

# Alcohols for Skin Antisepsis at Clinically Relevant Skin Sites<sup>∇</sup>

Mirja Reichel,<sup>1,2\*</sup> Peter Heisig,<sup>2</sup> Thomas Kohlmann,<sup>3</sup> and Günter Kampf<sup>1,4</sup>

*Bode Chemie GmbH, Scientific Affairs, Melanchthonstr. 27, 22525 Hamburg, Germany<sup>1</sup>; Department of Pharmaceutical Biology and Microbiology, Institute of Pharmacy, University of Hamburg, 20146 Hamburg, Germany<sup>2</sup>; Institute of Community Medicine, Ernst Moritz Arndt University, Walther-Rathenau-Str. 49a, 17487 Greifswald, Germany<sup>3</sup>; and Institute for Hygiene and Environmental Medicine, Ernst Moritz Arndt University, Walther-Rathenau-Str. 48, 17487 Greifswald, Germany<sup>4</sup>*

Received 30 April 2009/Returned for modification 17 June 2009/Accepted 31 August 2009

**The antiseptic efficacy of ethanol, isopropanol, and *n*-propanol at 60%, 70%, and 89.5% (all vol/vol) was analyzed after 2, 3, or 4 min of application to the forehead, back, and abdomen of 180 volunteers by the use of a standardized swab sampling method. Results of recolonization by the aerobic skin flora of the upper arms and backs of 20 volunteers were compared 72 h after treatment with 0.5%, 1%, or 2% chlorhexidine digluconate (CHG) in 89.5% *n*-propanol. The most effective alcohol at all skin sites was *n*-propanol, with a mean log<sub>10</sub> reduction of 1.82 after 2 min on the forehead. Efficacy against the aerobic flora of the forehead was mainly influenced by the type of alcohol ( $P < 0.001$ ), followed by the concentration ( $P < 0.001$ ) and the application time ( $P = 0.006$ ). Ethanol and isopropanol were significantly less effective (both  $P < 0.001$ ). Alcohol supplemented with 0.5% or more CHG was significantly more effective than alcohol alone in the suppression of recolonization ( $P < 0.05$ ). An 89.5% solution of *n*-propanol was the most effective alcohol for the reduction of populations of aerobic skin flora. Its combination with CHG is appropriate whenever recolonization of the skin must be limited. Further studies are needed to determine the most effective concentration of CHG in *n*-propanol to provide the best protection against recolonization of the skin, e.g., for catheter site care.**

In the United States and Europe, both ethanol and isopropanol are recognized active agents in medicinal products (7, 32), e.g., to prevent surgical-site infections as recommended by the Centers for Disease Control and Prevention (CDC). The U.S. Food and Drug Administration (FDA) assessed aqueous ethanol at 60% to 95% and isopropanol at 70% to 91.3% (vol/vol) as safe and effective for patient preoperative skin preparations (9). In Europe, *n*-propanol is also approved as an active ingredient in medicinal products for skin antisepsis.

For catheter site care, the CDC strongly recommends the use of a 2% chlorhexidine-based preparation (24). The suppression of the recolonization of the catheter site is important because of the permanent gap in the skin barrier and the long intervals between dressing changes. Chlorhexidine is more effective than other standard preparations in preventing catheter colonization when used for catheter site care (33). It is also more effective in reducing bloodstream infection rates, which are the most important clinical endpoints (6, 19, 22).

To the best of our knowledge, the levels of efficacy of the three alcohols used for skin antisepsis have never been studied systematically in vivo. Therefore, in this study, ethanol, isopropanol, and *n*-propanol were compared at three different concentrations and for three different application times at four clinically relevant skin sites to determine which of these antiseptic treatments is most effective in reducing the populations of aerobic skin flora. We then combined the most effective alcohol solutions identified with different concentrations of chlorhexidine digluconate (CHG) and analyzed their effects on

recolonization at two different skin sites after 72 h of sterile coverage.

## MATERIALS AND METHODS

**Antiseptic solutions.** For part 1 of the study, aqueous solutions of ethanol, *n*-propanol, and isopropanol, at concentrations of 60%, 70%, and 89.5% (all vol/vol) each, were used.

For part 2 of the study, 89.5% (vol/vol) aqueous *n*-propanol supplemented with 0.5%, 1.0%, or 2% (all wt/wt) CHG (Evonik Degussa GmbH, Hanau, Germany) was used. All formulations were manufactured by Bode Chemie GmbH, Hamburg, Germany.

