed for the challenge virus. Any significant difference (greater than ± 1.0 $\log_{10}$) in the viral infectivity titer is indicative of the test substance's and/or the neutralizer's ability to affect the viral susceptibility of the host cells. In such a case, a different neutralizer or alternative approaches to the removal of the test substance residues in the samples to be titrated for viral infectivity may be needed.

10.5 *Validation of Neutralization:*

10.5.1 *Handwash Agents*—While the virucidal activity of the test substance must be neutralized as soon as possible at the end of the exposure period for an accurate measure of the contact time (Test Method E1482), this is not readily attainable in testing handwash agents as described here. The treatment of the contaminated hands with such test substances is immediately followed by rinsing of the hands with 500 mL of hard water and then drying with paper towels. These additional steps, which are an integral part of a normal handwash procedure, may incrementally reduce the levels of any test substance remaining on the hands with a corresponding reduction in virucidal activity. Therefore, the eluates may not require any active neutralization before infectivity assays.

10.5.2 *Handrub Agents*—Add a suitable neutralizer to the eluent (EBSS-peptone or equivalent) when working with handrubs and validate that it can successfully quench the activity of the active ingredient(s) against the test virus(es).

10.5.2.1 Add a countable number of infective units of the test virus to 10 mL of the eluent with the neutralizer and hold the suspension for 10 min at room temp. For control, use the eluent without the neutralizer.

10.5.2.2 Assay the suspensions for infectious virus. The neutralization is considered to be validated if the level of infectious virus recovered from the eluents with neutralizer and the cell culture medium are similar (± 1.0 $\log_{10}$).

11. Cleaning and Decontamination of Hands Before Experimental Contamination

11.1 Immediately prior to the experimental contamination, instruct each subject to wash his/her hands with a mild, proven non-antimicrobial soap for 1 min under running tap water and then dry them thoroughly with paper towels.

11.2 Place approximately 5 mL of 80 % (v/v) ethanol in the cupped hands and instruct subject to rub it over the entire surface of both hands till the hands are thoroughly dry. To ensure a complete removal of any remaining residue, subjects' hands may further be rinsed with approximately 200 mL of sterile deionized water and dried by an air blower. This water-rinse step is optional.

12. Procedures for Viral Contamination of Hands and Application of Test Substance/Control Fluids

12.1 *Method 1 for Handwash Agents:*

12.1.1 *Viral Contamination of the Hands*—Immediately after washing hands as directed in Section 11, place 1.5 mL of the test virus suspension onto the palm of the cupped left hand of the subject and distribute the inoculum with washing movements over the entire surface of both hands (not on wrists). Spread suspension for 90 s and allow to dry another 90 s.

12.1.2 Treat hands with the test substance as follows: Moisten the hands with 10 mL of sterile hard water and then place the amount/volume of the test substance, as specified by the manufacturer or in the set test regimen, into the subject's cupped hands and spread with lathering movements for 20 s in a fashion similar to that described in Ref (**8, 9**). For a control, use an equivalent volume of EBSS or other appropriate medium as a replacement for the test substance.

12.1.3 Gradually pour 500 mL of sterile hard water over both hands of the subject for at least 15 s while the subject rubs hands together to simulate rinsing movements. Such rinsing should be with the hands held over a bowl containing at least 500 mL of a 1:10 dilution of domestic bleach (~5 % sodium hypochlorite) to capture and inactivate the virus. Dry subjects' hands thoroughly with either an air blower or sterile paper towel (the same drying method must be used for the entire test) for 15 s. Then, surviving virus on the hands can be recovered by either the Glove Juice Method (12.1.4) or the Rubbing and Rinsing Method (12.1.5).

