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

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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.

1.2 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 the 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 requirements prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:* [2]

E 1174 Test Method for Evaluation of the Effectiveness of Health Care Personnel or Consumer Handwash Formulations

E 1327 Test Method for Evaluation of Health Care Personnel Handwash Formulations by Utilizing Fingernail Regions

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.

3.2 This guide was 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.

3.3 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 **(1)**,[4] 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.

[1] This guide 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] 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.

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

3.4 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.

3.5 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 **(2)**. 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 **(3)**). 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 **(4)** 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."

3.6 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 E 1327, which samples around the fingernail region using a toothbrush, or the use of direct contact plating when washing is not involved **(5)**, 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.

3.7 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.

3.8 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 E 1327 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.

3.9 EN1500 is an adaptation of a test developed by Rotter known as the Vienna Model **(6)**.

3.10 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 these areas before and after treatment. The sampled areas are only portions of the areas treated. However, published results have shown consistent, statistically valid data. With the EN1500 test procedure, sampling is performed after a single use of the product (divided into two portions for application).

4. Significance and Use

4.1 The United States has concentrated attention and testing efforts on surgical scrubbing far more than on hand care in patient-to-patient routines. Great Britain, the originators of infection control nursing, have always had their focus on infection transmission. In the United States, published articles have documented the short exposure time for health care personnel who do wash their hands between patients. The average is less than 10 s. The ideal product for the reduction of transient flora is one that rapidly kills or removes or both the microbial load acquired during health care activities. The emphasis on rapidity is essential simply because health care personnel will not take the necessary time when using conventional hand-washing products. The use of products not intended for use with water has increased dramatically and their use is common in European countries largely because of convenience and effectiveness. A second characteristic is the level of antimicrobial action. The use of a rapid and potent active product to reduce work-acquired microbial flora is ideal.

4.2 Since the change from strictly in-vitro testing of topical antimicrobials for use on skin to simulated use testing in hand washing, prepping, site access testing, and sampling, emphasis has always been on washing hands, agitating, rubbing, and brushing with liquid on the skin site to estimate bacteria removed after testing.

4.3 The use of hard agitation has diminished with surgical scrubs without brushes or with only mild agitation and friction.

4.4 There is a history of microbial dispersal **(7)** and increase in surface bacteria from deeper layers resulting from showering **(8-10)**, washing, scrubbing, and agitation. In the normal situation on the skin, there is a superficial, surface flora and a deeper or hidden flora **(3)**. The proportion of one to the other has been addressed by Selwyn **(2)** and his judgment is that from 20 to 50 % of the flora is "deep." The intent in skin sampling has almost always been to scrub, agitate, and use surfactant to remove as many organisms as we can. In doing this, we have completely ignored the two types of flora.

4.5 Further, when the skin is treated with a cleansing agent or an antimicrobial that is subsequently rinsed away, the "deep" or "hidden" flora is pushed to the surface as the sebum replenishes the sebum from the sebaceous glands removed in