

Keywords*Skin antiseptics**Skin flora**Reference method**1-propanol**2-propanol**Non-inferiority test***Mirja Reichel^{1,2*}, Peter Heisig², Günter Kampf^{1,3}**¹ BODE Chemie GmbH, Scientific Affairs, Hamburg, Germany² Institute of Pharmacy, Department of Pharmaceutical Biology and Microbiology, University of Hamburg, Hamburg, Germany³ Institute of Hygiene and Environmental Medicine, Ernst-Moritz-Arndt-University, Greifswald, Germany

Comparison between the efficacy of 89.5 % 1-propanol in shorter application times and the reference method of the DGHM for skin antiseptics

Summary

Background: In Germany skin antiseptics are considered to be effective on sebaceous-rich skin sites if an equal effectiveness is demonstrated compared to the reference method (RM) of the German Society for Hygiene and Microbiology (DGHM); 10 minutes application time; 70 % 2-propanol. In a previous study we were able to show that 89.5 % (v/v) 1-propanol is more effective than 70 % 2-propanol [1]. Therefore we investigated in vivo if the efficacy of 89.5 % 1-propanol is non-inferior compared to the RM in shorter application times.

Methods: The efficacy of 89.5 % 1-propanol at 4, 3, 2 minutes as well as 1 minute application time was determined at the forehead, the abdomen, the upper back and the lumbar area compared to the RM. Quantification of the skin flora was carried out according to the standard test method of the DGHM. Statistical analysis was carried out by non-inferiority testing.

Results: 89.5 % 1-propanol showed non-inferiority to the RM at 3 minutes application time at all sites, and it was sometimes even superior. The lowest \log_{10} reductions were found at the forehead; at one minute application time 89.5 % 1-propanol showed non-inferiority [\log_{10} reduction; 1-propanol: 1.24 ± 0.77 ; RM: 1.51 ± 0.91]. At the upper back a minimum of 3 minutes application time was necessary to obtain non-inferiority; at the abdomen and the lumbar area 2 minutes were sufficient.

Conclusion: Compared to the RM, equal reduction of skin flora was obtained with 89.5 % 1-propanol

at 3 minutes application time. For establishment of an European standard test method for skin antiseptics, 1-propanol represents an interesting option for the reference method.

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Introduction

The prevention of nosocomial infections, such as postoperative wound infections and catheter-related infections, is of major importance in clinical practice. One crucial source of infection is the endogenous, resident skin flora of the patient. Hence, reducing the skin flora by using skin antiseptics is one important infection control measure [2, 3]. For using a preparation in practice, it is essential that its efficacy at defined application times has been proven previously.

There are three factors that are of major importance to the efficacy and therefore the patient protection: the type of alcohol, the concentration of the alcohol-based solution and the compliance with the necessary application time [1]. In Europe, ethanol, 2-propanol and 1-propanol are used in preparations for skin antiseptics. Up to now, there is no European standard for the test of skin antiseptics analogous to the standards for the efficacy tests in hand hygiene. In Germany, skin antiseptics are tested according to a standard method that

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was established by the DGHM, comprising practical *in vivo* tests with healthy volunteers [4]. This method is used for the determination of the efficacy against the resident skin flora.

In a cross-over comparison with 20 participants, the efficacy of the test preparation is compared with the efficacy of the DGHM reference alcohol (70 % (v/v) 2-propanol) on sebaceous-rich and sebaceous-poor skin. The method prescribes that the sebaceous-rich skin has to be kept moist with the reference alcohol and the test preparation for a time period of 10 minutes. On this account all antiseptics available on the German market had to fulfil the 10-minute application time on sebaceous-rich skin areas according to their authorization as a medicinal product until the end of 2008. The fact that drug approvals were based on the DGHM recommendation caused scientists to increasingly criticise the minimum application time of 10 minutes [5]. However, a more recent study proved that certain preparations reach an efficacy that equals the efficacy of the reference procedure within shorter application times [6]. Shortening the application times, while maintaining the protection of patients, offers many considerable advantages, and will lead to higher acceptance in clinical practice [7]. The key advantage in daily clinical routine is the time saved, which, for example, can be of particular importance to accident and emergency units. Another important factor is the cost savings.

