

Comparative Efficacy of Alcohol-Based Surgical Scrubs: The Importance of Formulation



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ABSTRACT

Alcohol-based surgical scrubs (ABSSs) are used to prevent surgical site infections. Chlorhexidine gluconate (CHG) often is added to enhance persistent germicidal activity. The aim of this study was to determine the influence of ABSS product formulation on efficacy. We evaluated three commercially available ABSS formulations and one control alcohol formulation according to the surgical scrub methodology specified by the US Food and Drug Administration (FDA). Only one ABSS formulation met FDA efficacy requirements when tested at the manufacturer's recommended dosage. In contrast, two ABSS formulations, one of which contained CHG, failed to meet the FDA acceptance criteria for a 3-log_{10} reduction on day 5, meaning the formulations did not sufficiently reduce bacteria levels on hands on the fifth day of product application. The data suggest that recommendations to include CHG in ABSS formulations should be reconsidered, and product efficacy, skin tolerability, and user acceptability should be evaluated on a case-by-case basis. *AORN J* 100 (December 2014) 641-650. © AORN, Inc, 2014. <http://dx.doi.org/10.1016/j.aorn.2014.03.013>

Key words: *surgical scrub, alcohol-based hand rub, chlorhexidine gluconate, antimicrobial.*

The purpose of preoperative hand disinfection is to eliminate transient microorganisms, reduce resident microorganisms from the hands, and maintain microorganism levels below baseline for the duration of surgery.¹ In the United States, surgical scrubs must meet both immediate kill and persistence requirements, according to the *Tentative Final Monograph for Healthcare Antiseptic Drug Products* published by the US Food and Drug Administration (FDA).²

The objective of this study was to evaluate the effect of the active ingredient and product

formulation on the antimicrobial efficacy of surgical scrubs. We asked three research questions:

- What are the relative contributions of ethanol and chlorhexidine gluconate (CHG) to both the immediate and persistent activity of surgical scrub preparations?
- Does the inclusion of CHG provide a microbiological benefit to alcohol-based surgical scrubs (ABSSs)?
- What influence does overall ABSS product formulation have on surgical scrub efficacy?

NURSING SIGNIFICANCE

The results of this study will help perioperative nurses make more informed choices for hand disinfection before donning sterile gloves prior to entering the OR or procedure room. These choices may affect patient safety as well as the risk of adverse skin reactions among nurses and other perioperative personnel.

LITERATURE REVIEW

The World Health Organization (WHO),³ Centers for Disease Control and Prevention,⁴ and AORN^{1,5} recommend using either an antimicrobial hand wash or an alcohol-based hand rub before donning sterile gloves to perform surgical procedures. Because the activity of alcohol-containing products is demonstrated to be superior to antimicrobial hand washes, the WHO guidelines state a preference for alcohol-based products.³

There is debate regarding the need for additional antimicrobial ingredients to provide added persistence activity to ABSSs. Although alcohol does not have true persistent activity, because of the extent of its immediate kill activity, regrowth of the resident microflora to baseline typically takes more than six hours.³ The WHO³ has concluded that because alcohol maintains microbial hand flora below baseline for that period, the need for a sustained effect of a product is “superfluous.” In contrast, the Centers for Disease Control and Prevention⁴ and AORN⁵ emphasize the need to use hand hygiene products with demonstrated persistent activity. The AORN “Recommended practices for hand hygiene in the perioperative setting,” last updated in 2009, states,

A standardized surgical hand scrub using an alcohol-based surgical hand rub product with demonstrated persistence and cumulative activity should be performed according to the manufacturer’s written directions for use. An alcohol and chlorhexidine product that is fast drying and has residual effect is preferred.^{5(p64)}

