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# Influence of Applied Volume on Efficacy of 3-Minute Surgical Reference Disinfection Method prEN 12791

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**For assessment of the efficacy of surgical hand disinfection, European reference method prEN 12791 prescribes that the hands must be kept wet with the reference alcohol for 3 min regardless of the applied volume. The aim of this study was to determine whether the applied volume of the reference disinfectant *n*-propanol (60%, vol/vol) influences the effect on the resident hand flora. Ten experiments with 200 reference disinfections were analyzed. Hands were washed for 1 min with soap. The bacterial prevalue was obtained by rubbing fingertips in tryptic soy broth for 1 min. After this, each subject treated the hands with *n*-propanol (60%, vol/vol) by using as many portions as necessary to keep hands wet for a total of 3 min. Bacterial postvalues (immediate effect) were obtained for one hand, and the other hand was gloved for 3 h. After the gloves were taken off, a second postvalue was obtained (sustained effect). Most surgical reference disinfections (73%) were achieved with 9 ml of the reference alcohol, followed by 12 ml (24%) and 6 ml (3%). There was no significant difference between the mean $\log_{10}$ reduction values for the three treatment groups, both in terms of the immediate effect ($P = 0.333$, as determined by analysis of variance) and in terms of the sustained effect ($P = 0.442$). A higher number of portions did not correlate with a higher reduction factor (for immediate effect, Pearson's correlation coefficient = −0.028 [$P = 0.689$]; for sustained effect, Pearson's correlation coefficient = 0.059 [$P = 0.404$]). If the hands were kept wet with the reference alcohol for the total application time, the applied volume could vary, but this did not alter the efficacy.**

With the publication of the new Centers for Disease Control and Prevention guideline on hand hygiene, new interest on this topic has emerged worldwide. For postcontamination treatment of hands a clear recommendation was given in favor of alcohol-based hand rubs (3). For preoperative treatment of hands, however, no clear recommendation was provided in the United States guideline (3), although remarkable differences in the effects on resident hand bacteria were described for different agents (6). In Europe, however, alcohol-based hand rubs are thought to have better efficacy and to result in better dermal tolerance and are therefore preferred to antimicrobial soaps (15). There are two principal options for preoperative treatment of hands: use of an alcohol-based hand rub (surgical hand disinfection) or use of an antimicrobial liquid soap (surgical hand scrub). Evaluation of the efficacy of preparations for surgical hand antisepsis is based on different test methods. In the United States, in 1994 a method was published which was designed to test preparations for surgical hand scrubbing but which is difficult to apply to hand rub formulations (1). No reference treatment is included in the test method. In the European reference test method prEN 12791 (4), a preparation, regardless of its type (hand rub or hand scrub), is always compared to a reference treatment, which is the application of a variable number of 3-ml portions of 60% (vol/vol) *n*-propanol for at least 3 min (4). The hands must be kept wet with the alcohol for the total application time, but a specific volume is not recommended and the volume may vary from 6 to 12 ml or even more (4). This treatment was chosen as the treatment with the greatest effect on resident skin bacteria (18). The test principle of including a reference treatment has been used successfully in various studies in order to evaluate the comparative efficacies of various preparations for surgical hand disinfection (5, 8, 16). That is why we investigated whether the applied volume has any effect on the efficacy of the reference alcohol applied for surgical hand disinfection.

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## MATERIALS AND METHODS

**Test principle and prerequisites.** A total of 10 experiments were carried out by using prEN 12791. The in vivo bactericidal efficacy of the reference alcohol *n*-propanol (60%, vol/vol) was assessed with 20 healthy volunteers per experiment (4). The volunteers were office workers and laboratory staff and were not the same for the different experiments. No skin breaks, such as cuts or abrasions, and no other skin disorders were present. The nails were short and clean. The volunteers did not use any substances with antibacterial activity or antibacterial soaps starting 1 week prior to testing. Between experiments there was a rest period of at least 1 week, which allowed reconstitution of the normal skin flora.

