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Evaluation of Two Methods of Determining the Efficacies of Two Alcohol-Based Hand Rubs for Surgical Hand Antisepsis

Günter Kampf,[1,2]* Christiane Ostermeyer,[3] Peter Heeg,[4] and Daryl Paulson[5]

Bode Chemie GmbH & Co., Scientific Affairs, Melanchthonstrasse 27, 22525 Hamburg, Germany[1]; Institute for Hygiene and Environmental Medicine, Ernst-Moritz-Arndt Universität, Walther-Rathenau-Str. 49a, 17489 Greifswald, Germany[2]; Bode Chemie GmbH & Co., Microbiology, Melanchthonstrasse 27, 22525 Hamburg, Germany[3]; Institute for Medical Microbiology and Hygiene, University Hospital, Elfriede-Aulhorn-Str. 6, 72076 Tübingen, Germany[4]; and BioScience Laboratories, Inc., 300 North Willson, Bozeman, Montana 59771-0190[5]

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The antimicrobial efficacies of preparations for surgical hand antisepsis can be determined according to a European standard (prEN 12791 [EN]) and a U.S. standard (tentative final monograph for health care antiseptic drug products [TFM]). The U.S. method differs in the product application mode (hands and lower forearms, versus hands only in EN), the number of applications (11 over 5 days, versus a single application in EN), the sampling times (0, 3, and 6 h after application, versus 0 and 3 h in EN), the sampling methods (glove juice versus fingertip sampling in EN), and the outcome requirements (absolute bacterial reduction factor [RF], versus noninferiority to reference treatment in EN). We have studied the efficacies of two hand rubs according to both methods. One hand rub was based on 80% ethanol and applied for 2 min, and the other one was based on 45% propan-2-ol, 30% propan-1-ol, and 0.2% mecetronium etilsulfate and applied for 1.5 min. The ethanol-based hand rub was equally effective as the 3-min reference disinfection of prEN 12791 in both the immediate (RFs, 2.97 ± 0.89 versus 2.92 ± 1.03, respectively) and sustained (RFs, 2.20 ± 1.07 versus 2.47 ± 1.25, respectively) effects. According to TFM, the immediate effects were 2.99 $\log_{10}$ (day 1), 3.00 $\log_{10}$ (day 2), and 3.43 $\log_{10}$ (day 5), and bacterial counts were still below baseline after 6 h. The propanol-based hand rub was even more effective than the reference disinfection of prEN 12791 in both the immediate (RFs, 2.35 ± 0.99 versus 1.86 ± 0.87, respectively) and sustained (RFs, 2.17 ± 1.00 versus 1.50 ± 1.26, respectively) effects. According to TFM, the immediate effects were 2.82 $\log_{10}$ (day 1), 3.29 $\log_{10}$ (day 2), and 3.25 $\log_{10}$ (day 5), and bacterial counts were still below baseline after 6 h. Some formulations have been reported to meet the efficacy requirements of one of the methods but not those of the other. That is why we conclude that, despite our results, meeting the efficacy requirements of one test method does not allow the claim that the requirements of the other test method are also met.

The presurgical antiseptic treatment of the hands of surgical staff is a standard procedure used worldwide in order to reduce the risk of surgical-site infection, which continues to be one of the most frequent types of nosocomial infection (3, 8). The fact that about 18.6% of sterile surgical gloves have been noted to perforate during surgical procedures (19) argues for the necessity of high reductions of bacteria on the hands before the gloves are donned (11). In the CDC guideline for hand hygiene, two different types of antimicrobial preparations are recommended for surgical hand antisepsis: alcohol-based hand rubs and antimicrobial soaps. In order to determine the efficacies of such preparations, two different test methods are commonly used. In the United States, the test method is described in the tentative final monograph for health care antiseptic products (TFM), including those for surgical scrubbing, based on ASTM method E 1115 (7). In Europe, a test standard, prEN 12791, was developed in 1997 (6), based on the test method developed by the German Society for Hygiene and Microbiology (27).

The two methods have in common that the antimicrobial efficacy is evaluated using resident hand flora (Table 1). In the European method, the efficacy of a single application is evaluated against a standardized reference treatment with 60% (vol/vol) propan-1-ol (*n*-propanol) for 3 min (14), which has been described to have maximum efficacy as a single alcohol on the resident flora (11, 29). A test formulation must be at least as effective as the reference treatment (Table 1). A single application meeting a set standard is justified in that each patient should be allowed to expect the same efficacy for an upcoming operation.

In the U.S. method, the efficacy of 11 repetitive product applications over a 5-day period is evaluated (Table 1). A reference treatment is recommended to ensure internal validity. The test product must reduce the resident flora by at least 1 $\log_{10}$ from baseline after application 1, by at least 2 $\log_{10}$ after application 2, and by at least 3 $\log_{10}$ after application 11.