**Study population.** The sample size was chosen to determine the efficacy of the alcohols at a specific concentration for a specific application time for 20 subjects. The three types of alcohol were tested at three different concentrations for three application times, resulting in a total of 180 subjects in the first part of the study. For all subjects, ethanol, *n*-propanol, and isopropanol were tested in parallel at a specific concentration and for a specific application time. All four skin sites of each subject were evaluated consecutively on the same day. Volunteers were allowed to participate multiple times in the study but only once each for the evaluation of a specific concentration at a specific application time. A treatment-free interval of at least 7 days between tests of the same subject was maintained. A total of 20 different subjects participated in the second part of the study. In both parts of the study, a sex ratio of 10 women to 10 men was preferred, but a ratio of 9 to 11 or 11 to 9 was accepted.

Volunteers who were younger than 18 years of age, had a skin disease(s) such as dermatitis at one or more of the tested skin sites, or had undergone antibiotic or local antiseptic treatment 7 days before the test were excluded.

**Study design.** A prospective, randomized, unicenter, double-blind study design was chosen. This study was approved by an ethics commission (Freiburger Ethik-Kommission GmbH International, Freiburg, Germany; approval code 07/2064). Informed consent was obtained from each volunteer in a signed consent form.

**Efficacy of alcohols.** The immediate effect of the different alcohols was tested on the forehead, upper back, abdomen, and lumbar area. The alcohol solutions (each identified only by a blinded code designation) were tested with an application time of 2, 3, or 4 min.

At each of these sites, four test areas (5 cm<sup>2</sup> each in size) were marked with a sterilized metal stamp and stamp pad ink before sampling. The four test areas were arranged side by side horizontally on the forehead and in each corner of a larger square on the other skin sites. The test areas on all skin sites of each subject were randomly assigned for baseline sampling (pretreatment) and for the

\* Corresponding author. Mailing address: Bode Chemie GmbH, Scientific Affairs, Melanchthonstr. 27, 22525 Hamburg, Germany. Phone: 49-40-54006276. Fax: 49-40-54006128. E-mail: mirja.reichel@bode-chemie.de.

<sup>∇</sup> Published ahead of print on 8 September 2009.

three types of alcohol by using a Latin-square design. The units were arranged in a four-by-four square such that each treatment appeared once in each row and each column. The rows of the square were allocated to specific skin sites, and the columns were allocated to the specific test areas. A new Latin square was constructed for each subject.

In each test area, the alcohol was applied once every minute with a rayon swab in a standardized manner. In order to ensure that the skin was kept moist during the minute, the entire test area was completely wiped three times. The swab was moistened prior to each use.

Both baseline sampling and posttreatment sampling were performed as follows. The test area was swabbed with a sterile rayon swab (BBL CultureSwab ohne medium; Becton Dickinson, Darmstadt, Germany), premoistened with broth, by pressing the swab as evenly as possible on the skin and rubbing the 5-cm<sup>2</sup> area with a total of 30 swab movements (during approximately 10 to 12 s). The tip of the swab was broken off into a sterile collection tube containing 5 ml of casein-peptone soymeal-peptone broth (Merck KgaA, Darmstadt, Germany) supplemented with 3% polysorbate 80 (Merck KgaA, Darmstadt, Germany), 0.3% lecithin (SERVA Electrophoresis GmbH, Heidelberg, Germany), 0.1% *l*-histidine, and 0.1% *l*-cysteine (both Merck KgaA, Darmstadt, Germany), and the sample was mixed for 30 s. The samples were stored at 2 to 8°C for a maximum of 3 h until spreading, because the numbers of bacteria do not change during this type of storage (16). Each sample was mixed for 5 s before dilution. Aliquots (0.5 ml) and serial dilutions were spread in duplicate on casein-peptone soymeal-peptone agar (CASO agar; Merck KgaA, Darmstadt, Germany) plates with a diameter of 90 mm.

After incubation of the CASO agar plates (aerobically for 48 h at 37 ± 2°C), the numbers of CFU were counted. For determination of the baseline bacterial density, all plates with CFU counts between 15 and 300 were evaluated. For posttreatment bacterial density determinations, all plates with CFU counts below 300, including those with less than 15 CFU, were evaluated to avoid false-positive efficacy results.