**Wash phase.** To remove transient bacterial flora and any foreign particles, volunteers' hands were washed with a nonmedicated soap (sapo kalinus). Five milliliters of the soft soap was poured into the cupped dry hands and rubbed vigorously onto the skin up to the wrists for 1 min in accordance with the standard procedure in order to ensure total coverage of the hands. The hands were then rinsed with running tap water and dried with a paper towel.

TABLE 1. Evaluation of 10 surgical reference disinfection experiments with 20 volunteers involving application of 60% (vol/vol) *n*-propanol for at least 3 min

| Expt | Prevalue for resident hand flora ($log_{10}$ CFU) | No. of volunteers treated with: | | | RF at: | |
|---|---|---|---|---|---|---|
| | | 6 ml | 9 ml | 12 ml | 0 h (immediate effect) | 3 h (sustained effect) |
| 1 | 4.48 ± 0.85[a] | 2 | 15 | 3 | 2.54 ± 1.12[a] | 2.59 ± 0.72[a] |
| 2 | 4.50 ± 0.69 | 3 | 10 | 7 | 2.93 ± 1.01 | 2.43 ± 1.23 |
| 3 | 4.40 ± 0.71 | 0 | 20 | 0 | 2.98 ± 0.90 | 2.56 ± 1.17 |
| 4 | 4.44 ± 0.92 | 0 | 13 | 7 | 2.58 ± 1.19 | 1.67 ± 0.99 |
| 5 | 4.39 ± 0.87 | 0 | 18 | 2 | 2.20 ± 1.32 | 1.53 ± 1.09 |
| 6 | 4.52 ± 0.61 | 0 | 17 | 3 | 2.86 ± 1.06 | 2.08 ± 1.26 |
| 7 | 4.43 ± 0.75 | 0 | 18 | 2 | 2.64 ± 1.09 | 2.08 ± 0.83 |
| 8 | 4.52 ± 0.68 | 0 | 9 | 11 | 2.86 ± 1.15 | 2.52 ± 1.05 |
| 9 | 4.44 ± 0.54 | 0 | 12 | 8 | 3.03 ± 1.09 | 2.26 ± 0.99 |
| 10 | 4.26 ± 1.10 | 1 | 14 | 5 | 2.32 ± 1.41 | 2.27 ± 1.23 |
| All | 4.44 ± 0.77 | 6 | 146 | 48 | 2.70 ± 1.15 | 2.20 ± 1.10 |

[a] The values are means ± standard deviations. The *P* values, derived from a comparison of the 10 means by ANOVA (SPSS), were as follows: prevalue, $P = 0.994$; RF at 0 h, $P = 0.271$; and RF at 3 h, $P = 0.016$.

**Determination of the prevalues.** The distal phalanges of the right and left hands, including the thumbs, were rubbed separately for 1 min onto two petri dishes (diameter, 9 cm) containing 10 ml of tryptic soy broth (TSB) as described in prEN 12791 (4). This is the same sampling technique that is used in other European test methods which are used for preparations for hygienic hand disinfection (EN 1500) and hygienic hand washing (EN 1499) (9, 10). A 1:10 dilution of the sampling fluid obtained from each hand was prepared in TSB. Aliquots were taken from the sampling fluid (1 and 0.1 ml) and the dilution (0.1 ml) and spread onto tryptic soy agar dishes with a sterile glass spatula. No more than 30 min elapsed between the time of sampling and the time of seeding. The dishes were incubated for a total of 48 h at 36 ± 1°C, and the number of CFU counted ranged from 15 to 300 colonies per plate.

**Disinfection phase.** Each of the volunteers was treated with a reference product, 60% (vol/vol) *n*-propanol, for at least 3 min. The number of 3-ml portions which was necessary to keep the hands wet with the reference alcohol was counted for each application.