Taking the differences into account, we studied the efficacies of two alcohol-based hand rubs according to both methods and analyzed the major differences between the test methods based on our results.

MATERIALS AND METHODS

Two alcohol-based hand rubs commonly used for surgical hand disinfection were evaluated in this study. Both preparations were manufactured by Bode Chemie GmbH & Co., Hamburg, Germany. Sterillium is used in Europe and is based on propan-2-ol (isopropanol; 45%, wt/wt), propan-1-ol (30%, wt/wt), and mecetronium

* Corresponding author. Mailing address: Bode Chemie GmbH & Co., Scientific Affairs, Melanchthonstrasse 27, 22525 Hamburg, Germany. Phone: 49 (0) 40 54006-0. Fax: 49 (0) 40 54006-128. E-mail: guenter.kampf@bode-chemie.de.

TABLE 1. Comparison of various criteria of the U.S. test method according to the tentative final monograph (7) and the European test method prEN 12791 (6) to determine the efficacies of preparations for surgical hand antisepsis

Main criterion	Detail	U.S. method	European method
Design		Randomized, blinded parallel arm design	Randomized, reference-controlled crossover design
Target of hand antisepsis		Resident hand flora	Resident hand flora
Subjects	Prerequisites	No evidence of dermatoses, no antibiotics or other antimicrobial preparations for at least 2 wk prior to testing	Healthy skin on hands, no cuts or abrasions, short and clean fingernails, no use of antimicrobial soaps or creams for at least 1 wk prior to testing
	Sample size	Calculation of sample size using the formula $n \geq [(S^2)(Z_{\alpha/2} + Z_{\beta})^2]/\delta^2$, taking into account the variance (S^2), the significance level ($Z_{\alpha/2}$), the power of the test (Z_{β}), and the clinical difference of significance (δ)	18–20, with a discrimination between means of $\geq$6 $\log_{10}$ and a power of 0.95 (26, 28)
Treatment of hands before baseline sampling		Wash hands with a liquid soap containing no antimicrobials for 30 seconds, rinse hands for 30 seconds	Wash hands with a defined soap (sapo kalinus) for 1 min
Sampling	Type of method	Glove juice method for 1 min (whole hand)	Petri dish method for 1 min (fingertips)
	No. of samplings	Three baseline samplings on three separate days prior to study; samples immediately, 3 h, and 6 h following first product application on test days 1, 2, and 5	Three samplings per subject and expt (baseline and 0 and 3 h after treatment)
Antiseptic hand treatment	Reference treatment	An FDA-approved formulation is acceptable, but not required, as a positive control	Treatment with 60% propan-1-ol for 3 min
	Product	As recommended by the manufacturer or 10 min, followed by a 1-min final rinse for scrub products	As recommended by the manufacturer
	Number	Eleven treatments per subject and expt (one treatment on days 1 and 5 and three treatments on days 2, 3, and 4)	One treatment per subject and expt
	Treated skin area	Both hands and lower two-thirds of forearms	Both hands up to the wrists
Neutralization of residual activity	Necessity	Recommended, but not required, in sampling fluid; required in dilution fluid and agar plates	Required in sampling and dilution fluid, not required in agar plates
	Validation of neutralization	Required according to ASTM E 1054	Required according to prEN 12054
Calculation	Aim of calculation	Determine total no. of bacteria recovered from both hands	Determine no. of bacteria per ml in the sampling fluid
Requirement	Baseline value	Minimum of 1.5×10^5 bacteria after the first and second baseline samplings from both hands	Mean $\log_{10}$ baseline value shall be at least 3.5
	Immediate efficacy (0 h)	Day 1 (after application 1), $\geq$1 $\log_{10}$ reduction per hand; day 2 (after application 2), $\geq$2 $\log_{10}$ reduction per hand; day 5 (after application 11), $\geq$3 $\log_{10}$ reduction per hand	Not significantly less effective than the reference treatment (Wilcoxon matched pairs signed rank tests)
	Sustained efficacy (3 or 6 h)	Days 1, 2, and 5 after 6 h, bacterial cell count does not exceed baseline	Not significantly less effective than the reference treatment after 3 h (Wilcoxon matched pairs signed rank test)

etilsulfate (0.2%, wt/wt). It was applied for 1.5 min. Sterillium Rub is used in the United States and is based on ethanol (80%, wt/wt). It was applied for 2 min. In the experiments done according to the TFM method, each hand rub was allowed to air dry after the application. In the tests done according to the European test method, a reference alcohol, propan-1-ol (60%, vol/vol) applied for 3 min, was included.

