Designation: E2613 – 08

StandardTest Method for Determining Fungus-Eliminating Effectiveness of Hygienic Handwash and Handrub Agents Using Fingerpads of Adults[1]

This standard is issued under the fixed designation E2613; 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

Human hands are frequently in contact with other surfaces and can readily acquire transient microbial contamination. Fungi are common among these types of contaminants (**1, 2**),[2] particularly in healthcare settings and where food is handled. Standardized methods to assess the fungus-eliminating potential of handwash and handrub agents have not been available and this test method addresses the gap.

1. Scope

1.1 This test method is designed to assess the ability of hygienic handwash and handrub agents to reduce levels of fungal contamination on hands (**3**). This test method is not meant for use with surgical hand scrubs (Test Method E1115) or preoperative skin preps (Test Method E1173).

1.2 Performance of this procedure requires the knowledge of regulations pertaining to human experimentation.[3]

1.3 The test method should be performed by persons with training in microbiology in facilities designed and equipped for work with infectious agents at biosafety level 2 (**4**).

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

1.5 *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:*[4]

D1129 Terminology Relating to Water

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E1115 Test Method for Evaluation of Surgical Hand Scrub Formulations

E1173 Test Method for Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations

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

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

3. Terminology

3.1 *Definitions*—For definitions of general terms used in this test method, refer to Terminology D1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *active ingredient, n*—a substance added to a formulation specifically for the inhibition or inactivation of microorganisms.

[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.

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

[3] Federal Register, Vol 46, No. 17, Jan. 27, 1991.

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

3.2.2 *dry control*—a control to determine the number of colony forming units (CFU) of the test fungus remaining viable after the initial drying of the inoculum on the skin.

3.2.3 *handrub, n*—a liquid, gel, or foam, which is applied by rubbing to decontaminate lightly soiled hands between handwashings and generally does not require a post-treatment water rinse; such agents usually contain alcohol alone or with other active ingredients.

3.2.4 *hard water, n*—water with a defined level of hardness as calcium carbonate.

3.2.5 *hygienic handwash agent, n*—an agent generally used for handwashing by personnel in hospitals, other health-care facilities, day-care centers, nursing homes, and food-handling establishments to eliminate transient microorganisms from intact skin.

3.2.6 *input control, n*—a control to determine the number of colony forming units of the test fungus placed on each digit.

3.2.7 *neutralization*—a process which results in quenching the antifungal activity of a test material. This may be achieved through dilution of the test material(s) to reduce the antifungal activity, or through the use of chemical agents, called neutralizers, to eliminate antifungal activity.

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

3.2.9 *soil lead, 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.

3.2.10 *test formulation, n*—a formulation which incorporates antimicrobial ingredients.

3.2.11 *test organism, n*—an applied inoculum of an organism that has characteristics which allow it to be readily identified. The test organism is used to simulate a transient topical fungal contaminant. It may also be referred to as a marker organism, fungal simulant/surrogate or fungal contaminant.

3.2.12 *test vehicle, n*—the test agent without an active ingredient.

4. Summary of Test Method

4.1 This test method is conducted using 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. 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 fungal suspension is placed on a demarcated area on each fingerpad and the inoculum allowed to dry. The contaminated area is then exposed to the control (standard hard water) or test agent for the desired contact time and organisms remaining on the fingerpad are eluted and the eluates assayed for fungal CFU. Reductions in the numbers of CFU after treatment with the control and test agents are then determined. 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.

5. Significance and Use

5.1 This *in vivo* procedure is designed to test the ability of hygienic handwash or handrub agents to eliminate fungal contamination from experimentally-contaminated hands. Since the two thumbpads and all eight fingerpads can be used in any given test, it allows for the incorporation of an input control (two), control for viable cells of the test fungus remaining after the inoculum has been allowed to dry (two), fungal cells eliminated after treatment with a control or reference solution (two), and up to four replicates to assess the fungus-eliminating efficiency of the formulation under test. No more than 100 µL of the test fungal suspension is required to complete one test.

5.2 Whereas this test method is designed to work with fungi, similar ASTM standards exist for testing against viruses (Test Method E1838) and vegetative bacteria (Test Method E2276).

5.3 Infectious microorganisms left on hands after washing can be reduced further by drying the washed hands with paper, cloth, or warm air (**5**). A step for the drying of fingerpads after exposure to the control or test solution, therefore, has not been included to avoid fungal removal by the drying process itself.

5.4 This test method is not meant for testing surgical hand scrubs or preoperative skin preps.

5.5 The level of contamination with viable fungi on each fingerpad after the drying of the inoculum should be at least 10^4 CFU so that it would permit the detection of up to a 4-$\log_{10}$ reduction in the viability titer of the test organism by a test formulation under the conditions of this test. This in itself does not represent the product performance criterion, which may vary depending on the jurisdiction and the nature of the formulation being evaluated.

6. Equipment and Apparatus

6.1 *Colony counter,* any of several types may be used, for example, Quebec Colony Counter.