12.1.4 *Recovery of Virus after Treatment with the Test Agent—Glove Juice Method*—Within 5 min of test substance treatment, place loose-fitting sterile disposable gloves on both the right and left hands. Add 40 mL of EBSS-peptone or other elution medium (with neutralizer if necessary) to each glove and secure the glove above the wrist. Massage all surfaces of each hand for one minute in a uniform manner with particular attention paid to the subungual areas. Then remove the elution medium aseptically from each glove, combine the eluates, and transfer to a sterile tube and analyze for infectious virus. No less than 8 mL (~10 %) of the eluate shall be assayed for viral infectivity

12.1.5 *Recovery of Virus after Treatment with the Test Agent—Rubbing and Rinsing Method*—Place a sterile funnel (27-cm diameter) in a 200-mL sterile beaker. Instruct the subject to place hands over the funnel and gradually pour 20 mL of EBSS-peptone or other elution medium (with neutralizer, if necessary) over them while he/she rubs hands together to elute any remaining virus. No less than 2 mL (~10 %) of the eluate shall be assayed for viral infectivity.

12.1.6 At the end of the elution process, each subject shall decontaminate the hands by placing 5 mL of a 1:10 water dilution of domestic bleach (~5 % sodium hypochlorite) or 80 % (v/v) ethanol into the cupped hands of the subject and requiring him/her to rub the bleach or ethanol thoroughly over the surface of both hands for at least 2 min. This is to be followed by a thorough washing of hands with plain soap and drying with paper towel.

12.2 *Method 2 for Handwash Agents:*

12.2.1 In this alternative procedure, only the eight fingerpads are contaminated and eluted after treatment allowing the use of a smaller volume of eluent.

12.2.2 *Viral Contamination of the Hands*—Instruct the subject to wash and decontaminate hands as described in Section 11. Face the palms upward and with fingers and thumbs outstretched, apply 20 µL of virus suspension on each fingerpad. The subject will then rub each fingerpad against the thumbpad of the same hand over a total period of 40 ± 5 s. Allow the inoculum to dry for 90 ± 5 s before exposure to the test substance or control fluid.

12.2.3 Perform application of test substance or control fluid (EBSS or vehicle) in the same manner as described in 12.1.2.

12.2.4 After the hands are dry, place one finger at a time along with the thumb into a sterile vial containing 10 glass beads in 10 mL of EBSS-peptone. While holding several of the beads between the digits, rub the entire surface of the fingerpad against the thumbpad vigorously for 20 s. Drain as much of the eluent as possible back into the vial. Repeat the procedure with the remaining fingerpads. Assay at least 2 mL (~20 %) of the eluate for infectious virus.

12.2.5 Instruct the subject to decontaminate hands (12.1.6) before leaving the test area.

12.3 *Method for Handrubs:*

12.3.1 Contaminate the subject's hands using one of the two methods described in 12.1.1 or 12.2.2.

12.3.2 *Do not* pre-moisten the hands.

12.3.3 Place the volume of the test substance, as specified by the manufacturer or in the set test regimen, into the subject's cupped hands and instruct the subject to rub the test substance over the entire surface of both hands for 20 s. For a control, use an equivalent volume of EBSS instead.

12.3.4 Do not wash hands in water or dry them with paper towel.

12.3.5 Elute any remaining virus from the hands in accordance with the whole-hand (12.1.4 and 12.1.5) or fingerpad (12.2.4) contamination procedure described.

12.3.6 Decontaminate the subject's hands as described in 12.1.6.

12.4 *Assaying for Infectious Virus*— Assay the eluates and controls for infectious virus in monolayers of susceptible host cells using a plaque assay or a method based on 50 % cell culture infective dose ($TCID_{50}$).

13. Calculation of Reduction in Virus Infectivity

13.1 For handwash agents, the $\log_{10}$ reduction on the hands after the application of the test substance and post-treatment water-rinse is compared to that recovered after the application of hard water only. In the case of handrub agents, the comparison should be between the hands treated with test substance and the EBSS or other medium control.