**Determination of postvalues.** After disinfection, each volunteer rubbed the distal phalanges of one hand (randomly selected) for 1 min in a petri dish containing 10 ml of TSB supplemented with neutralizers (immediate effect). The following neutralizers were used: 3% Tween 80, 3% saponin, 0.1% histidine, and 0.1% cysteine (7). The other hand was gloved for 3 h for assessment of the sustained effect. After the glove was taken off, sampling was done by using the same method that was used for the immediate effect. A 1:10 dilution in TSB was prepared from the sampling fluid obtained from each hand. Aliquots of the sampling fluid (1 and 0.1 ml) and the dilution step (0.1 ml) were removed and spread onto tryptic soy agar dishes with a sterile glass spatula. The dishes were incubated at 36 ± 1°C for a total of 48 h, and the number of CFU counted ranged from 15 to 300 colonies per plate.

For each dilution the mean number of CFU was calculated. The mean was then multiplied by the dilution factor in order to obtain the number of CFU per milliliter of sampling liquid.

All pre- and postvalues were expressed as $log_{10}$ values. For calculation purposes, values of 0 ($\log 0 = -\infty$) were reset to 1 ($\log 1 = 0$). If values in the range that could be entered into calculations were obtained from more than one dilution, the mean was used as the final logarithm value. For each volunteer the logarithmic reduction factor (RF) was obtained by determining the difference between the $log_{10}$ prevalue and $log_{10}$ postvalues.

Statistical analysis of multiple means was performed by using the analysis of variance (ANOVA) model (SPSS for Windows, version 11.5.1; SPSS Inc., Chicago, Ill.). Pearson's correlation coefficient was determined to evaluate a possible correlation between the number of 3-ml disinfectant portions used for the reference disinfection and the mean RF (SPSS for Windows, version 11.5.1, SPSS Inc.).

## RESULTS

A total of 10 experiments were performed, resulting in a total of 200 surgical reference disinfections. The mean prevalue for the resident hand bacteria before application of the reference alcohol was 4.44 ± 0.77 $log_{10}$ CFU (range, 4.26 ± 1.10 to 4.52 ± 0.68 $log_{10}$ CFU) (Table 1). The 10 mean prevalues were not significantly different ($P = 0.994$, as determined by ANOVA). The mean $log_{10}$ RF immediately after the reference disinfection (zero hour value) was 2.70 ± 1.15 (range, 2.20 ± 1.32 to 3.03 ± 1.09) (Table 1). The 10 mean RFs were not significantly different ($P = 0.271$, as determined by ANOVA). The mean $log_{10}$ RF 3 h after the reference disinfection (3-h value) was 2.20 ± 1.10 (range, 1.53 ± 1.09 to 2.59 ± 0.72) (Table 1), and the 10 mean RFs were significantly different ($P = 0.016$, as determined by ANOVA).

Most surgical reference disinfections were achieved with 9 ml of the reference alcohol ($n = 146$; 73%), followed by 12 ml ($n = 48$; 24%) and 6 ml ($n = 6$; 3%) (Table 1). The mean $log_{10}$ RFs with 6 ml of reference alcohol were 2.18 ± 0.88 (immediate effect) and 1.65 ± 0.79 (sustained effect). Use of 9 ml of reference alcohol yielded mean $log_{10}$ RFs of 2.76 ± 1.11 (immediate effect) and 2.20 ± 1.11 (sustained effect). Application of 12 ml of reference alcohol resulted in mean $log_{10}$ RFs of 2.57 ± 1.27 (immediate effect) and 2.26 ± 1.09 (sustained effect) (Table 2). The mean $log_{10}$ RFs for the three treatment groups were not significantly different, both for the immediate effect ($P = 0.333$, as determined by ANOVA) and for the sustained effect ($P = 0.442$). A higher number of portions did not correlate with a higher $log_{10}$ RF, both for the immediate effect (Pearson's correlation coefficient, −0.028; $P = 0.689$) and for the sustained effect (Pearson's correlation coefficient, 0.059; $P = 0.404$).