**Efficacy of supplementary CHG.** To test the immediate and residual effects of antiseptic containing CHG, six test areas were chosen on the upper arms and upper back. The skin sites were primarily chosen for their clinical relevance for catheter site care. The upper back is very close to the puncture site of subclavian catheters and can easily be sampled and covered with sterile dressings. The upper arm is very close to the antecubital fossa and can also easily be sampled and covered with sterile dressings. On each upper arm, three 5-cm<sup>2</sup> areas were marked, one below the other; on the upper back, two cranial areas were marked side by side, and four areas were marked just below them. The areas in the middle of each upper arm and the cranially located areas on the upper back were used to analyze the baseline counts and the immediate antiseptic effects. The allocation of different sites of the body was balanced across the groups of participants.

The baseline counts and immediate effects of the antiseptics were determined as described for the first part of the study. Casein-peptone soymeal-peptone broth containing 3% polysorbate 80, 0.3% lecithin, 0.1% *l*-histidine, and 0.5% sodium thiosulfate (Merck KgaA, Darmstadt, Germany), 3% saponin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), and 1% sodiumlaurylthethersulfate (Cognis GmbH, Mohnheim am Rhein, Germany), previously described as suitable (14), and CASO agar containing 0.1% *l*-histidine, 0.3% lecithin, and 3% polysorbate 80 (Merck KgaA, Darmstadt, Germany) were used. The neutralization of the alcohol solution supplemented with 2% CHG was validated according to ASTM E 1054-02 (2) with five aerobic challenge microorganisms, all of which are found on human skin (25), to ensure that the carryover of residual antiseptic in vitro did not lead to false-positive efficacy results.

The other four test areas were treated randomly with 89.5% *n*-propanol with or without CHG. The application times were 30 s on the upper arms and 3 min on the back, as described for the first part of the study. After the skin had been dried, each test area was covered with a sterile, water-resistant, vapor-permeable adhesive dressing for 72 ± 2 h. The dressings were then removed and the samples collected, handled, and incubated as described for the baseline samples.

**Determination of efficacy.** Each efficacy evaluation was based on the mean baseline log<sub>10</sub> CFU/cm<sup>2</sup> count at the corresponding skin site. For each type of treatment, the mean log<sub>10</sub> CFU/cm<sup>2</sup> counts for all duplicate analyses were calculated. When the results of two different dilution steps were within the defined CFU range, the weighted means were calculated.

The log<sub>10</sub> reduction was calculated by subtracting the mean log<sub>10</sub> CFU/cm<sup>2</sup> count after each treatment from the mean baseline log<sub>10</sub> CFU/cm<sup>2</sup> count at each skin site.

**Statistical analysis.** All computer-based statistical calculations were made with SPSS 13.0 or 15.0 (SPSS Inc., Chicago, IL).

In the first part of the study, differences between the treatment groups regard-

ing different types of alcohol for a specific application time at a specific concentration were determined (using analysis of variance or, if applicable, the Welch test). When the differences were significant ( $P < 0.05$ ), Tukey's honestly significant difference test or Tamhane's T2 test was used for post hoc analyses.

In the second part of the study, the differences in the mean log<sub>10</sub> reductions between all the treatment groups were analyzed with the Friedman test. When the  $P$  value was lower than 0.05, the Wilcoxon-Wilcox test was applied.

## RESULTS

**Efficacy of alcohols.** The average age of the 180 volunteers was 33 ± 12 years in the first part of the study. The highest density of aerobic flora was on the forehead (mean log<sub>10</sub> CFU/cm<sup>2</sup>, 3.69 ± 1.00), followed by the upper back (3.00 ± 0.90), the abdomen (2.98 ± 0.74), and the lumbar area (2.35 ± 0.70).

Overall, the most effective type of alcohol for reduction of populations of flora was *n*-propanol at 89.5% (vol/vol). The application time was of minor relevance. The results are summarized in Table 1.

All three parameters were ranked regarding their respective degrees of influence on the log<sub>10</sub> reduction at each skin site. Overall, the type of alcohol had the strongest effect (Table 2).

Post hoc analyses of the results obtained for the forehead, the upper back, and the abdomen showed that *n*-propanol was more effective at reducing populations of flora than ethanol (all  $P < 0.001$ ) and isopropanol ( $P < 0.001$  for forehead and upper back;  $P = 0.001$  for abdomen), regardless of the concentration or application time.