European test method, prEN 12791. (i) Test principles and prerequisites. The antimicrobial efficacy was assessed with 20 healthy volunteers per experiment (6, 26). No skin breaks, such as cuts or abrasions, were present on the hands or arms of subjects, nor were other skin disorders. Nails were short and clean. Starting 1 week prior to testing, volunteers did not use any substances on the hands and arms that are known to affect the normal skin microbial populations. Between each experiment, a rest period of at least 1 week was allowed to elapse in order to allow reconstitution of normal skin flora.

(ii) Wash phase. To remove transient bacterial flora that would bias the analysis, as well as any foreign particles, the volunteers washed their hands with a nonmedicated soft soap (sapo kalinus). Five milliliters of the soft soap was dispensed into the cupped, dry hands, and the subjects rubbed the soap vigorously onto the skin of the hands up the wrists for 1 minute, in accordance with the standard procedure, to ensure total coverage of the hands. Hands were then rinsed with running tap water and blotted dry with a paper towel.

TABLE 2. Scale for grading the skin of the hands

Score	Description
0	No visible damage, "perfect" skin
1	Slight dryness, ashen appearance, usually involving dorsum only
2	Marked dryness, slight flaking, involving dorsum only
3	Severe dryness of dorsum, marked flaking, possibly fissures in webs
4	Severe flaking of dorsum, surface fissures, possibly with slight palmar dryness
5	Open fissures, slight erythema (>10% of dorsal and interdigital surface), with or without severe dryness, no bleeding
6	Bleeding cracks, deep open fissures, or generalized erythema (>25% of area)

(iii) Determination of population baseline values. The distal phalanges of the right and left hands were rubbed separately, including thumbs, for 1 minute on two petri dishes (diameter of 9 cm), each containing 10 ml of tryptic soy broth (TSB) (6). This is the same sampling technique used in other European methods for testing hand hygiene preparations (15, 16). From the sampling fluid obtained from each hand, a 1:10 dilution was prepared in TSB. Aliquots taken from the sampling fluid (1 and 0.1 ml) and from the dilution step (0.1 ml) were transferred to petri plates of tryptic soy agar (TSA) and spread evenly over the agar surface with a sterile glass spatula. No more than 30 min elapsed between sampling and inoculation of the TSA plates. The plates were incubated for a total of 48 h at 36°C ± 1°C, and the CFU from plates bearing between 15 and 300 colonies per plate were counted.

(iv) Disinfection phase. Each hand rub was tested in a separate experiment against the reference disinfectant in a crossover design. At the beginning of each experiment, half of the volunteers used a hand rub, and the remaining subjects carried out the reference treatment (random assignment). After a rest period of at least 7 days, each volunteer used the other preparation, thereby completing the second phase of the crossover testing.

The reference treatment consisted of applying 60% propan-1-ol to both hands and wrists for 3 min. Sterillium was applied to both hands and wrists in two applications of 3 ml (total of 6 ml) for 45 seconds each (total application time of 1.5 min). Sterillium Rub was applied to both hands and wrists in as many 3-ml aliquots as necessary to keep both hands wet with the preparation for a total of 2 min.

(v) Determination of microbial populations after product application. After the hand disinfection procedure, volunteers rubbed the distal phalanges of one hand (randomly selected) for 1 minute in a petri plate containing 10 ml of TSB supplemented with product neutralizers (3% Tween 80, 3% saponin, 0.1% histidine, and 0.1% cysteine) (12). The unsampled hand was gloved for 3 h to simulate the real situation in the operating room. This allows determination of the bacterial population kinetics under the surgical glove with time, which is known to be the persistent or sustained antimicrobial effect. After the 3-hour wear time, the glove was removed and the second hand sampled in the same manner as was the first. A 1:10 dilution of the sampling fluid obtained from each hand was prepared in TSB. Aliquots from the sampling fluid (1 ml and 0.1 ml) and the dilution step (0.1 ml) were spread onto TSA plates with a sterile glass spatula, and the plates were incubated as described for the baseline samples. At each dilution, the mean of the CFU was calculated, and this value was then multiplied by the dilution factor in order to obtain the number of CFU per ml of sampling liquid. All pre- and postapplication values were expressed as $\log_{10}$ values. For calculation purposes, plate count values of 0 were reset to 1, because the $\log_{10}$ of 0 is undefined and the $\log_{10}$ of 1 is 0. If values in the 15- to 300-CFU range were obtained from more than one dilution, their combined mean was used as the final logarithm value. For each sample from each volunteer, the logarithmic reduction factor (RF) was calculated as the difference between the $\log_{10}$ baseline value and the $\log_{10}$ postapplication values.

U.S. test method, TFM. The number of subjects was estimated from the following equation per TFM requirements: $n \geq [(S^2)(Z_{\alpha/2} + Z_{\beta})^2]/\delta^2$. S^2 is the estimate of variance and was set at 0.6. $Z_{\alpha/2}$ corresponds to the level of the test and was 1.96 for an α error of 0.05. Z_{β} corresponds to the power of the test and was 0.842 for an 80% power. The clinical difference of significance, δ, was set at 0.5 $\log_{10}$. The antimicrobial efficacy was therefore assessed for a total of 40 healthy volunteers per experiment.

