

How effective are hand antiseptics for the postcontamination treatment of hands when used as recommended?

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Background: Alcohol-based hand antiseptics are often tested using 3 or 5 mL per application, but smaller volumes are likely to be applied in clinical practice. For that reason, we investigated the efficacy of 2 different volumes of 4 marketed hand rubs when applied to contaminated hands.

Methods: Hands of 16 volunteers were contaminated with *Serratia marcescens*. Hand rub A (85% ethanol), hand rub B (60% ethanol), hand rub C (62% ethanol), and hand rub D (61% ethanol) were applied as blinded formulations, each in single applications of 2.4 or 3.6 mL. Hibiclens (4% chlorhexidine gluconate) served as the reference treatment. Each hand rub was rubbed into the hands until dry. Preintervention and postintervention bacterial populations were obtained by the glove juice method. Neutralization of residual activity was validated.

Results: A 2.4-mL aliquot of a hand rub product was sufficient to cover both hands in 96.9% of the subjects. Applied in that volume, hand rubs produced a \log_{10} -reduction in bacterial populations of 2.79 for hand rub A, 2.26 for hand rub C, 1.96 for hand rub D, and 1.90 for hand rub B. Application of 3.6 mL was significantly more effective for hand rubs B, C, and D. The reference treatment reduced test bacteria by 2.39 \log_{10} . Analysis of variance revealed that both the type of hand rub and the applied volume had a highly significant influence on the mean \log_{10} reduction on artificially contaminated hands ($P < .001$).

Conclusions: Hand rubs applied in amounts sufficient to cover both hands may not reduce the bacterial density by even 2 \log_{10} steps. Based on our findings, the general trend toward alcohol-based hand rubs should not overlook evidence of significant differences in efficacy that appear to be related primarily to a product's overall concentration of alcohol. (*Am J Infect Control* 2008;36:356-60.)

Hand hygiene in US hospitals has changed in recent years since the use of alcohol-based hand rubs was recommended by the Centers for Disease Control and Prevention (CDC) guideline for routine decontamination of hands.¹ The recommendation for alcohol-based hand rubs is based on their broader spectrum of antimicrobial activity, faster efficacy on hands, better dermal tolerance, and rather positive effect on the rate of compliance with hand hygiene protocols compared with antimicrobial soaps.²

In the US, ethanol in a concentration between 60% and 95% is considered safe and effective for the postcontamination treatment of hands.³ Most formulations used in US hospitals contain ethanol at a quite low concentration (60% to 70%).⁴ Many of the formulations with relatively low ethanol concentrations have been found to be significantly less effective than the

European reference treatment specified in EN 1500, with 3 mL applied for 30 seconds.⁵ These findings have raised concerns about the general suitability of such formulations for use in hospitals,⁵ because their efficacy is basically similar to that of a hand wash with plain soap.⁶ Many manufacturers recommend that their product be applied in the following way: "Wet hands thoroughly and rub until dry" or "wet hands thoroughly and allow to dry." The amount of hand rub necessary to achieve sufficient coverage of both hands is not really known, however. In Europe, most formulations are tested and recommended for use in a volume of 3 mL per application, an amount considered sufficient to cover most hands.^{5,7,8} Reports supporting formulations commonly used in US hospitals indicate that a similar or larger volume (eg, 5 mL) is often applied in tests of efficacy. To the best of our knowledge, smaller volumes have not been evaluated. The present study aimed to determine the efficacy of 4 ethanol-based hand antiseptics applied in volumes of 2.4 and 3.6 mL, and also to assess whether the volume applied was sufficient to completely cover both hands.

