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Standard Guide for Evaluation of the Effectiveness of Hand Hygiene Topical Antimicrobial Products using *ex vivo* Porcine Skin[1]

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INTRODUCTION

Use of *ex vivo* animal skin models such as pigskin has widely been used as surrogate for human skin. Pigskin model is a safe, inexpensive, accurate, and reliable platform of testing antiseptic efficacy.[2,3,4] The test guide described here utilizes sterilized pigskin to evaluate the effectiveness of hand hygiene topical antimicrobial products. The pigskin substrate is used to overcome limitations posed by exposure of human subjects to potentially pathogenic microorganisms, while offering the benefit of applicability to a wide variety of hand-washing conditions that cannot be simulated in test tubes. The microbial reduction is the difference in $\log_{10}$ value obtained from artificially contaminated pigskins after use of test formulation to the $\log_{10}$ value obtained from contaminated pigskins not exposed to the test formulation.

1. Scope

1.1 This guide is designed to demonstrate the effectiveness of hand hygiene topical antimicrobial products using pigskin as a surrogate model.

1.2 Knowledge of microbiological techniques is required for these procedures.

1.3 This standard guide can be used to evaluate topical antimicrobial handwash or handrub formulations.

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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

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

2. Referenced Documents

2.1 *ASTM Standards:*[5]

E1054 Practices for Evaluation of Inactivators of Antimicrobial Agents

E1174 Test Method for Evaluation of the Effectiveness of Healthcare Personnel Handwash Formulations

E1874 Test Method for Recovery of Microorganisms From Skin using the Cup Scrub Technique

3. Terminology

3.1 *Definitions:*

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

3.1.2 *neutralization, n*—the process for inactivating or quenching the activity of a microbicide, often achieved through physical (for example, filtration or dilution) or chemical means.

3.1.3 *resident microorganisms, n*—microorganisms that survive and multiply on the skin, forming a stable population.

[1] This guide 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] Woolwine, J. D., and Gerberding, J. L., "Effect of Testing Method on Apparent Activities of Antiviral Disinfectants and Antiseptics," *Antimicrobial Agents and Chemotherapy*, Vol 39, 1999, pp. 921–923.

[3] Bush, L. W., Benson, L. M., and White, J. H., "Pigskin as a Test Substrate for Evaluating Topical Antimicrobial Activity," *J. Clinical Microbiology*, Vol 24, 1986, pp. 343–348.

[4] McDonnel, G., Haines, K., Klein, D., Rippon, M., Walmsley, R., and Pretzer, D., "Clinical Correlation of a Skin Antisepsis Model," *J. Microbiological Methods*, Vol 35, 1999, pp. 31–35.

[5] 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.

3.1.4 *transient microorganisms, n*—microorganisms that contaminate the skin but do not form a stable population.

3.1.5 *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 microbial contaminant. It may also be referred to as a marker organism, bacterial simulant, or bacterial contaminant.

3.1.6 *test substance, n*—a leave-on or wash-off product or formulation which incorporates antimicrobial ingredient(s).

4. Summary of Guide

4.1 This guide describes procedures for testing using non-living pigskin substrates that have been shaved to remove the hair and sterilized using gamma radiation prior to use in testing. The specific procedures allow use of a test microorganism of the investigator's choice. Activity of a test substance is measured by comparing the number of test microorganisms recovered from artificially contaminated pigskins after use of an antimicrobial formulation to the number of test microorganisms recovered from contaminated pigskins not exposed to the test formulation. The antimicrobial activity of the test substance can be measured following a single wash or multiple washes, or both, in a single day. Sampling is performed using the cup scrub technique and a fluid shown to neutralize effectively the antimicrobial activity of the test formulation, and to be non-toxic to the test microorganism.

5. Significance and Use

5.1 The guide may be used to demonstrate the effectiveness of topical antimicrobial products using pigskin as a surrogate for human skin and the cup scrub technique for sampling.

5.2 The techniques described can be used to simulate Test Method E1174 and will use the pigskin substrate to overcome limitations posed by exposure of human subjects to potentially pathogenic microorganisms, while offering the benefit of applicability to a wide variety of hand-washing conditions that cannot be simulated in test tubes.

