ABHR Efficacy Revisited: Influence of Alcohol Level and Product Application Volume

Poster # P273

D. R. Macinga, PhD[1]*, Esther Campbell[2], Sarah L. Edmonds, MS[1], Joseph D. Rutter[1], David J. Shumaker[1], James W. Arbogast, PhD[1]

[1]GOJO Industries, Inc., [2]Bioscience Laboratories, Inc.

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Introduction

Previous studies have established the effective concentration range for ethanol to be from 60 to 95% by volume.[1-4] Within this concentration range, activity has been demonstrated to be fairly constant.[1,2] The *in vivo* efficacy of ABHR formulations is currently evaluated using either EN1500 or ASTM E1174.[5,6] In these methods hands are contaminated using large volumes of challenge bacteria which introduces significant soil load and moisture that can inhibit the activity of the alcohol. The WHO, U.S. CDC, and others have noted the shortcomings of the current methods, and each have emphasized the need to develop better *in vivo* test methods for ABHR that more closely represent in use conditions.[3,7,8]

We hypothesized that recent studies showing greater activity from ABHR containing higher concentrations of alcohol may have made erroneous conclusions due to biases in the test methodologies and study designs.[9] To test this hypothesis, we have re-investigated the relationship of ethanol concentration and antimicrobial activity using *in vivo* methods that greatly reduce or eliminate the soil load and moisture introduced onto to the subjects' hands, more closely simulating the in-use conditions of ABHR.[10,11]

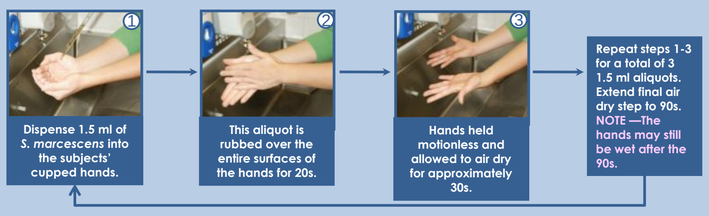
Materials and Methods

***In vitro* Time-Kill Studies:** *In vitro* time-kill studies were conducted according to ASTM E2783-10 using *Serratia marcescens* (ATCC 14756) and *Staphylococcus aureus* (ATCC 6538).[12] Test samples (9.9 ml) were exposed to 0.1 ml of the bacterial suspension for 15 seconds. Following exposure, the test product/challenge suspension was diluted 10-fold in BBP+ to neutralize antimicrobial activity, serially diluted in BBP+, and plated in duplicate on TSA with neutralizer. Resulting data were fit using a sigmoidal dose-response (variable slope).

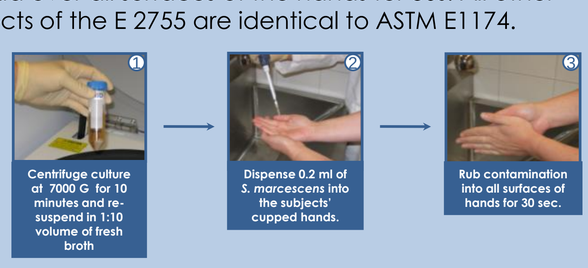
***In vivo* studies:**

Human Subjects: Studies using human subjects were conducted according to an IRB approved protocol and all subjects signed a Study Description and Informed Consent Form. Twelve (12) subjects were tested in each arm of each study.

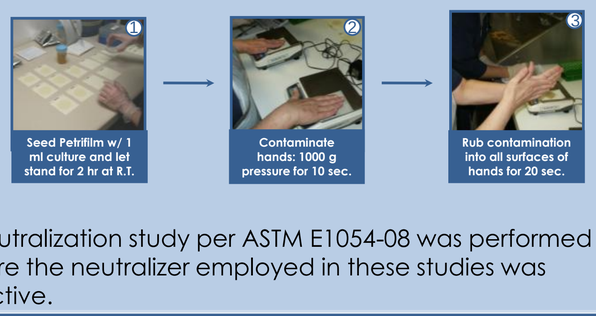
ASTM E1174 Methodology: Studies were performed according to the published method.[6] Hands were contaminated with 4.5 ml from an overnight culture of *S. marcescens* (ATCC 14756) in 3 successive 1.5 ml aliquots followed by a 90 second air dry.



ASTM E2755 Methodology: Studies were carried out following ASTM E2755-10 using 12 subjects for each test product.[10] E2755 utilizes a "Low-Volume" contamination procedure where hands are contaminated with 0.2 ml of a concentrated challenge suspension (*S. marcescens*) which is spread over all surfaces of the hands for 30s. All other aspects of the E 2755 are identical to ASTM E1174.



