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Evaluation of the bactericidal effect of five products for surgical hand disinfection according to prEN 12054 and prEN 12791

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Summary Surgical hand disinfection (with an alcohol-based hand rub) and surgical handwash (with an antiseptic-based liquid soap) are accepted measures to reduce the risk for surgical site infections. The new European Standards allow a comparison of their antimicrobial efficacy. The bactericidal activity of surgical hand rubs [Sterillium and Softaman, (active ingredient = alcohols)] and handwashes [Derman plus (triclosan), Hibiscrub (chlorhexidine) and Betadine (PVP-iodine)] was tested according to the prEN 12054 suspension test using *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus hirae*, and to prEN 12791 for the effect on resident skin flora in comparison with 1-propanol, 60% (v/v). All five products achieved a reduction of test bacteria within 3 min of $>10^5$ -fold so fulfilling prEN 12054. However, only Hibiscrub, Sterillium and Softa Man met the requirements of prEN 12791, giving a mean reduction of resident micro-organisms (immediate and sustained effect) which was not significantly lower than the reference alcohol ($P > 0.1$; Wilcoxon matched-pairs signed-rank test). Sterillium was significantly more effective than the reference alcohol (immediate and sustained affect). Products for surgical hand disinfection may have equal antimicrobial activity in suspension tests but show large differences under practical conditions. Healthcare workers should not rely on results from suspension tests when deciding on a product for surgical hand disinfection.

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Introduction

Surgical site infections (SSI) are still among the most common hospital-acquired infections worldwide

despite significant developments in surgical technique.¹⁻³ Surgical hand disinfection has long been used to prevent SSI,^{4,5} especially as perforation rates of surgical gloves are as high as 17%,⁶ and many perforations are unnoticed by the surgeon.⁷ Disinfection can be achieved using an alcohol-based hand rub or a surgical handwash with an antiseptic liquid soap (based on chlorhexidine, PVP-iodine or triclosan). Products for surgical hand disinfection

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should pass two European Standards (currently in the pr or preliminary phase) for bactericidal efficacy: prEN 12054 is a suspension test using four different test bacteria to determine a general bactericidal activity, and prEN 12791 which is a test used to determine the bactericidal efficacy in vivo.⁸⁻¹⁰ A product can be regarded as suitable for surgical hand disinfection if it passes the requirement of prEN 12054 ($>10^5$ -fold-reduction within 5 min for a hand rub, $>10^3$ -fold-reduction within 5 min for a handwash) and prEN 12791 (not significantly less effective after 0 and 3 h than the reference alcohol). We have evaluated five products using these standards.⁸

Materials and methods

Products tested

The following hand rubs were tested: Sterillium (45% 2-propanol, 30% 1-propanol, 0.2% mectetronium etilsulphate) and Softa Man (45% ethanol, 18% 1-propanol). The following handwashes were tested: Derman plus (1% triclosan), Hibiscrub (4% chlorhexidine), and Betadine (7.5% PVP-iodine).

prEN 12054

The following test strains were used: *Escherichia coli* (K12) NCTC 10536, *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538 and *Enterococcus hirae* ATCC 10541.

The handwashes were diluted (55%) in hard water, and the rubs were tested undiluted. A suspension of test bacteria was added and after specified contact times at 20°C aliquots of the mixture were neutralized. The surviving bacteria were counted and numbers compared with those in the initial suspensions, and \log_{10} reduction factors (RF) calculated. For surgical hand rubs the product should demonstrate a minimum RF of 5, and for a handwash the diluted product RF of 3 within 5 min.¹⁰

The following neutralizers were used:

1. Polysorbate 80 (3%), saponin (0.3%), histidine (0.1%) and cysteine (0.1%) for Sterillium, Softa-man, Derman plus and Hibiscrub.
2. Polysorbate 80 (3%), lecithin (0.3%), histidine (0.1%), sodium thiosulfate (0.5%) and bovine serum albumin (0.1%) for Betadine.

