

***In Vivo* Efficacy of
Novel Alcohol-Based Hand Rubs
Utilizing the Standard Test Method ASTM E 2755
to Represent In-Use Conditions**

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ABSTRACT

Background

The current U.S. standard for evaluating the *in vivo* activity of alcohol-based hand rubs (ABHR) is the Health Care Personnel Hand Wash (HCPHW) method, ASTM E 1174. The HCPHW method was developed for the evaluation of hand wash products and does not reflect the in-use conditions of ABHR. ASTM E 2755, a recently approved Standard Test Method specifically designed for evaluation of hand rubs, is more reflective of normal use conditions.

Objective

Evaluate the *in vivo* efficacy of two novel alcohol-based hand rubs using ASTM E 2755.

Methods

Test products were a novel ABHR gel (Product A) and a novel ABHR foam (Product B) containing 70% ethanol and formulated for optimized antibacterial efficacy and skin feel. The Control Product was an NDA approved ABHR gel based on 61% ethanol and 1% chlorhexidine gluconate (CHG). Test products were evaluated on adult human hands using ASTM E 2755. Hands were contaminated by spreading 200 ml of a concentrated suspension of *Serratia marcescens* for 30 seconds. Test Product A and the Control were evaluated at an application volume of 2 ml and Test Product B was evaluated at 1.6 ml. Bacterial log reductions

were calculated after a single application and after ten consecutive product applications. Statistical analysis was conducted using one-way ANOVA ($\alpha=0.05$).

Results

Products A and B, and the Control achieved log reductions of 3.14, 3.54, and 3.37 respectively, after a single product application and were statistically equivalent ($P>0.05$). After ten consecutive hand contamination and product application cycles, Products A and B, and the Control achieved log reductions of 5.11, 3.92, and 0.75 respectively. Both Test products were statistically superior to the Control after ten product applications ($P<0.001$).

Conclusions

When tested according to E 2755 to more realistically simulate in-use conditions, two novel ABHR products were equivalent to an NDA approved product after a single use, and were superior after ten uses. Despite the presence of 1% CHG, efficacy of the Control declined after 10 uses, whereas efficacy of Test Products A and B improved. These results demonstrate that product efficacy under high frequency usage cannot be extrapolated from single use data and highlight the need to evaluate product performance after repeated use. Finally, total product formulation is a critical determinant of antibacterial efficacy.

INTRODUCTION

In the U.S., ABHR are regulated by the FDA under the 1994 Tentative Final Monograph for Health Care Antiseptic Drug Products (TFM) and are included in the product category termed Antiseptic Handwashes or Healthcare Personnel Handwashes.¹ The ASTM test method E 1174 is used to evaluate ABHR in the U.S.² E 1174 was originally designed in the 1970s to evaluate antimicrobial hand washing agents, which are lathered with the aid of water and then rinsed off. E 1174 presents several technical issues when evaluating ABHR. Most importantly, the large volume of challenge organism typically remains wet on the hands when the test product is applied thus diluting the active ingredient, compromising activity.^{3,4}

Both the CDC and WHO have noted the limitations of the current methods, and each have emphasized a need to develop better *in vivo* test methods for ABHR.^{5,6} In 2010, ASTM International approved a new standard test method specifically developed to evaluate ABHR and to more closely simulate the in-use conditions of ABHR (i.e. dry hands which are minimally soiled).⁷ This method, designated E 2755, follows the same overall design of E 1174 with the exception that hands are contaminated with a greatly reduced volume of challenge organism (200 microliter). The reduced volume leaves the hands dry and minimally soiled when product is applied, allowing typical product volumes to be tested at more realistic product dry times.

Studies were conducted to determine the efficacy of two novel ABHR formulations in comparison to an FDA approved ABHR and pre-surgical rub based on 61% ethanol and 1% CHG.

MATERIALS AND METHODS

ASTM E 2755 Methodology

Studies were carried out following ASTM E 2755-10 (Standard Test Method for Determining the Bacteria-Eliminating Effectiveness of Hand Sanitizer Formulations Using Hands of Adults) using 12 subjects for each test product.⁷ E 2755 utilizes a "Low-Volume" contamination procedure where hands are contaminated with 0.2 ml of a concentrated suspension as illustrated in Figure 1. All other aspects of the E 2755 are identical to the Healthcare Personnel Handwash method (ASTM E 1174).² A total of eleven product applications were performed and hands were sampled for surviving bacteria after the first test product application and after 10 consecutive hand contamination / product application cycles. Neutralizer was incorporated into the sampling fluid and a neutralization study per ASTM E 1054-02 was performed to ensure the neutralizer employed in this study was effective.

