



Standard Guide for Testing Leave-On Products Using In-Situ Methods¹

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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.

1.1.1 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.

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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

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 Hand Sanitizer 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 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.

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.

3.2 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 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 (9) and increase in surface bacteria from deeper layers resulting from showering (10-12), washing, scrubbing, and agitation. In the normal situation on the skin, there is a superficial, surface flora and a deeper or hidden flora (5). The proportion of one to the other has been addressed by Selwyn (4) 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 washing. Many early investigators have looked at simpler sampling methods that we now recognize were sampling primarily the superficial transient flora.

5. Published Testing Methods for Leave-On Products

5.1 Low Volume Contamination:

5.1.1 Prior to 2010, ASTM did not have a standard test method specifically designed for testing leave-on hand hygiene products. In the absence of such a method, products were tested according to the Healthcare Personnel Handwash methodology, Test Method E1174, which was originally designed to test water-aided handwash products.

5.1.2 Test Method E1174 measures the reduction of a transient marker organism (*Serratia marcescens*) introduced to the hands in the form of a 24-h saturated liquid broth culture (4.5 mL total). Hands are sampled via the glove juice procedure. Test products are evaluated after a single application and after ten consecutive hand contamination and product application cycles. The method presents several technical issues when evaluating leave-on products. The large volume of challenge

organism often remains wet on the hands when the test product is applied thus diluting the active ingredient and leading to excessive product rub in times. Hand wetness is exacerbated over the course of the study and can result in a decline in product efficacy over multiple application cycles. Additionally, the high soil load present in the challenge suspension can further compromise the activity of the ABHR which are intended to be applied to dry, unsoiled hands.

5.1.3 In 2010, ASTM International approved a new standard test method specifically developed to evaluate ABHR and to more closely estimate the in use conditions of leave-on products (that is, dry hands which are minimally soiled). This method, designated E2755, follows the same overall design of Test Method E1174 with the exception that hands are contaminated with a greatly reduced volume of a concentrated challenge suspension (200 µL). By reducing the volume of challenge organism applied to the hands, the hands are dry and minimally soiled when product is applied. This modification enables leave-on products to be tested at typical product volumes and results in more realistic product dry times (13). Additionally soil load buildup over the course of multiple hand contamination and product application cycles is minimized.

5.2 Hygienic Hand Rub—Vienna Model:

5.2.1 When viable organisms are captured in the sampling fluid after exposure to a test product, sampling like that used in the glove juice test uses a much larger amount of fluid followed by microbiological analysis on a small sample. While in the test method in EN1500 and Rotter’s procedure (8), the volume in the plate after rubbing the fingertips on the plate’s bottom is either cultured in toto or sampled and diluted.

5.2.2 With this in-situ procedure, only the fingertips are sampled in contrast to the whole hand in the glove juice procedure. The agitation to the fingertips in the in-situ testing is more intense than 1 min of massage of the whole hand.

5.2.3 This test method has been legally mandated as the official CEN method for their member countries. It is described in this international standard as simulating practical conditions for whether a product designed as a hygienic hand rub reduces the release of transient flora in use. The criteria specified in the standard require that the mean reduction shall not be less than achieved by a reference hand rub with propan-2-ol, 60 % (v/v).

5.2.4 Rotter, in Austria, has published numerous articles describing the development of this hand-rub procedure as well as comparative studies. It has been adopted as a standard in Germany and Austria and may now be replaced with the CEN standard. Other tests with a product, for example, in-vitro microbiological testing, are required before use, depending on the specified use pattern. Users may want to examine the methodology published in the many trials described by Rotter et al (14) for more details than those described the European standard. High correlation in the reductions in counts (reduction factor) was found from subject to subject in the many published studies.

5.3 Sampling Procedure Using Fingernail Regions:

5.3.1 Mahl (15) published a sampling procedure for the subungual and fingernail regions of the hand, which is also an ASTM International standard, Test Method E1327. Again, this methodology samples a portion of the entire hand using a

technique to enhance the recovery from a difficult-to-sample area. Fingers artificially contaminated with marker organisms that can be enumerated after sampling are used. The fingernail areas are sampled with a toothbrush (manual or electric) in 7 mL of recovery fluid in a petri dish. This methodology is similar in concept to that used by Rotter (8) and the method codified in EN1500.

5.3.2 This test method offers a procedure for reliably sampling a difficult area, for instance, compared to the fingertip sampling of Rotter (8) and in EN1500. Furthermore, individual fingers or combinations of fingers can be used to test more than one test product.

5.3.3 Any of the test methods for sampling the microflora of the skin recovers only a small fraction of that flora. For example, the most often used cup-scrub procedure recovers approximately 15 %; template swabbing 3 to 20 %; and impression plating with contact plating, velvet block/pad, or tape transfer, all under 0.5 %. Selwyn (4) developed these data by comparing a sampling method to the culture results of a full thickness biopsy of the skin designated as 100 %. Another technique developed and published by Leyden et al (16) may enhance the use of some contact methods. This test method involves improved counting methodology using computer imaging of colonies on contact plates from fingerprint/handprint techniques.

