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CHEMICAL DISINFECTANTS AND ANTISEPTICS: EVALUATION OF THE EUROPEAN TEST METHOD EN 12791 OF DETERMINING THE ANTIMICROBIAL EFFICACY OF PRODUCTS FOR SURGICAL HAND DISINFECTION

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Abstract

For surgical hand disinfection a fast and strong immediate antibacterial effect is desired to enable the surgeon to begin his work without delay with “safe” hands. The reduced bacterial release should last for the duration of an operation to keep a potential microbial inoculum into the wound below an infection-generating dose in case the surgical glove becomes damaged. The evaluation of the antibacterial efficacy is of great importance for the selection of safe products. This is done in Europe according to EN 12791 on the hands of volunteers in comparison to a standardized reference dsinfection procedure which is concomitantly performed with the same volunteers in a second experimental test-run in a cross-over design. There, hands are sampled 0 and 3 h after application to account for immediate and sustained effects. Although in clinical practice, surgeons will treat their hands AND forearms as recommended by several guidelines, in EN 12791 only hands are treated because the highest bacterial density is found there and most gloves are perforated on the fingers.

In this study, good reproducibility and workability of EN 12791 was demonstrated. Because most surgeries last less than 3 h, the vast majority of surgeries are finished in a span of time covered by EN 12791 and the contribution of bacteriostatic supplements to delay bacterial re-growth on gloved hands appears rather minor, if only a product exerts an immediate effect as strong as that of the reference disinfection procedure. It was demonstrated that the efficacy of hand antiseptics is significantly associated with the duration of application, emphasising the importance of validation before a product is introduced into clinical practice. By contrast, the mode of application, in terms of the inclusion of forearms, has no significant influence on the reduction of the hand flora, hence, the mode of application as described in EN 12791 needs no alteration in this respect.

Keywords:

surgical hand disinfection, EN 12791, mode of application, duration of application

Kurzfassung

Für die chirurgische Händedesinfektion wird eine starke und rasche antibakterielle Wirkung gefordert, damit das OP-Team seine Arbeit mit „sicheren Händen“ ohne Verzögerung beginnen kann. Die Abgabe hauteigener Bakterien von den Händen soll für die Dauer einer OP so weit verringert werden, dass im Falle einer OP-Handschuhperforation das in die Wunde eingebrachte Inokulum unter der infektionserzeugenden Dosis bleibt. Da die Auswahl sicherer Produkte wichtig erscheint, wird in Europa deren antibakterielle Wirkung gemäß der Europäischen Norm EN 12791 an den Händen von Probanden im Vergleich zu einem parallel an denselben Personen, im Überkreuzdesign durchgeführten Referenzverfahren erhoben und die Wirkung sofort und 3 h nach Behandlung beurteilt. Entsprechend EN 12791 werden nur die Hände desinfiziert weil an diesen die Bakteriendichte am höchsten ist und OP-Handschuhe vorwiegend an den Fingerspitzen perforiert werden. Diese Anwendung entspricht jedoch nicht der klinischen Praxis, wo Hände UND Unterarme desinfiziert werden.

In dieser Studie konnte die Machbarkeit und Reproduzierbarkeit der Europäischen Testmethode EN 12791 eindeutig bewiesen werden. Die in EN 12791 geprüfte Zeitspanne kann als klinisch relevant betrachtet werden, da die Mehrzahl der gängigen OPs nicht länger als 3 h dauert. Ein Zusatz von Wirkstoffen, die eine Langzeitwirkung unter dem Handschuh vermitteln, scheint unbedeutend, wenn nur die Sofortwirkung des Antiseptikums stark genug ist. Es wurde gezeigt, dass die antibakterielle Wirksamkeit signifikant mit der Desinfektionsdauer korreliert, was in Hinblick auf die empfohlene Anwendungsdauer eines Produktes die Wichtigkeit des Wirksamkeitsnachweises vor Einführung desselben in die klinische Praxis unterstreicht. Im Gegensatz dazu hat die Anwendungsart, konkret die zusätzliche Desinfektion der Unterarme, keinen signifikanten Einfluss auf die Reduktion der residenten Händeflora. Eine Adaptierung des in EN 12791 beschriebenen Verfahrens ist daher nicht nötig.