Irrespective of the application time or type of alcohol, post hoc analyses of the different alcohol concentrations showed that a 60% concentration was less effective than 70% or 89.5% ( $P < 0.001$  and  $P = 0.006$ ) on the forehead. Furthermore, a 70% concentration was less effective than 89.5% ( $P = 0.003$ ) on the forehead and the upper back ( $P = 0.027$ ). On the upper back and abdomen and in the lumbar area, a concentration of 60% was significantly less effective than 89.5% ( $P = 0.01$  for the upper back;  $P = 0.017$  for the abdomen;  $P < 0.001$  for the lumbar area).

An application time of 2 min was significantly less effective than 3 min and 4 min on the forehead and the upper back ( $P = 0.048$  and  $P = 0.023$  for the forehead;  $P = 0.013$  and  $P < 0.001$  for the upper back), regardless of the type of alcohol or its concentration.

**Efficacy of 89.5% *n*-propanol supplemented with 0.5%, 1%, or 2% CHG or with no supplementation.** The average age for the group of 20 volunteers was 36 ± 13 years. The baseline count, the immediate effect of *n*-propanol, and the log<sub>10</sub> reductions in the populations of flora induced by the supplemented and unsupplemented alcohol solutions after 72 h are shown in Table 3.

The effects of the four antiseptic solutions differed significantly at both skin sites, as is consistent with the maintenance of recolonization by aerobic skin flora below the baseline level after 72 h of sterile coverage ( $P = 0.001$  for the upper arms and  $P = 0.005$  for the upper back by the Friedman test).

On the upper arms, all supplemented antiseptic solutions were significantly more effective than *n*-propanol without CHG in maintaining the recolonization of the aerobic skin flora below the baseline level after 72 h (0.5% CHG,  $P < 0.01$ ; 1% CHG,  $P < 0.05$ ; 2% CHG,  $P < 0.01$  [paired analyses with the Wilcoxon-Wilcox test]).

TABLE 1. Mean log<sub>10</sub> reduction of the skin flora on different skin sites by various alcohols after different application times

