Testing of the World Health Organization recommended formulations in their application as hygienic hand rubs and proposals for increased efficacy

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Hygienic hand antisepsis
WHO formulation

Background: In Central Europe, alcohol-based hand rubs have been the preferred choice for hand hygiene, whereas, in other countries, other preparations have been used that are based on other active agents. Recently, a move towards alcohol-based hand rubs has begun, but they may be costly and unaffordable to some. Therefore, the World Health Organization (WHO) has recommended 2 hand rub formulations (WHO I and WHO II) for local production in health care settings where commercial products are not available or are too expensive.

Objectives: WHO I, based on ethanol 80% (vol/vol), and WHO II, based on isopropanol 75% (vol/vol), were investigated for their bactericidal efficacy in their application as hygienic hand rubs.

Methods: The investigation took place at the Institute for Hygiene and Applied Immunology, Medical University Vienna, Austria, as a prospective, randomized, in vivo laboratory study, comparative in crossover design. Both formulations were tested according to the European Standard EN 1500 in 2 applications (1/3 mL/30 seconds or 2/3 mL/2 × 30 seconds). Additionally, modifications with increased alcohol concentrations (weight instead of volume percent) were tested in the short application. Bactericidal efficacies were compared with those of the respective reference procedure “R,” ie, rubbing 2 × 3 mL 60% vol/vol isopropanol for 2 × 30 seconds onto hands artificially contaminated with Escherichia coli K12.

Results: The short application of either WHO formulation resulted in bacterial reductions significantly inferior to the respective ones of R. However, prolonging the contact time to 60 seconds or increasing the alcohol content produced reductions similar to those of R.

Conclusion: Both WHO-recommended formulations meet the efficacy requirements of EN 1500 within 60 seconds but not within 30 seconds. Increasing the respective alcohol concentrations from 80% vol/vol to 80% wt/wt and 75% vol/vol to 75% wt/wt renders the formulations sufficiently active to conform to the norm also within 30 sections.

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guideline includes alternatives for 2 different hand rub formulations that have been recommended for use in health care settings where commercial products are not available or too expensive. One formulation (WHO I) is based on ethanol 80% (vol/vol); the other one (WHO II) is based on isopropanol 75% (vol/vol). Whereas alcohol is the active component in both these formulations, a low concentration of hydrogen peroxide is incorporated to help in eliminating contaminating bacterial spores during storage because short-chain, aliphatic alcohols are known to lack sporocidal activity. In addition, glycerol is added as a humectant to improve the acceptability of the formulations.

The WHO Guidelines indicate that the microbicidal activity of the 2 WHO-recommended formulations was tested by a WHO reference laboratory according to (the forthcoming amendment of) the European Standard EN 1500, which is an in vivo laboratory model on the artificially contaminated hands of volunteers to evaluate hand rubs for their use in hygienic (general) hand antisepsis. (Their suitability to serve as presurgical hand rubs has been tested before by us and others). In these tests, the bacterial reductions achieved with the formulations are compared with those obtained from a reference hand antisepsis procedure consisting of 2 successive hand rubs, each with 3 mL isopropanol 60% vol/vol for 30 seconds, thus a total of 60 seconds. Volunteers are randomly assigned to 2 groups where one applies the test formulation (WHO I) is based on ethanol 80% (wt/wt); the other (WHO II) is based on isopropanol 75% (wt/wt); the other group the reference solution.

Furthermore, it is stated in the WHO guidelines (part I, section 12.1.4.1) that the activity of the formulations was found to be equivalent to that of the reference alcohol (isopropanol 60% vol/vol) for hygienic hand antisepsis, although the details on methods and the exact results are not presented. It was felt, therefore, that these data should be made available to the involved scientific community. The purpose of this study was to test whether an increase in the alcohol concentrations in these formulations by changing the volume percentage (vol/vol) concentrations prescribed in the guidelines into weight percentage (wt/wt) concentrations could improve the bactericidal effect such that the duration of application can be considerably shortened. It is of note that the weight concentration of 80% wt/wt ethanol equals approximately a volume concentration of 85% vol/vol and that of isopropanol 75% wt/wt equals a volume concentration of 80% vol/vol.

