Introduction

The European standard regulating testing of chemical disinfectants and antiseptics, EN 1500, deems products used for hygienic hand disinfection to be sufficiently effective if their antibacterial efficacy is not significantly lower than that of a reference disinfection procedure tested in parallel on the same subject [1]. The reference disinfection procedure involves twofold application of EN 1500.

Impact of shortening the duration of application and the standardized rubbing sequence as well as the reduction of the disinfectant volume used for the hygienic hand rub with 2-propanol (60 % V/V)

Method:

On the basis of the reference hand rub procedure with 2-propanol 60 % V/V as described in EN 1500, the impact of the following modifications was studied and compared with the result of the standard procedure: duration of hand rub of 15 and 30 s with a concomitant reduction of the disinfectant to halve its volume; amounts of alcohol put onto the hands of 1 and 2 ml versus the volume of 3 ml as required by the norm, although with a short duration of application of only 15 s; reduction of the sequence of hand movements standardized in EN 1500 from 6 steps to 5 or 4 steps and, with the latter two, reduction of the required repetitions of each step to three times instead of the required 5 times.

Results:

Shortening of the duration of the hand rub was associated with a highly significant reduction of the mean reduction factors (RF) from 4.5 lg with a 60 s rub to 3.5 with one of 15 s. At a duration of only 15 s, lowering of the alcohol volume taken onto the hands was accompanied by a highly significant reduction of the mean lg RFs from 3.5 with 3 ml to 2.9 with 1 ml. Furthermore, it turned out that even with a volume as small as 1 ml the mean duration of drying the hands was 23 s, hence longer than the duration of the hand rub. To shorten the standardized technique of hand rubbing from 6 to 5 or 4 steps and decreasing the number of repetitions from 5 to 3 times per step had only small effects, but with the 4-step sequence the bacterial reduction was less pronounced such that this procedure cannot be considered equivalent to the standard.

Conclusion:

Shortening of the duration of hygienic hand disinfection with 2-propanol 60 % V/V together with a reduction to halve the disinfectant volume leads clearly to a reduction of bactericidal efficacy. When considering the assessment of products by application of EN 1500, shortening of the duration of a hand rub to 15 s should, according to present knowledge, be avoided despite of the clinical practice of a possibly very short hand rubbing procedure. Simplification of the standardized hand rubbing technique by carrying out only 5 steps instead of 6 seems to be possible with a rather small loss of efficacy.

Summary

Background: The performance of hygienic hand rub in clinical reality and the request for a shortening to 15 s of the duration of hygienic hand rubs which is accepted by scientific societies dealing with hospital hygiene – combined with an appropriate evaluation of bactericidal efficacy – have prompted us to investigate the impact of variations in the standard procedure by laboratory tests according to EN 1500.

Methods: On the basis of the reference hand rub procedure with 2-propanol 60 % V/V as described in EN 1500, the impact of the following modifications was studied and compared with the result of the standard procedure: duration of hand rub of 15 and 30 s with a concomitant reduction of the disinfectant to halve its volume; amounts of alcohol put onto the hands of 1 and 2 ml versus the volume of 3 ml as required by the norm, although with a short duration of application of only 15 s; reduction of the sequence of hand movements standardized in EN 1500 from 6 steps to 5 or 4 steps and, with the latter two, reduction of the required repetitions of each step to three times instead of the required 5 times.

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3 ml 2-propanol (isopropanol) 60 % V/V for 30 s. There are by all means formulations available on the market capable of assuring the required efficacy already after 30 s, but because of the time pressures faced in the clinical setting, there is always the desire to develop products that can meet that requirement already after 15 s. In a first series of tests we therefore investigated how shortening the application duration of the reference procedure specified in EN 1500, and based on 2-propanol 60 % V/V, to 1 × 15 s and 1 × 30 s, compared with the reference procedure of 2 × 30 s, i.e. a total of 60 s, would affect microbial reduction. Since on using 3 ml portions of disinfectant, the time needed by the alcohol to dry on the hands would exceed the shortened application duration of only 15 and 30 s, the disinfectant volumes prescribed in the standard had to be reduced from 2 × 3 ml to 1 × 3 ml because the excess alcohol would flow away from the hands without making any contribution to bactericidal efficacy.

Based on our experience, after wetting hands with 3 ml alcohol they are not yet dry after 15 s. But since in practice engaging in any other activities while the hands are still wet with alcohol is unpleasant or impossible, it is feared that, in the everyday clinical setting when using a shorter disinfection duration, a volume of less than 3 ml of the product will be used to, in turn, shorten the evaporation duration of the alcohol. We therefore investigated in a second series of tests the effects of reduced disinfectant volumes of 1 and 2 ml compared with 3 ml on the efficacy of a 15 s hand disinfection with 2-propanol 60 % V/V.

