Designation: E2361 – 13 (Reapproved 2021)

Standard Guide for Testing Leave-On Products Using In-Situ Methods[1]

This standard is issued under the fixed designation E2361; 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.

1. Scope

1.1 This guide covers test methods and sampling procedure options for leave-on products for consumer and hospital personnel. Leave-on products, such as alcohol hand rubs and lotions containing antimicrobial ingredients, are increasingly marketed and used by consumers and health care personnel. These products are distinguished from conventional washing and scrubbing preparations in that they do not rely on the rinsing, physical removal, and antimicrobial action in determining their effectiveness. Although agitation and friction may serve to release organisms from the skin and folds and crevices, organisms are then killed in situ and are not rinsed from the skin surface before sampling. Appropriate test methods for the hands have been published, while other sampling methods will be needed for testing body areas other than the hands.

1.1.1 Researchers have described techniques to identify the expanded flora we now know can be present on the skin. It is impractical, if not prohibitive to attempt to recover and identify these varieties of organisms with each test. At some point in the design of a test, a decision is necessary for defining the target organisms. Should the sampling be designed to recover as much of the microflora as possible or a particular portion of it? Consideration of transient and resident, superficial and deep, or aerobic and anaerobic flora must be included in defining the objective in testing products. The recovery methods selected for any testing must be based on the projected use of the product type being tested.

1.2 Methods of recovery after application of the contaminating organisms to a part of the body other than by the agitation/rubbing of the hands against a glass petri plate also need examination. Consideration should be given to contact plating, controlled swabbing with a template, and cup scrubbing (detergent/agitation used) since the target organisms for recovery are likely to be on the superficial layers of skin.

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

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

1.5 *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:*[2]

E1174 Test Method for Evaluation of the Effectiveness of Health Care Personnel Handwash Formulations

E1327 Test Method for Evaluation of Antimicrobial Handwash Formulations by Utilizing Fingernail Regions

E2755 Test Method for Determining the Bacteria-Eliminating Effectiveness of Healthcare Personnel Hand Rub Formulations Using Hands of Adults

2.2 *European Standard:*[3]

EN1500 Chemical Disinfectants and Antiseptics-Hygienic Handrub-Test Method Requirements (phase 2/step 2) approved by CEN (Comité Européen de Normalisation)

3. Summary of Guide

3.1 In this guide, choices of recovery techniques after the use of antimicrobial products will be considered. By the nature of the distribution of the skin flora, these sampling techniques estimate the flora remaining after antimicrobial use; some of it is superficial and some hidden. An appropriate sampling method can be selected depending on product use and the importance of superficial (transient) and hidden or deep (mostly resident) flora. Recent publications have revealed a

[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.

Current edition approved Oct. 15, 2021. Published October 2021. Originally approved in 2004. Last previous edition approved in 2013 as E2361 –13. DOI: 10.1520/E2361-13R21.

[2] 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] Available from British Standards Institute (BSI), 389 Chiswick High Rd., London W4 4AL, U.K.

greater variety of organisms that populate the skin and comprise the skin microbiome (**1, 2**).[4] This information requires a larger selection of recovery media. For certain applications, such as acne studies or when recovery of the greatest diversity of organisms is desired, specific anaerobic/microaerophilic media should be used.

3.2 This guide was originally written because ASTM Subcommittee E35.15 worked on its own test method for leave-on products used without water, but found that the EN1500 protocol encompassed the test method that had been developed. In 2010, a new standard test method specifically designed to evaluate the efficacy of leave-on product was approved under the designation Test Method E2755. This guide has now been updated to cover Test Method E2755.

3.3 ASTM has Test Method E1174 to test water-aided handwash products for health-care personnel. This test method includes both wash-off and leave-on products. It has been revised (E1174–13) to include special instructions for leave-on products to use another Test Method E2755(–11) that has been published for testing leave-on hand treatment products.

