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Ethanol in pre-surgical hand rubs: concentration and duration of application for achieving European Norm EN 12791

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SUMMARY

In Europe, ethanol is a common active agent in hand rub formulations and nowadays it is also recommended in guidelines for hand hygiene published by the Centers for Disease Control and Prevention and by the World Health Organization. However, data on the range of concentrations and durations of application providing a basis for passing the efficacy test of the European norm EN 12791 are still lacking. Therefore, the bactericidal efficacy of rubbing clean hands with pure ethanol in volume concentrations of 95%, 85% or 75% during 3 min was compared with that of the reference procedure of EN 12791 employing *n*-propanol 60% v/v for 3 min, immediately and 3 h after disinfection. Ethanol 85% was also tested at a 5 min application. A Latin-square design was used with 20 randomly allotted volunteers. Whereas the mean immediate bacterial reductions caused by ethanol at concentrations of 75% (log RF 1.68) and 95% (log RF 2.70) were significantly less efficacious compared to that of the reference (log RF 3.27), at 85% they were not significantly less active with both applications, 3 and 5 min (log RFs 2.90 and 3.12, respectively). Three hours after antisepsis, the bacterial reduction on the gloved hand was only significantly less efficacious than that of the reference when 75% ethanol was used. It is concluded that ethanol-based hand rubs have a good chance of meeting the EN 12791 requirements if their ethanol concentration is >75% v/v but <95% v/v and if they are applied for at least 3 min.

Introduction

Although hand hygiene is considered the single most important measure to reduce the transmission of nosocomial pathogens via the hands of healthcare workers, compliance with and knowledge about hand hygiene treatments are still not satisfactory.[1] To improve this condition new guidelines for hand hygiene in healthcare settings were published in 2002 by the Centers for Disease Control and Prevention (CDC) and in 2009 by Health Canada.[2,3] Recognising the global need to improve hand hygiene, the World Health Organization (WHO) has recently published *WHO Guidelines on Hand Hygiene in Health Care*.[4] Each of these guidelines recommends the use of alcohol-based hand rubs – at least, if hands are not visibly soiled – because of their stronger efficacy, faster action and better dermal tolerance, which latter property is considered beneficial for compliance with hand hygiene protocols.[1,5,6] All alcohol-based rubs contain short-chain aliphatic alcohols such as ethanol, isopropanol or *n*-propanol, or combinations of these alcohol species. Their microbicidal efficacy against the resident microbial hand flora depends on the alcohol species and on the concentration as well as on the duration of application.[5,7,8] Although, at the same concentration, ethanol is less efficacious than isopropanol, which, in turn, is less efficacious than *n*-propanol, it is the preferred alcohol for hand antisepsis in many countries, e.g. the USA or the Netherlands.[5,9] As early as the 1940s it was appraised as the most efficacious pre-surgical hand rub by the American surgeon P.B. Price.[10] In 1994, the *Tentative Final Monograph for Health Care Antiseptic Products (TFM)* published by the Food and Drug Administration (FDA) considered ethanol in concentrations between 60% and 95% as generally safe and effective for use in hand hygiene products.[11] Furthermore, ethanol 80% v/v (plus glycerol and hydrogen peroxide) is the active agent in one of the two hand-rub formulations recommended by the WHO for healthcare settings where commercial products are not available or too expensive.[4]

The aim of this *in vivo* laboratory study with volunteers was to generate – in a well-controlled and homogeneous fashion and in

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direct comparison with the reference alcohol of the European Norm EN 12791 – data on the bactericidal efficacy of three concentrations of ethanol (without any additives) when used for pre-surgical hand antisepsis during 3 min (which is the duration of application prescribed by EN 12791), in order to provide a basis for ethanol-based hand rubs to pass the efficacy requirement of the mentioned norm. The most efficacious of the three concentrations was also to be tested for 5 min (which is the longest application allowed by the norm for a product) to explore the maximum bactericidal efficacy of ethanol within the allowed time frame.

Methods

Volunteers

Twenty volunteers were included in this study. Exclusion criteria were: aged >18 years, pregnancy, skin breaks such as cuts, abrasions or other skin disorders on the hands. Nails were short and clean and the volunteers agreed not to take or use any antibacterial substance or antibacterial soap during the trial, starting one week prior to testing. All gave their written informed consent.