The non-toxicity of neutralizers and the inactivation of dilution-neutralization method⁹ were validated beforehand. Validation of neutralization

was considered to be particularly important for chlorhexidine.¹¹ The method was also valid under the experimental conditions with a 55% (v/v) dilution with hard water.

prEN 12791

The in vivo bactericidal efficacy of the five products was assessed in 20 healthy volunteers aged >20 years. The skin of the volunteers was free from cuts or abrasions and no other skin disorders were present. Nails were short and clean. Volunteers did not use any substances with antibacterial activity or antibacterial soaps for one week before testing.

Wash phase

To remove transient bacterial flora and foreign particles volunteers' hands were washed with a non-medicated soap (sapo kalinus). Ten millilitres of the soap were poured into the cupped dry hands and rubbed vigorously on to the skin up to the wrists in accordance with the standard procedure to ensure total coverage of the hands, which were then rinsed in running tap water and dried with a sterile paper towel.

Determination of the pre-values

The distal phalanges of the right and left hand were rubbed separately, including thumbs, for 1 min on to two 9 cm Petri dishes containing 10 mL tryptic soy broth (TSB). Two 0.1 mL aliquots, as well as the same volume of 1:10 and 1:100 dilutions, were seeded in TSB. Sampling fluids were spread over tryptic soy agar (TSA) dishes with a sterile glass spatula. Two dishes were used for each dilution. No more than 30 min elapsed between sampling and seeding. Dishes were incubated for 24 h at $36 \pm 1^\circ\text{C}$. After an initial count of the colony-forming units (cfu) dishes were incubated for another 24 h to detect slow-growing colonies.

Disinfection phase

Each volunteer was treated with a reference product [n-propanol, 60% (v/v)]¹² as well as with all the other products. Between each product application a period of at least one week elapsed to allow reconstitution of normal skin flora. For surgical hand rubs volunteers rubbed their hands with 3 mL of test product following the same standard rub-in procedure used for washing. As soon as the hands were dry, an identical aliquot was used to keep the hands moist while proceeding with the rubbing procedure. For surgical handwashes 3 mL of the test product was used. Total application time was 3 min for all disinfection procedures.

After surgical handwash hands were rinsed with running tap water for 15 s and dried with a sterile paper towel.

Determination of post values

After disinfection the volunteer rubbed the distal phalanges of one hand (randomly selected) for 1 min in a Petri dish containing 10 mL of TSB supplemented with neutralizers (immediate effect). The other hand was gloved for 3 h for the assessment of the sustained effect. After removing the glove sampling was done as for the immediate effect. From the sampling fluid two 1 mL and two 0.1 mL aliquots were seeded, each in two Petri dishes with solidified TSA. A 1:10 dilution of the sampling fluid in TSB was prepared and two 0.1 mL aliquots of this were seeded as above. Dishes were incubated at $36 \pm 1^\circ\text{C}$ for 24–48 h.

For each dilution the mean number of colony-forming units scored in duplicate dishes was calculated. This was multiplied by the dilution factor in order to obtain the number of colony-forming units per millilitre of sampling liquid.

Calculation

Pre- and post-values were expressed as \log_{10} values. For calculation purposes values of 0 ($\log_{10}0 = -\infty$) were reset to 1 ($\log_{10}1 = 0$). If values in the range that could be entered into calculations were obtained from more than one dilution their mean was used as the final logarithm value. For each volunteer the RF was obtained from the difference between the \log_{10} pre-value and \log_{10} post-values.

The mean of the \log_{10} values (RF) of the reference alcohol 1-propanol were compared with the corresponding values for each product for a paired analysis of the immediate and sustained effect.

For a product to be considered effective for surgical hand disinfection the mean of the RF of both immediate and sustained effect has to be not significantly lower than that produced by the reference alcohol. Statistical analysis of means

was performed using the Wilcoxon matched-pairs signed-rank test with one-tailed level of significance, i.e. $P = 0.1$. A more comprehensive statistical analysis (not included in the standard) was done with an analysis of variance (ANOVA) model and the Tukey test.