Schlüsselwörter:

Chirurgische Händedesinfektion, EN 12791, Art der Applikation, Dauer der Applikation

CONTENTS

1 INTRODUCTION

Although the role of hands in the transmission of puerperal fever had been recognized by Alexander Gordon in 1795 (Stewart & Williams, 1996) and by Oliver Wendell Holmes in 1843 (Fischoff, 1982), the Hungarian obstetrician Ignaz Philipp Semmelweis was the first to demonstrate the role of hand hygiene in the prevention of person-to-person transmission of infections and who took the necessary steps to interrupt infection chains. During the years 1841-1847, puerperal fever caused maternal mortality rates of up to 18% in one of the two maternity departments of the General Hospital in Vienna, Austria, whereas that in the other department averaged around 3% (Rotter, 1998). Semmelweis was able to produce the epidemiological evidence, that hands of doctors and students could act as vectors for the fatal etiologic agent imported from the autopsy-room and introduced into the maternal birth canal during vaginal examination (Rotter, 1998). He found the reason for the much lower mortality rate at the other department in the fact, that deliveries there were performed mainly by midwives who never actively took part in post mortem examinations. He further observed that normal hand washing did not always prevent the spread of fatal infection and recommended hand disinfection in a solution of chlorinated water before each vaginal examination. His introduction of hand disinfection had a great effect on the mortality which dropped to <3% (Rotter, 1999).

Some years later, the Scottish surgeon Sir Joseph Lister tested and proved the hypothesis of the French chemist Louis Pasteur, that microorganisms not only cause fermentation and putrefaction but may also be the cause for suppuration in living tissues and converted it into effective preventive measures. By inactivating and destroying the causative microorganisms in the air and in the environment of the surgical wound by using antiseptic measures such as carbolic acid, he successfully prevented postoperative wound infection (Godlee, 1921). Among other vehicles and sources, he also recognized the importance of the surgeons´ hands and consequently tried to eliminate their microbial skin flora before surgery.

Since then, hand hygiene gradually became accepted as one of the most important measures for preventing transmission of pathogens in health-care settings (WHO, 2006).

2 MICROBIAL SKIN FLORA

As early as 1938, the American surgeon Price (Price, 1938) divided the microbial flora recovered from human skin into two groups, those that permanently reside on the skin (resident flora) and those that happen to be there as contaminants and which lodge only temporarily (transient flora).

In addition, a third group was described (infectious flora), including pathogens which are frequently isolated from septic lesions of the skin such as abscesses, whitlows, paronychia, or infected eczema (Rotter, 1999).

2.1 Resident flora

The human skin and mucous membranes are permanently colonized by various microorganisms, mainly bacteria. These bacteria are usually not regarded as pathogens on intact skin. They may, however, cause infections in sterile body cavities, in the eyes, or on non intact skin (Lark et al., 2001).

The normal skin flora fulfills a protective function by preventing other and potentially more pathogenic organisms from colonizing on skin surfaces.

The composition of the normal skin flora varies quantitatively and qualitatively with location, sex, age, health condition, hospitalisation, season of the year and frequency of hand washing (Kligman, 1965).

On the hands, the population density of resident skin bacteria ranges from 10^2 to 10^3 cfu/cm² (Marples, 1974) and remains stable in a person over long periods of time (Price, 1938; Blank, 1965). The dominant species is *Staphylococcus epidermidis*, which is found on almost every hand (Lee et al., 1994; Rayan & Flourney 1987; Slight et al., 1987) and other coagulase-negative staphylococci, followed by coryneform bacteria such as propionibacteria, and micrococci (Evans et al., 1950; Leyden & McGinley, 1993; Noble, 1993).

The resident flora is difficult to remove by mechanical means. Washing hands with water and soap for 5 minutes only halves the release of normal bacterial skin flora (Price, 1938).