Skin site	Type of alcohol <sup>a</sup>	Concn (%)	Mean ± SD log <sub>10</sub> reduction and <i>P</i> value at indicated time (min) <sup>a</sup>					
			2	<i>P</i> value	3	<i>P</i> value	4	<i>P</i> value
Forehead	prop-1	60	1.54 <sup>A/AA</sup> ± 0.94	<0.001	1.93 <sup>B/BB</sup> ± 0.73	<0.001	1.74 <sup>C</sup> ± 1.26	0.044
	prop-2		0.67 <sup>AA</sup> ± 0.65		0.78 <sup>BB</sup> ± 0.79		1.05 ± 0.88	
	eth		0.40 <sup>A</sup> ± 0.54		0.79 <sup>B</sup> ± 0.60		0.83 <sup>C</sup> ± 1.31	
Upper back	prop-1		1.90 <sup>D</sup> ± 0.69	0.020	2.08 ± 0.75	0.292	2.16 ± 0.97	0.596
	prop-2		1.38 ± 0.95		1.72 ± 0.92		1.87 ± 0.97	
	eth		1.09 <sup>D</sup> ± 1.02		1.69 ± 0.95		2.08 ± 0.80	
Abdomen	prop-1		2.25 ± 0.59	0.434	2.63 <sup>E</sup> ± 0.74	0.003	2.56 ± 0.77	0.182
	prop-2		2.16 ± 0.77		2.13 ± 1.07		2.14 ± 0.94	
	eth		1.94 ± 0.97		1.67 <sup>E</sup> ± 0.65		2.10 ± 0.86	
Lumbar area	prop-1		1.93 ± 0.60	0.754	1.81 ± 0.55	0.631	1.82 ± 0.78	0.595
	prop-2		1.79 ± 0.59		1.84 ± 0.57		1.97 ± 0.80	
	eth		1.87 ± 0.61		1.67 ± 0.60		1.72 ± 0.77	
Forehead	prop-1	70	1.73 <sup>F</sup> ± 0.92	0.024	1.90 <sup>G/GG</sup> ± 0.81	0.001	1.89 <sup>H</sup> ± 0.91	0.026
	prop-2		1.21 ± 0.65		1.26 <sup>GG</sup> ± 0.54		1.18 <sup>H</sup> ± 0.84	
	eth		0.98 <sup>F</sup> ± 0.71		1.08 <sup>G</sup> ± 0.64		1.23 ± 0.98	
Upper back	prop-1		1.81 ± 0.64	0.623	1.97 ± 1.36	0.806	2.53 <sup>J/JJ</sup> ± 0.91	0.002
	prop-2		1.69 ± 0.80		1.81 ± 0.99		1.67 <sup>JJ</sup> ± 0.83	
	eth		1.56 ± 1.00		1.74 ± 1.05		1.49 <sup>J</sup> ± 1.07	
Abdomen	prop-1		2.40 ± 0.64	0.245	2.69 ± 0.89	0.235	2.73 <sup>K/KK</sup> ± 0.81	0.002
	prop-2		2.43 ± 0.72		2.23 ± 0.95		1.69 <sup>KK</sup> ± 1.11	
	eth		2.07 ± 0.83		2.55 ± 0.77		2.02 <sup>K</sup> ± 0.80	
Lumbar area	prop-1		2.18 ± 0.51	0.364	2.10 ± 0.65	0.628	2.00 ± 0.86	0.731
	prop-2		2.12 ± 0.53		1.91 ± 0.60		1.90 ± 1.12	
	eth		1.94 ± 0.58		1.93 ± 0.74		1.76 ± 1.01	
Forehead	prop-1	89.5	1.82 ± 1.24	0.132	2.09 ± 1.33	0.403	2.38 <sup>L</sup> ± 1.17	0.002
	prop-2		1.44 ± 1.03		1.70 ± 0.93		1.88 ± 0.88	
	eth		1.17 ± 0.71		1.60 ± 0.94		1.34 <sup>L</sup> ± 0.52	
Upper back	prop-1		2.25 <sup>M</sup> ± 0.75	0.006	2.13 ± 0.53	0.445	2.59 ± 0.90	0.068
	prop-2		1.89 ± 0.91		2.00 ± 0.59		1.91 ± 1.07	
	eth		1.40 <sup>M</sup> ± 0.74		2.27 ± 0.85		2.02 ± 0.93	
Abdomen	prop-1		2.54 ± 0.71	0.888	2.53 ± 0.97	0.722	2.70 ± 0.97	0.070
	prop-2		2.43 ± 0.71		2.51 ± 0.85		2.29 ± 0.90	
	eth		2.50 ± 0.78		2.32 ± 0.98		1.99 ± 1.02	
Lumbar area	prop-1		2.14 ± 0.55	0.679	2.05 ± 0.69	0.793	2.36 ± 0.59	0.302
	prop-2		2.03 ± 0.52		2.15 ± 0.54		2.26 ± 0.61	
	eth		1.98 ± 0.68		2.01 ± 0.77		2.06 ± 0.65	

<sup>a</sup> prop-1, *n*-propanol; prop-2, isopropanol; eth, ethanol.

<sup>b</sup> Different superscript uppercase characters (A to M) represent significant differences in post hoc analysis results between the different types of alcohol. Single and double superscript uppercase characters are used whenever a significant difference was found between two pairs of means. Each mean value represents data from 20 different subjects.

TABLE 2. Ranking of parameters with influence on the reduction of skin flora on different skin sites (*n* = 180)

Skin site	Parameter ranking (highest influence to lowest influence)
Forehead.....	Type of alcohol ( <i>P</i> < 0.001) > concn ( <i>P</i> < 0.001) > application time ( <i>P</i> = 0.006)
Upper back.....	Type of alcohol ( <i>P</i> < 0.001) > application time ( <i>P</i> < 0.001) > concn ( <i>P</i> = 0.006)
Abdomen.....	Type of alcohol ( <i>P</i> < 0.001) > concn ( <i>P</i> = 0.024) > application time (n.s. <sup>a</sup> )
Lumbar area.....	Concn ( <i>P</i> < 0.001) > type of alcohol (n.s.)/application time (n.s.)

<sup>a</sup> n.s., not significant.

On the upper back, the antiseptic solutions supplemented with 0.5% and 2% CHG were significantly more effective than the *n*-propanol solution without CHG in suppressing the recolonization by the aerobic skin flora after 72 h (both *P* < 0.05).

No significant differences were observed among the three CHG-supplemented solutions at both skin sites (all paired analyses; *P* > 0.05 [Wilcoxon-Wilcox test]).