METHODS

Products and application

Four hand rub preparations were used, with the following active agent concentrations: 85% (w/w)

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ethanol, coded as hand rub A; 62% ethanol (hand rub C); 61% ethanol (hand rub D), and 60% ethanol (hand rub B). In all of the hand rubs, the ethanol concentration was within the safe and effective limits (ie, 60% to 95%) set by the Food and Drug Administration (FDA).⁵ The hand rubs were manufactured by (in alphabetical order) Advanced Sterilization Products, Irvine, CA; Bode Chemie GmbH & Co KG, Hamburg, Germany; Pfizer Consumer Healthcare, Morris Plains, NJ; and 3M Health Care, St Paul, MN. The 4 alcohol-based hand rubs were blinded to the investigator and coded as formulations A, B, C, and D. Each hand rub was applied by subjects in volumes of 2.4 and 3.6 mL, in separate experiments. Hibiclens (5 mL, applied for 30 seconds, followed by a 30-second rinse with water) was used as an FDA-approved reference treatment,⁵ to allow comparison of the data with previously obtained data. After the final data analyses, the 4 hand rubs were unblinded.

Study design

The study was performed at BioScience Laboratories Inc, Bozeman, MT. Sample size was determined using the formula provided in the FDA's *Tentative Final Monograph for Health Care Antiseptic Products: Proposed Rule*.⁵ Handwashing was done using a nonmedicated soap to remove dirt and oil from the hands. A technician instructed the subjects in the appropriate handwashing technique and verified its proper execution. The temperature of the water used for this and any subsequent handwashings was controlled at 40°C ± 2°C.

On the test day, a 5-mL aliquot of a suspension containing approximately 1.0×10^8 colony-forming units per milliliter (cfu/mL) of *Serratia marcescens* (ATCC 14756) was transferred into each subject's cupped hands in 3 aliquants of approximately 1.5, 1.5, and 2 mL. *S marcescens* was used as a test organism because it is recommended for efficacy testing in the FDA's *Tentative Final Monograph for Health Care Antiseptic Products*.⁵ The inoculum was then distributed evenly over both hands, not reaching above the wrists, by gentle continuous massage for 45 seconds. After a timed 2-minute air-dry, the glove juice sampling procedure was performed. This first contamination cycle provided the baseline bacterial population level. This was followed by a 30-second handwashing using nonmedicated soap.

Subsequently, 5 mL of the microbial inoculum was again distributed evenly over both hands, not reaching above the wrists, through gentle continuous massage for 45 seconds. After a timed 2-minute air-dry, the subjects applied their randomly assigned test product or the control product according to their assigned configuration of application. For application of the hand rub products, 2.4 or 3.6 mL was placed in each subject's

cupped hands. The subject rubbed the product into the hands until dry, covering the entire surface, including the nails. A technician assessed the product coverage on the subject's hands and recorded "yes" if the product volume was sufficient to completely cover the subject's hands or "no" if it was not.

The control product was applied according to the label instructions. After the subject wetted the hands and forearms, 5 mL of the control product was dispensed into the subject's cupped hands. The subject rubbed the hands together and lathered on hands and forearms for 30 seconds, followed by a 30-second rinse. For the sampling procedure, the hands were gloved while wet.

Determination of the preintervention and postintervention values

The subject's hands were sampled after the first contamination (baseline value) and immediately after each product application. For sampling, 75 mL of sterile stripping fluid was instilled into the gloves. The wrists were secured, and an attendant massaged the hands through the gloves in a standardized manner for 60 seconds. Then 5-mL aliquots of the glove juice were removed and diluted in 5 mL of Butterfield's phosphate-buffered saline (PBS) solution with product neutralizers (BBP++), followed by serial dilution in BBP++.

Duplicate spread plates and automated spiral plates were prepared from each of these dilutions on tryptic soy agar with product neutralizers and incubated at 30° ± 2°C for 48 h. Colonies were counted and data recorded using the computerized Q-COUNT colony-counting system.

The estimated log₁₀ number of viable microorganisms recovered from each hand was designated the *R* value, the adjusted average log₁₀ colony count measurement from each subject at each sampling time. Each *R* value was determined using the following formula:

$$R = \log_{10} [F \times C_i \times 10^{-D}],$$

where *F* is the amount of sterile sampling solution instilled into a glove (75 mL), *C_i* is the arithmetic average colony count from the 2 plates for each subject at a particular dilution level, and *D* is the dilution factor.