Contact Contamination Methodology: Hands were contaminated by touching an agar surface (3M Petrifilm) seeded with the challenge bacteria (*S. marcescens*) for 10 seconds and then spreading the organism over all surfaces of the hands for 20s.[11] This process transfers minimal soil and moisture to the hands.



A neutralization study per ASTM E1054-08 was performed to ensure the neutralizer employed in these studies was effective.

References

1. Harrington, C. and Walker, H. The Germicidal Action of Alcohol. Boston Medical and Surgical Journal CXLVIII, 548-552 (1903).
2. Price, P. B. Reevaluation of ethyl alcohol as a germicide. Arch. Surg. 60, 492-502 (1950).
3. WHO Guidelines on Hand Hygiene in Health Care. World Health Organization. 2009.
4. Food and Drug Administration. 1994. Tentative final monograph for healthcare antiseptic drug products: proposed rule. Fed. Regist. 59:31441–31450.
5. EN 1500. 1997. Chemical disinfectants and antiseptics-Hygienic handrub—test method and requirements (phase 2).
6. ASTM International. E-1174-06: standard test method for evaluation of the effectiveness of health care personnel or consumer handwash formulations. 2006. West Conshohocken, PA, ASTM International.
7. Boyce J, Pittet D. Guideline for Hand Hygiene in Health-Care Settings: Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. 51 ed. 2002.
8. Rotter, M., S. Sattar, S. Dharan, B. Allegranzi, E. Mathai, and D. Pittet. 2009. Methods to evaluate the microbicidal activities of hand-rub and hand-wash agents. J. Hosp. Infect 73:191-199.
9. Kramer, A., Rudolph, P., Kampf, G. and Pittet, D. Limited efficacy of alcohol-based hand gels. Lancet 359, 1489-1490 (2002).
10. ASTM International. E-2755-10: Standard test method for determining the bacteria-eliminating effectiveness of hand sanitizer formulations using hands of adults. 2002. West Conshohocken, PA, ASTM International.
11. Macinga, D. R. and C. M. Beausoleil. 2008. Development of a Standard Test Method for the Clinical Evaluation of Alcohol-Based Hand Sanitizers. 48th Annual ICAAC/IDSA 46th Annual Meeting, Washington, DC.
12. ASTM International. E-2783-10: Standard test method for assessment of antimicrobial activity for water miscible compounds using a time-kill procedure. 2010. West Conshohocken, PA, ASTM International.

For additional information contact:
David Macinga,
GOJO Industries, Inc.
T: 330.255.6292
email: macingad@gojo.com

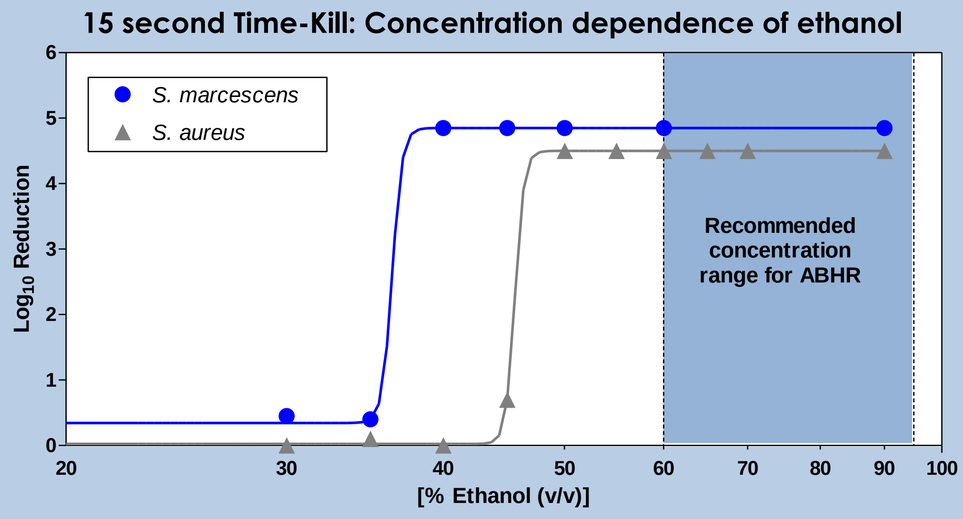
Summary

- **The minimum ethanol concentration required for rapid bactericidal activity was well below concentrations typically used in ABHR formulations.**
- **When *in vivo* test methods representative of ABHR use-conditions (ASTM E2755 and "contact contamination") were used, the activity of ABHR was not dependent on alcohol concentration within the range of 50% to 90%.**
  - The apparent dependence of activity on alcohol level when ASTM E1174 was used was due to soil load and moisture on the hands, biasing the method to favor higher alcohol concentrations.
- **Log_{10} reductions produced by ABHR correlated strongly with the volume applied to hands when tested according to E2755.**

