American Journal of Infection Control xxx (2015) 1-4

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Major article

Comparison of 3 in vivo methods for assessment of alcohol-based hand rubs

Sarah Edmonds-Wilson MS [a,*], Esther Campbell BS [b], Kyle Fox BS [a], David Macinga PhD [a]

[a] Research & Development GOJO Industries, Inc, Akron, OH
[b] BioScience Laboratories, Bozeman, MT

Key Words:
Hand hygiene
ASTM
Test methods
Hand sanitizer

Background: Alcohol-based hand rubs (ABHRs) are the primary method of hand hygiene in health-care settings. ICPs increasingly are assessing ABHR product efficacy data as improved products and test methods are developed. As a result, ICPs need better tools and recommendations for how to assess and compare ABHRs.

Methods: Two ABHRs (70% ethanol) were tested according to 3 in vivo methods approved by ASTM International: E1174, E2755, and E2784. Log_{10} reductions were measured after a single test product use and after 10 consecutive uses at an application volume of 2 mL.

Results: The test method used had a significant influence on ABHR efficacy; however, in this study the test product (gel or foam) did not significantly influence efficacy. In addition, for all test methods, log_{10} reductions obtained after a single application were not predictive of results after 10 applications.

Conclusions: Choice of test method can significantly influence efficacy results. Therefore, when assessing antimicrobial efficacy data of hand hygiene products, ICPs should pay close attention to the test method used, and ensure that product comparisons are made head to head in the same study using the same test methodology.

Alcohol-based hand rubs (ABHRs) are typically evaluated for efficacy in a clinical laboratory setting using standards set by ASTM International. The most commonly used method in North America, the health care personnel handwash method (ASTM E1174), was originally developed to evaluate efficacy of antimicrobial handwashing products before widespread use of ABHR. ASTM E1174 has characteristics that limit its ability to accurately evaluate ABHRs.[1,2] Specifically, increasing hand wetness and a buildup of soil load from repeated application of the bacterial suspension dilutes the alcohol, which reduces alcohol's antimicrobial activity. Both the Centers for Disease Control and Prevention and the World Health Organization acknowledge weaknesses in the methods and emphasize the need to develop more effective methods.[3,4] For this reason new methods have been developed by ASTM in recent years, including ASTM E2755 and ASTM E2784, which aim to make hand contamination and product assessment more realistic.[1,5–7]

Before sale of new ABHRs into health care settings, manufacturers must evaluate their antimicrobial efficacy performance. Whereas the Food and Drug Administration currently requires evaluation of ABHR by a previous version of E1174,[8] recent articles have questioned the use of E1174 for assessment of ABHR[9] and have implied that use of the newer methods (eg, E2755) would provide different results that would invalidate conclusions made with the widely accepted E1174 standard. However, there are no published studies to date that directly compare the efficacy of an ABHR when tested by the 3 in vivo ASTM methods. The purpose of our study was to directly compare the antimicrobial efficacy results of ABHRs tested by E1174, E2755, and E2784 standards.

MATERIALS AND METHODS

Test products were Purell Advanced Instant Hand Sanitizer and Purell Advanced Instant Hand Sanitizer Foam, both containing 70% ethanol as the active ingredient, and manufactured by GOJO Industries, Inc.

Participants were recruited from the general population according to standard operating procedures for BioScience

* Address correspondence to Sarah Edmonds-Wilson, MS, GOJO Industries Inc, PO Box 991, Akron, OH 44309.
E-mail address: edmondss@gojo.com (S. Edmonds-Wilson).
Conflicts of interest: None to report.

0196-6553/$36.00 - Copyright © 2015 by the Association for Professionals in Infection Control and Epidemiology, Inc. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ajic.2015.01.025

Table 1
Comparison of in vivo test method inoculum application instructions

ASTM Method	Inoculum application instructions
E1174	Three aliquots of 1.5 mL (4.5 mL total) containing ~ 1×10^9 CFU/mL *Serratia marcescens* were placed in participant's cupped hands and distributed evenly over both hands after each application for 20 sec, then allowed to dry for 30 sec between applications and 90 sec after final application
E2755	A 0.2-mL aliquot containing ~1×10^{10} CFU/mL *S marcescens* was transferred into the participant's cupped hands and distributed evenly over both hands for 30 sec
E2784	Two 30-mL aliquots containing ~1×10^9 CFU/mL *S marcescens* were distributed and allowed to evenly saturate the surface of 2 sterile paper towels. The participant centered each hand directly above the individual paper towels, and pressed down firmly for 5 sec. The entire palm, fingers, and finger pads were in contact with the saturated towel. Hands were then held motionless and allowed to dry for 90 sec.