The 14 days prior to the baseline portion of the study constituted the pretest conditioning period. During this time, subjects avoided the use of medicated soaps, lotions, and shampoos, as well as skin contact with solvents, detergents, acids, and bases. Nonantimicrobial personal hygiene products were supplied to the subjects, and these were used exclusively throughout the period of study. Prior to being sampled, subjects were questioned regarding their adherence to protocol restrictions. Subjects also were questioned as to whether they complied with a requirement not to wash their hands or apply any type of lotion, moisturizer, or ointment within 2 hours prior to sampling. This regimen allowed for stabilization of the normal microbial populations residing on the hands. Following the pretest period, prospective subjects proceeded into the baseline week.

TABLE 3. Block group sampling schedule

Subject	Hand[a] sampled at time:		
	Immediate	3 h	6 h
1	R	L	
2	L		R
3		L	R
4	L	R	
5	R		L
6		R	L

[a] R, right hand; L, left hand.

(i) Baseline week. On day 1 of the baseline week, the skin of subjects' hands was evaluated for dryness. Subjects with scores of ≤2.0 (Table 2) proceeded with the study. Baseline sampling was then performed on days 1, 3, and 5 (baseline week). The following procedures were followed under technician supervision. (i) Subjects clipped fingernails to ≤1 mm free edge, if necessary. All jewelry was removed from hands and arms. (ii) All washes/rinses were performed in tap water regulated at 40°C ± 2°C. (iii) Subjects rinsed hands, including the lower two-thirds of the forearms, under running tap water for 30 seconds. During this rinse, fingernails and cuticles were cleaned with a nail cleaner. (iv) Subjects washed their hands and forearms with 5 ml of nonmedicated soap for 30 seconds, using water as required to develop lather. The hands were positioned higher than the elbows during this procedure. (v) Subjects rinsed hands and forearms thoroughly for 30 seconds under running tap water to remove all lather.

This was followed by performance of the glove juice procedure (see below) for baseline sampling. For a subject to continue into the experimental period, baseline populations of both hands were required to be $\geq 1 \times 10^6$ CFU/ml. Using the formula presented above (Table 1), the sample size for each of the two tests was calculated to be 18.

(ii) Experimental period (test week). The subjects accepted into the study were assigned randomly to a test product application or to application of the reference product (surgical scrub with a total of 10 ml Hibiclens over a total of 3 min, followed by a final rinse). Each of the groups of 18 subjects was subdivided randomly into three block groups of six each, and hands were sampled according to the schedule shown in Table 3.

(iii) Product application. The products were used by subjects once on test days 1 and 5 and three times on test days 2, 3, and 4. At least 1 hour elapsed between each of the three applications on days 2, 3, and 4, which were performed under supervision of a technician. Prior to application of a test product, subjects clipped fingernails to ~1 mm free edge if necessary, removed all jewelry from hands and arms, and cleaned under fingernails with a nail cleaner.

(iv) Test product application. Each product was applied to the hands, with particular attention paid to fingers, cuticles, and interdigital spaces. One-half palmful of approximately 2 to 3 ml of product was dispensed into the palm of one of a subject's hands, and the subject dipped the fingers of the opposite hand into the palm and rubbed product into fingernails and around the hand. This procedure was repeated with the other hand. A third 2- to 3-ml volume of product was dispensed into either palm, and product was rubbed into both hands, including the lower one-third of the forearms. The procedure lasted approximately 2 min (Sterillium Rub) or 1.5 min (Sterillium), during which the hands were maintained moist with product. Following application, the hands were rubbed together until dry. Subjects with scores of >4 (Table 2) were discontinued from further treatment.

(v) Glove juice procedure. One of a subject's hands was sampled immediately following the first product application procedure on days 1, 2, and 5. The other hand, or both hands if no immediate sample was taken, was gloved and sampled 3 and/or 6 h following product application. Powder-free, sterile latex gloves were placed on both of the subject's hands. For sampling, 75 ml of sterile stripping fluid without product neutralizers was instilled into a glove. The wrist was

TABLE 4. Reduction of bacterial baseline counts on hands by surgical hand antisepsis with two hand rubs,[a] according to the TFM (U.S. test method)