The results of the dissertation, which formed the basis for the DGHM method [8], already suggest that the efficacy and reduction of the skin flora respectively depends on the type of alcohol. For this reason and for optimising skin antiseptics, we previously performed a systematic study and determined the efficacy of three alcohols in three different concentrations at three different application times (≤ 4 minutes) to identify the most effective alcohol solution [1]. The study also comprised the reference alcohol used in the DGHM method; however, the 89.5 % 1-propanol solution yielded a significant higher efficacy.

The question that arose from this study was whether the 89.5 % 1-propanol solution can reach an efficacy that is not inferior to the reference procedure within application times that are shorter than 10 minutes. Hence, we investigated the effi-

cacy of this antiseptic solution at the forehead, the abdomen, the upper back and in the lumbar area at application times of 1, 2, 3 and 4 minutes, and compared it with the DGHM reference method.

Materials and Methods

Alcohol-based solutions

89.5 % 1-propanol and 70 % (v/v) 2-propanol manufactured by BODE Chemie GmbH, Hamburg, Germany.

Participants

20 volunteers participated in each of both trial parts. Criteria for exclusion were:

- age under 18 years,
- existing skin diseases on the skin areas to be tested,
- undergoing an antibiotic therapy or an antiseptic treatment within 7 days before the test.

In both parts of the study a balanced gender ratio of 10 women to 10 men was preferred, in order not to possibly influence the results due to the different skin colonisation of men and women [9], but a ratio of 9 to 11 or 11 to 9 was accepted. In this study, both parts were performed with 11 women and 9 men. Each participant was allowed to take part twice, but only once in each part of the study, so that each mean value was based on the results of 20 different participants.

Study design

Prospective, randomised, monocentric study. This study was approved by an ethics committee (Freiburg Ethics Commission GmbH International, Freiburg, study number 07/2064). Before each test, informed consent was obtained from each volunteer in a signed consent form.

Sampling

The immediate effect of 89.5 % 1-propanol was determined in a first study part with application times of 4 and 3 minutes and in a second part with application times of 2 minutes and 1 minute, and was compared with the 10-minute reference procedure with 70 % (v/v) 2-propanol in both parts of the study. Both alcohols were applied to the forehead, abdomen, upper back and the lumbar area of 20 participants. On each of these four skin sites, we

marked four sampling areas that were 5 cm² in size (arrangement: side by side on the forehead; rectangular on all other skin sites). The test areas on all skin sites were randomly assigned for baseline sampling (pre-treatment) and for the application of the test products to avoid sample site bias [9]. If necessary, hair was pinned up for the tests on the forehead. The DGHM method also prescribes sampling after 30 minutes. As several other studies showed that there are no significant differences to the immediate effect and that these values usually lead to the same overall result [6, 10], this study does not include sampling after 30 minutes.

The different alcohol-based solutions were applied with a rayon swab (BBL CultureSwab without medium, Becton and Dickinson, Darmstadt, Germany) in a standardised way. The standardised application at least once per minute ensured that all test fields were kept moist during the entire application time. Both baseline and posttreatment sampling of skin microorganisms was carried out as follows: prior to each sampling, test sites were marked by using a sterilised metallic stamp and stamping ink. For 10–12 seconds, the 5 cm² sampling sites were rubbed with a swab, previously saturated with broth, 30 times by applying steady pressure. Afterwards, the swab tip was put into a sterile test tube containing 5 ml CSL [4], and was then vortexed for 30 seconds. The broth was supplemented with a neutralisation mixture consisting of 3 % polysorbate 80, 0.3 % lecithin, 0.1 % L-histidine, and 0.1 % L-cysteine [4]. Until the plating, the samples were stored at 2 to 8 °C for a maximum of 3 hours [11, 12]. Each sample was vortexed for 5 seconds before preparing the dilution series; 0.5 ml of the sample and aliquots of corresponding dilutions were plated on casein-peptone soymeal-peptone agar (CASO Agar, Merck KGaA, Darmstadt, Germany) in duplicates.