While not required by the standard (nor can it be advocated either), many infection control teams are calling for standardisation of the hand rubbing sequences used for hand disinfection, as stipulated in EN 1500 for determination of the efficacy of products. The disinfection duration can also be shortened if the 6-step rubbing sequence described in the standard is reduced to a 5- or 4-step sequence. At the same time the procedure could be simplified by repeating each step only three times instead of five times. In a 3rd test series we therefore investigated how such a modification of the disinfection technique would affect reduction of the bacterial count on the artificially contaminated hands of subjects who had received training in this modified technique in advance.

### Materials and Methods

#### Disinfection duration (1st test series)
Testing was conducted in strict conformance with the test procedures of EN 1500, providing for disinfection of hands artificially contaminated with *Escherichia coli* K12, with bacterial release from the finger tips before and after disinfection being noted by kneading them in 10 ml trypticase soybean broth as a collecting liquid, followed by culture on trypticase soybean agar, to which 0.05 % sodium desoxycholate had been added. Fifteen subjects who met the inclusion or exclusion criteria of EN 1500 were randomly assigned to three groups, each of which comprised five persons. Each group complied with the guideline given in EN 1500 for conductance of the reference procedure (twofold application of 3 ml 2-propanol 60 % V/V for 30 s in each case), but additionally using disinfection times of 15 s and 30 s with a single application of 3 ml, and with subjects rubbing the alcohol on their hands using the standard technique described in EN 1500. All three groups concomitantly conducted the respective test activity using a Latin square, so that after three test runs each group had tried out each disinfection duration once. No provision was made for neutralisation of the disinfectant in the collecting and dilution liquids for registrations of the postdisinfection bacterial release from the finger tips before and after disinfection being noted by kneading them in 10 ml trypticase soybean broth as a collecting liquid, followed by culture on trypticase soybean agar, to which 0.05 % sodium desoxycholate had been added. The results of the 1st and 2nd test series were analysed to identify differences in the mean values using non-parametric methods such as Friedman’s [2] variance analysis and, in the event of significant results, post-hoc comparisons for significance were performed using Wilcoxon-Wilcox tests [3]. For both tests a significance level of p = 0.05, using a two-sided (two-tailed) test was agreed. For statistical corroboration of significant non-inferiority of the disinfectant techniques compared with the standard (3rd test series), a non-parametric non-inferiority test as per Hodges and Lehman [4] was performed. A significance level of p = 0.025, one-sided, with a one-sided (one-tailed) test and a safety margin of D = 0.6 log, was used.

#### Disinfectant volumes (2nd test series)
Using one of the experimental set-ups described above, disinfectant volumes of 1, 2 or 3 ml of the reference alcohol, 2-propanol 60 % V/V, was rubbed for 15 s onto the artificially contaminated hands using the standard rubbing sequences specified in EN 1500. Furthermore, it was not only the reduction in the microbial count but also, in another independent experiment where no artificial contamination was used – albeit only with five persons from the aforementioned patient group – how changing the amount (volume) of disinfectant applied affected the mean drying duration of the hands was recorded. The end of adequate wetting was defined by noting a marked increase in the resistance encountered when rubbing the hands together.

#### Shortening the standardised rubbing sequences (3rd test series)
In two tests the effects of two shorter disinfection techniques on the extent of microbial reduction were compared with the standard technique specified in EN 1500. Otherwise, apart from the following modifications, the same procedure as specified in the standard was used:

1. The total number of subjects was 20, since this test was also aimed at investigating the implications of a shorter disinfection technique when evaluating the disinfectant as per the amendment to EN 1500 currently under preparation. That calls for a large sample size following changes to the statistical methods used.
2. As opposed to the currently used technique, the individual steps of the shorter sequence were not repeated five times but instead only three times per step.
3. The originally last 6th step (Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa) was now used as the 2nd step of the shortened sequences (Figure 2).

In the 1st test series the standardised 6-step rubbing sequence (Figure 1) was compared with the 5-step sequence (Figure 2) in a crossover experimental design. To that effect, ten subjects were randomly assigned to two groups, one of which performed the 6-, and the other the 5-step, sequence. Following the first test run, the groups exchanged roles, so that after completion of the 1st test series each group had carried out both rubbing sequences. Similarly, in the 2nd test run the 6-step was compared with a 4-step shorter rubbing sequence.