Recent studies suggest that the overall product formulation may be a more important determinant of efficacy than the inclusion of CHG. Kampf and Ostermeyer⁶ compared the efficacy of two waterless surgical hand scrubs and found that an 80% ethanol-only product met European efficacy requirements for presurgical hand antisepsis when tested according to the EN 12791 standard,⁷ whereas a product composed of 61% ethanol and 1% CHG did not. Rotter et al⁸ compared the activity of three ABSS formulations, attributing an immediate “fast and strong” effect entirely to the alcohol content of one of the products. In the same study, a preparation containing CHG provided some persistent effect, but the investigators noted that it was not significant and concluded that the contribution of CHG to delaying bacterial regrowth on gloved hands was “minor.”⁷ In contrast, Olson et al⁹ showed superior persistence of an ABSS containing 1% CHG after five days of use when tested according to the FDA-recommended method (ASTM E1115).¹⁰ However, the investigators failed to mention that none of the products in the study met FDA efficacy requirements for a 3-log₁₀ reduction immediately after use on day 5; thus, legitimate conclusions regarding the superiority of one product versus the others cannot be made.⁸

METHODS

The Gallatin Institutional Review Board in Bozeman, Montana, approved our protocol before subject enrollment. Using nonspecific advertising, we recruited participants from the general population who were healthy adults. We asked them to sign an informed consent form before participation, and we compensated those who completed the entire study with \$300 for their time.

Study Design

We conducted the study as described in the FDA *Tentative Final Monograph for Healthcare Antiseptic Drug Products*.² We calculated sample

size according to the tentative final monograph guidelines with Cronbach α of 0.05 to a power of 80%. Assuming a standard deviation of 0.5 based on preliminary experiments, we concluded that 16 participants were required for our sample to be sufficient. Because we conducted testing in blocks of six participants, we targeted 18 participants per study arm. Participants completed a 14-day pretest conditioning period, during which they refrained from using antimicrobial products or harsh chemicals; a five-day baseline period consisting of hand sampling for counts of resident microflora to establish baseline population values from their hands; and a five-day test period.

During the test period, participants used a product or product configuration 11 times:

- once on day 1;
- three times each on days 2, 3, and 4; and
- once on day 5.

At least one hour elapsed between the second and third product application on day 2. There was no time restriction between the first and second scrub because the second scrub occurred either 3 or 6 hours after the first scrub. Participants gloved immediately after performing the first scrub on day 2. We sampled each hand either immediately, at three hours, or at six hours after gloving. The subjects were assigned to be sampled at two of the three postscrub sampling times. At least one hour elapsed between each of the three product applications on days 3 and 4. We sampled the participants' hands for bacterial recovery and enumeration as described in the following section on days 1, 2, and 5 immediately and six hours after product application.

Study Procedures

Test products and controls are listed in [Table 1](#). We acquired study products through normal sales and distribution channels. During the baseline period, the participants rinsed their hands, including the

TABLE 1. Test Products and Controls

Test product	Active ingredient(s)	Product format
Chlorhexidine gluconate (CHG)	4% CHG	Rinse-off liquid
Alcohol plus CHG rub	61% ethanol, 1% CHG	Leave-on gel
Alcohol rub A	70% ethanol	Leave-on gel
Alcohol rub B	80% ethanol	Leave-on liquid
Alcohol control	70% ethanol	Leave-on liquid

lower two-thirds of their forearms, under running tap water for 30 seconds. During this rinse, they cleaned their fingernails and cuticles using a nail cleaner. Participants then washed their hands and forearms with 5.0 mL liquid, nonmedicated soap for 30 seconds, using water as required to develop lather. We asked them to position their hands higher than their elbows during this procedure. Participants rinsed their hands and forearms thoroughly for 30 seconds under running tap water to remove all lather, and then we performed the glove juice sampling procedure as described in the section on bacterial recovery and enumeration.

During the test period, we randomly assigned participants to use one of the test products and applied it according to the product's specific instructions for use. Different participants were assigned to each arm of the study. For the first test article in phase 1 of the study, we dispensed a total of two applications of 2 mL of alcohol rub A into the subjects' hands as follows:

- We dispensed the first application into the cupped palm of one hand, and the participant dipped the fingertips of his or her opposite hand into the product and worked it under the nails.
- The subject then spread the remaining product evenly over his or her hands and the lower two-thirds of one forearm, paying particular attention to the nails, cuticles, and interdigital spaces.

- Participants repeated this process with the second aliquot of 2 mL with the opposite hand and forearm.
- The product was allowed to air dry completely.

For the second test configuration, alcohol rub A was applied the same way except that a third application of 2 mL was used. After applying the first and second aliquots as described in the preceding text, the third aliquot was then dispensed and the subject spread the product evenly over both hands, paying particular attention to the nails, cuticles, and interdigital spaces after the other applications had dried.