Sampling time	Ethanol-based hand rub (2 min)		Propanol-based hand rub (1.5 min)	
	CFU (mean ± SD)	Mean $\log_{10}$ RF	CFU (mean ± SD)	Mean $\log_{10}$ RF
Baseline	6.36 ± 0.26	NA[b]	6.27 ± 0.24	NA
Day 1				
Immediate	3.37 ± 0.45	2.99	3.45 ± 0.52	2.82
3 h	4.17 ± 0.77	2.19	3.86 ± 0.51	2.41
6 h	4.82 ± 0.79	1.54	4.25 ± 0.94	2.02
Day 2				
Immediate	3.36 ± 0.58	3.00	2.98 ± 0.34	3.29
3 h	3.77 ± 0.89	2.59	3.75 ± 0.63	2.52
6 h	4.51 ± 0.95	1.85	4.70 ± 0.79	1.57
Day 5				
Immediate	2.93 ± 0.55	3.43	3.02 ± 0.57	3.25
3 h	3.61 ± 0.49	2.75	3.75 ± 0.87	2.52
6 h	4.23 ± 0.61	2.13	3.84 ± 1.00	2.43

[a] Hands were treated with a 2-min application of a hand rub based on 80% ethanol and a 1.5-min application of a hand rub based on 45% propan-2-ol, 30% propan-1-ol, and 0.2% mecetronium etilsulfate.
[b] NA, not applicable.

secured, and an attendant massaged the hand through the glove in a standardized manner for 60 seconds. A 5-ml aliquot of the glove juice was removed and diluted in 5 ml of Butterfield's phosphate buffer solution with product neutralizers (10^0 dilution). The 10^0 dilution was then serially diluted in Butterfield's phosphate buffer solution with product neutralizers, as appropriate.

(vi) Data collection. Duplicate spread plates and/or automated spiral plates were prepared from each of these dilutions on tryptic soy agar with 0.07% lecithin (wt/vol) and 0.5% Tween 80 (wt/vol) and incubated at 30°C ± 2°C for up to 72 h or until sufficient growth was observed. Colonies were counted and data recorded using the computerized q-count plate counting system. If 10^0 plates gave an average count of 0, the average plate count was expressed as 1.00.

(vii) Data handling. The estimated $\log_{10}$ number of viable microorganisms recovered from each hand was designated the "*R* value." It is the adjusted average $\log_{10}$ colony count measurement for each subject at each sampling time. Each *R* value was determined using the formula $\log_{10}(F \times C_i \times 10^{-D})$, where *F* is the amount of sterile sampling solution instilled into glove (75 ml), C_i is the arithmetic average colony count from the two plates for each subject at a particular dilution level, and *D* is the dilution factor.

(viii) Statistical analysis. The MiniTab statistical computer package was used for all statistical calculations. Descriptive statistics and confidence intervals were generated using the 0.05 level of significance for type I (α) error.

RESULTS

When tested according to the U.S. test method, a 2-min application of the ethanol-based hand rub reduced, on average, the mean baseline bacterial count of 6.36 ($\log_{10}$) by 2.99 $\log_{10}$ on day 1, by 3.00 $\log_{10}$ on day 2, and by 3.43 $\log_{10}$ on day 5 (all immediate effects) (Table 4). At 3 and 6 hours after treatment with the hand rub, periods during which the hands were occluded within surgical gloves, the bacterial density slowly increased with time but remained at least 1.54 $\log_{10}$ less than baseline. The test preparation fulfilled the efficacy requirements of the U.S. test method. The results obtained with the reference procedure were comparable to those in previously published reports (24), indicating internal data validity (data not shown).

When the ethanol-based hand rub was tested using the European test method, comparable results were obtained. The mean baseline count was between 4.41 and 4.56 $\log_{10}$ (Table 5). Application of the ethanol-based hand rub for 2 minutes reduced the number of resident hand bacteria to a similar extent (immediate effect, reduction by 2.97 $\log_{10}$; sustained effect, reduction by 2.20 $\log_{10}$). The 3-minute treatment with the reference, 60% propan-1-ol, reduced the number of resident hand bacteria, on average, by 2.92 $\log_{10}$ (immediate effect) and 2.47 $\log_{10}$ (sustained effect). There was no significant difference between the mean reduction of hand bacteria obtained following the 3-minute reference disinfection and that obtained following the 2-minute application of the ethanol-based hand rub ($P > 0.1$) (Table 5). The preparation therefore met the efficacy requirements of the European test method with a 2-minute application time.

In testing according to the U.S. method, the propanol-based hand rub applied for 1.5 min reduced, on average, the mean baseline bacterial count by 2.82 $\log_{10}$ on day 1, by 3.29 $\log_{10}$ on day 2, and by 3.25 $\log_{10}$ on day 5 (all immediate effects) (Table 4). At 3 and 6 hours after treatment with the hand rub, the bacterial density slowly increased with time but remained at least 1.57 $\log_{10}$ less than baseline. The preparation fulfilled the efficacy requirements of the U.S. test method. The results obtained with the reference procedure were comparable to those in previously published reports (24), indicating internal data validity (data not shown).