13.2 At this time, there are no criteria for the $\log_{10}$ reduction required for a claim for effectiveness.

14. Keywords

14.1 adenovirus; antisepsis; antiseptics; calicivirus; handrubbing; handwashing; *in vivo*; norovirus; rhinovirus; rotavirus; skin sampling; viral infection; virucidal activity; virus-eliminating activity

APPENDIX

(Nonmandatory Information)

X1. VIRUSES AND THEIR HOST CELLS RECOMMENDED FOR USE IN THIS TEST PROTOCOL

X1.1 The selection of the following test viruses is based on their (*a*) relative safety to the subjects as well as experimenters, (*b*) ability to grow to titers sufficiently high for testing, (*c*) ability 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 handwash and handrub agents. Other strains or types of viruses may be substituted provided they meet the above 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 hygienic handwash agents. Therefore, caution must be exercised when substituting viruses as this may lead to variations in results from one laboratory to another.

X1.2 Human Rotavirus Wa (ATCC VR-201 8) with CV-1 (ATCC CCL-70) or MA-104 (ATCC CRL-2378.1) cells as hosts. Prior to rotavirus inoculation, cell monolayers must be washed at least three times with EBSS or Phosphate Buffered Saline (PBS) to remove the serum from the growth or seeding medium. All diluents, maintenance media, and agar overlays (if applicable) must also be free from serum. Most rotaviruses also require the presence of trypsin in the medium for infection.

X1.3 Human Rhinovirus Type 37 (ATCC VR-1147) or 14 (ATCC VR-284) and MRC-5 (ATCC CCL-171), H1-HeLa (ATCC CRL-1958), or WI-38 (ATCC CCL-75) cells for culture and infectivity assays.

X1.4 Feline calicivirus (ATCC VR-782); the cell line recommended is CrFK (ATCC CCL-94). This virus is used as a surrogate for human norovirus.

X1.5 Murine Norovirus (Washington University or other appropriate source); the cell line recommended is RAW 264.7 (ATCC TIB-71). This virus is used as another surrogate for human norovirus.

X1.6 Human Adenovirus Type 5 (ATCC VR-1516) and cell line 293 (ATCC CRL-1573) for making virus pools and Vero cells (ATCC CCL-81) for infectivity titrations.

REFERENCES

(1) 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 & bacteria: Experiments with rotavirus & Escherichia coli," *Appl. Environ. Microbiol*, Vol 55, No. 12, 1989, pp. 3113–3118.

(2) Sattar, S.A., Abebe, M., Bueti, A., Jampani, H., Newman, J., and Hua, S., "Activity of an alcohol-based hand gel against human adeno-, rhino-, and rotaviruses using the fingerpad method," *Infect. Control & Hosp. Epidemiol*, Vol 21, 2000, pp. 516–519.

(3) *Biosafety in Microbiological and Biomedical Laboratories*, 5th Edition, U.S. Department of Health and Human Services, Washington, D.C., CDC-NIH, 2010.

(4) *Laboratory Biosafety Guidelines*, 3rd Edition, Health Canada, Ottawa, ON, Canada, 2004.

(5) *Protection of Laboratory Workers from Instrument Biohazards and Infectious Diseases Transmitted by Blood, Body Fluids and Tissues* , NCCLS, 1998.

(6) *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections*, N.J. Schmid and R.W. Emmons, Eds., 6th Edition, American Public Health Association, Washington, D.C. 1989.

(7) Blackwell, J.H., Chen, J.H.S., "Effects of Various Germicidal Chemicals, on Hepatitis 2 Cell Culture and Herpes Simplex Virus, *Journal of Association of Analytical Chemists*, Vol 53, 1970, pp. 1229–1236.

(8) Bellamy, K., Alcock, R., Babb, J.R., Davies, S.G., and Ayliffe, G.A.J.,"A Test for the Assessment of "Hygienic" Hand Disinfection Using Rotavirus," *Hospital Infec.*, Vol 24, pp. 201–210.

(9) Casewell, M.W., Desai, N. "Survival of Multiply-Resistant *Klebsiella aerogenes* and other Gram-Negative Bacilli on Fingertips," *J. Hospital Infection*, Vol 4, 1983, pp. 350–360.