Alcohols and application

The study was carried out by rubbing ethanol (pro analysi; Merck, Darmstadt, Germany) at volume concentrations of 95%, 85% or 75% on to both hands for 3 min. After the bactericidal efficacy of the three concentrations was known, the most efficacious one, 85% v/v, was also used for 5 min. In a similar fashion, *n*-propanol 60% v/v was rubbed on to the hands for 3 min as specified by EN 12791. The bacterial reduction achieved by this procedure served as a reference for bactericidal activity against the resident microbial hand flora.

Neutralising agents

In this study, neutralising agents were not used because we had known from many earlier validation tests with the pure short-chain, aliphatic alcohols tested here that even their dilution with the sampling and dilution fluids is sufficient to neutralise any antimicrobial effect.

Test method and experimental design

The method for testing the above-mentioned hand rubs followed EN 12791.[12] A Latin-square design was used with five groups, each of four randomly allotted volunteers; as many experimental runs were performed as there were test situations including the reference procedure. In each run, all five situations were concurrently tested. At the end of the whole series of five test runs, each volunteer had used each antiseptic procedure once. The individual test runs were spaced by at least one week to allow re-growth of the normal skin flora.

Initial wash phase

To remove transient bacterial flora and any foreign material, a preparatory hand wash was carried out as described in EN 12791 by washing hands with soft soap (sapo kalinus, 20%). For this, 10 mL of soap was poured into the pre-wetted, cupped hands and rubbed vigorously for 1 min. Hands were then rinsed with running tap water and dried with paper towels.

Assessment of pre-treatment values

Immediately after drying, samples for bacterial counts were taken by kneading the fingertips, including the thumbs, of both hands for 1 min on the base of a Petri dish (diameter 9 cm) containing 10 mL of tryptone soya broth (TSB) without neutraliser. A separate dish was used for each hand. From these sampling fluids dilutions of 10^{-1} and 10^{-2} were prepared in TSB. From each dilution an aliquot of 0.1 mL was then spread over tryptone soy agar (TSA) with sterile glass spreaders. Plates were incubated for a total of 48 h at 36 ± 1 °C and colony-forming units (cfu) were counted by an electronic colony counter (Fisher colony counter, Model 480, Artek Systems Corp., Farmingdale, NY, USA).

Antiseptic treatment

Thereafter, surgical hand antisepsis was performed according to the standardised hand rub procedure described in EN 12791.[12] This consists of applying and rubbing as many 3 mL portions of product on to both hands up to the wrists as is necessary to keep the hands wet for a total of 3 min or, in the case of ethanol 85%, for 5 min.

Assessment of post-treatment values

Immediately after the antiseptic procedure, volunteers rubbed the fingertips of one randomly selected hand for 1 min at the bottom of a Petri dish containing 10 mL TSB to assess the immediate effect. The other, unsampled hand was covered with a sterile surgical glove for 3 h, before samples were collected as described above to evaluate the 3 h effect.

From all sampling fluids and their decimal dilutions, quantitative surface cultures were done on TSA. Counting plates were incubated at 36 ± 1 °C for a total of 48 h.

Assessment of used volumes of the study products

The norm requires recording the volumes of antiseptic used by each volunteer and product. This has been done by counting and recording the number of 3 mL volumes put on the hands of each volunteer necessary to keep them wet for the pre-set duration.

Statistical analysis

Viable bacteria counts were processed as described in EN 12791.[12] All pre- and post-application values were expressed as $\log_{10}$ values. Pre-application values were tested for significant differences by non-parametric Friedman analysis of variance (ANOVA) at an agreed level of significance of 5%. For each volunteer, logarithmic reduction factors (log RFs) were calculated as the intraindividual differences between $\log_{10}$ pre-treatment and $\log_{10}$ post-treatment values, separately for the immediate and after 3 h effects.[13] According to EN 12791, a product is considered to be effective for surgical hand antisepsis if the mean log RFs of the immediate and 3 h effects are not significantly below the respective ones obtained with the reference antiseptic procedure. According to EN 12791, mean log RFs of the five test products were tested for significant differences by Friedman ANOVA at an agreed level of significance of $P = 0.05$, followed by Wilcoxon–Wilcox tests for pairwise *post hoc* comparisons at significance levels of $P = 0.1$ (one-sided) for immediate and of $2P = 0.05$ (two-sided) for 3 h effects.[14] If the latter effect happened to be greater than that of the reference, statistical significance was tested at $P = 0.01$ (one-sided) as specified by the norm. All statistical calculations have been done manually using tables.