2.2 Transient flora

The transient skin flora consists of bacteria, fungi and viruses, occasionally with a high pathogenic potential, that may be found on skin only at times as a contamination from the environment (Price, 1938). This flora usually does not multiply on the skin and does not survive for very long but long enough to be transferred, for example, from person to person.

The transmissibility of transient bacteria depends on the species, the number of bacteria on the hands, their survival on skin, and the dermal water content (Jaques et al., 1983; Marples & Towers, 1979; Patrick et al., 1997).

In contrast to the resident microbial skin flora, the transient flora is easily removable by mechanical means, such as hand washing. A one minute hand wash reduces the bacterial release by two to three orders of magnitude (Lowbury et al, 1964; Ayliffe et al., 1975; Mittermayer & Rotter, 1975).

2.3 Infectious flora

This group includes etiologic agents, such as *Staphylococcus aureus* or beta-haemolytic streptococci, from actual skin infections.

No antiseptic treatment of suppurative lesions will render hands save, because this flora tends to remain until lesions are healed (Rotter, 1999).

3 STRATEGIES OF HAND HYGIENE

Strategies for the prevention of hand-associated microbial transfer will vary between different situations taking into consideration that it is much easier to reduce the release of transient flora from the hands than that of resident flora and that infectious lesions on the hands are an absolute contraindication for any direct activity with for instance patients, pharmaceuticals and foodstuff.

Therefore, the choice of preventive measures depends on which group of microbial skin flora is to be attacked:

3.1 Strategies against transmission of transient flora

3.1.1 Protection of the hands

For any activity for which microbial contamination is to be expected, the strategy should be to “keep hands clean”, because this is much easier to achieve than to “make hands clean”. For this purpose, the non-touch technique, using instruments instead of fingers and the use of protective gloves are suitable measures. Both instruments and gloves must, of course, be changed after every patient or activity.

3.1.2 Elimination of transient flora

If hands are known to be or suspected to be contaminated, an appropriate post-contamination treatment is necessary to eliminate the undesired potential pathogens or to reduce their release from the hands to an acceptable level, to render them save for the next patient contact. This may be achieved by a social hand wash, a hygienic hand wash or a hygienic hand disinfection.

These post-contamination treatments differ with respect to their antimicrobial efficacy, safety, time, economy, comfort and user preference. The higher the risk, the more important is it to use a post-contamination treatment that is effective and safe.

3.1.2.1 Hand washing

The objective of a normal hand wash with plain, unmedicated soap and water (with or without using a brush) is the mechanical removal of dirt and loosely adherent microorganisms which includes the majority of the transient skin flora.

A one minute social hand wash reduces the release of transient bacteria from artificially contaminated hands by 2.7 to 3.0 log (Rotter, 1999). Longer wash periods are unrealistic and, with respect to the relatively poor effect, not worth the effort.

It should be mentioned, that hand washing possibly disperses the microorganisms to be washed off (Börnstein, 1915; Namura et al., 1994) or also those from a contaminated sink drain (Döring et al., 1991) into the surrounding area of the wash basin and onto the washing person.

Wash basins should be conveniently located, no plugs or overflows are necessary as only running tap-water should be used. Mixer taps to provide water of comfortable temperature and operation of the water flow without using hands may be desirable in certain critical areas (e.g. intensive care or newborn units). Suitable refillable dispensers for soap, disinfectant, hand lotion and one-way towels (paper or textile) should be standard. A container for used towels is also necessary.

3.1.2.2 Hygienic hand wash

The objective of a hygienic hand wash with antimicrobial or medicated soap and water is the mechanical removal of dirt and loosely adherent and the inactivation of strongly adherent microorganisms of the transient bacterial skin flora.

Active agents used are detergent preparations containing iodophors, chlorhexidine gluconate, triclosan, biphenylol and chloroxylenol.

A one minute hygienic hand wash with a povidone-iodine- (0.75%), a chlorhexidine- (4%) or a triclosan-based (0.1%) soap reduces the release of transient bacteria from artificially contaminated hands by 3.5, by 3.1 or by 2.8 log, respectively (Rotter & Koller, 1991).

