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

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Received 21 November 2005/Accepted 18 March 2006

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 ethilsulfate 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 log10 (day 1), 3.00 log10 (day 2), and 3.43 log10 (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 log10 (day 1), 3.29 log10 (day 2), and 3.25 log10 (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 log10 from baseline after application 1, by at least 2 log10 after application 2, and by at least 3 log10 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...
Starting 1 week prior to testing, volunteers did not use any substances on the arms of subjects, nor were other skin disorders. Nails were short and clean. Antimicrobial efficacy was assessed with 20 healthy volunteers per experiment.

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 \( n = \frac{\left( \frac{1}{2} \right)}{6} \), taking into account the variance (6), the significance level (Z), the power of the test (Z), and the clinical difference of significance (6). 18–20, with a discrimination between means of \( \geq 0 \) 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 (sape 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 \( 1.5 \times 10^5 \) 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 test).

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).

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

<table>
<thead>
<tr>
<th>Main criterion</th>
<th>Detail</th>
<th>U.S. method</th>
<th>European method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Design</td>
<td>Randomized, blinded parallel arm design</td>
<td>Randomized, reference-controlled crossover design</td>
<td></td>
</tr>
<tr>
<td>Target of hand antisepsis</td>
<td>Resident hand flora</td>
<td>Resident hand flora</td>
<td></td>
</tr>
<tr>
<td>Subjects</td>
<td>Prerequisites</td>
<td>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</td>
<td></td>
</tr>
<tr>
<td>Sample size</td>
<td>Calculation of sample size using the formula ( n = \frac{\left( \frac{1}{2} \right)}{6} ), taking into account the variance (6), the significance level (Z), the power of the test (Z), and the clinical difference of significance (6). 18–20, with a discrimination between means of ( \geq 0 ) log_{10} and a power of 0.95 (26, 28).</td>
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<td>Treatment of hands before baseline sampling</td>
<td>Wash hands with a liquid soap containing no antimicrobials for 30 seconds, rinse hands for 30 seconds.</td>
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<td></td>
</tr>
<tr>
<td>Sampling</td>
<td>Type of method</td>
<td>Glove juice method for 1 min (whole hand). Petri dish method for 1 min (fingertips).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. of samplings</td>
<td>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).</td>
<td></td>
</tr>
<tr>
<td>Antiseptic hand treatment</td>
<td>Reference treatment</td>
<td>Treatment with 60% propan-1-ol for 3 min.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Product</td>
<td>An FDA-approved formulation is acceptable, but not required, as a positive control. As recommended by the manufacturer or 10 min, followed by a 1-min final rinse for scrub products.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Number</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treated skin area</td>
<td>Both hands and lower two-thirds of forearms. Both hands up to the wrists.</td>
<td></td>
</tr>
<tr>
<td>Neutralization of residual activity</td>
<td>Necessity</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Validation of neutralization</td>
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</tr>
<tr>
<td>Calculation</td>
<td>Aim of calculation</td>
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<td></td>
</tr>
<tr>
<td>Requirement</td>
<td>Baseline value</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Immediate efficacy (0 h)</td>
<td>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 test).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sustained efficacy (3 or 6 h)</td>
<td>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).</td>
<td></td>
</tr>
</tbody>
</table>
(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 2 min). Sterillium Rub was applied to both hands and wrists in as many 3-ml applications of 3 ml (total of 6 ml) for 45 seconds each (total application time 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 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.

The experiment was repeated for 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. 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 ≥1 × 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.

(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, according to the TFM (U.S. test method)

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Ethanol-based hand rub (2 min)</th>
<th>Propanol-based hand rub (1.5 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFU (mean ± SD)</td>
<td>Mean log_{10} RF</td>
</tr>
<tr>
<td>Baseline</td>
<td>6.36 ± 0.26</td>
<td>NA</td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediate</td>
<td>3.37 ± 0.45</td>
<td>2.99</td>
</tr>
<tr>
<td>3 h</td>
<td>4.17 ± 0.77</td>
<td>2.19</td>
</tr>
<tr>
<td>6 h</td>
<td>4.82 ± 0.79</td>
<td>1.54</td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediate</td>
<td>3.36 ± 0.58</td>
<td>3.00</td>
</tr>
<tr>
<td>3 h</td>
<td>3.77 ± 0.89</td>
<td>2.59</td>
</tr>
<tr>
<td>6 h</td>
<td>4.51 ± 0.95</td>
<td>1.85</td>
</tr>
<tr>
<td>Day 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediate</td>
<td>2.93 ± 0.55</td>
<td>3.43</td>
</tr>
<tr>
<td>3 h</td>
<td>3.61 ± 0.49</td>
<td>2.75</td>
</tr>
<tr>
<td>6 h</td>
<td>4.23 ± 0.61</td>
<td>2.13</td>
</tr>
</tbody>
</table>

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.

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, each compared to the 3-min reference treatment, according to prEN 12791

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Ethanol-based hand rub (2 min)</th>
<th>Propanol-based hand rub (1.5 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFU (mean ± SD)</td>
<td>Mean log_{10} RF</td>
</tr>
<tr>
<td>Baseline</td>
<td>4.41 ± 0.61</td>
<td>NA</td>
</tr>
<tr>
<td>Immediate</td>
<td>1.49 ± 1.05</td>
<td>2.92 ± 1.03</td>
</tr>
<tr>
<td>Baseline</td>
<td>4.52 ± 0.69</td>
<td>NA</td>
</tr>
<tr>
<td>3 h</td>
<td>2.05 ± 1.41</td>
<td>2.47 ± 1.25</td>
</tr>
</tbody>
</table>

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 choose 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