#### Statistical Methods
The results of the 1st and 2nd test series were analysed to identify differences in the mean values using non-parametric methods such as Friedman’s [2] variance analysis and, in the event of significant results, post-hoc comparisons for significance were performed using Wilcoxon-Wilcox tests [3]. For both tests a significance level of p = 0.05, using a two-sided (two-tailed) test was agreed. For statistical corroboration of significant non-inferiority of the disinfectant techniques compared with the standard (3rd test series), a non-parametric non-inferiority test as per Hodges and Lehman [4] was performed. A significance level of p = 0.025, one-sided, with a one-sided (one-tailed) test and a safety margin of D = 0.6 log, was used.
Shortening of the standardised rubbing sequence (3rd test series)
Table 3 shows that no inferior disinfectant action was achieved, compared with the standard procedure, by omitting the 4th step of the standardised 6-step rubbing sequence (Back of fingers to opposing palms with fingers interlocked, Figure 1) while repeating each step five times, shortening to 5 steps and repeating only three times as well as incorporation of the original 6th step (Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa, Figure 2). It was possible to reject the hypothesis of inferiority on using a one-sided 2.5 % significance level.

But that was not the case when the disinfection sequence was reduced by a further

Disinfectant volumes (2nd test series)
Table 2 shows that on using a short disinfection duration of only 15 s, the volume of disinfectant applied to the hands was of paramount importance in determining the bactericidal efficacy (Friedman’s test $p < 0.01$). The mean reduction in the bacterial count following disinfection dropped from 3.5 log on using 3 ml to 3.2 log for 2 ml and then dropped to 2.9 log on applying a volume of only 1 ml. The differences are significant between 2 and 3 ($p < 0.05$) as well as between 1 and 3 ml ($p < 0.001$). The mean drying duration for the alcohol-wet hands under the experimental conditions for volumes of 3, 2 and 1 ml was around 49, 35 and 23 s, respectively.

Results

Disinfection duration (1st test series)
As can be seen from Table 1, the shortened duration of hygienic hand disinfection (in combination with half the disinfectant volume) led to a reduction in the bactericidal efficacy of 2-propanol on the hands (Friedman’s test $p < 0.001$). In our tests this reduction in efficacy was statistically significant between 30 and 60 s ($p < 0.01$) as well as between 15 and 60 s ($p < 0.001$). For an application duration of only 15 s, the bactericidal efficacy compared with that seen for 60 s during the reference procedure was reduced by one entire order of magnitude. However, there was no change in the variance of the log reduction factors (RFs).

Figure 1: Standard procedure of the 6-step rubbing technique as per EN 1500. Each step is repeated five times.

Figure 2: Shortened disinfection technique with five or four steps, with each step being repeated three times and the original 6th step integrated into the rubbing sequence as the 2nd step.
step, i.e. on omitting the original 2nd step of the standard technique (Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa, Figure 1). This gave rise to a small, but important, reduction in the mean log RFs from 4.8 to 4.5.

Table 1: Hygienic hand disinfection with 2-propanol 60% V/V – shortening of the disinfection duration and reduction in the test bacteria count.

<table>
<thead>
<tr>
<th>Disinfectant duration</th>
<th>15 s</th>
<th>p*</th>
<th>30 s</th>
<th>p*</th>
<th>60 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>On using mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 vs 60 s</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 vs 3 ml</td>
<td>1 × 3 ml</td>
<td>1 × 3 ml</td>
<td>2 × 3 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean log RF (sd)*</td>
<td>3.5 (0.8)</td>
<td>&lt; 0.001</td>
<td>3.7 (0.8)</td>
<td>&lt; 0.01</td>
<td>4.5 (0.8)</td>
</tr>
</tbody>
</table>

* Wilcoxon-Wilcox, p = 15

Table 2: Hygienic hand disinfection with 2-propanol 60% V/V during 15 s – reduction of the disinfectant volume applied to the hands and reduction of the test bacteria count and drying times on the hands after application of such volumes.