3.1.2.3 Hygienic hand disinfection

The objective of a hygienic hand disinfection (“rub”) with an antiseptic preparation without the addition of water is the reduction of the transient bacterial skin flora by

killing it rather than removing it, with maximum efficacy and speed. It should be mentioned, that the hygienic hand rub does not completely replace hand washing, because dirt and emollients that build up during multiple applications must be washed away from time to time.

A hygienic hand disinfection is performed by rubbing 3-5 ml of a fast-acting disinfectant onto both hands until dryness or for a preset duration recommended by the manufacturer (usually 30 to 60 seconds). Agents used for hygienic hand rubs are highly concentrated alcohols (used alone or mixed with other antiseptics such as quaternary ammonium and ampholytic compounds), aqueous solutions containing chlorine-releasing agents, iodine and iodophors or chlorhexidine.

Depending on the microbial species and the antiseptic agent, bacterial reductions of up to more than 5 log are possible within 1 minute (Wewalka et al., 1977).

3.2 Strategies against transmission of resident flora

3.2.1 Protection of the wound

In surgical practice, the transmission of microbial skin flora from the hands into a sterile body cavity or into the surgical wound is undesirable, thus, the rule is to prevent any microbial release from the hands. This is best achieved with surgical gloves, which are unfortunately vulnerable to injury. Perforations are found, on average, in 18.2% of surgical gloves, and more than 80% of cases go unnoticed by the surgeon (Kralj et al, 1999). Even when double-gloving is used, the frequency of perforation has been reported to occur in 4.2% of operations (Thomas et al., 2001).

3.2.2 Elimination of resident flora

For the above reason, a pre-operative treatment of hands with antiseptics is usually performed as an additional safety measure before donning the gloves. Two options are available, a surgical hand wash (“scrub”) or a surgical hand disinfection (“rub”).

3.2.2.1 Surgical hand wash

The objective of a surgical hand scrub - which is the cleaning of hands with antimicrobial soap and water - is to reduce the release of skin bacteria from the surgical team's hands to sub infective levels for the duration of an operation in case the surgical glove gets punctured or torn.

Surgical hand washes are performed with antiseptic detergents according to the instructions of the manufacturer. But this should not take longer than 5 minutes. Drying hands with sterile drapes or towels is necessary before donning surgical gloves.

The most commonly used agent is chlorhexidine gluconate contained in detergent, usually at concentrations of 2 or 4%. Triclosan can also be found in medicated soaps, usually at a concentration of 1%.

A surgical hand wash with a chlorhexidine-based detergent can reduce the number of resident hand flora by 0.4 to 2.3 log, the use of triclosan yields mean log reductions between 0.3 and 0.8 (Kampf & Kramer, 2004).

3.2.2.2 Surgical hand disinfection

Excepting the cleaning function, the objective of a surgical hand rub with the application of an antiseptic preparation without the addition of water is the same as mentioned above. If performed with a well formulated antiseptic, surgical hand disinfection can be very effective in reducing the resident skin flora. In addition, hands need not be dried afterwards. Because the cleaning function of a surgical rub is missing, hands and forearms should be washed up to the elbows with unmedicated soap for approximately 1 minute prior to the first operation of the day. Cleaning of the subungual spaces with soft wooden sticks is obligatory, also a soft brush may be used for them but never for other hand surfaces. Fingertips should always point upward, with elbows down, to avoid recontamination of the fingers by water running down from contaminated proximal areas. Thereafter, drying hands and forearms is of great importance, especially if an alcohol-based hand rub is to be used afterwards to avoid its dilution. Finally, a surgical hand rub is performed by pouring small volumes of 3 to 5 ml of a suitable antiseptic into the cupped dry hands and rubbing it vigorously all over the hands and forearms, which must be kept wet for the recommended time period by applying further portions as necessary. Times of 3 to 5 minutes are common today for surgical hand rubs. The

duration of any pre-operative treatment of the surgical team's hand should be kept as short as possible but as long as necessary to attain a low bacterial load under the glove (Rotter, 1999).