MATERIALS AND METHODS

Subjects

Subjects included in this study were 20 volunteers employed at the Institute for Hygiene and Applied Immunology, Medical University Vienna. Exclusion criteria were less than 18 years of age, pregnancy, skin breaks such as cuts, or abrasions or other skin disorders on the hands. Nails were short and clean, and the volunteers agreed not to take or use any antibacterial agent or soap during the trials, starting 1 week prior to testing. All subjects provided written informed consent. The study protocol was approved by the Institutional Ethics Committee of the Medical University of Vienna, Vienna, Austria.

Formulations

The following formulations were used in this study:

WHO I: ethanol (pro analysis, 1.00983; Merck, Darmstadt, Germany) 80% (vol/vol), hydrogen peroxide (pro analysis, 1.07210; Merck, Darmstadt, Germany) 0.125% (vol/vol), and glycerol (pro analysis, 1.04092; Merck, Darmstadt, Germany) 1.45% (vol/vol);

WHO II: isopropanol (pro analysis, 1.09634; Merck, Darmstadt, Germany) 75% (vol/vol), hydrogen peroxide 0.125% (vol/vol), and glycerol 1.45% (vol/vol);

WHO I-modified: ethanol 80% (wt/wt), hydrogen peroxide 0.125% (vol/vol), and glycerol 1.45% (vol/vol);

WHO II-modified: isopropanol 75% (wt/wt), hydrogen peroxide 0.125% (vol/vol), and glycerol 1.45% (vol/vol).

Isopropanol (pro analysis, 1.09634; Merck, Darmstadt, Germany) 60% (vol/vol) served as the reference alcohol as specified in EN 1500.

A solution of 20% (vol/vol) non-medicated soft soap (APOCA, Vienna, Austria) was used for the required preparatory hand wash.

Test strain

The specified apathogenic strain of Escherichia coli K12 (NCTC 10538) was used for artificial contamination.

Nutrient media

Tryptic soy broth (Cas broth, 1.05459; Merck, Darmstadt, Germany) served as a nutrient medium for the culture of the test strain and was also employed for sampling and dilution fluids; tryptic soy agar (TSA; Cas agar, 1.05458; Merck, Darmstadt, Germany) complemented with sodium-desoxycholate (0.05%, 1.06504; Merck, Darmstadt, Germany) to inhibit the growth of skin staphylococci was used for the counting plates. In these tests, neutralizing agents were not necessary for any of the tested WHO formulations because even dilution with the pure broth without supplement was shown to neutralize any antimicrobial effect.

Test methods and experimental design

The test method of the forthcoming amended European test standard EN 1500 for the evaluation of the microbicidal efficacy of hand hygiene products in their application for the hygienic hand rub was used. This standard differs from its former version by replacing the original comparative test model with a noninferiority test model with the necessary consequence of changing statistical tests and increasing the sample size (=number of subjects) from 12-15 to 18-24. In 6 individual experimental runs, the bactericidal efficacies of the 2 formulations (WHO I and WHO II) and the 2 modified formulations (WHO I modified and WHO II modified) were tested in a crossover design against a standardized reference hand rub procedure which, in accordance with EN 1500, includes rubbing hands thoroughly in a standardized fashion with 3 mL isopropanol 60% (vol/vol) for 30 seconds and repeating this procedure for another 30 seconds.

In short, the test method was carried out by the following steps: freshly washed and dried hands of volunteers—who were randomly allotted into 2 groups—were artificially contaminated by a short (5 seconds) immersion up to the mid-carpsals into a bacterial suspension containing approximately 2 × 10⁶/mL colony-forming units of an apathogenic strain of Escherichia coli K12. Hands were then briefly drained into a bowl and air-dried for 3 minutes by slow and supination in a horizontal position to avoid formation of droplets at the fingertips. Next, hands were sampled for the assessment of pretreatment bacterial release by kneading and rubbing the fingertips (including the thumbs) for 1 minute at the bottom of a Petri dish filled with sampling fluid, one for each hand. Immediately thereafter, the hands of a group of volunteers were treated with one of the WHO formulations for either 30 seconds or 2 × 30 seconds, whereas the other group used the reference
procedure. After completion of these procedures, hands were sampled again as above for the assessment of post-treatment bacterial release. Subsequently and after thorough handwashing with soft soap, both groups of volunteers repeated the tests but with the respective other procedure. The sampling fluids were diluted as necessary and cultivated on the surface of tryptic soy agar with added sodium deoxycholate preventing the growth of resident gram-positive microbial skin flora. The plates were then incubated at 36°C ± 1°C for a total of 48 hours and colony-forming units were counted by means of an electronic colony counter (Fisher colony counter, Model 480; Artek Systems Corporation, Farmingdale, NY).