After incubation (aerobically; 48 hours at 37 ± 2 °C), the colony-forming units (CFU) on each plate were quantified. To determine the baseline density of microorganisms, all plates with a density of 15 to 300 CFU were included. To preclude false positive efficacy results, all results < 15 CFU were also included for the determination of the posttreatment bacterial density.

Determination of the efficacy

Each efficacy evaluation was based on the mean baseline \log_{10} CFU/cm² count at the corresponding skin site. The efficacy of each procedure was determined for each skin site by calculating the \log_{10} reduction from the difference between the mean \log_{10} CFU/cm² count after each treatment (posttreatment) and the mean \log_{10} baseline CFU/cm².

Statistical evaluation

Data of all disinfection procedures were checked for normal distribution by the Kolmogorov-Smirnov test. The main objective of the study was to determine the non-inferiority of the test procedure in comparison to the reference method at each skin site and for each application time. For this, a one-sided 97.5 % confidence interval was calculated following the Feller method [13]. The type I error was set to 0.025; the non-inferiority margin to 0.7. In case the mean value of the test procedure was higher than the mean value of the reference method [14], it was determined with a two-sided t-test whether the difference between the efficacies was significant. The significance level for the one-sided examination was set to $p < 0.05$.

Results

The tables 1 and 2 show the mean baseline-values and \log_{10} reductions with the corresponding standard deviations for all skin sites and application times of both trial parts. Normal distribution was demonstrated for all datasets.

At the forehead, non-inferiority to the reference procedure was shown at all four time periods for the antiseptic treatment with 89.5 % 1-propanol. With a 2-minute application time, the efficacy of the antiseptic solution even exceeded the 10-minute reference method (2-minute application time: $p=0.018$; t-test). With an application time of 1 minute, the \log_{10} reductions of both procedures were very low; especially the \log_{10} reduction of the new procedure was about one \log_{10} step lower compared to the \log_{10} reduction of the 2-minute application time.

At the abdomen, the 1-propanol solution needed a minimum application time of 2 minutes to reach an efficacy equivalent to the reference procedure; an equivalent efficacy could not be proved for an

application time of 1 minute. With an application time of 4 minutes, this antiseptic procedure was even superior to the reference procedure ($p = 0.038$; t-test).

At the upper back, non-inferiority of the 1-propanol solution could not be proved at application times of less than 3 minutes. However, at application times of 3 minutes and longer the optimised procedure even yielded a superior efficacy to the reference procedure (3-minute application time: $p = 0.021$; 4-minute application time: $p = 0.027$).

At the lumbar area, the \log_{10} reductions were marginally different from the reference procedure at an application time of 3 minutes and longer. The antiseptic treatment with 1-propanol yielded an efficacy equivalent to the reference procedure at an application time of 2 minutes.

Discussion

In this study, we could demonstrate that, within significantly shorter application times, the 89.5 % 1-propanol solution reaches \log_{10} reductions of the skin flora that are at least equivalent to the DGHM reference method. A minimum application time of 3 minutes was sufficient on all four skin sites; on the forehead, on the abdomen and in the lumbar area non-inferiority could even be proved within application times < 3 minutes.

Until the end of 2008, the DGHM recommended an application time of at least 10 minutes on sebaceous-rich skin, for all skin antiseptics available on the German market, due to in vivo tests with healthy volunteers. Drug approvals that were based on this recommendation stipulated the minimum application time of 10 minutes for all preparations. Scientists increasingly criticised this [5]. Since the beginning of 2009, the Association for Applied Hygiene (VAH) list also has included skin antiseptics with much shorter application times [15], because a study showed that, for example, within 2.5 minutes an 89 % (m/m) ethanol solution reaches a \log_{10} reduction that equals the reference method [6].

In this study, the application time necessary to prove non-inferiority depended on the skin site. A possible explanation could be the different skin flora density on the one hand [16–19]; and on the other hand the different physiological

conditions of the skin sites may also play a role [20]. We found the highest microbial density at the forehead; however, the reduction achieved with the different procedures was the lowest here.