When tested according to the European test method, a similar result was obtained. The mean baseline count was between

TABLE 5. Reduction of bacterial baseline counts on fingertips by surgical hand antisepsis with two hand rubs,[a] each compared to the 3-min reference treatment, according to prEN 12791

Sampling time	Ethanol-based hand rub					Propanol-based hand rub				
	Reference treatment (3 min)		Hand rub (2 min)			Reference treatment (3 min)		Hand rub (1.5 min)		
	CFU (mean ± SD)	Mean $\log_{10}$ RF	CFU (mean ± SD)	Mean $\log_{10}$ RF	*P* value	CFU (mean ± SD)	Mean $\log_{10}$ RF	CFU (mean ± SD)	Mean $\log_{10}$ RF	*P* value
Baseline	4.41 ± 0.61	NA[b]	4.56 ± 0.63	NA	NA	4.55 ± 0.57	NA	4.90 ± 0.36	NA	NA
Immediate	1.49 ± 1.05	2.92 ± 1.03	1.59 ± 1.15	2.97 ± 0.89	>0.1	2.70 ± 0.89	1.86 ± 0.87	2.54 ± 0.91	2.35 ± 0.99	>0.1
Baseline	4.52 ± 0.69	NA	4.45 ± 0.70	NA	NA	4.47 ± 0.77	NA	4.91 ± 0.37	NA	NA
3 h	2.05 ± 1.41	2.47 ± 1.25	2.24 ± 1.18	2.20 ± 1.07	>0.1	2.98 ± 0.99	1.50 ± 1.26	2.74 ± 0.94	2.17 ± 1.00	0.1

[a] Hands were treated with a 2-min application of a hand rub based on 80% ethanol and a 1.5-min application of a hand rub based on 45% propan-2-ol, 30% propan-1-ol, and 0.2% mecetronium etilsulfate.
[b] NA, not applicable.

4.55 and 4.90 $\log_{10}$ (Table 5). Application of the propanol-based hand rub for 1.5 min reduced the number of resident hand bacteria to a similar extent (immediate effect, reduction by 2.35 $\log_{10}$; sustained effect, reduction by 2.17 $\log_{10}$). The 3-minute reference treatment reduced the number of resident hand bacteria, on average, by 1.86 $\log_{10}$ (immediate effect) and 1.50 $\log_{10}$ (sustained effect). The difference between the mean reduction obtained with the 3-minute reference disinfection and that obtained with the 1.5-min application of the propanol-based hand rub was significant for the sustained effect ($P = 0.1$) (Table 5). The preparation therefore also fulfilled the efficacy requirements of the European test method with a 1.5-min application time.

DISCUSSION

In this study, we were able to show that two well-formulated alcohol-based hand rubs fulfill the efficacy requirements for surgical hand antisepsis when tested according to two completely different test methods, even when quite brief applications of 2 minutes (ethanol-based hand rub) or 1.5 min (propanol-based hand rub) are used.

Meeting the test criteria of one method, however, does not automatically guarantee that a product will meet those of the other test method. A surgical scrub based on 4% chlorhexidine gluconate, for example, met both the European requirements (22) and the U.S. requirements (24). A surgical scrub based on 7.5% povidone iodine, however, failed to pass the European efficacy requirements (22). Varied success can be seen for alcohol-based hand rubs. Hand rubs based on 80% ethanol (13) or 85% ethanol (10) fulfilled the efficacy requirements. A hand rub based on a total of 75% propanol (mixture of 45% propan-2-ol and 30% propan-1-ol) still significantly exceeded the European efficacy requirements in 3 min (22) and 1.5 min (17) and fulfilled the U.S. requirements with an application time of 1.5 min (Table 4). Conversely, a hand rub based on 61% ethanol and 1% chlorhexidine gluconate failed to meet the European efficacy requirements (13) but met the U.S. efficacy requirements (24).

One major difference between the U.S. and the European methods is the procedure for application of the surgical hand antiseptic. According to the European test standard, only the hands are treated because most gloves perforate on the fingers and the highest bacterial density is found on hands, whereas according to the U.S. standard, hands and lower forearms are treated. In clinical practice, surgeons and perioperative personnel will treat both the hands and the forearms, in conformity with the recommendations of various national guidelines for hand hygiene (2, 5, 20). Hence, the U.S. test method more closely reflects the actual procedure of application in the surgical theater than does the European test method. For the efficacy on the resident hand flora in our study, however, it did not make a difference for the two test formulations.