**DISCUSSION**

We have shown for the first time in vivo that the type of alcohol is the most important factor, followed by its concen-

TABLE 3. Mean log<sub>10</sub> reduction and suppression of recolonization of skin flora after 72 h of coverage (*n* = 20)

Skin site	Mean log <sub>10</sub> baseline count (CFU/cm <sup>2</sup> )	Application time	Immediate log <sub>10</sub> reduction of <i>n</i> -propanol (89.5%)	Suppression of recolonization (expressed as log <sub>10</sub> difference from baseline count after 72 h of sterile coverage) in <i>n</i> -propanol solution (89.5% [vol/vol]) supplemented with <sup>a</sup> :			
				No supplementation	0.5% CHG	1% CHG	2% CHG
Upper arms	2.36 ± 0.77	30 s	2.06 ± 0.59	0.41 <sup>Δ/****</sup> ± 1.34	1.33 <sup>Δ</sup> ± 1.29	1.16* ± 1.23	1.24** ± 0.96
Upper back	2.94 ± 0.94	3 min	2.14 ± 0.85	0.28 <sup>▲/°</sup> ± 1.46	1.07 <sup>▲</sup> ± 1.81	1.42 ± 1.43	1.30 <sup>°</sup> ± 1.56

<sup>a</sup> Different superscript characters represent significant differences of means for each skin site in post hoc analysis results.

tration and application time, in producing the best aerobic skin flora reduction.

In our study, *n*-propanol was the most effective alcohol in reducing the aerobic skin flora at all skin sites tested. To achieve the same reduction as *n*-propanol, ethanol or isopropanol must be applied at higher concentrations or for longer times or both. No systematic studies have compared the efficacy of these alcohols in skin antiseptics, although similar findings have been reported for hand hygiene (but a less systematic approach was taken in those studies) (13, 27, 31). Despite the evidence identifying *n*-propanol as the most effective alcohol, it was the only alcohol in our study that is not approved by the FDA as safe and effective (category I) for skin antiseptics (32).

We found that the skin site itself has an impact on the reduction of the skin flora by antiseptics. This is explicable by the range of densities of skin flora at different sites (4, 18, 20, 23) and probably the physiological condition of the treated skin area (15). For example, the mean baseline counts per square centimeter on the abdomen and the upper back were similar, but the log<sub>10</sub> reductions on the upper back were considerably lower.

The highest bacterial density was found on the forehead, where the log<sub>10</sub> reductions were lower those at the other skin sites. This can be explained by the high density of sebaceous glands, which may create a protecting barrier (4, 8, 10, 18, 20, 21). Nevertheless, the greatest differences in the efficacy were observed at this site. Alcohol solutions that were effective on the forehead were also highly effective at the other skin sites. These results challenge the idea that testing antiseptics on the groin and abdomen, as recommended by the FDA and the ASTM E 1173-01 methods (3, 9), provides acceptable results for surgical procedures performed on the head, e.g., in neurosurgery.

In our study the lowest colony counts were found at the lumbar site. All treatments achieved an irreducible minimum density of the aerobic skin flora within 2 min. This area does not appear to be as critical as previously thought in Germany, where a standard application time for skin antiseptics is 10 min at the lumbar site, although there may be severe consequences if bacteria gain access to the cerebrospinal fluid during puncture (26, 28, 29).

The composition of the skin microflora and their sensitivity to antiseptic agents also depend on the skin site. Skin with many sebaceous glands is highly colonized by anaerobes, and skin with few sebaceous glands mainly harbors staphylococci and aerobic coryneforms (5, 18, 21). We did not distinguish between different floral species. Therefore, the composition of

the resident flora may also explain the variable levels of efficacy at different skin sites.

The findings with regard to the effects of CHG on the persistence of antimicrobial efficacy on human skin are supported by substantial evidence (6, 19). However, efficacy data for CHG must be assessed very carefully, because false positive results are possible when neutralization is insufficient (12). Combining CHG with alcohol is advantageous, because the slowness with which CHG takes effect is of little importance due to the immediate effect of the alcohol (8).

We found that CHG (0.5% or higher) in 89.5% *n*-propanol significantly slowed down the recolonization of the skin when it was covered for 72 h. The unsupplemented alcohol solution had only a marginal effect. This finding is supported by the results of other studies (11, 17); on the abdominal skin, 2% CHG in 70% isopropanol provided a significantly more persistent antimicrobial activity than did isopropanol alone (11). It has been shown effective in the prevention of colonization of peripheral venous catheters (1, 11, 30).