Conclusions

- **The volume of ABHR applied to the hands has a much greater impact on efficacy than does total alcohol concentration.**
- **To ensure maximal efficacy from ABHR, healthcare workers should apply enough product to ensure adequate hand coverage and contact time.**
  - Less emphasis should be placed on the total alcohol concentration of ABHR products.
- **Further research is necessary to fully understand others factors that can influence ABHR efficacy.**

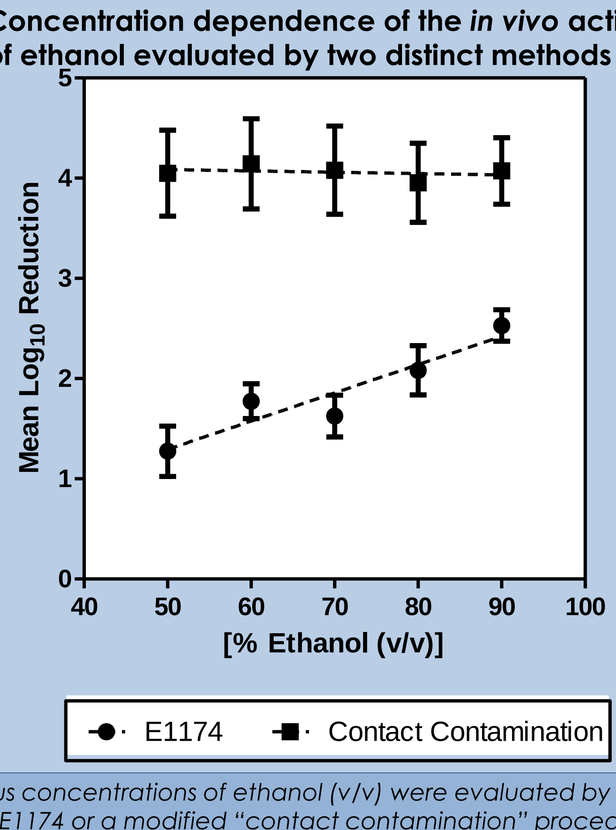
Alcohol concentrations used in ABHR are well above the bactericidal threshold concentration



Various concentrations of ethanol were challenged with S. marcescens and S. aureus in 15-s time-kill experiments . A "threshold concentration" for each organism was revealed, below which little to no activity was observed, and above which activity was maximal. Across the concentration range recommended for ABHR (60-95%), bactericidal activity was maximal.[3,4]

When using *in vivo* test methods that are more reflective of ABHR use-conditions, the activity of ABHR is not determined by alcohol level

Concentration dependence of the *in vivo* activity of ethanol evaluated by two distinct methods



Various concentrations of ethanol (v/v) were evaluated by either ASTM E1174 or a modified "contact contamination" procedure that greatly reduced the moisture and soil load introduced to the hands. When the more realistic method was used, log_{10} reductions were not dependent on ethanol concentration across the range of 50% and 90%.

Concentration dependence of the in vivo activity of ABHR evaluated by ASTM E2755

Test Product Active Ingredient	N[a]	Log_{10} Reduction
None (Vehicle)	12	1.56±0.46[b]
62% Ethanol	12	2.41±0.76
70% Ethanol	12	2.41±0.96
85% Ethanol	12	2.61±0.86

[a]*N* = number of test subjects; [b]error = Std. Dev.

Identical ABHR test formulations containing either 62%, 70% or 85% ethanol or no alcohol (Vehicle) were evaluated at 1.5 ml according to ASTM E2755. When this more realistic method was used, no differences in log_{10} reductions were observed between the test products by one-way ANOVA. All test formulation were superior to the test Vehicle.

ABHR efficacy is directly proportional to the volume of product applied to the hands

- *Various volumes of a 62% ABHR gel were evaluated according to ASTM E2755-10.*
- *Linear regression analysis of the data yielding a slope of 0.84 log_{10}/ml.*
- N = *12 subjects for each test volume and error bars represent the standard error.*