Alcohol-wet hands should not be gloved but must be air-dried before donning surgical gloves to avoid skin damage. A surgical hand rub should never be followed by a hand wash, because this lessens the effect of the hand rub considerably (Lilly et al., 1979).

Several antimicrobial effects of agents for surgical hand rubs have been described (Michaud et al., 1976), depending on the frequency of contacts with the antiseptic: the *immediate* effect, which describes the reduction of microbial flora immediately after a procedure for surgical hand antisepsis; the *sustained* effect, which after a single contact maintains or further reduces the bacterial count under the glove for a while; a *cumulative* effect building up with multiple applications; and a *persistent* effect, which is a progressive reduction of skin flora over longer periods of time.

With respect to these various antimicrobial effects, a strong immediate effect is of greatest importance, because the surgeon's hands should be safe already at the time of the first operation on the list. In contrast, cumulative or persistent effects are dispensable. In addition, a sustained effect may be useful to keep the bacterial load under the glove low during an operation. Because most operations are completed within 3 hours or gloves have to be changed during more prolonged surgery, this time span seems to be a reasonable duration of sustained activity.

The antimicrobial spectrum of a surgical rub need not cover mycobacteria, fungi and viruses, because pathogens belonging to these groups of microorganisms do not usually cause surgical wound infections. However, agents for surgical hand disinfection must be active against the resident hand flora and bacteria associated with surgical site infections.

There is only a limited list of possible agents that can be used for pre-surgical hand preparation. Antimicrobial solutions prove to be much more effective than antiseptic detergents. When rubbed onto the hands for 1 to 5 minutes they reduce the release of resident hand flora by 0.4 to 2.9 log (Rotter, 1999). Alcohol solutions achieve by far the strongest effects, which is positively correlated with their concentration and the duration of application. N-propanol is the most active agent, followed by isopropanol, ethanol and povidone-iodine solution.

4 METHODS USED FOR EVALUATING THE ANTIMICROBIAL EFFICACY OF PRODUCTS FOR HAND HYGIENE (LABORATORY TESTS)

In the field of hand hygiene, European regulations exist, or are under development, for assessing the antimicrobial efficacy of products for hand hygiene. These norms use a step-wise procedure.

First, the agent is evaluated *in vitro* in a suspension test for demonstrating bactericidal activity according to the tentative European pre-norm prEN 12054 (1997) using *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli K12* and *Enterococcus hirae*. There is also the intention to develop standards to prove activity against yeasts (*Candida albicans*) and mycobacteria (*Mycobacterium terrae* and *Mycobacterium avium*), but they are still in preparation (Rotter, 2004), and will need to be demonstrated only with products for the hygienic hand rub or if specific claims for activity against these microorganisms are made, whereas a positive result of a test according to prEN 12054 is obligatory for each hand decontamination preparation. According to this norm, a 5 log reduction is required for the rubs, whereas for the washing products only a 3 log is necessary.

Finally, when an agent has passed the *in vitro* test, it is evaluated with volunteers in the laboratory by *in vivo* tests simulating practical conditions.

In vivo tests such as these exist for the post-contamination treatments, hygienic hand wash (EN 1499) and hygienic hand disinfection (EN 1500), and for the pre-operative hand treatments, surgical hand wash or rub (EN 12791).

In EN 1499 (1997) and 1500 (1997), the hands of 12 to 15 subjects, who are randomly allotted to two groups, are artificially contaminated with a suspension of a non-pathogenic strain of *Escherichia coli K12*. After contamination, hands are allowed to dry and then sampled for assessment of the pre-treatment bacterial release by kneading the fingertips for one minute at the bottom of a Petri dish filled with sampling fluid, one for each hand. Immediately after that, the hands of one group of volunteers are treated with one of two procedures, either with the product under test according to the instructions of the manufacturer for a maximum of 60

seconds or with a reference procedure, whereas the other group uses the respective other procedure. According to EN 1499, the reference procedure is a one minute hand wash with unmedicated soap, the one according to EN 1500 is a one minute hand rub with isopropanol 60% (vol/vol). After completion of these procedures, hands are sampled again as above for assessment of post-treatment bacterial release. Subsequently and after thorough hand washing, both groups of subjects repeat the test, but with the respective other procedure (cross-over design). After quantitative culture of the sampling fluids, viable counts are established per ml sampling fluid, transferred into logarithmic values and logarithmic reduction factors (log RFs) are calculated as the difference of log pre-treatment minus log post-treatment values for each subject. Log RFs obtained from the procedure with the product under test are compared individually with those obtained with the reference procedure tested with the same subjects, on the same day and under comparable conditions.