The DGHM method stipulates the forehead as test site for the test on sebaceous-rich skin [4]. There are two reasons for this: 1. in Germany, sebaceous-rich skin is considered as a critical site in terms of antiseptic efficacy, and 2. this site is easy to sample due to its accessibility. The categorisation of sebaceous-rich and sebaceous-poor skin areas, like commonly done in Germany, is not unproblematic, as there is no definite border between these areas. Therefore, it is not always clear which application times need to be observed in clinical practice. The figure of the human body in the VAH list clearly illustrates this [21], as it highlights sebaceous-rich sites, but does not demarcate them definitely from the adjacent skin sites.

Our results show that the forehead is a suitable test site for skin antiseptics, as all alcohols that were efficient on this skin site also reached a very good efficacy on all other selected skin sites. In addition, this was the site where the different alcohol-based solutions showed the most significant differences in efficacy, facilitating a categorisation of efficient and less efficient antiseptics [1].

Compared to the reference method, the optimised alcohol solution consisting of 1-propanol in a concentration of 89.5 % proved to yield very good efficacies particularly on the forehead. Even with the shortest application time of 1 minute, the solution was not inferior to the reference procedure at this site. The \log_{10} reductions, however, show a significant increase in efficacy between the 1-minute and the 2-minute application time, so that the proof of non-inferiority can be related to the reference method's comparably low efficacy. In our trial, the 10-minute reference method still yielded a reduction of 1.5 \log_{10} steps only. As 89.5 % 1-propanol even exceeded the reference method's efficacy at an application time of 2 minutes, this procedure proved to be particularly suitable for the reduction of the skin flora.

Instead of the upper arms, which the DGHM method uses for the test on sebaceous-poor skin [4], we selected the abdomen, the upper back and the lumbar area for this study. From a clinical point of view, the abdomen is a relevant skin

Table 1: Comparison of 89.5 % 1-propanol at application times of 4 and 3 minutes with the DGHM reference method (70 % 2-propanol; 10-minute application time); (n=20).

Skin site	Baseline density (log ₁₀ KBE/cm ²)	Mean log ₁₀ -reduction ± SD		
		4 min	3 min	10 min (Ref.)
Forehead	3,75 ± 1,05	2,00 ± 0,99*	2,03 ± 0,72*	1,51 ± 0,96
Abdomen	3,17 ± 0,80	2,92 ± 0,75*/**	2,75 ± 0,90*	2,36 ± 0,89
Upper back	2,86 ± 0,96	2,31 ± 0,77*/**	2,35 ± 0,81*/**	1,74 ± 0,79
Lumbar area	2,35 ± 0,75	2,11 ± 0,72*	1,99 ± 0,86*	2,28 ± 0,68

* Non-inferiority (89.5 % 1-propanol) to the reference procedure (ref.); ** Superiority ($p < 0.05$; t-test).

Table 2: Comparison of 89.5 % 1-propanol at application times of 2 minutes and 1 minute with the DGHM reference method (70 % 2-propanol; 10-minute application time); (n=20).

Skin site	Baseline density (log ₁₀ KBE/cm ²)	Mean log ₁₀ -reduction ± SD		
		2 min	1 min	10 min (Ref.)
Forehead	3,75 ± 1,04	2,26 ± 1,00*/**	1,24 ± 0,77*	1,51 ± 0,91
Abdomen	3,12 ± 0,78	2,26 ± 1,03*	1,85 ± 0,62	2,37 ± 0,68
Upper back	3,10 ± 0,92	2,16 ± 0,98	1,80 ± 0,89	2,53 ± 0,69
Lumbar area	2,42 ± 0,61	1,84 ± 0,75*	1,67 ± 0,53	2,23 ± 0,51

* Non-inferiority (89.5 % 1-propanol) to the reference procedure (ref.); ** Superiority ($p < 0.05$; t-test).