Results

prEN 12054

All the products reduced the counts of all four test strains by $\text{RF} = 5$ within 3 min, thereby fulfilling the requirement for bactericidal activity specified in prEN 12054.

prEN 12791

The reference alcohol revealed a mean immediate RF of 0.83 ± 0.52 , and a mean sustained RF of 0.50 ± 0.84 (Table I). Betadine and Derman Plus had an immediate mean RF significantly lower than the reference alcohol (Betadine: 0.59 ± 0.47 ; Derman plus: -0.2 ± 0.30) and so failed. Hibiscrub, Sterillium and Softa Man revealed mean RFs (immediate and sustained), which were not significantly lower than the reference alcohol. Sterillium was the only product with mean RFs (immediate effect: 1.45 ± 0.88 ; sustained effect: 0.84 ± 0.93) significantly higher than the reference alcohol (Table I).

Comparison of mean RFs using an ANOVA model revealed a significant difference among the products ($F = 13.42$; $P < 0.0001$). The following comparisons were performed:

1. Reference product, Betadine, Hibiscrub and Softa-Man: no significant difference ($F = 1.04$; $P = 0.383$);
2. Reference product, Derman Plus and Sterillium:

Table I Comparison of the bactericidal efficacy of five products for surgical hand disinfection with the reference alcohol (n-propanol, 60%) according to prEN 12791

Product	Immediate value	P-value	Requirement	3 h Value	P-value	Requirement
n-propanol (60%)	0.83 ± 0.52			0.50 ± 0.84		
Hibiscrub	0.82 ± 0.50^a	>0.1	Pass	0.53 ± 0.98	>0.01	Pass
Betadine	0.59 ± 0.47	<0.1	Fail	0.29 ± 0.84	<0.1	Fail
Derman Plus	-0.2 ± 0.30	<0.1	Fail	-0.01 ± 0.46	<0.1	Fail
Sterillium	1.45 ± 0.88^b	<0.01	Pass	0.84 ± 0.93^b	<0.01	Pass
Softa Man	1.06 ± 0.68	>0.01	Pass	0.38 ± 0.72	>0.1	Pass

Mean with standard deviation of 20 subjects.

^a The mean is below the reference treatment and assessed not to be significantly lower at $P = 0.1$.

^b The mean is above the reference treatment and assessed to be significantly higher at $P = 0.01$.

this test showed a significant difference ($F = 42.74$; $P < 0.0001$).

Sterillium gave a reduction of bacterial skin flora shedding significantly higher than the reference product (Tukey test $P = 0.0041$) and Derman Plus (Tukey test $P = 0.0001$). The latter exhibited an efficacy significantly lower than 1-propanol (Tukey test $P = 0.0001$).

ANOVA tests showed a significant difference between the immediate and sustained effects for all the products ($F = 10.17$; $P < 0.0048$). The immediate effect was always significantly greater than the sustained effect.

Discussion

As European preliminary standards are now available a standardized direct comparison of the bactericidal efficacy of products for surgical hand disinfection can be performed. Products should pass prEN 12054 (suspension tests) and prEN 12791 (in vivo test). Our main finding is that significant differences under practical conditions were observed in vivo despite all of the products passing the in vitro suspension tests. Betadine and Derman plus failed the in vivo test. This is an important finding although the clinical relevance is hard to assess because only limited data relating SSI to the type of surgical hand disinfection are available.^{13,14} A similar result was obtained using the EN1500 standard for hygienic hand disinfection; significant differences were described even within 30 s application time.¹⁵ The accuracy of EN1500 has been described to be very good.^{16,17}

Our best results were achieved with the alcohol-based hand rubs. This is not surprising as their efficacy in the pre-operative treatment of hands is well known,¹⁸ and a surgical handwash may even contaminate the local environment.¹⁹ Nevertheless many surgeons remain reluctant to switch from an antiseptic soap to an alcohol-based hand rub. Our data should help to show that alcohol-based rubs are not only more effective,^{20,21} but also better for the skin.^{22,23} Sterillium had the best bactericidal efficacy in vivo. The excellent sustained effect may be explained by the presence of mecetronium etilsulphate (MES), a non-volatile quaternary ammonium compound. In addition MES has a known skin smoothing effect²⁴ and a mild antiperspirant effect.²⁵

Traditions die hard, but evidence is accumulating in favour of alcohol-based hand rubs for the pre-operative treatment of hands.

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