<table>
<thead>
<tr>
<th>Disinfectant volume</th>
<th>1 ml</th>
<th>p*</th>
<th>2 ml</th>
<th>p*</th>
<th>3 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs 3 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean log RF (sd)*</td>
<td>2.9 (0.6)</td>
<td>&lt; 0.001</td>
<td>3.2 (0.7)</td>
<td>&lt; 0.05</td>
<td>3.5 (0.6)</td>
</tr>
<tr>
<td>Mean drying time in s (sd) +</td>
<td>23.4 (4.8)</td>
<td>35.0 (9.4)</td>
<td>49.4 (12.4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Wilcoxon-Wilcox, n = 15, + n = 5

Discussion

In the methodology used in the 1st test series, it was necessary to change, in addition to the disinfection duration, a second variable compared with the reference procedure, i.e. the volume of alcohol applied to the hands. Twofold application of, in each case, 3 ml of 2-propanol 60 % V/V, i.e. in total 6 ml, as required by the reference procedure of the respective standard was deemed not to be suitable for this study because applying a total of 6 ml to even large hands within the short disinfection duration would only result in the excess alcohol flowing away, and this amount would not be available for disinfection purposes. From our efficacy testing of disinfectants for surgical hand antisepsis we know that it has no bearing on a disinfectant’s efficacy whether 3 or 5 ml per application is used so long as the hands are kept continuously wet for the entire duration of the disinfection time (which called for several applications in the case of surgical antisepsis for applications of between 1.5 and 5 min). But for short exposure times of 30 or even only 15 s, it is well known that the hands remain wet throughout the entire period on using only a single application of 3 ml. It has therefore been postulated that doubling the 3 ml volume used in the study would not be advisable for short disinfection periods. That is incidentally the reason why the manufacturers of commercially available products that meet the requirements of EN 1500 already in 30 s, in general recommend only a single application of a volume of between 3 and 5 ml. The WHO guideline which is to be soon published, “WHO Guidelines for Hand Hygiene in Health Care” recommends for hygienic hand disinfection: “Apply a palmful of alcohol-based handrub and cover all surfaces of the hands… Rub until dry” [5] (One of the authors (M. Rotter) interprets a ‘palmful’ to mean a volume of around 3 ml.

In the everyday clinical setting, shortening of alcohol-based hand rub hygienic hand disinfection would be desirable, since it has been revealed that medical personnel disinfect, or must disinfect, their hands up to more than 15 times per hour [6], and in intensive care units (ICUs) hand disinfection may be needed up to 30 times per hour [7,8] and even that may not suffice, and this thus raises the question as to whether 100% compliance, at least in certain patient care areas, is at all possible due to time constraints [9,10]. However, the desire for a shorter disinfection duration is hampered, at least in the case of products endowed with an efficacy profile comparable with that of 2-propanol 60 % V/V, by the fact that, first, the efficacy specified by EN 1500 is not achieved, as clearly borne out in Table 1, and on applying e.g. 3 ml as recommended by the majority of manufacturers, the hands continue to feel wet after 15 s. But since it takes between 30 und 60 s for the alcohol to dry on the hands when using such a disinfectant volume (Table 2) and during this interval other manual tasks are unpleasant or impossible, it is possible that efforts will be made to apply smaller volumes to expedite evaporation of the disinfectant. But that would detract from the disinfectant efficacy, as can be seen from Table 2, and also shown in the case of soap when reducing the volume from 3 to 1 ml [11]. In the everyday clinical setting it must therefore be expected that the reduction in transient bacterial flora following hygienic hand disinfection with a product endowed with an efficacy comparable with that of the reference alcohol will only be of an order magnitude of between 3.0 and 3.5 log, rather than of that which can be achieved with the reference procedure specified in EN 1500, which is expected to be around one log level higher. It must therefore be expected that in the everyday clinical setting the reduction achieved in the transient bacterial flora on using hygienic hand disinfection is comparable with that seen on using a product endowed with an efficacy level similar to that of the reference procedure is only between 3.0 and 3.5 log. This must not necessarily be seen as a drawback so long as the clinical effect of hygienic hand disinfection is achieved and consistent use is accompanied by a lower quota of hand-mediated
hospital infections. Nonetheless, apart from the shortcoming that the hands still feel wet after 15 s, there is the risk of diminished, or even in some cases, no disinfectant effect, hence an exposure time of only 15 s for hand disinfection must be rejected based on current scientific knowledge. Even if the shortening of the standardised 6-step rubbing sequence specified in EN 1500, which continues to be recommended to date for clinical practice, does not lead to shortening of the disinfection duration because the entire procedure must be repeated until the end of the recommended application duration, it makes that ritual easier. That in turn could improve compliance with this rubbing sequence. But, as can be seen from Table 3, any greater simplification – reduction by two out of six steps – could result in a minor, but significant, reduction in the disinfection action. Whether this difference is clinically relevant cannot of course be predicted without carrying out relevant tests in a clinical setting.

**Conflict of Interest**

The authors declare that there is no conflict of interest as understood by the International Committee of Medical Journal Editors.

**References**