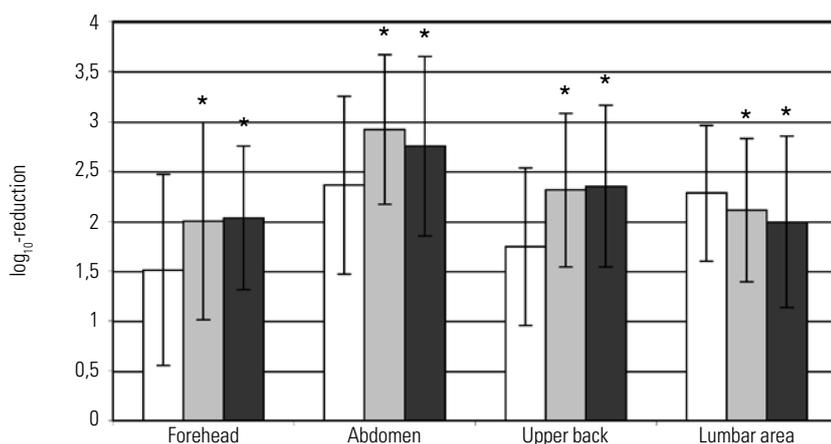


Figure 1: Comparison of the mean log₁₀ reduction of 89.5 % 1-propanol at application times of 4 and 3 minutes with the DGHM reference method; (n=20). White: 70 % 2-propanol; 10-minute application time (reference method) / Light grey: 89.5 % 1-propanol; 4-minute application time / Dark grey: 89.5 % 1-propanol; 3-minute application time * Non-inferiority (89.5 % 1-propanol) to the reference procedure.

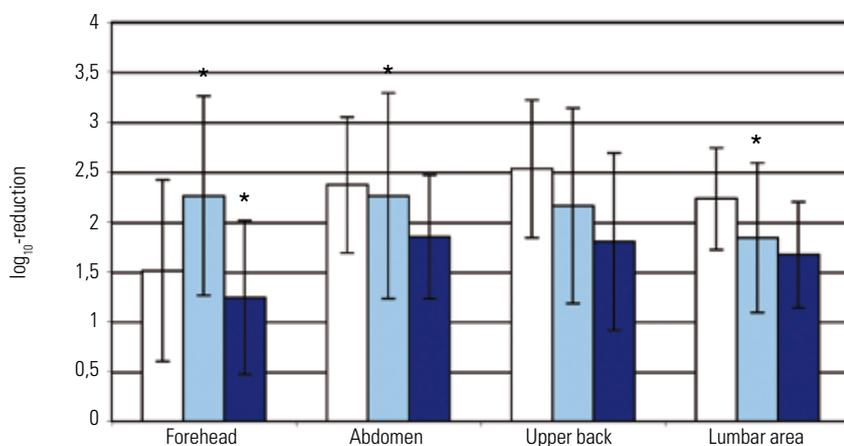


Figure 2: Comparison of the mean log₁₀ reduction of 89.5 % 1-propanol at application times of 2 minutes and 1 minute with the DGHM reference method; (n=20). White: 70 % 2-propanol; 10-minute application time (reference method) / Light blue: 89.5 % 1-propanol; 2-minute application time / Dark blue: 89.5 % 1-propanol; 1-minute application time * Non-inferiority (89.5 % 1-propanol) to the reference procedure.

site. For the back, there were so far no studies if there are any differences in efficacy depending on the location in this skin site. The lumbar area still belongs to the sites with sebaceous-rich skin and is considered especially critical for skin antiseptics in Germany. But our results could not confirm this: the skin flora density was particularly low in this site and even with very short application times we could prove a very good efficacy. However, it has to be kept in mind that with the rectangular arrangement of the test fields to the right and left of the spine we – according to the illustration of the VAH list [21] – sampled the transition region between sebaceous-rich and sebaceous-poor skin. Due to the very good efficacy on the sebaceous-rich forehead, this fact can however be disregarded.