3.4 This CEN type of test methodology is widely used in European and Scandinavian countries but has not been widely used in the United States, although the use of alcohol/alcohol gel hand rubs has expanded greatly here in the last few years. The underlying question is whether a test method designed for a leave-on product like alcohol or the conventional hand washing followed by sampling in a glove or plastic bag is more appropriate. There have been criticisms of test methods, such as EN1500, which was based on Rotter's methods (**3**), but published data confirm that the test is highly reliable in showing consistent reduction levels with low variation from subject to subject. Leave-on products that are not rinsed or washed off in use are primarily represented by alcohol-based hand rubs. However, other leave-on formulations have been introduced and, undoubtedly, their number will increase in the future. Often test methods designed for washing/rinsing procedures have been used for these products. When different more specific methods are required for testing, questions of methodology become clearer, and the selection of a new or different sampling method is necessary.

3.5 When a typical hand-washing product is used, the hands are wet; scrubbing and manipulation are pursued, often vigorously; and rinsing follows. Agitation here is to remove organisms and particulate and oily soil physically. Any residue of active ingredient remaining on the skin is a small fraction of the amount applied and assumed to be attached to the stratum corneum. The residual may also be absorbed over time. Ultimately, the reduction in microbial count is a combination of kill from the antimicrobial and the physical removal by agitation and rinsing.

3.6 In contrast, leave-on products, such as alcohol products intended to be applied and not rinsed off, present a different situation. There are two distinct techniques when sampling: (*1*) sampling by washing target organisms off with detergent, assuming that most of removal is transient flora, and (*2*) sampling in situ, for example, the cup scrub, swab, contact plate, or velvet block/pad that sample bacteria by impression and contact or by using fluid to remove samples so that the volume of the sample is restricted to a very small size. These different sampling methods disturb the deep or hidden flora to differing degrees. There has been an overwhelming concentration of the cup-scrub sampling method as various test methods have been developed. The combination of detergent and agitation attempts to remove as much remaining flora as possible. The best effort, however, only removes about 15 % of the full thickness flora (**4**). When other contact sampling or tape stripping are used, the distribution of bacterial colonies on the skin are mirrored as they occur; whereas, if detergent/scrubbing techniques are used, the microcolonies are dispersed yielding higher counts. Washing/scrubbing methods stir up the cells and bacteria from the deeper skin layers and release more of the hidden flora (described by Reybrouck (**5**)). This is also true of the cup scrub method that uses detergent/surfactants to detach bacteria from the skin. Contact methods sample the flora that can easily be transferred and that is conceded to be the most important in disease transmission. Williams (**6**) has stated that, "although the distinction between residents and transients must certainly be a real one, it is not to allocate the various bacterial species to one or other class with regularity."

3.7 There has been a long-time focus on the cup-scrub technique only, and it would be beneficial to look at sampling specific areas, such as Test Method E1327, which samples around the fingernail region using a toothbrush, or the use of direct contact plating when washing is not involved (**7**), as in skin prepared for surgery. This guide is intended to assess the effectiveness of application of products rubbed into the skin or on the hands when these sites are not washed between uses.

3.8 Superficially, the testing method is the same as with products that are used to scrub and wash the hands or skin in that the hands are contaminated with a recoverable transient organism and the test product applied. The similarity ends here.

3.9 If the hands are sampled after application of organisms and the test product in sequence, they are dried or gloved wet and are sampled after extensive rinsing. The stripping solution is then added for sampling to increase the release of viable organisms to be recovered. In contrast, in testing for hand rubs or leave-on products, glove sampling would seem appropriate only if sampling were performed after each contamination and product application. Since changes have been made in Test Method E1327 to sample only after the first and last applications, the applicability of this test method for products rubbed into the skin and used repeatedly without water may not be applicable for these leave-on products.

3.10 EN1500 is an adaptation of a test developed by Rotter known as the Vienna Model (**8**).

3.11 There are many publications describing and evaluating fingertip-sampling methods. One of the major criticisms of the methods is the procedure used for sampling. The tips of the fingers and thumb are sampled by rubbing against the bottom of a glass petri dish to release contaminating bacteria from

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