5.3 Use of the pigskin surrogate offers less expensive and higher throughput screening.

6. Apparatus

6.1 *Colony Counter*—Any of several types may be used, for example, Quebec Colony Counter or similar devices.

6.2 *Incubator*—Any incubator capable of maintaining desired temperature range.

6.3 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterilization.

6.4 *Timer (Stop-Clock)*—Type that can be read for minutes and seconds.

6.5 *Sink*—A sink of sufficient size to permit the washing of pigskins without touching the sink surface.

6.6 *Water Faucet(s)*—To be located above the sink at height which permits the rubbing of the pigskins during the washing procedure. Faucet should maintain a constant flow rate.

6.7 *Water Temperature Regulator and Temperature Monitor*—To set and maintain the water temperature at 40 °C ± 2 °C.

6.8 *Vortex Mixer*—Any suitable vortex mixer capable of mixing sample and diluent.

6.9 *Spectrophotometer*—An instrument that can measure optical density at a wavelength of 620 nm.

7. Reagents and Materials

7.1 *Sterile Bacteriological Pipettes*—10 mL capacity; 1 mL capacity and 0.1 mL capacity.

7.2 *Inoculating Loops or Sterile Swabs.*

7.3 *Gamma Sterilized Pigskins*—Pig hides can be obtained from local source, defatted, washed with water and sterilized by gamma-irradiation (or other acceptable method) and stored in a freezer (–20 °C) prior to use.

7.4 *Industrial Grade Adhesive*—Any of several types can be used; for example, epoxy or other suitable glue.

7.5 *Suitable Carrier*—For holding pigskin in place allowing mechanical manipulation (washing, rubbing, and so forth); for example, phenolic caps/closures.

7.6 *Scalpel*—Or any other appropriate cutting tool.

7.7 *Tissues or Paper Towels*—Any sterile tissue or paper towel that can be used to dry the pigskins.

7.8 *Sterile Container*—Any sterile or sterilizable container having the capacity to culture the volume of inoculum required for testing.

7.9 *Scrub Cups*—Sterile plastic/polypropylene or other suitable cylinders, height approximately 2.5 cm, inside diameter approximately 4.2 cm. Useful sizes range from approximately 1.5 cm to 4.0 cm.

7.10 *Sterile Polytetrafluoroethylene (PTFE) Scraper*—Can be fashioned in the laboratory or purchased.

7.11 *Sterile Culture Tubes,* or equivalent.

7.12 *Appropriate Bacterial Cultures.*

7.13 *Test Formulation/Substance*—Manufacturer directions for use of the test substance should be utilized and, prior to testing, the appropriate volume to be applied to the surface area of the pigskin substrate should be calculated as a ratio of the estimated volume that would exist on the hands of a human subject. If directions are not available, the investigator must determine an appropriately realistic volume.

7.14 *Sampling and Dilution Fluid*—Sterile Butterfield's phosphate buffered diluent,[6] containing an antimicrobial inactivator specific for the test formulation.

NOTE 1—Neutralizer validation should be conducted according to Practice E1054 prior to the study. Practice E1054 provides a list of neutralizers appropriate for commonly used antimicrobial agents. In some cases neutralization may be achieved by dilution alone.

[6] Horowitz, W., (Ed.), *Official Methods of Analysis of the AOAC*, 17th Ed., Sec. 6.3.03 A.(f), Chapter 6, 2000, p. 10. Official Methods of Analysis of AOAC International, Gaithersburg, MD.

7.15 *Plating Medium*—Soybean-casein digest agar or other solid media appropriately validated to support growth of the test organism, with effective neutralizers, if needed.

8. Test Substrate

8.1 The pigskin substrate is prepared by removing hair with electronic clippers and/or razor blades prior to irridiation. Care should be taken not to damage the skin or introduce any ointments or gels during preparation.

8.2 Using a sterile scalpel or other suitable tool, cut the sterile pigskin hides into applicable shapes and equal sizes.

NOTE 2—Shape and size should accommodate the coverage area of the scrub cup (7.9).

8.3 Mount the pigskin substrates to the surfaces of pre-cleaned carriers, by applying the epoxy or other suitable adhesive to the carrier. Follow the adhesive manufacturer's instructions for opening and usage of the product.