To evaluate the CHG scrub, the participants wet their hands and the lower two-thirds of their forearms, after which we dispensed an application of 5 mL of product. The participants applied it using a scrub brush for 1.5 minutes per hand (ie, three minutes total) followed by a 30-second rinse, after which we applied another 5 mL with a scrub brush for participants to scrub 1.5 minutes per hand (ie, three minutes total), followed by a one-minute rinse per hand (two minutes total). In phase 2 of the study, we evaluated alcohol rub A, alcohol plus CHG rub, and the alcohol control using three applications of 2 mL as described for the second configuration in the previous paragraph. During the test period, we randomly assigned participants in groups (see tables for exact numbers per phase) to use one of the test products and applied it according to the product's specific instructions for use.

To evaluate alcohol rub B, two applications of 2 mL were dispensed into the palm of the participants' hands, and they followed the same protocol as the first configuration described previously with the following modification: additional product was dispensed into the palm of either of the subject's hands as needed to ensure that the hands remained wet for the entire application time, which lasted approximately two minutes. The subject then spread the product evenly over both hands up to the wrist, paying particular attention to the nails, cuticles, and interdigital spaces. The amount of product dispensed

for alcohol rub B was, on average, 8 mL per scrub procedure.

Bacterial recovery and enumeration. Immediately after product application, we placed oversized, powder-free sterile latex gloves on each participant's hands. One hand for each participant was randomly assigned to be sampled either immediately or six hours after product application. We dispensed 75 mL of sterile stripping fluid consisting of 0.4 g of KH_2PO_4 , 10.1 g of Na_2HPO_4 , and 1.0 g of isooctylphenoxypolyethoxyethanol in 1 L of distilled water, with an adjusted pH of 7.8, into each glove. After a 60-second massage of the hands through the gloves, we removed a 5-mL aliquot sample from each glove and diluted this in 5 mL of Butterfield's phosphate buffer solution with product neutralizers (BBP++) and then serially diluted the sample. We plated these dilutions on tryptic soy agar with product neutralizers (TSA+) and incubated them at 30° C (86° F) for 72 hours or until we observed sufficient growth. We counted colonies and recorded data using the Computerized Q-Count Plate-Counting System®.¹¹

Neutralization. We conducted a neutralization effectiveness study according to ASTM E1054-08, Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents (ASTM),¹⁰ with the exception that we added the microorganism to the neutralizer before the addition of the test formulations. The current ASTM -13 version of the method as well as the -08 version of the method specify adding the microorganism to the neutralizer before adding the product. Only the -02 version of the method specifies adding product before the neutralizer. At the time of the study, the FDA recommended performing the evaluation this way.¹⁰

Data analysis. We determined the log transformed number of viable microorganisms recovered from each hand (ie, the R value) using the formula $R = \log(75 \times C_i \times 10^D \times 2)$, where 75 is the volume (in mL) of stripping solution instilled into each glove, C_i is the arithmetic average colony

count of the plate counts at a particular dilution, D is the dilution factor, and 2 is the neutralization dilution. We generated statistical calculations of means and standard deviations on the log₁₀ recovery data from baseline samples, postproduct application samples, and the log₁₀ differences between baseline and postproduct application samples using Minitab® 15 statistical software.¹² We calculated descriptive statistics and confidence intervals using the 0.05 level of significance for type I (α) error. Test product comparisons were performed using a one-way analysis of variance with Bonferroni post hoc analysis whereby α = 0.05 using GraphPad Prism 5.04.¹³

RESULTS

Table 2 presents the results of phase 1 of this study, during which we compared the efficacy of a CHG scrub and an ABSS formulation. Both test products met all FDA efficacy requirements; however, performance of the CHG scrub and alcohol rub A differed considerably throughout the study. The immediate activity of the CHG scrub was relatively low on day 1 (mean log₁₀ reduction = 1.35), but we found that it increased each day during the course of the study such that the mean immediate log₁₀ reduction on day 5 (3.77) was significantly higher than that on days 1 and 2 (P < .001). In contrast, the mean log₁₀ reductions for each configuration of alcohol rub A started out higher than that for the CHG scrub on day 1 but did not increase significantly during the course of the study. When we compared the activities of the two test products, we noted significant differences. On day 1, the immediate log₁₀ reduction for alcohol rub A when applied with three applications of 2 mL was significantly higher than that for the CHG scrub (P < .0001). The persistent activity of alcohol rub A in both configurations, as measured by the log reductions at six hours, was significantly greater than that of the CHG scrub on days 1 (P < .0001) and 2 (P < .05). There were no significant differences in persistent activity on day 5.