Although we found that 1% CHG in 89.5% *n*-propanol had the greatest effect on the upper back after 72 h, we found no statistically significant difference compared to the results obtained with unsupplemented alcohol. This is a surprising finding to us, and we can only explain it with the chosen statistical test, which was a pairwise comparison. But we do not doubt its biological relevance.

Our data indicate that 0.5% CHG was already effective in slowing down recolonization at two skin sites. Vallés et al. found no difference between 0.5% alcoholic CHG and 2% aqueous CHG in preventing catheter colonization (33). With alcohol used as a solvent, even a <0.5% concentration of CHG may be sufficient to slow down recolonization. Further studies are needed to clarify whether CHG concentrations lower than 2% in alcohol solutions provide equivalent results for the prevention of catheter-related bloodstream infections.

**Conclusions.** An 89.5% concentration of *n*-propanol was the most effective alcohol solution in reducing the aerobic skin flora. A combination with at least 0.5% CHG slowed down the recolonization of the skin flora. But further studies are needed to determine the most effective CHG-concentration in *n*-propanol for providing the best protection against recolonization of the skin, e.g., for catheter site care.

#### ACKNOWLEDGMENTS

The study was funded by Bode Chemie GmbH, Hamburg, Germany. Mirja Reichel and Günter Kampf are employees of Bode Chemie GmbH, Hamburg, Germany. The independent statistical analysis was carried out by Thomas Kohlmann. No compensation or funding was

received for conducting the statistical analyses. Thomas Kohlmann, who is independent of the commercial sponsor, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Peter Heisig has no potential conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript.