6.2 *Freezer,* a freezer at –70°C or lower is required to store fungal stocks.

6.3 *Handwashing sink,* a sink of sufficient size to permit subjects to wash hands without touching hands to sink surface.

6.4 *Incubator,* any incubator that can maintain a temperature suitable for the growth of *Candida albicans* and *Aspergillus niger*.

6.5 *Laminar flow cabinet,* a Class II biological safety cabinet.

6.6 *Magnetic stirrer and magnets,* Llarge enough to hold a 5-L beaker or Erlenmeyer flask for preparing culture media or other solutions.

6.7 *Membrane filtration system,* a membrane filtration system and membranes with a pore diameter of 0.45 µ m or 0.22 µm are required to sterilize heat-sensitive media/solutions.

6.8 *Positive displacement pipette,* a pipette and pipette tips that accurately can dispense 10-µL volumes.

6.9 *Refrigerator,* a refrigerator at 4 ± 2°C for storage of prepared culture media and reagents.

6.10 *Sterilizer,* any suitable steam sterilizer that produces the conditions of sterilization is acceptable.

6.11 *Timer (stop clock),* one that can be read for minutes and seconds.

6.12 *Tap water temperature regulator and temperature monitor,* to monitor and regulate water temperature at 40 ± 2°C.

6.13 *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.

7. Reagents and Materials

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

7.2 *Culture plates,* Petri plates of 100 mm diameter for culturing the fungus.

7.3 *Soil Load:*

7.3.1 A tripartite soil load, prepared from the following three stock solutions, as an alternative to serum.

7.3.2 Add 0.5 g of tryptone to 10 mL of phosphate buffer.

7.3.3 Add 0.5 g of bovine serum albumin (BSA) to 10 mL of phosphate buffer.

7.3.4 Add 0.04 g of bovine mucin to 10 mL of phosphate buffer.

7.3.5 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.

7.3.6 To obtain a 500-µL inoculum of the test inoculum, add to 340 µL of the fungal suspension 35 µL of tryptone (7.3.2), 25 µL BSA (7.3.3), and 100 µL mucin (7.3.4) stock solutions. This mixture contains approximately 2 g of total protein/L, which is roughly equivalent to the protein content of a 5 % solution of fetal bovine serum.

7.4 *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* (**6**) 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 fungal 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 activity against the test fungus. If water with a higher level of hardness is used for making the use-dilution of the test formulation, this change must be clearly justified and documented in the report.

7.5 *Test agents,* at least two samples of the test formulation shall be evaluated.

NOTE 1—Water with a standard level of hardness is also recommended as a control in this test procedure to determine any mechanical removal of the test organism(s) from the skin.

7.6 *Diluent for fungal titration,* normal saline (0.85 % NaCl) at pH 7.2 to 7.4 or an appropriate buffer.

7.7 *Eluent for fungal recovery from fingerpads,* normal saline.

7.8 *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.

7.9 *Miscellaneous laboratory ware,* automatic pipettes, pipette tips, and plastic vials for storing stock cultures.

7.10 *Broth,* trypticase soy broth (TSB) or equivalent to prepare the inoculum of *Candida albicans*.

7.11 *Agar,* sabouraud dextrose agar or equivalent to prepare the inoculum of *Aspergillus niger* and to recover and count the colonies of the two test organisms in control and test samples. The addition of any neutralizer(s) in such recovery media must first be properly validated (Test Methods E1054).

8. Test Fungi

8.1 The selection of the following test fungi 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*) ease of recovery and identification in the laboratory, and (*d*) potential to spread through contaminated hands. Other strains or types of fungi may be substituted provided they meet the preceding criteria and are approved by the relevant institutional review board.

8.1.1 *Candida albicans* (ATCC 10231). This represents non-filamentous fungi (yeast-like) and is among the most common species of *Candida* recovered as clinical isolates(**7**).

8.1.2 *Aspergillus niger* (ATCC 64958). This is an example of a filamentous fungus that is considered safe for the experimental contamination of the skin of adult subjects.

8.1.3 Other fungal species may be used provided they are safe for contamination of the hands of adult subjects and only after permission has been received for their use from the relevant institutional review board.

9. Preparation of Test Inoculum

9.1 To prepare *C. albicans* test inoculum, add 0.1 mL of a frozen stock culture to 10 mL of TSB (7.10) and incubate for 48 ± 4 h at the appropriate temperature.

9.2 To prepare a suspension of the conidia of *A. niger*, inoculate the center of a sabouraud dextrose agar plate with 0.1 mL of a frozen stock suspension and incubate for 10 days at 28°C. Harvest mycelial mats from the agar surface, homogenize with sterile glass beads in normal saline, and filter through sterile gauze to remove the hyphae.

9.3 Add soil load (7.3) to the test fungal cell suspension, if required.

9.4 Swirl, vortex, or shake the test fungal cell suspension before withdrawal of each aliquot.