Validation of neutralization

The neutralizing agent was evaluated according to ASTM E 1054-02, "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents."⁹ This test comprised the following steps:

- Preparation of inoculum. A test organism suspension was prepared in PBS through the transfer of cultures from tryptic soy agar (TSA) by a loop. The concentration was adjusted to between 3×10^8 and 1×10^9 cfu/mL. Five dilutions (1:10) in PBS were prepared, resulting in a final test organism concentration of 3×10^3 to 1×10^4 cfu/mL.
- Initial cell count and test organism viability. In this step, 0.1 mL of the inoculum was transferred to 9.9 mL of PBS. Within 1 min (initial cell count) or within 30 min (test organism viability), 1 mL of the 10^{-2} dilution was plated in duplicate on TSA (total of 4 replicates).
- Sampling fluid toxicity. Here, 0.1 mL of the inoculum was transferred to 9.9 mL of sampling fluid. Within 1 min and 30 min, 1 mL of the 10^{-2} dilution was plated in duplicate on TSA (total of 4 replicates).
- Neutralizer toxicity. In this step, 0.1 mL of the inoculum was transferred to 9.9 mL of BBP++ containing the neutralizing agent. Within 1 min and 30 min, 1 mL of the 10^{-2} dilution was plated in duplicate on TSA (total of 4 replicates).
- Test material control. Here, 0.1 mL of the inoculum was transferred to 8.9 mL of PBS, followed by 1 mL of product. Within 1 min, 1 mL of the 10^{-2} dilution was plated in duplicate on TSA (total of 4 replicates).
- Neutralizer effectiveness. Here, 0.3 mL of product was transferred to 7.5 mL of sampling fluid and vortexed thoroughly. Then 0.1 mL of the inoculum was transferred to 4.9 mL of PBS containing the neutralizing agent and vortexed thoroughly. Immediately after vortexing, 5 mL of the product in the sampling fluid was added to the test tube containing inoculum and neutralizing agents and vortexed. Within 1 min, 1 mL of the 10^{-2} dilution was plated in duplicate on TSA (total of 4 replicates).

All plates were incubated at 36°C for 24 to 48 h.

Statistics

Validation of neutralization. The mean cfu/mL for any test was compared with the mean cfu/mL of the initial cell count (*t*-test). A difference was considered significant if the *P* value was $< .05$ or the difference of means was $> 0.25 \log_{10}$ -steps.

Efficacy test. Any application of a hand rub with a mean reduction of bacterial density $\geq 2 \log_{10}$ -steps was considered to fulfill the FDA's efficacy requirements for antiseptic health care products (application 1). For comparison, the mean \log_{10} -reductions of bacterial density were analyzed using analysis of variance (ANOVA; comparison of multiple means, ie, among all 4 products) and *t*-tests for independent samples (comparison of 2 means, ie, between both volumes). An analysis of variance was performed to identify the influence of the

type of product and the applied volume on the overall efficacy. *P* values $< .05$ were considered significant.

RESULTS

Validation of neutralization

The initial cell count for validation of neutralization was 2.23 ± 0.07 (Table 1). The test organism proved to be viable for 30 minutes. The sampling fluid, neutralizing agents, and 4 alcohol-based hand rubs (1:10 dilution) demonstrated no toxic effects on the test organism. Only Hibiclens (1:10 dilution) inhibited multiplication of the test organism within 1 minute. The chosen neutralizing agent was found to effectively neutralize residual activity for each hand antiseptic.

Efficacy test

The mean baseline bacterial density (\log_{10}) was between 8.84 and 9.02, indicating very high reproducibility (Table 2). The reference treatment with Hibiclens reduced the bacterial density on hands by $2.39 \pm 0.49 \log_{10}$ -steps (Table 2). A volume of 2.4 mL of alcohol-based hand rub was sufficient for most volunteers to completely cover both hands, irrespective of the selected formulation (overall, 96.9%; Table 2). A slightly lower rate was found with a volume of 3.6 mL (overall, 93.6%).