TABLE 2. Evaluation of 10 surgical reference disinfection experiments with 60% (vol/vol) *n*-propanol and 3-min treatments

| No. of portions | Applied vol (ml) | No. of procedures | Prevalue for resident hand flora ($log_{10}$ CFU) | RF at: | |
|---|---|---|---|---|---|
| | | | | 0 h (immediate effect) | 3 h (sustained effect) |
| 2 | 6 | 6 | 4.62 ± 0.61[a] | 2.18 ± 0.88[a] | 1.65 ± 0.79[a] |
| 3 | 9 | 146 | 4.50 ± 0.69 | 2.76 ± 1.11 | 2.20 ± 1.11 |
| 4 | 12 | 48 | 4.20 ± 1.02 | 2.57 ± 1.27 | 2.26 ± 1.09 |
| All | | 200 | 4.44 ± 0.77 | 2.70 ± 1.15 | 2.20 ± 1.10 |

[a] The values are means ± standard deviations. The *P* values, derived from a comparison of the three means by ANOVA (SPSS), were as follows: RF at 0 h, $P = 0.333$; and RF at 3 h, $P = 0.442$.

## DISCUSSION

For the first time, we were able to show that in surgical hand disinfection with a standard 3-min rub-in procedure the applied volume of the formulation has no impact on the reduction of resident skin bacteria. This result is not a surprise, because as long as the skin is covered with the alcohol, an effect on the resident hand bacteria can be expected regardless of the volume that is applied. This result is in line with data from another study, in which two 3-ml portions of a propanol-based hand rub were applied in one part of the study for a total of 60 s and in another part of the study for 90 s. The same dose led to sufficient efficacy compared with the reference treatment after 90 s but not after 60 s, indicating that the application time has the main impact on the efficacy (11).

Further evaluation of the reference disinfection revealed that the prevalues for 10 experiments were highly reproducible (variance coefficient, 0.17). Although there was no artificial contamination of the hands and although the people were not the same in the experiments, the volunteers nevertheless provided a standard amount of resident hand bacteria prior to application of the reference alcohol. The reproducibility of the number of resident hand bacteria is similar to that observed for artificial contamination with a specific test organism (9, 10). It can therefore be concluded that the resident hand flora is suitable for studying the efficacy of a preparation for preoperative treatment of hands. The immediate effect on the resident skin bacteria also yielded good reproducibility (variance coefficient, 0.43), but it was lower than the reproducibility of the prevalues. The sustained effect showed a significant difference for all 10 experiments ($P = 0.016$, as determined by ANOVA) and had the highest variance coefficient (0.50). This finding may have various explanations. After 3 h in a surgical glove the microbial density on the skin depends on various factors, like the distribution and abundance of skin bacteria (14) and the number and activity of sweat and sebaceous glands, which may bring up more resident skin bacteria out of the ducts onto the stratum corneum (2, 17). Although the sweat glands do not contain any bacteria themselves (19), the ducts of the sweat glands do. Increased sweat production may bring up the bacteria of the ducts to the skin. The activity of the sweat glands also depends on the physical activity of the volunteer. Currently, what a volunteer does or does not do during the 3 h is not standardized. In addition, microorganisms may also recolonize the skin from the hair follicles. Also, variations in microbial density after 3 h may also be explained by the specific flora of a volunteer, which may recover faster or slower for different volunteers depending on the composition of different species (12, 13). If all these factors are taken into account, it becomes quite evident that the variation is likely to be greatest after 3 h despite a standard treatment.

Overall, reference disinfection with 60% (vol/vol) *n*-propanol for 3 min for evaluation of the efficacy of a preparation for surgical hand disinfection is an accepted principle in Europe. If hands are kept wet with the disinfectant for the total application time, the applied volume may vary, but this does not alter the efficacy.

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