Table 3 presents the results of phase 2 of this study. All of the test products, including the 70%

TABLE 2. Phase 1 Results

Sample	Application instructions	n ^a	Day 1		Day 2		Day 5	
			Immediate LR (95% CI) ^b	6-Hour LR (95% CI)	Immediate LR (95% CI)	6-Hour LR (95% CI)	Immediate LR (95% CI)	6-Hour LR (95% CI)
Alcohol rub A	3 × 2 mL	19	3.08 (2.56-3.60)	2.29 (1.75-2.84)	3.38 (3.11-3.66)	2.51 (1.91-3.11)	3.02 (2.78-3.25)	2.64 (2.15-3.13)
Chlorhexidine gluconate scrub	2 × 2 mL	18	2.31 (1.85-2.77)	2.18 (1.73-2.64)	2.92 (2.42-3.43)	2.46 (2.05-2.88)	3.15 (2.71-3.59)	2.87 (2.43-3.32)
US Food and Drug Administration criteria	—	—	1.35 (0.89-1.80)	0.49 (0.21-0.76)	2.33 (1.71-2.96)	1.33 (0.93-1.72)	3.77 (3.22-4.32)	2.75 (2.08-3.41)
			≥ 1	> 0	≥ 2	NA	≥ 3	NA

LR = log reduction; CI = confidence interval; NA = not applicable.
^a The number of participants who completed all configurations for all days.
^b Log₁₀ reduction of bacteria (95% CI).

TABLE 3. Phase 2 Results

Sample	n ^a	Day 1		Day 2		Day 5	
		Immediate LR (95% CI) ^b	6-Hour LR (95% CI)	Immediate LR (95% CI)	6-Hour LR (95% CI)	Immediate LR (95% CI)	6-Hour LR (95% CI)
Alcohol control	19	1.76 (1.41-2.12)	0.50 (0-1.06)	1.91 (1.66-2.16)	0.26 (0-0.58)	2.07 (1.75-2.39)	0.68 (0.25-1.10)
Alcohol rub A	18	2.71 (2.41-3.01)	2.55 (2.26-2.83)	2.87 (2.59-3.15)	2.57 (2.37-2.77)	3.06 (2.84-3.28)	2.53 (2.15-2.91)
Alcohol rub B	19	2.13 (1.93-2.31)	0.88 (0.61-1.16)	2.43 (2.17-2.69)	1.07 (0.76-1.38)	2.43 (2.14-2.73)	1.48 (1.07-1.89)
Alcohol plus chlorhexidine gluconate rub	19	2.21 (1.86-2.56)	2.65 (2.27-3.03)	2.34 (2.02-2.67)	2.76 (2.36-3.17)	2.70 (2.38-3.03)	3.06 (2.78-3.33)
US Food and Drug Administration criteria	—	≥ 1	> 0	> 2	NA	> 3	NA

LR = log reduction; CI = confidence interval; NA = not applicable.
^a The number of participants who completed all configurations for all days.
^b Log₁₀ reduction (95% CI).

alcohol control, met FDA immediate log₁₀ reduction requirements on days 1 and 2, and all met the FDA persistence requirement of maintaining microbial counts below baseline for six hours on day 1. However, alcohol rub A was the only product to meet the FDA requirement of a 3-log₁₀ reduction immediately after use on day 5. A summary of the statistical analysis is presented in Table 4. In comparison to the 70% alcohol control, alcohol rub A exhibited significantly greater immediate log₁₀ reductions on each day. None of the other products exhibited immediate activity that was significantly different from the alcohol control. At six hours, both alcohol rub A and alcohol plus CHG exhibited significantly greater log₁₀ reductions than both the alcohol control and alcohol rub B on all three days. Alcohol rub A and alcohol plus CHG were not significantly different at any time point.