Application of a volume of 2.4 mL reduced the bacterial density by $2.79 \pm 0.54 \log_{10}$ -steps for hand rub A, by $2.26 \pm 0.67 \log_{10}$ -steps for hand rub C, by $1.96 \pm 0.68 \log_{10}$ -steps for hand rub D, and by $1.90 \pm 0.59 \log_{10}$ -steps for hand rub B. The differences in reduction produced by the 4 hand antiseptics were statistically significant ($P < .001$; ANOVA). A pairwise comparison revealed a significantly better efficacy for hand rub A ($P < .001$ compared with hand rubs B, C, and D; *t*-test for independent samples) and hand rub C ($P = .02$ compared with hand rub B).

The efficacy of all 4 hand rubs was better when a volume of 3.6 mL was applied: $3.04 \pm 0.81 \log_{10}$ -steps for hand rub A, $2.85 \pm 0.51 \log_{10}$ -steps for hand rub C, $2.63 \pm 0.59 \log_{10}$ -steps for hand rub D, and $2.53 \pm 0.60 \log_{10}$ -steps for hand rub B. The difference was statistically significant only for hand rubs B, C, and D ($P < .001$; *t*-test for independent samples), not for hand rub A ($P = .117$).

Again, the differences in reductions attributable to all 4 hand antiseptics were statistically significant ($P = .006$; ANOVA). A pairwise comparison revealed a significantly better efficacy for hand rub A compared with hand rubs B and D ($P = .003$ and $.021$, respectively; *t*-test for independent samples) and for hand rub C compared with hand rub B ($P = .019$).

An analysis of variance revealed that both the type of hand rub and the volume applied have highly significant

Table 1. Validation of neutralization of residual activity of the tested hand antiseptics (diluted 1:10) against the test organism *S marcescens*

Validation step	Test to which organism was exposed	Exposure time, min	cfu/mL, mean ± standard deviation	P value
Initial cell count	Not applicable	1	2.23 ± 0.07	Not applicable
Test organism viability	Saline	30	2.15 ± 0.03	.094
Sampling fluid toxicity	Sampling fluid	1	2.23 ± 0.03	.953
		30	2.22 ± 0.03	.810
Neutralizer toxicity	Neutralizers	1	2.26 ± 0.06	.514
		30	2.25 ± 0.02	.582
Test material control	Hand rub A	1	2.05 ± 0.04	.011*
	Hand rub B	1	2.05 ± 0.04	.012*
	Hand rub C	1	2.11 ± 0.02	.043*
	Hand rub D	1	2.11 ± 0.06	.048*
	Hibiclens	1	0 ± 0	< .001
Neutralizer effectiveness	Hand rub A (neutralized)	1	2.21 ± 0.03	.553
		30	2.15 ± 0.05	.123
	Hand rub B (neutralized)	1	2.18 ± 0.01	.275
		30	2.18 ± 0.01	.275
	Hand rub C (neutralized)	1	2.19 ± 0.01	.351
		30	2.19 ± 0.01	.319
	Hand rub D (neutralized)	1	2.19 ± 0.02	.361
		30	2.18 ± 0.01	.221
	Hibiclens (neutralized)	1	2.18 ± 0.01	.221
		30	2.16 ± 0.02	.135

*No significant difference despite $P < .05$, because the difference from the mean initial count was $> 0.25 \log_{10}$.

Table 2. Mean \log_{10} -reduction obtained with the 4 alcohol-based hand antiseptics and the reference treatment on hands artificially contaminated with *S marcescens*

Type of hand antiseptic	Applied volume, mL	Mean baseline bacterial density	Mean \log_{10} -reduction	Applied volume was sufficient to completely cover both hands
Hibiclens	5	8.98 ± 0.23	2.39 ± 0.49	Not applicable
Hand rub A	2.4	8.97 ± 0.18	2.79 ± 0.54	16 of 16 subjects (100%)
	3.6	9.01 ± 0.19	3.04 ± 0.81	15 of 16 subjects (94%)
Hand rub B	2.4	8.99 ± 0.18	1.90 ± 0.59	15 of 16 subjects (94%)
	3.6	8.84 ± 0.27	2.53 ± 0.60	16 of 16 subjects (100%)
Hand rub C	2.4	8.93 ± 0.22	2.26 ± 0.67	15 of 16 subjects (94%)
	3.6	8.94 ± 0.19	2.85 ± 0.51	13 of 16 subjects (81%)
Hand rub D	2.4	9.02 ± 0.16	1.96 ± 0.68	16 of 16 subjects (100%)
	3.6	8.94 ± 0.23	2.63 ± 0.59	16 of 16 subjects (100%)

effects on the mean \log_{10} reduction in bacterial populations on artificially contaminated hands ($P < .001$).