A second major difference between the test methods is the procedure for the recovery of surviving bacteria from the hands of volunteers after product application. According to the European standard, only the fingertips are sampled, as they have a higher density of bacteria than all other parts of the hand (23), whereas according to the U.S. standard, the whole hand is sampled. In clinical practice, perioperative personnel cover the entire hand with the surgical glove. Although most perforations of the surgical glove are described to occur around the fingertips of the nondominant hand (which is explained, for example, by careless suturing), perforations do also occur on other parts of the glove (1, 21). That is why, for the sampling of surviving bacteria, the U.S. test method reflects the actual "risk area" better than the European test method does. For efficacy on the resident hand flora, however, it did not seem to make a difference in outcomes for our two test formulations. The ethanol-based hand rub yielded, for example, immediate effects of 2.97 $\log_{10}$ (European method) and 2.99 $\log_{10}$ (U.S. method). Similar results were seen with the propanol-based hand rub. Based on this finding, the two different modes of sampling may not have a significant impact on the overall outcome.

A third difference between the two test methods is the number and timing of samplings. In the U.S. test method, hands are sampled at three different time points postapplication on a single day to account for immediate kill and persistence of activity and on three separate days to determine if a cumulative antimicrobial effect had developed. In the European test method, samplings are performed on a single day only at times 0 and 3 h, accounting for immediate kill and persistence. However, no measurement of a cumulative effect can be made. Most but not all surgeries last less than 3 hours (3). Hence, the vast majority of surgeries are performed in a span of time consistent with the European test method (9). All data from both sampling points are used for the efficacy assessment per the criteria of the European test method. In the U.S. test method, however, the population data obtained 3 hours after the treatment of hands are not used to evaluate the efficacy of the surgical hand antisepsis procedure (7).

The requirements imposed by the two test methods may represent the most important difference. In the U.S. test method, the efficacy of 11 repetitive applications is evaluated and is not directly controlled by comparison with a reference standard. The reference product, Hibiclens, is quite often used as an internal validity control, although it might be better to chose a simple active agent without excipients because the formulation of a finished product may be changed and the efficacy may also change. A test preparation is expected to reduce the resident flora by at least 1 $\log_{10}$ unit after the single application on day 1, by at least 2 $\log_{10}$ units after the first application on day 2 (second of the 11 applications), and by at least 3 $\log_{10}$ units after the single application on day 5 (last of the 11 applications) (7). Per the European test method, a hand rub or scrub for surgical hand antisepsis must be at least as effective as a standard reference treatment known to have a specific effect on the resident hand flora; this may be variable depending on the panel of subjects (6, 14), which can also be seen in our own study (approximately 1 $\log_{10}$ difference between both reference procedures). Hence, in Europe, each patient can expect a high efficacy with each application, which is almost as high as the U.S. one after 11 specific applications. In the United States, a patient having surgery on Monday morning may have to accept a lower reduction of resident hand bacteria on the surgeon's hands (e.g., 1.6 to 1.8 $\log_{10}$ reduction) (25) than a corresponding patient in Europe (approximately 2.7 $\log_{10}$ reduction) (14). The bacterial reduction in the United States may also be much lower than the required 3

$\log_{10}$ on a Friday afternoon if during the week fewer than 11 procedures for surgical hand antisepsis are performed by the surgeon or the cumulative effect of chlorhexidine gluconate is neutralized due to hand contact with various agents such as anionic detergents (4) or other neutralizing agents (18).

Overall, different hand antiseptics may well pass the efficacy requirements of both methods despite many methodological differences. Whether the differences may affect patient safety cannot be determined based on our study. Only a prospectively conducted clinical trial on surgical-site infection surveillance will allow determination of which of the two methods is a better predictor of surgical-site infection rates following use of a particular product.