Laboratories following approval of the protocol by the Gallatin Institutional Review Board. Seventy-two participants were assigned randomly using the Minitab statistical computer package (Minitab Inc., State College, PA) to evaluate 1 of the 2 test products by 1 of the 3 ASTM methods, for a total of 12 participants per test configuration. For all test methods the indicator microorganism, *Serratia marcescens* (ATCC #14756), was prepared as described previously.[2,5,6] Table 1 describes the hand contamination steps for each method. For all test configurations hands were contaminated 11 times, with the first hand contamination followed by a sample for baseline microbial counts and the remaining 10 contaminations followed by product applications. Microbial samples were taken after baseline and product applications 1, 3, 7, and 10. Samples taken at product applications 3 and 7 were not processed for microbial enumeration. For product application, 2 mL gel product and 3 pumps of the foam product (about 2 mL) were dispensed into participants' cupped hands, then product was rubbed by the participant over the entire surface of the hands and fingers until dry. Samples were taken using the Glove Juice Sampling Procedure, where powder-free sterile latex gloves were placed on participants' hands, 75 mL sampling solution (previously described[2,5,6]) was introduced into each of the gloves, and hands were massaged for 60 seconds. Samples were serially diluted in a dilution solution with neutralizer (previously described[2,5,6]) and plated on tryptic soy agar plates incubated at 35°C until sufficient growth was observed. Only colonies producing a red pigment indicative of *S marcescens* were counted.

Statistical comparisons were made between test products, ASTM methods, and combinations of each to evaluate efficacy. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC) and R version 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria) with a type I error rate of 5%. Mean $\log_{10}$ reductions were calculated using $\log_{10}$ reduction values for both the left and right hand of each participant. Paired *t* tests were used to determine the differences between $\log_{10}$ reductions for application 1 and application 10 for each product and method. Two-way analysis of variance (ANOVA) models that combined gel and foam product were created to specifically examine the effects of the ASTM test method at different applications. Two-way ANOVA with interaction at both application 1 and application 10 was used to determine if product and/or test method had the greatest influence on the outcome $\log_{10}$ reduction. Student-Newman-Keuls tests and Tukey's method tests were performed to evaluate the direction and magnitude of pairwise combinations of product and test. P values $< .05$ were considered to be significant.

Table 2
Comparison of products by method: Mean baseline $\log_{10}$ recovery summary statistics among each product by ASTM International method with Student-Newman-Keuls test comparison

Product	N	Method	Mean $\log_{10}$ recovery	Standard deviation	95% Confidence interval	*P* value
Foam	12	E1174	9.21	0.250	(9.05-9.37)	<.0001*
		E2755	9.11	0.201	(8.98-9.24)	
		E2784	7.44	0.368	(7.21-7.67)	
Gel	12	E1174	9.28	0.171	(9.17-9.38)	<.0001*
		E2755	9.14	0.316	(8.94-9.34)	
		E2784	7.28	0.445	(7.00-7.56)	

*Statistically significant at the 95% confidence level.

RESULTS

Table 2 illustrates a summary of baseline results by product and ASTM method. The Student-Newman-Keuls test value for each individual mean $\log_{10}$ reduction allows for pairwise comparisons among ASTM methods. Statistically significant differences are observed where $\log_{10}$ recoveries obtained with ASTM E1174 and ASTM E2755 were equivalent, and the baseline values for the ASTM E2784 were significantly lower.

Table 3 summarizes test product $\log_{10}$ reductions by product, application, and method. Efficacy was found to increase significantly from application 1 to application 10 for the ABHR gel using all 3 test methods. Similarly, for the ABHR foam, $\log_{10}$ reductions were significantly higher at application 10 than at application 1 when tested according to E1174 and E2755. However, no significant difference ($P = .0940$) in $\log_{10}$ reduction for foam was observed from application 1 to application 10 when E2784 was used. The Student-Newman-Keuls test value for each individual mean $\log_{10}$ reduction allows for pairwise comparisons among ASTM methods at applications 1 and 10. Statistically significant differences were observed according to specific combinations of product, test, and method as shown in Table 3. These results demonstrate that efficacy is influenced by multiple factors, including product formulation, application number (1 or 10), and test method. There was a significant interaction between ASTM method and product ($P = .0272$), suggesting that it is not possible to understand efficacy by only knowing the test product; one must also consider the method. In addition, when evaluating application 10, main effect results from a 2-way ANOVA, including ASTM method ($P = .0010$) and product ($P = .2589$), are indicative of a stronger effect of the test method and a nonsignificant effect of the specific product formulation for this study.

DISCUSSION

The results presented in Table 3 indicate that in this study the ASTM method used has a significant influence on the observed efficacy of ABHR formulations. These differences are likely attributable to differences in the hand contamination procedures between the 3 methods, as highlighted in Table 1. For example, the baselines obtained with the E2784 method were significantly lower than the baselines for E1174 and E2755, and E2784 was associated with the largest $\log_{10}$ reductions. Starting with lower baseline levels of bacteria, and therefore a lower bioburden, may make killing microorganisms easier, particularly after multiple uses because there would be less influence from microorganism buildup. Furthermore, previous studies[10] have shown that rubbing the inoculum into hands makes the bacteria more difficult to kill. The E1174 and E2755 methods both include a rub-in step during inoculum application, whereas E2784 does not. This also likely