DISCUSSION

The results presented here show the importance of product formulation on surgical scrub efficacy and further call into question the presumed benefit of CHG in surgical scrub formulations. The results of phase 1 demonstrate the superior immediate activity of a waterless ABSS formulation compared with a water-aided CHG-based scrub and highlight the need to use CHG-based scrubs several times before the immediate activity becomes equivalent to that of ethanol-based formulations. Furthermore, the results show that despite the lack of true “persistence,” properly formulated ABSSs can reduce and maintain microbial counts to a better degree than CHG-based scrubs. These results are consistent with previous findings that an ABSS formulation was significantly more effective than a 4% CHG surgical scrub at reducing bacteria counts on hands for surgeries lasting more than three hours.¹⁴ The apparent “persistence” of alcohol-based formulations is likely because of the sublethal effects of alcohol, which have been shown to slow regrowth of surviving organisms.^{15,16} It is important to note that in our study, the CHG scrub did not achieve persistent activity equivalent to alcohol rub A, as

TABLE 4. Summary of Statistical Analysis for Phase 2

Statistical comparison ^a	Day 1		Day 2		Day 5	
	Immediate	6 Hours	Immediate	6 Hours	Immediate	6 Hours
Alcohol rub A versus alcohol control	$P < .05$	$P < .0001$	$P < .01$	$P < .0001$	$P < .01$	$P < .0001$
Alcohol rub B versus alcohol control	NS	NS	NS	$P < .05$	NS	NS
Alcohol plus chlorhexidine gluconate (CHG) rub versus alcohol control	NS	$P < .0001$	NS	$P < .0001$	NS	$P < .0001$
Alcohol rub A versus alcohol rub B	NS	$P < .0001$	NS	$P < .0001$	NS	$P < .001$
Alcohol plus CHG rub versus alcohol rub B	NS	$P < .0001$	NS	$P < .0001$	NS	$P < .0001$
Alcohol rub A versus alcohol plus CHG rub	NS	NS	NS	NS	NS	NS

NS = not significant.
^a The test listed on the left is statistically superior where statistical significance is indicated.

measured by the six-hour \log_{10} reduction, until after the 11th product application on day 5. Considering both the immediate and six-hour reductions, we found that the efficacy of the CHG scrub was inferior to that of alcohol rub A until the fifth day of use.

In clinical practice, surgical scrubs should provide a high level of antimicrobial kill after single use and not require multiple uses for the efficacy to build up to achieve that high level. It is important to note that “because chlorhexidine is a cationic molecule, its activity can be reduced by natural soaps, various inorganic anions, nonionic surfactants, and hand creams containing anionic emulsifying agents.”^{4(p13)} Because the aforementioned ingredients are nearly ubiquitous in personal care, hand hygiene, and the environmental cleaning products that health care workers encounter on a daily basis, both in their workplace and household, it is likely that residual CHG on the skin could be inactivated before five consecutive days of use, and therefore the reductions observed in this controlled study are highly unlikely to be realized in clinical settings.

The results of phase 2 highlight the influence of ABSS formulation on efficacy. Alcohol rub A contains the same active ingredient (70% ethanol) as the alcohol control but was statistically superior

with regard to efficacy compared with the control at each time point. Furthermore, alcohol rub B did not exhibit superior immediate efficacy compared with the 70% alcohol control despite having a higher ethanol concentration. These data demonstrate that overall product formulation has a greater effect on efficacy than alcohol concentration alone. These data are consistent with the findings of Suchomel et al,¹⁷ which demonstrated that when levels of a moisturizer in an ethanol-based ABSS were too high, both immediate and three-hour sustained activity were inhibited. The inclusion of 1% CHG in the alcohol plus CHG rub did not enhance immediate efficacy, as demonstrated by the fact that immediate \log_{10} reductions were not significantly different from those of the alcohol control on any of the test days. In fact, the alcohol plus CHG rub failed to meet FDA efficacy requirements for immediate kill on day 5, corroborating previous reports that the immediate efficacy of this product may be inadequate.^{6,9} The inclusion of 1% CHG in the alcohol plus CHG rub did provide added persistence, as shown by the fact that \log_{10} reductions at six hours were statistically greater than those for the alcohol control and alcohol rub B. However, the \log_{10} reductions at six hours for alcohol plus CHG and alcohol rub A were not significantly different. These data support the

KEY TAKEAWAYS FOR CLINICAL PRACTICE

CHG Does Not Improve the Efficacy of Alcohol-Based Surgical Scrubs

Why Did We Do This Research?

