

Bacterial reduction of alcohol-based liquid and gel products on hands soiled with blood

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The antibacterial efficacy of three alcohol-based products (liquid and gel) were tested on the hands with blood and contaminated with *Serratia marcescens* (ATCC 14756), using EN 1500 procedures in 14 healthy volunteers. The alcohol-based products tested, either gel or liquid-based, reached bacterial reduction levels higher than 99.9% in the presence of blood and did not differ significantly (ANOVA test; $P = 0.614$).

Key Words: Analysis of variance; anti-infective agents; local agents/administration and dosage; alcohols; Brazil; colony count; microbial; cross-over studies; ethanol; gels; handwashing; hygiene/standards; *Serratia marcescens*.

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Alcohol is the preferred agent for hand hygiene because of antimicrobial activity, requiring less time, resulting in less skin irritation, and improving hand hygiene adherence. Health care workers (HCW) should wash hands with soap and water when hands are visibly dirty with blood or other organic material (OM).¹⁻³ Studies have shown HCW low adherence to the standard precautions, and their hands may have contact with blood/OM.⁴⁻⁸

It has been described that liquid-based alcohol products have antibacterial activity in the hands with blood.^{9,10} We evaluated the antibacterial activity of 3 alcohol-based products (2 gel based and 1 liquid) on the hands pre-exposed to blood, and we hypothesized

that gel-based alcohol also has antibacterial activity in the hands with blood.

METHODS

This was a controlled, experimental, laboratory-based study with a cross-over design, conducted on the same day, and blinded for the volunteers as well as for the microbiology personnel. The alcohol-based products tested (A, B, and C) in the dirty hands with sterile sheep blood and contaminated with *Serratia marcescens* ATCC 14756 were all ethanol based: product A, 62% gel-based (Purell, GOJO, Akron, OH); product B, 70% gel based (Álcool Gel Dr Clean, Dr Clean, São Paulo, Brazil); product C, 70% liquid based with 2% glycerol (Álcool glicerinado; Rio Química, São Paulo, Brazil) (all products provide free of charge by manufacturers).

To assess the effect of blood on the antimicrobial activity of the alcoholic products, either gel and liquid based, we applied the procedure of the European Standard EN1500 to determine the initial count (IC), the final count (FC), and the reduction factor (RF). EN 1500 requires 12 to 15 volunteers and is described elsewhere.¹¹⁻¹³

The analysis of variance (ANOVA) was used to assess differences in mean \log_{10} RF among the test products, considering $P < .05$ as statistically significant. The volunteers enrolled were 14 healthy adults with no skin problems, age ranging between 18 and 55 years (mean, 31; median, 25); 1 participant was male; 6 nursing students, 3 administrative personnel, and 5 HCW (physician/administrative nurses). They participated in training sessions and signed the consent form.

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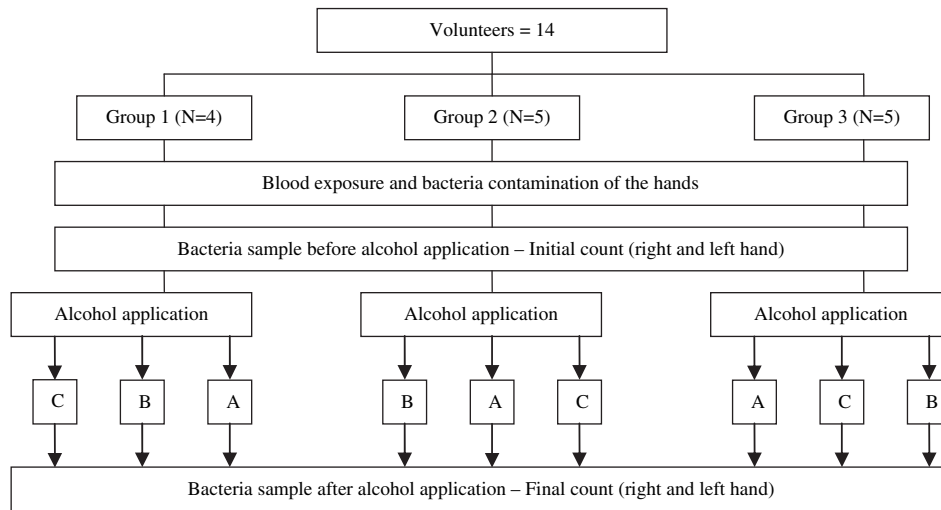


Fig 1. General study scheme to obtain the initial and final count of *S Marcescens* (224 samples). (A) 62% Ethanol gel based. (B) 70% Ethanol gel based. (C) 70% Ethanol liquid based with 2% glycerol.