8.4 The area to be sampled is delineated by the scrub cup/sampling cylinder.

8.5 Allow enough time for the pigskin to adhere to the surface prior to step 9.2.

9. Procedure

9.1 *Test Microorganism(s):*

9.1.1 *Preparation of Test Microbial Suspension(s):*

9.1.1.1 Test species representative of the bacterial flora encountered under the conditions of use should be selected for testing.

9.1.1.2 Transfer culture(s) 2 times (once every 18 h to 24 h) into appropriate liquid growth medium. The second transfer must be into a volume of medium that will be sufficient to testing.

9.1.1.3 Alternatively, the second transfer can be to an agar plate or slant.

9.1.1.4 If preparing the challenge suspension from broth, wash culture two times by means of centrifugation and resuspend in Butterfield's phosphate buffered water.

9.1.1.5 If preparing the challenge suspension from agar plate or slant, resuspend organisms in Butterfield's phosphate-buffered diluent or equivalent.

9.1.1.6 Using a spectrophotometer or other comparator, adjust the final titer of the challenge species to 1.0×10^7 cfu/mL to 1.0×10^9 cfu/mL. Inoculum should be well mixed to disperse clumps.

9.1.1.7 Determine the titer of the challenge suspension by a standard plate count method, or equivalent.

9.2 *Treatment of Test Samples:*

9.2.1 Contaminate each test pair of pigskin substrates with an appropriate volume of the challenge species. The investigator shall determine the appropriate number of replicates to be used to achieve the desired confidence level.

9.2.2 Immediately, rub the pigskins together for 15 s to evenly distribute the inoculum while avoiding excessive loss due to dripping. Allow appropriate dry-time.

9.2.3 Apply the appropriate volume of test formulation on the pair of pigskins. See Appendix X1.

9.2.4 Immediately rub the pigskins together for 30 s to evenly distribute the product while avoiding excessive loss due to dripping. Expose for the specified contact time.

9.2.5 For leave-on products, proceed to step 9.2.9.

9.2.6 For wash-off products, rinse for 30 s under tap water regulated at 40 °C ± 2 °C.

9.2.7 Use a paper towel or tissue to blot the pigskins dry.

9.2.8 Sample using the cup scrub technique (Section 10).

9.2.9 If multiple sampling intervals are desired, procedures can be repeated using separate pairs of skin to examine each sampling interval.

9.3 *Treatment of Control Samples:*

9.3.1 Contaminate each control pair of pigskin substrates with an appropriate volume of the challenge species.

9.3.2 Rub the pigskins together for 15 s and allow appropriate dry-time.

9.3.3 Sample using the cup scrub technique (Section 10).

NOTE 3—When necessary, conduct a sterility control on a randomly selected, prepared pigskin substrate by following the procedure outlined in Section 10.

10. Sampling

10.1 Quantitative microbial counts are obtained using the cup scrub technique (see Test Method E1874). This procedure is used for test and control determination.

10.2 The treated pigskins are positioned on a flat surface.

10.3 The cylinder is pressed firmly against the pigskin surface during sampling to ensure that the sampling fluid does not leak from the delineated sampling site.

10.4 A specified volume of sterile sampling fluid (7.14) is transferred into the cylinder on elapse of the contact time.

10.5 The pigskin is then scrubbed with moderate pressure for 60 s ± 6 s using a sterile polytetrafluoroethylene (PTFE) scraper. After scrubbing, an aliquot of the sampling fluid is transferred into a sample tube and serially diluted, as necessary. This procedure is repeated for all samples.

10.6 Care must be taken during the sampling process to prevent the fluid from spilling.

11. Microbial Counts

11.1 Each sample is mixed thoroughly. Ten-fold serial dilutions of each sample are prepared in dilution fluid, as necessary. Appropriate dilutions are pour- or spread-plated in duplicate using the appropriate medium with suitable neutralizer and incubated at the appropriate temperature, ±2 °C, for 24 to 72 h, or until colonies are countable.

12. Determination of Microbial Reduction

12.1 Convert plate counts to Log_{10} values, and average the values from each pair for each sampling interval.

12.2 Determine Log_{10} reductions at each sampling interval using the following formula:

$$Log_{10}\ Reduction\ at\ Sampling\ Interval = Log_{10}\ Control\ recovery - Log_{10}\ Post-Treatment\ recovery$$