5.3.4 At any rate, we are sampling only a fraction of the total skin flora and sampling the hands presents even a bigger dilemma. There are nail folds, cuticles, and fingernail spaces that collect bacteria. Price (17) himself showed that bacteria are released in each of a sequential series of twelve basins, so that a technique like the glove fluid sampling method is still only releasing a fractional portion of the total flora. The fingertips are the part of the hand most frequently in contact with people and hard surfaces. The important question becomes, “Is the fraction removed consistent?” for both pre- and post-sampling. The procedure in EN1500 does give consistent recovery, when statistical analyses are performed.

5.3.5 In selecting a test method for assessing products used with repeated applications, the reason and frequency for the applications must be considered. The following standards are published test methods that have been used to test leave-on products: Test Method E1174 and EN1500.

5.4 *Other In-Situ Sampling Methods*—In the study of the microbial flora of the skin and test methods to reduce it, a variety of in-situ sampling techniques have been used. Selwyn (4) has hypothesized and used a technique to estimate 100 % of the microflora to include both the superficial and deep flora. The very best test method compared to the skin full thickness punch biopsy was swabbing (approximately 3 to 25 %). All other procedures sampled a small percentage less than 1 %. Swabbing with a detergent and pressure may sample some of the deeper flora. The dispersible, superficial, in-situ flora is inactivated when a non-rinse product is applied. There is little agitation when a contact plate, velvet block, or tape strip is used. It is important to define the goal of the sampling. In some testing, the goal is not to recover the very last bacteria that can be found on the layer or structure of the skin.

5.5 *Contact Agar Plate (Rodac):*

5.5.1 This procedure has a history of use for sampling hard surfaces. It has been adapted for use on skin surfaces (4, 7, 18-22). These plates can be prepared with neutralizer(s). Two critical factors in this sampling method are the preparation of the agar plate so that the meniscus extends above the side of the plate and specific training in the sampling technique.

5.5.2 Comments have been made that contact sampling is not highly reproducible. On skin surfaces, the microflora are in microcolonies and not singly dispersed as occurs when detergent and agitation are used. This technique has been used in effectiveness studies in clinical settings when samples are taken at the surgical site before and after the surgical procedure.

5.5.3 A somewhat similar test method has been used involving pressing the whole hand onto an agar surface. This procedure has been upgraded (16) by use of computer imaging to enhance the reliability of counting colonies on the agar surface or from contact sampling that also might be used with velvet block or pad sampling. Many earlier studies, especially in England (4, 18), have used these contact methods.

5.5.4 We have had a tendency to believe that bacteria are uniformly distributed over the surface of the body, when, in fact, the types and numbers vary wildly over the body, often in the most inaccessible locations. Nutritional substances, humidity, and heat greatly affect the populations. Wet surface sampling used with varying surfactants and volumes of liquid sample varying portions of the skin flora. These include cup scrub, swabbing in a defined area, water pik (23) or Thram gun (similar to the water pik) devices used to automate sampling.

5.5.5 Sampling methods discussed in publications supporting a new and better test method are often highly critical of superficial sampling methods; however, these contact methods have been used in studies with success. The reader is referred to the published descriptions of how to use and prepare these mechanical samplers.

5.6 *Velvet Pad or Block*—This procedure is another contact method that involves a piece of velvet fabric attached to a carrier or a wooden block and sterilized (24). This, of course, is related to replicate plating. There are potential improvements, such as a second or sequential sample that could be implemented when this test method is used.

5.7 *Tape Stripping*—Tape stripping involves the use of transparent tape applied to a skin area with subsequent culturing of the tape to count superficial flora removed (25, 26). A 5-cm square of pressure-sensitive tape is applied to the skin, rubbed down with a square of sterile paper, removed, and placed adhesive side up in a petri dish and cultured. This procedure can be repeated (up to 20 strips have been used), but the skin becomes irritated as the process proceeds. It has been hypothesized that only one layer of skin cells is removed with one strip. The microcolony characteristics of the skin population can be observed when the tape is cultured. An evaluation of superficial flora can be made if only a few strips are used before trauma changes the skin condition.

5.8 *Cardboard Plug*—Seeberg et al. (27) used a moistened cardboard plug (6 cm²) rotated against the skin and then replica-plated on agar with repeated stamping with good count

correlation in a clinical trial of preoperative showering. This technique is a variant of replica plating with velvet.

6. Summary

6.1 In summary, Marples (28) has said that “The variety of methods employed in the investigation and quantification of skin bacteria suggest no one method is outstanding.” The investigator must look at the target population, what the intent of the sampling is and the most effective technique to use. The

total flora cannot be effectively sampled routinely. The sample is always some fraction of the total population, and an effective technique must reliably sample that fraction removed by the selected sampling method.

7. Keywords

7.1 antimicrobial products; in-situ methods; leave-on products; recovery techniques

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