Statistical analysis

For statistical evaluation, the pre- and post-treatment colony counts per milliliter sampling fluid were expressed as decadic logarithms (log_{10}); the mean values of right and left hands were formed; and, from these, logarithmic reduction factors (log_{10} RFs) were calculated per volunteer as the intraindividual difference between log_{10} pretreatment minus log_{10} post-treatment value. Additionally, intraindividual differences between these log_{10} RFs of study formulations minus those from the reference procedures were calculated. These were then tested for significance by a parameter-free noninferiority statistical test according to Hodges-Lehmann.8 The new requirement in the amended norm is that a hand rub be noninferior to the reference treatment. Noninferiority is supposed if the Hodges-Lehmann upper 97.5% confidence limit for the individual differences in log_{10} bacterial reductions between test formulation and reference is greater than 0.75 log_{10} (= agreed inferiority margin).

RESULTS

The results are shown in Tables 1 and 2. To make the illustration clearer, the results of both the mean log RFs achieved with the study formulations and those with the appropriate references as well as those of the mean individual differences of these log_{10} RFs are depicted, although the statistical tests have been done with the latter results. As shown, with a 30-second duration of application, neither formulation (WHO I based on 80% [vol/vol] ethanol or WHO II formulation is isopropanol 75% [vol/vol] plus hydrogen peroxide 0.125% [vol/vol] plus glycerol 1.45% [vol/vol]).

Table 1

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Application</th>
<th>Test</th>
<th>Reference</th>
<th>Mean (n = 20) log reduction ± SD</th>
<th>Statistical result</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO I</td>
<td>1 × 3 mL/1 × 30 seconds (s)</td>
<td>3.59 ± 0.89</td>
<td>4.29 ± 1.00</td>
<td>0.70 ± 0.58</td>
<td>0.99*</td>
</tr>
<tr>
<td></td>
<td>2 × 3 mL/2 × 30 s</td>
<td>4.55 ± 0.80</td>
<td>4.49 ± 1.02</td>
<td>-0.06 ± 0.56</td>
<td>0.23</td>
</tr>
<tr>
<td>WHO II</td>
<td>1 × 3 mL/1 × 30 s</td>
<td>3.57 ± 0.82*</td>
<td>4.11 ± 1.10</td>
<td>0.54 ± 0.63</td>
<td>0.90*</td>
</tr>
<tr>
<td></td>
<td>2 × 3 mL/2 × 30 s</td>
<td>4.54 ± 0.84</td>
<td>4.57 ± 0.81</td>
<td>0.03 ± 0.59</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Application</th>
<th>Test</th>
<th>Reference</th>
<th>Mean (n = 20) log reduction ± SD</th>
<th>Statistical result</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO I-modified</td>
<td>1 × 3 mL/1 × 30 seconds</td>
<td>4.40 ± 0.52</td>
<td>4.79 ± 0.69</td>
<td>0.39 ± 0.59</td>
<td>0.70</td>
</tr>
<tr>
<td>WHO II-modified</td>
<td>1 × 3 mL/1 × 30 seconds</td>
<td>4.36 ± 0.75</td>
<td>4.62 ± 0.79</td>
<td>0.27 ± 0.75</td>
<td>0.55</td>
</tr>
</tbody>
</table>

DISCUSSION

To the best of our knowledge, no data have, as yet, been formally published on the efficacies of these 2 WHO-recommended formulations in their application for the hand hygiene. Therefore, we present here these results on the bactericidal activity of both formulations stemming from tests according to the method of the officially approved European test standard EN 1500.5 Because the WHO guideline has not specified the exact duration of application, we have tested both WHO formulations with 2 modes of application: 1 × 3 mL for 1 × 30 seconds and 2 × 3 mL for 2 × 30 seconds. To meet modern scientific requirements on study design and statistical evaluation of the results, we have used the new test methodology of the forthcoming amended EN 1500 using a larger sample size and noninferiority testing rather than the comparative test model of the former version of the norm. Under these conditions, the null hypothesis states that the study procedure is inferior to the reference procedure, whereas the alternative hypothesis assumes noninferiority. For the purpose of testing the bactericidal efficacy of hand antiseptic agents, the expert group working on the norm has agreed that the 1-sided formulation of the question—noninferiority
rather than equivalence with the reference hand rub procedure—is the appropriate approach. Because of the sometimes even not symmetrical—not to speak of normal—distribution of the individual difference values of the log_{10} RFs between study and reference procedure, the nonparametric approach by Hodges-Lehmann\(^8\) was chosen for significance testing. Noninferiority was accepted to be probable if the Hodges-Lehmann upper 1-sided 97.5% confidence limit for the intraindividual differences in log_{10} bacterial reductions between study and reference formulation is smaller than the agreed inferiority margin of 0.75 log_{10}.