Test Product and Application Procedure

Product A (PURELL® Advanced Instant Hand Sanitizer) and Product B (PURELL Advanced Instant Hand Sanitizer Foam) are novel, patent pending formulations containing 70% ethanol and formulated for optimized antibacterial efficacy and skin feel. The Control Product was an NDA approved pre-surgical and ABHR gel based on 61% ethanol and 1% chlorhexidine gluconate (CHG). Test Product A and the Control were evaluated at an application volume of 2 ml and Test Product B was evaluated at 1.6 ml.

MATERIALS AND METHODS (continued)

Figure 1: Low-Volume Contamination Procedure



Grow *S. marcescens* at 35°C with vigorous shaking (~10¹⁰ CFU/ml)



Centrifuge culture at 7000 G for 10 minutes and re-suspend in 1:10 volume of fresh broth



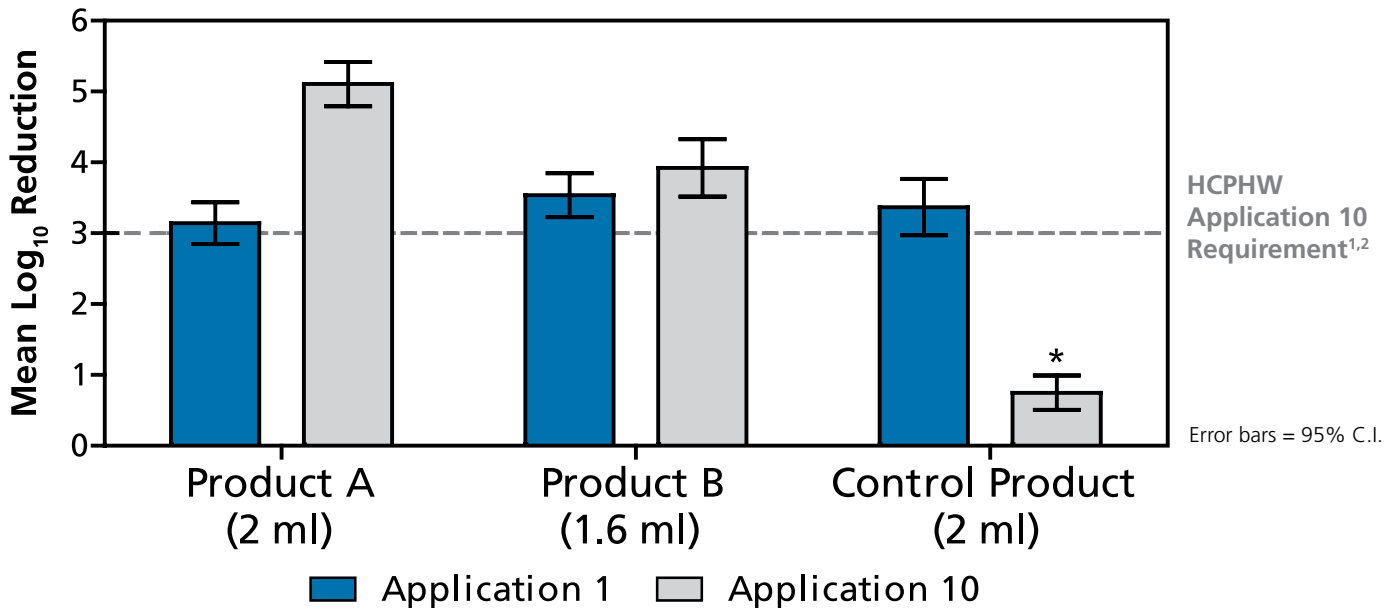
Dispense 0.2 ml of *S. marcescens* into the subjects' cupped hands



Rub contamination into all surfaces of hands for 30 sec.

RESULTS

Figure 2: Comparison of Novel ABHR to an FDA Approved Alcohol +1% CHG Control Product by ASTM E 2755



*Indicates statistical inferiority to products A and B at Application 10

SUMMARY

- When tested according to E 2755 to simulate in-use conditions, the antimicrobial efficacies of two novel ABHR were equivalent to an NDA approved control product after a single use, and were superior after ten repeated uses.
- Despite the presence of 1% CHG, efficacy of the Control Product declined after 10 uses, whereas efficacy of Test Products A and B improved.
 - Buildup of thickeners and/or other excipients may have contributed to the decline of the Control Product.
 - Further studies are needed to understand this phenomenon.

CONCLUSIONS

- Product efficacy under high-frequency usage cannot be extrapolated from single use data, highlighting the need to evaluate product performance after repeated use.
- Total product formulation is a critical determinant of antibacterial efficacy as excipient ingredients can have either a positive or negative influence on efficacy.
- To facilitate the adoption of E 2755 by regulatory agencies, appropriate efficacy standards/criteria need to be defined.

References

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