The objective of this study was to see how the active ingredient and product formulation affect the antimicrobial effects of alcohol-based surgical scrubs (ABSSs).

What Did We Find?

- Including chlorhexidine gluconate (CHG) in ABSS formulations is not needed. The most important criteria for choosing an ABSS is that it has a demonstrated ability to meet US Food and Drug Administration (FDA) or other regulatory agency efficacy criteria, skin tolerability, and end-user acceptance—not whether it contains CHG.
- ABSS formulations and practices can negate the effects of CHG.

How Can Clinicians Use These Results?

- **Clinicians:** Nurses should reconsider including CHG as an ABSS choice criterion and evaluate ABSS formulations carefully to ensure they meet FDA requirements.
- **Managers:** Members of a committee that includes managers, infection control personnel, perioperative team members, and medical staff should evaluate all ABSSs in use in the OR and determine which scrubs will be used based on product formulation and the research that supports their use.
- **Educators:** Educators should provide information to staff members about choosing ABSS formulations and the effects of hand hygiene practices on the efficacy of ABSSs.

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conclusions of Rotter et al⁸ that the persistent effect of CHG may not be necessary and further demonstrate that the inclusion of CHG does not guarantee that an ABSS will meet efficacy requirements.

LIMITATIONS

This study used a standardized, FDA-accepted, laboratory-based clinical study method. However, the sample size was relatively small, which limited the ability to identify statistical differences between treatment groups. Furthermore, the alcohol-only and alcohol plus CHG rubs evaluated were commercial products containing different alcohol concentrations and different nonactive ingredients, which likely affected overall efficacy. To fully investigate the relative effects of alcohol and CHG on surgical scrub efficacy, a series of test products differing only by the presence or absence of alcohol and CHG at fixed concentrations would be needed. It is unknown whether the differences in antimicrobial efficacy observed in this study would translate to differences in surgical site infection rates. In clinical practice, surgical site infection rates are influenced by many factors beyond the inherent

efficacy of surgical hand disinfectants. Finally, we did not investigate other properties beyond antimicrobial efficacy, such as skin tolerability and end-user acceptability, which may directly affect end-user compliance and indirectly affect surgical site infection rates.

RECOMMENDATIONS

The data presented here add to a growing body of evidence calling into question a long-held assumption that inclusion of CHG in ABSSs provides an added benefit.^{6,8,9} Perioperative nurses should be diligent when evaluating ABSS formulations for use in the OR to ensure that the product, first and foremost, meets FDA efficacy requirements. Without evidence to clearly demonstrate that CHG provides significant microbiological or clinical benefit, and with the possibility of negative effects of CHG, such as irritation, sensitization, and antimicrobial resistance,^{4,18-20} perioperative nurses should reconsider the inclusion of this agent as a criterion for choosing an ABSS.

These findings highlight the need for continued education regarding how to scrutinize the data

supporting formulations of ABSS products. Further education regarding the benefits and risks of hand hygiene active ingredients also is warranted. Studies should be performed in a clinical setting to further evaluate the effects of CHG and overall ABSS product formulation on effectiveness, skin tolerability, and end-user acceptability.

CONCLUSION

Our data show that overall product formulation has the greatest effect on the efficacy of surgical scrubs and that inclusion of CHG in ABSS formulations is not necessary. Therefore, the choice of a surgical scrub should not be based on the presence or absence of CHG. The most important criteria for choosing a surgical scrub are, and should remain, a demonstrated ability to meet efficacy criteria established by the FDA (or other regulatory agency), skin tolerability, and end-user acceptance—whether or not the product contains CHG. **AORN**

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- You can create a basic promotional flyer by clicking a button.
- Easy access to more than a dozen different parts of the application at any time allows you to complete information as you receive it.
- Paperwork and filing are eliminated because your approved application and post-activity reports are stored online, and the certificate of attendance and evaluation forms are generated automatically.

For more information, visit www.aorn.org/Education/CEApprovalProcess.