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World Health Organization-recommended hand-rub formulations do not meet European efficacy requirements for surgical hand disinfection in five minutes

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SUMMARY

The World Health Organization (WHO) has recommended two hand-rub formulations for local production based on 80% ethanol or 75% isopropanol (both v/v). We have looked at their efficacy according to EN 12791. Twenty-six subjects treated their hands with the reference procedure (*n*-propanol, 60%) for 3 min or with one of the two formulations for 1.5, 3 or 5 min (Latin square design). Post-values (immediate effect) were taken from one hand, the other hand was gloved for 3 h. After the glove had been taken off, the second post-value was taken (3 h effect). The mean $\log_{10}$ reduction of each hand rub at all three application times was compared to Hodges and Lehmann's reference procedure for non-inferiority. In the first block the reference procedure reduced bacterial load by 2.43 $\log_{10}$ (immediate effect) and 2.22 $\log_{10}$ (3 h effect). The efficacy of the ethanol-based formulation (e.g. immediate efficacy of 1.41 $\log_{10}$ at 5 min) was inferior to the reference procedure at all application times [lower 95% confidence interval (CI): less than −0.75]. In the second block the reference procedure reduced bacterial load by 2.72 $\log_{10}$ (immediate effect) and 2.26 $\log_{10}$ (3 h effect). The efficacy of the isopropanol-based formulation (e.g. immediate efficacy of 2.05 $\log_{10}$ at 5 min) was also inferior to the reference procedure at all application times (lower 95% CI: less than −0.75). Both WHO-recommended hand-rub formulations failed to meet the EN 12791 efficacy requirements for surgical hand disinfection within 5 min. A higher concentration of the active ingredients may improve the efficacy.

Introduction

Alcohol-based hand rubs are widely used for surgical hand disinfection in many countries.[1,2] They are considered to have better antimicrobial efficacy and dermal tolerance, regarded as two clear advantages in comparison to antimicrobial soaps.[3–6] In most countries, preparations are used which are commercially available and which are supported by efficacy and tolerance data provided by the manufacturer. In the World Health Organization (WHO) guideline on hand hygiene it is also recommended that in countries which may not be able to purchase hand rubs from manufacturers, two specific formulations may be prepared locally and used by healthcare workers.[7] Formulation 1 contains as the active ingredient ethanol at 80% (v/v). Formulation 2 is based on isopropanol at 75% (v/v). Both formulations also contain 1.45% glycerol and 0.125% hydrogen peroxide (both v/v). No data have yet been published on the efficacy of these two hand-rub formulations. This study is therefore designed to determine the efficacy of the two WHO-recommended hand-rub formulations for surgical hand disinfection according to EN 12791 (surgical hand disinfection; test method and requirement; phase 2, step 2).[8]

Methods

Products and application

The following preparations were used: 1-propanol (60%, v/v) as reference alcohol of EN 12791,[8] WHO formulation 1 which contains 80% (v/v) ethanol, 1.45% glycerol and 0.125% hydrogen peroxide (all v/v), and WHO formulation 2 which contains 75% isopropanol, 1.45% glycerol and 0.125% hydrogen peroxide (all v/v). The two

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WHO formulations were prepared by Bode Chemie GmbH, Hamburg, Germany, according to the WHO recommendation. The reference alcohol was applied to hands for 3 min according to EN 12791.[8] The WHO formulations were applied for 1.5, 3 or 5 min with both hands kept wet with the hand-rub for the entire application time. An application time of 5 min was chosen because it is the longest acceptable application time in EN 12791 for surgical hand rub preparations.[8] A 3 min application time was chosen because it has been the standard duration of surgical hand disinfection with alcohol-based hand rubs in many European countries for approximately two decades.[9] A 1.5 min application time was chosen because recent evidence suggests that well-formulated alcohol-based hand rubs may be equally effective in 1.5 and 3 min.[10,11] The applied volume as a multiple of 3 mL was noted for each subject and application.

Design

Each WHO formulation was tested with the three application times (1.5, 3 and 5 min) in one block in comparison to the 3 min reference procedure resulting in a total of four study arms per block. In each block there were a total of four test days, with one week between each test day. All three application times of a WHO formulation were tested in a Latin square design against the reference treatment in order to prevent a test day bias for any of the treatments. On each test day, one-quarter of the 26 subjects performed one of the four different applications. Three samples were taken from each subject on each test day: one baseline value per hand (one hand for the immediate effect, one hand for the 3 h effect) before an application, one immediate effect value after an application (one hand), and one sustained effect value 3 h after an application (other hand was gloved).

The reference alcohol was applied for 3 min, as described in EN 12791, in both applications with a preceding 1 min hand wash. The WHO formulations were also applied after a 1 min hand wash period for the particular application time.