REFERENCES

1. **Aarnio, P., and T. Laine.** 2001. Glove perforation rate in vascular surgery—a comparison between single and double gloving. Vasa **30:**122–124.
2. **Anonymous.** 2000. Händehygiene. Bundesgesundheitsblatt **43:**230–233.
3. **Anonymous.** 2003. National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1992 through June 2003. Am. J. Infect. Control **31:**481–498.
4. **Benson, L., L. Bush, and D. LeBlanc.** 1990. Importance of neutralizers in the stripping fluid in a simulated healthcare personnel handwash. Infect. Control Hosp. Epidemiol. **11:**595–599.
5. **Boyce, J. M., and D. Pittet.** 2002. 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. Morb. Mortal. Wkly. Rep. **51:**1–45.
6. **Comité Européen de Normalisation.** 1997. Chemical disinfectants and antiseptics. Surgical hand disinfection. Test method and requirement (phase 2, step 2). prEN 12791. Comité Européen de Normalisation, Brussels, Belgium.
7. **Federal Register.** 1994. Tentative final monograph for healthcare antiseptic products; proposed rule. Fed. Regist. **59:**31401–31452.
8. **Kampf, G., P. Gastmeier, N. Wischnewski, J. Schlingmann, M. Schumacher, F. Daschner, and H. Rüden.** 1996. Nosokomiale Infektionen in Deutschland—Erfassung und Prävention. NIDEP Studie, Teil 1: zur Prävalenz in der Chirurgie. Chirurg **67:**637–642.
9. **Kampf, G., P. Goroncy-Bermes, A. Fraise, and M. Rotter.** 2005. Terminology in surgical hand disinfection—a new Tower of Babel in infection control. J. Hosp. Infect. **59:**269–271.
10. **Kampf, G., and M. Kapella.** 2003. Suitability of Sterillium Gel for surgical hand disinfection. J. Hosp. Infect. **54:**222–225.
11. **Kampf, G., and A. Kramer.** 2004. Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs. Clin. Microbiol. Rev. **17:**863–893.
12. **Kampf, G., B. Meyer, and P. Goroncy-Bermes.** 2003. Comparison of two test methods for the determination of sufficient antimicrobial efficacy of three different alcohol-based hand rubs for hygienic hand disinfection. J. Hosp. Infect. **55:**220–225.
13. **Kampf, G., and C. Ostermeyer.** 2005. Efficacy of two distinct ethanol-based hand rubs for surgical hand disinfection—a controlled trial according to prEN 12791. BMC Infect. Dis. **5:**17.
14. **Kampf, G., and C. Ostermeyer.** 2004. Influence of applied volume on efficacy of 3-minute surgical reference disinfection method pr EN 12791. Appl. Environ. Microbiol. **70:**7066–7069.
15. **Kampf, G., and C. Ostermeyer.** 2003. Inter-laboratory reproducibility of the EN 1500 reference hand disinfection. J. Hosp. Infect. **53:**304–306.
16. **Kampf, G., and C. Ostermeyer.** 2002. Intra-laboratory reproducibility of the hand hygiene reference procedures of EN 1499 (hygiene hand wash) and EN 1500 (hygienic hand disinfection). J. Hosp. Infect. **52:**219–224.
17. **Kampf, G., C. Ostermeyer, and P. Heeg.** 2005. Surgical hand disinfection with a propanol-based hand rub: equivalence of short application times. J. Hosp. Infect. **58:**304–310.
18. **Kampf, G., M. Shaffer, and C. Hunte.** 2005. Insufficient neutralization in testing chlorhexidine-containing ethanol-based hand rub can result in a false positive efficacy assessment. BMC Infect. Dis. **5:**48.
19. **Kralj, N., M. Beie, and F. Hoffman.** 1999. Surgical gloves—how well do they protect against infections? Gesundheitswesen **61:**398–403.
20. **Labadie, J. C., G. Kampf, B. Lejeune, M. Exner, O. Cottron, R. Girard, M. Orlick, M. L. Goetz, J. C. Darbord, and A. Kramer.** 2002. Recommendation for surgical hand disinfection—requirements, implementation, and need for research. A proposal by representatives of SFHH, DGHM, and DGKH for a European discussion. J. Hosp. Infect. **51:**312–315.
21. **Malhotra, M., J. B. Sharma, L. Wadhwa, and R. Arora.** 2004. Prospective study of glove perforation in obstetrical and gynecological operations: are we safe enough? J. Obst. Gynecol. Res. **30:**319–322.
22. **Marchetti, M. G., G. Kampf, G. Finzi, and G. Salvatorelli.** 2003. Evaluation of the bactericidal effect of five products for surgical hand disinfection according to prEN 12054 and prEN 12791. J. Hosp. Infect. **54:**63–67.
23. **McGinley, K. J., E. L. Larson, and J. J. Leyden.** 1988. Composition and density of microflora in the subungual space of the hand. J. Clin. Microbiol. **26:**950–953.
24. **Mulberry, G., A. T. Snyder, J. Heilman, J. Pyrek, and J. Stahl.** 2001. Evaluation of a waterless, scrubless chlorhexidine gluconate/ethanol surgical scrub for antimicrobial efficacy. Am. J. Infect. Control **29:**377–382.
25. **Paulson, D. S.** 2004. Hand scrub products—performance requirements versus clinical relevance. AORN **80:**225–234.
26. **Paulson, D. S.** 2004. Applied statistical designs for the researcher. Marcel Dekker, New York, N.Y.
27. **Rotter, M., and M. Skopec.** 2003. Entwicklung der Händehygiene und die Bedeutung der Erkenntnisse von Ignaz Ph. Semmelweis, p. 1–27. *In* G. Kampf (ed.), Händ-Hygiene im Gesundheitswesen. Springer, Berlin, Germany.
28. **Rotter, M. L.** 2004. European norms in hand hygiene. J. Hosp. Infect. **56:**S6–S9.
29. **Rotter, M. L.** 1999. Hand washing and hand disinfection, p. 1339–1355. *In* C. G. Mayhall (ed.), Hospital epidemiology and infection control, 2nd ed. Lippincott, Williams & Wilkins, Philadelphia, Pa.