The requirement of noninferiority was found to hold true for the 2 × 30 seconds application of both WHO-recommended formulations but not for the short one of 1 × 30 seconds. When applied for a total of 60 seconds, the efficacy of each formulation was rather similar to that of the respective reference value, whereas the shorter duration of application (30 seconds) resulted in significantly lower mean log_{10} reductions. This is, in fact, not surprising, because the efficacy of alcohol-based hand rubs is affected by several factors, one of which is the duration of application.\(^2,9\)

Another factor is the alcohol concentration in the antiseptic solutions as is clearly evident from the results of this study (Table 2). When increasing this concentration from 80% vol/vol ethanol to 80% wt/wt (equaling 85% vol/vol) or from 75% vol/vol isopropanol to 75% wt/wt (equaling 80% vol/vol), then the efficacy of the modified WHO-formulations is conforming with the requirement of the norm, including the short application of 30 seconds. Even, if in clinical practice, a hand rub of 30 seconds might seem as lasting too long, this appears, nevertheless, to be an important improvement in efficacy over the WHO-recommended formulations because the aim of hygienic hand antisepsis is to render hands safe as quickly as possible. Furthermore, it may be supposed that after using an alcohol-based solution a hand rub will probably prevent health care workers from touching anything as long as the fingers are wet with the antiseptic solution. Drying takes usually approximately 30 seconds. Finally, to prevent criticism on the argument of having the WHO formulations tested with a contact time of 30 seconds—which (because taking too long) appears not relevant for hospital practice—it should be realized that it was not the aim of this study to evaluate the suitability of the norm to represent real hospital practice, a quality which, by the way, is not offered by any existing officially approved norm, to date. Instead, it was rather intended to use an approved standard test method to evaluate the microbicidal efficacy of the WHO formulations as is demanded in the WHO Guidelines. In fact, to follow this demand, we were bound to use these contact times because the norm does not allow any other duration of application than within the limits between 30 to 60 seconds. The European test standard EN 1500 is such an approved method, in which, however, for the sake of high sensitivity to detect unsuitable formulations at a bearable sample size (=number of volunteers), the reference hand antisepsis procedure takes an unrealistically long time of 2 × 30 seconds (thus, a total of 60 seconds). However, this reference procedure is by no means meant for practical use, but the size of bacterial reduction resulting from its use serves as a measure for efficacy in the laboratory. Of note, there are some commercial alcohol-based hand rubs in Europe in which the bactericidal efficacy is non-inferior to that of the reference even when used for only 15 seconds, but, because of the necessary high alcohol concentration and the most efficacious alcohol type contained in them, i.e., n-propanol, which is not accepted everywhere in a hand rub, they could not serve as WHO-recommended formulations.

In the context of increased alcohol concentrations, the question of the impact on the production costs arises. With ethanol, the increase from 80% to 85% vol/vol amounts to 6.2%; with isopropanol, the increase from 75% to 80% vol/vol is 6.6%. It is true that, with the increased alcohol content, the production costs will also proportionally go up by these amounts. However, in view of the advantage of using formulations with the efficacy that complies with the requirements of an officially approved test method, this increase of costs seems justifiable.

In conclusion, the results of this study demonstrate that the WHO-recommended alcohol-based hand rub formulations proposed in the recently published WHO Guidelines\(^1\) for Hand Hygiene in Health Care meet the efficacy requirement of the European Norm EN 1500 for hygienic hand rubs at an application of 2 × 30 seconds but not at 1 × 30 seconds. For harmonizing the efficacy of the WHO-recommended formulations with the efficacy requirement of the norm (also with this shorter application), increasing the alcohol concentrations would render them sufficiently active: with ethanol from 80% vol/vol to 80% wt/wt (equaling 85% vol/vol) and with isopropanol from 75% vol/vol to 75% wt/wt (equaling 80% vol/vol).

References
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