DISCUSSION

We were able to show for the first time that the application of 2.4 mL of an alcohol-based hand antiseptic is sufficient to wet both hands but is not necessarily sufficient to meet the FDA efficacy requirements for the first application. The application of 2.4 mL of 2 preparations with ethanol concentrations of 60% and 61% failed to achieve the FDA-required 2 \log_{10} reduction after the first application. All 3 hand antiseptics with ethanol concentrations of up to 62%, when

applied in volumes of 2.4 mL, were less effective than Hibiclens. Only the hand rub containing 85% ethanol was more effective than the reference treatment with Hibiclens. These findings confirm data from testing of hand antiseptics according to EN 1500. Gels with a total alcohol concentration $< 70\%$ were all significantly less effective than the European reference treatment of application of 60% (v/v) isopropanol for 60 seconds,⁵ which usually reduces *Escherichia coli* on hands by 4.6 \log_{10} -steps.^{10,11} Preparations with higher concentrations of ethanol or propanol ($\geq 75\%$) usually fulfill the efficacy requirements.^{5,7} The FDA's *Tentative Final Monograph for Health Care Antiseptic Products* considers an ethanol content of 60%

to 95% to be “effective” for the postcontamination treatment of hands. Based on our findings with *S marcescens* and on published evidence obtained versus *E coli*,⁵ many products with ethanol concentrations of around 60% no longer can be classified as “effective,” especially when a 2 log₁₀ reduction is not achieved when the product is used as recommended. Furthermore, we found it quite surprising that the efficacy of the 2 hand rubs with the lowest ethanol concentrations (application of 2.4 mL) yielded a lower reduction than produced by antimicrobial soap. Hand rubs with higher alcohol concentrations usually demonstrate superior efficacy to antimicrobial soaps^{5,12} and thus are recommended for routine decontamination of hands. Hand rubs with lower efficacy than antimicrobial soaps do not provide improved infection control and should be evaluated very critically.⁶

A surprising finding was that a higher volume of hand rub (3.6 mL) did not necessarily yield a higher rate of complete coverage of both hands. The overall coverage was 96.6% with a volume of 2.4 mL and slightly lower (93.6%) with a volume of 3.6 mL. In the present study, complete coverage of both hands was determined by a technician, not by the user. The subjective perception of complete coverage by the user may have been different but was not evaluated in the present study. Incomplete coverage of the hands possibly may be due to insufficient rub-in technique.¹³ In this case, even a higher volume, such as 3.6 mL, apparently is not sufficient to overcome the deficit of incomplete coverage.

We have provided results after a single application of each hand antiseptic. The test method described in the FDA's *Tentative Final Monograph for Health Care Antiseptic Products* requires a total of 10 applications within 24 hours, each following contamination with *S marcescens*. Repetitive contamination followed by hand rub application results in an increasingly thick and sticky layer on hands containing residual broth, bacteria, and cell debris¹⁴ and provides data of only limited practical relevance.¹⁵ In real life, such contamination of HCWs' hands would be classified as “visible dirt” requiring a simple handwashing according to the CDC guideline for hand hygiene,¹ not an additional contamination of hands followed by an antiseptic treatment. That is why we have chosen to study the effects of a single product application, which logically is a

more appropriate conceptualization of the practical use of an alcohol-based hand antiseptic. Based on our findings, the general trend toward the use of alcohol-based hand rubs in health care should take into account evidence of significant differences in efficacy related primarily to a product's overall alcohol concentration.

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