## REFERENCES

- Adams, D., M. Quayum, T. Worthington, P. Lambert, and T. Elliott. 2005. Evaluation of a 2% chlorhexidine gluconate in 70% isopropyl alcohol skin disinfectant. *J. Hosp. Infect.* **61**:287–290.
- Anonymous. 2002. ASTM E 1054-02. Standard test method for evaluation of inactivators of antimicrobial agents. ASTM, Villanova, PA.
- Anonymous. 2002. ASTM E 1173-01. Standard test method for evaluation of preoperative, precatheterization, or preinjection skin preparations. ASTM, Villanova, PA.
- Bibel, D. J., and D. J. Lovell. 1976. Skin flora maps: a tool in the study of cutaneous ecology. *J. Invest. Dermatol.* **67**:265–269.
- Bojar, R. A., and K. T. Holland. 2002. The human cutaneous microflora and factors controlling colonisation. *World J. Microbiol. Biotechnol.* **18**:889–903.
- Chaiyakunapruk, N., D. L. Veenstra, B. A. Lipsky, and S. Saint. 2002. Chlorhexidine compared with povidone-iodine solution for vascular catheter-site care: a meta-analysis. *Ann. Intern. Med.* **136**:792–801.
- Council of Europe. 2007. European Pharmacopoeia 6.0. Directorate for the Quality of Medicines & Health Care of the Council of Europe (EDQM), Strasbourg, France.
- Dzubow, L. M., A. C. Halpern, J. J. Leyden, D. Grossman, and K. J. McGinley. 1988. Comparison of preoperative skin preparations for the face. *J. Am. Acad. Dermatol.* **19**:737–741.
- Federal Register. 1994. Tentative final monograph for health care antiseptic products; proposed rule. *Fed. Regist.* **59**:31401–31452.
- Halpern, A. C., J. J. Leyden, L. M. Dzubow, and K. J. McGinley. 1988. The incidence of bacteremia in skin surgery of the head and neck. *J. Am. Acad. Dermatol.* **19**:112–116.
- Hibbard, J. S., G. K. Mulberry, and A. R. Brady. 2002. A clinical study comparing the skin antiseptics and safety of ChlorPrep, 70% isopropyl alcohol, and 2% aqueous chlorhexidine. *J. Infus. Nurs.* **25**:244–249.
- Kampf, G. 2009. Effect of chlorhexidine probably overestimated due to lack of neutralization after sampling. *Infect. Control Hosp. Epidemiol.* **30**:77–79.
- 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.
- Kampf, G., M. Shaffer, and C. Hunte. 2005. Insufficient neutralization in testing a chlorhexidin-containing ethanol-based hand rub can result in a false positive efficacy assessment. *BMC Infect. Dis.* **5**:48.
- Kearney, J. N., E. Ingham, W. J. Cunliffe, and K. T. Holland. 1984. Correlation between human skin bacteria and skin lipids. *Br. J. Dermatol.* **110**:593–599.
- Keswick, B. H., and D. Frank. 1987. Modified scrub technique for sampling infant skin microflora. *J. Clin. Microbiol.* **25**:2400–2401.
- Leyden, J. J., K. J. McGinley, A. N. Foglia, J. E. Wahrman, C. N. Gropper, and B. R. Vowels. 1996. A new method for in vivo evaluation of antimicrobial agents by translocation of complex dense populations of cutaneous bacteria. *Skin Pharmacol.* **9**:60–68.
- Leyden, J. J., K. J. McGinley, K. M. Nordstrom, and G. F. Webster. 1987. Skin microflora. *J. Invest. Dermatol.* **88**:65s–72s.
- Maki, D. G., M. Ringer, and C. J. Alvarado. 1991. Prospective randomised trial of povidone-iodine, alcohol and chlorhexidine for prevention of infection associated with central venous and arterial catheters. *Lancet* **338**:339–343.
- Marples, M. J. 1969. Life on the human skin. *Sci. Am.* **220**:108–115.
- McGinley, K. J., G. F. Webster, and J. J. Leyden. 1978. Regional variations of cutaneous propionibacteria. *Appl. Environ. Microbiol.* **35**:62–66.
- Mimoz, O., S. Villeminey, S. Rago, C. Dahyot-Fizelier, L. Laksiri, F. Petitpas, and B. Debaene. 2007. Chlorhexidine-based antiseptic solution vs alcohol-based povidone-iodine for central venous catheter care. *Arch. Intern. Med.* **167**:2066–2072.
- Nobel, W. C. 1975. Dispersal of skin microorganisms. *Br. J. Dermatol.* **93**:477–485.
- O'Grady, N. P., M. Alexander, E. P. Dellinger, J. L. Gerberding, S. O. Heard, D. G. Maki, H. Masur, R. D. McCormick, L. A. Mermel, M. L. Pearson, I. I. Raad, A. Randolph, and R. A. Weinstein. 2002. Guidelines for the prevention of intravascular catheter-related infections. *Infect. Control Hosp. Epidemiol.* **23**:759–769.
- Reichel, M., P. Heisig, and G. Kampf. 2008. Pitfalls in efficacy testing-how important is the validation of neutralization of chlorhexidine digluconate? *Ann. Clin. Microbiol. Antimicrob.* **7**:20.
- Reihsaus, E., H. Waldbaur, and W. Seeling. 2000. Spinal epidural abscess: a meta-analysis of 915 patients. *Neurosurg. Rev.* **23**:175–204.
- Rotter, M. L., R. A. Simpson, and W. Koller. 1998. Surgical hand disinfection with alcohols at various concentrations: parallel experiments using the new proposed European standards methods. *Infect. Control Hosp. Epidemiol.* **19**:778–781.
- Sato, S., T. Sakuragi, and K. Dan. 1996. Human skin flora as a potential source of epidural abscess. *Anesthesiology* **85**:1276–1282.
- Sendi, P., T. Bregenzer, and W. Zimmerli. 2008. Spinal epidural abscess in clinical practice. *QJM* **101**:1–12.
- Small, H., D. Adams, A. L. Casey, C. T. Crosby, P. A. Lambert, and T. Elliott. 2008. Efficacy of adding 2% (w/v) chlorhexidine gluconate to 70% (v/v) isopropyl alcohol for skin disinfection prior to peripheral venous cannulation. *Infect. Control Hosp. Epidemiol.* **29**:963–965.
- Suchomel, M., W. Koller, M. Kundi, and M. L. Rotter. 2009. Surgical hand rub: influence of duration of application on the immediate and 3-hours effects of n-propanol and isopropanol. *Am. J. Infect. Control* **37**:289–293.
- U.S. Pharmacopeial Convention. 2009. National formulary 2009, vol. USP 32-NF 27. U.S. Pharmacopeia, Rockville, MD.
- Vallés, J., I. Fernandez, D. Alcaraz, E. Chacon, A. Cazorla, M. Canals, D. Mariscal, D. Fontanals, and A. Moron. 2008. Prospective randomized trial of 3 antiseptic solutions for prevention of catheter colonization in an intensive care unit for adult patients. *Infect. Control Hosp. Epidemiol.* **29**:847–853.