Subjects

The subjects were lay people who volunteered to participate. All of them had healthy skin on their hands. There were no cuts or abrasions. The fingernails were short and clean. The subjects were not allowed to use antimicrobial soaps or creams including local or systemic antibiotics for at least one week prior to testing. They were trained on the correct application procedure and supervised by a technician during each application.

Wash phase

When hands were washed, it was done with a non-medicated soft soap (sapo kalinus). Sapo kalinus is a standard soft soap, described in EN 12791 and contains linseed oil (10%), potassium hydroxide 1.9%, ethanol (1.4%) and water. Thereafter hands were rinsed with running tap water and dried with a non-sterile paper towel.

Determination of the pre-values and post-values

Sampling, cultivation and calculation of values were done according to EN 12791. In order to obtain the pre-values, volunteers rubbed the distal phalanges of both hands for 1 min on to two Petri dishes (diameter 9 cm) containing 10 mL of tryptic soy broth (TSB). After application of the hand antiseptic, one hand was randomly selected to obtain the post-value (immediate effect). The other hand was allowed to dry and thereafter gloved (sterile surgical glove) for 3 h for assessment of the sustained effect, obtained after removal of the glove. In order to obtain the post-value, TSB with neutralisers was used. The neutralisers were 3% Tween 80, 3% saponin, 0.1% histidine and 0.1% cysteine. Sampling was done in a similar way to the immediate effect.

From the sampling fluid obtained from each hand, dilutions between 1:10 and 1:10 000 were prepared in TSB. Aliquots were taken from the sampling fluid (1 and 0.1 mL) and the dilution steps (0.1 mL) and spread over tryptic soy agar (TSA) dishes with a sterile glass spatula. No more than 30 min elapsed between sampling and seeding. Dishes were incubated for a total of 48 h at 36 ± 1 °C and the colony-forming units (cfu) counted between 15 and 300 colonies per plate. All pre- and post-values were expressed as $\log_{10}$ values. If values in the 15–300 cfu range were obtained from more than one dilution, their weighted mean was used as the final logarithm value. For each sample from each volunteer, the logarithmic reduction factor (RF) was calculated as the difference between the $\log_{10}$ pre-value and the $\log_{10}$ post-values.

Statistics

A product is currently considered effective for surgical disinfection if the mean of the RF of both the immediate and sustained effect is not significantly lower than the corresponding mean RF of the reference treatment.[8] Differences in the immediate and sustained effects between the reference treatment and the three variations of product application were therefore investigated by analysis of variance (ANOVA). A post-hoc analysis was performed with Tukey's honestly significant difference (HSD) test. $P < 0.05$ was chosen to indicate a significant difference. Due to the forthcoming change in the statistical analysis in EN 12791, the efficacy of the WHO hand-rub formulations was also assessed for non-inferiority to the reference treatment according to the test of Hodges and Lehmann.[12] A non-inferiority margin of at least −0.75 has been proposed for EN 12791 and was therefore used in our study.

Results

WHO formulation based on 80% ethanol (v/v)

Baseline bacterial density (cfu/mL) before surgical hand disinfection was between 4.09 ± 0.81 $\log_{10}$ and 4.30 ± 0.59 $\log_{10}$ (Table I). The reference disinfection (3 min) reduced the bacterial density by 2.43 ± 1.10 $\log_{10}$ steps (immediate efficacy). The ethanol-based WHO formulation yielded a remarkably lower efficacy between 1.41 ± 0.97 (5 min) and 1.50 ± 0.75 $\log_{10}$ steps (3 min). There was a highly significant difference between all four treatments ($P < 0.001$; ANOVA). The post-hoc analysis revealed that the ethanol-based WHO formulation was significantly less effective in comparison to the reference procedure, irrespective of the application time of 1.5, 3 or 5 min ($P \leq 0.001$; Tukey's HSD). In addition, the immediate efficacy of the ethanol-based WHO formulation was not non-inferior at each application time [lower 95% confidence interval (CI): between −1.37 and −1.465; Table I]. After 3 h under the surgical glove following the 3 min reference disinfection, bacterial density increased somewhat but was still 2.22 ± 1.00 $\log_{10}$ steps below baseline. With the ethanol-based WHO formulation, the bacterial density was between 0.87 ± 0.80 (1.5 min) and 1.08 ± 1.04 $\log_{10}$ steps (5 min) below baseline. There was a highly significant difference between all four treatments ($P < 0.001$; ANOVA). The post-hoc analysis revealed that the ethanol-based WHO formulation was significantly less effective in comparison to the reference procedure irrespective of the application time of 1.5, 3 or 5 min ($P < 0.001$; Tukey's HSD), but no