In the paired comparison of the results yielded on the upper back, non-inferiority could not be proved for the 2-minute application time, although the values of the 2-minute and 3-minutes application time were quite similar. A possible reason might be that, in contrast to all other skin sites, the log₁₀ reductions of the reference method on the upper back differed by about 0.8 log₁₀ steps between the two trial parts.

For the lumbar area, the minimum application time of 10 minutes is still recommended for the most commercially available skin antiseptics according to their authorization as a medicinal prod-

uct. In clinical practice, this long time period might pose a problem, e.g. in emergency situations. The proof that antiseptic preparations can reach sufficient efficacy within shorter application times while maintaining the patient safety is a major improvement for the daily routine in clinics. In this study, an application time of 2 minutes sufficed to proof non-inferiority to the reference method at this site. Both procedures yielded similar efficacies. In contrast to all other skin sites, superiority of the 1-propanol solution to the reference procedure could not be proved. Possible reasons might be that the low baseline density of microorganisms and the fact that in general there were very few microorganisms at this site after the antiseptic treatment. This might mean that at this site the skin flora is quickly reduced to an irreducible minimum that cannot be reduced further.

Additional systematic studies are necessary to clarify the question whether alcohol-based solutions that are different from the reference alcohol of the DGHM yield an equivalent reduction of the aerobic skin flora at different application times. In a previous study with 180 participants we could show that the type of alcohol is the most important influencing factor in skin antiseptics; 2-propanol was inferior to 1-propanol and an alcohol concentration of 70 % lead to a lower reduction than 89.5 % [1].

Surgical hand disinfection in accordance with the European standard EN 12791 [22] uses 60 % 1-propanol as reference alcohol. In 1998, Rotter et al. showed that 1-propanol was more efficient against the resident skin flora of hands than 2-propanol. Only in a concentration of as much as 90 % 2-propanol reached an equivalent efficacy compared to 60 % 1-propanol [23]. Suchomel et al. confirmed the superior efficacy of 1-propanol against the resident flora of hands in a study that compared 60 % 1-propanol and 70 % 2-propanol [24]. The results of this study suggest that the less efficient 70 % 2-propanol can also yield better efficacies when it is used with significant longer application times.

A non-inferiority test was carried out to compare the effect of the application of 89.5 % 1-propanol at shorter application times and the current reference method. Up to now, this procedure is not

destined for the DGHM method for determining the efficacy for skin antiseptics, but found its way to the European method for determining the efficacy of hand disinfection (EN 1500) and surgical hand disinfection (EN 12791). The Wilcoxon signed-rank test for paired differences, which is described in the DGHM method for skin antiseptics, is suitable for proving the superiority of an antiseptic procedure in comparison to the reference method. However, this study should show that a skin antiseptics procedure is at least equivalent (i.e. not inferior) to the reference method. Hence, it should be tested for non-inferiority. For this, the Wilcoxon signed-rank test for paired differences is not suitable. To perform non-inferiority testing, a non-inferiority limit σ has to be defined. A test procedure reaching this limit can be considered as at least equivalent to the reference method. We decided to set this limit to 0.7 for this study, i.e. a mean deviation of the \log_{10} reduction of up to 0.7 is considered as equivalent to the reference method. The current draft of the EN 1500, for example, includes a non-inferiority limit of 0.6; the one for surgical hand disinfection (EN 12791) will most likely be 0.75 (Suchomel 2010; personal communication). In any case, it should be checked, whether the data show normal distribution. If not, a nonparametric test (e.g. according to Hodges and Lehmann) should be used.

Conclusions

The conclusion of this study is that, at significantly shorter application times, 89.5 % 1-propanol solution yields a reduction of the aerobic, resident skin flora on the forehead, the abdomen, the upper back and in the lumbar area that is equivalent to the 10-minute reference method. Compared to the reference procedure, an application time of 3 minutes lead to a reduction of the skin flora that was at least equivalent and partly even significantly higher. For the establishment of a European standard method for testing the efficacy of skin antiseptics, 1-propanol represents an interesting option as reference procedure.

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Conflict of interest statement

The authors Günter Kampf and Mirja Reichel are employees of Bode Chemie GmbH, Hamburg, Germany.

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