Volunteers were randomized in 3 groups, and each one used the 3 products in different sequences (Fig 1).

The procedures described were followed by each volunteer¹¹: Hands were washed with soft soap, rinsed, and dried. Sterile sheep blood (1.2 mL) was applied on the palm of the hands by a sterile syringe, rubbed over both hands, and air-dried for 2 minutes and immersed halfway to the metacarpals in the fluid containing 2.0×10^8 colony-forming units (CFU)/mL of *S marcescens* for 5 seconds and air-dried for 3 minutes. Next, the fingertips of each hand were rubbed for 1 minute in each Petri dish containing 10 mL of tryptone soya broth to obtain the IC.

Each one of the products was applied onto the hands, at a time described in Figure 1. Three milliliters of product A, B, or C was applied by sterile syringe and vigorously rubbed thoroughly over their entire surface (not exceeding 60 seconds). Next, the fingers were rinsed in running tap water for 5 seconds, and the excess water was shaken off.

FC was obtained as described to obtain IC. The CFU were counted after 24 and 48 hours of incubation at 36°C ($\pm 1^\circ\text{C}$) and transformed to the decimal logarithm. The \log_{10} counts of the left and right hands of each participant were averaged separately for IC and FC. The \log_{10} RF for each subject was established (individuals' \log_{10} IC – \log_{10} FC), and the arithmetic means of all subjects' log RF were calculated for each test product.

RESULTS

The IC of the hands revealed successful and equal contamination with *S marcescens* for the 3 products (6.30-7.65 \log_{10}) (Friedman test; $P = .1102$). The 3 alcohol-based products tested on the hands artificially

Table 1. Bacterial mean \log_{10} reduction factor comparison of the test products used on the hands with blood

| Product | Initial count, mean (SD) | Final count, mean (SD) | Reduction factor, mean (SD) |
|---------|--------------------------|------------------------|-----------------------------|
| A | 6.95 (0.36) | 3.65 (0.61) | 3.29 (0.77) |
| B | 6.97 (0.29) | 3.62 (0.69) | 3.36 (0.75) |
| C | 7.05 (0.37) | 3.49 (0.60) | 3.56 (0.72) |

NOTE. $P = .614$ (A, B, and C; not significant). Product A, 62% ethanol gel based; product B, 70% ethanol gel based; product C, 70% ethanol-liquid-based with 2% glycerol. SD, standard deviation.

dirty with blood showed a bacterial mean RF higher than 3 \log_{10} . The product C showed higher RF \log_{10} than B, which in turn was higher than A, but there was no significant difference among them (ANOVA test; $P = .614$) (Table 1).

DISCUSSION

The 3 products tested produced bacterial reduction in presence of blood by more than 99.9%. Alcohol-based products may be affected by a number of factors including the presence of OM on the hands.¹⁴ In our study, we used sterile sheep blood to simulate OM, as also done by 2 studies.^{9,10} One evaluated 7 hand hygiene products in colonizing flora on the hands with and without sterile sheep blood and found that both liquid-based alcohol products (70% isopropyl alcohol and 70% ethanol alcohol plus 0.5% chlorhexidine gluconate) led to greater reduction in colonizing flora of hands exposed to blood when compared with detergent-based products.⁹ Another study used artificial contamination on the hands with *Escherichia coli*

to test 6 liquid-based alcohol products, with and without sterile sheep blood in different exposure times (30 seconds and 1 minute), showing that longer exposures to hand hygiene products improved bacterial reduction in hands with and without blood.¹⁰

Both studies found that alcohol-based products have antimicrobial activity when blood is present, but they did not test a gel-based alcohol product. Although there is some concern on the limited efficacy of gel-based product containing alcohol up to 70%,¹⁵ our study did not show any significant difference between 70% liquid-based alcohol and 62% gel-based product ($P = .614$).

In conclusion, alcohol-based products, including the gel based, have activity against *S marcescens* even in the presence of blood and could be used when hands have contact with blood or OM and handwashing is not possible.

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