Whereas by means of EN 1499 the superiority of the product under test over the reference hand wash must be demonstrated (P = 0.01, one-sided), with EN 1500 a hand rub must prove not to be significantly inferior to the reference treatment (P = 0.1, one sided).

According to EN 12791 (2005), the efficacy of a test product is tested - after an initial pre-wash with soap and water - in a similar way as described above for post contamination treatments but on clean, not contaminated hands of 18-20 subjects. Here, the reference disinfection procedure consists of applying and rubbing as many portions of 3 ml n-propanol 60% (vol/vol) into both hands and up to wrists as necessary to keep hands wet for a total of 3 minutes. For assessing the immediate effect of the product under test or the reference procedure, one randomly selected hand is sampled immediately after disinfection and, for the assessment of a possible sustained effect, the other, meanwhile gloved hand is sampled 3 hours after disinfection (split-hand model by Michaud et al., 1976). The tests are also performed using a cross-over design, but the two experimental runs are spaced by an interval of at least one week to allow re-growth of transient flora.

The requirement of EN 12791 is that the reduction achieved by the product under test shall not be significantly inferior to that of the reference disinfection procedure (immediate: P = 0.1, one-sided; 3-hours: 2P = 0.01, two-sided). The claim of any

sustained activity must be demonstrated by the product under test achieving a significantly greater bacterial reduction after 3 hours as compared to the reduction of the reference disinfection procedure (P = 0.01, one-sided).

A specific feature of the *in vivo* test models described in EN 1499, EN 1500 and EN 12791 is that the efficacy of a hand wash or hand disinfection procedure is intraindividually compared with that of a reference treatment. This proceeding has several advantages: 1) with the reference, an individual performance standard is set which has to be met by a test preparation, 2) every test person acts as his/her own control thereby fulfilling statistical requirements with smaller sample sizes than with separate test populations, 3) the test results (= log RFs) of different laboratories can be standardized and therefore be made comparable.

5 REVIEW OF PREPARATIONS USED FOR HAND HYGIENE

This section summarizes the agents most often used for hand hygiene:

5.1 Plain, unmedicated soap

Nonmedicated soaps are detergent-based products that contain esterified fatty acids and sodium or potassium hydroxide. They are available in various forms including bar soap, tissue leaflet, and liquid preparations. Their cleaning activity can be attributed to their detergent properties. Plain soaps do not contain any active ingredient with an antimicrobial activity.

However, hand washing with plain soap can remove and reduce loosely adherent transient flora by 0.5 to 2.8 log (Rotter, 1999), but has no significant effect on the resident hand flora. The dermal tolerance of frequent use is more or less poor (Kampf & Kramer, 2004).

5.2 Alcohols

The majority of alcohol-based antiseptics contain either ethanol, isopropanol, n-propanol, or a combination of two of these alcohol-species. Although being also a member of this group, methanol is seldom used. Low concentrations of higher alcohols such as butanol and aromatic alcohols such as benzylalcohol are sometimes contained in alcoholic preparations as synergistic supplements (Rotter, 1999). Alcohol-based hand rubs are available as low viscosity rinses, gels, and foams. Limited data are available regarding the relative efficacy of various formulations.

The general antimicrobial activity of alcohols can be attributed to their ability to denature proteins (Larson & Morton, 1991). Alcohol solutions containing 60 to 95% alcohol are most effective, and higher concentrations are less potent (Larson & Morton, 1991; Price 1939; Harrington & Walker, 1903) because proteins are not denatured easily in the absence of water. Alcohols have excellent and the most rapid bactericidal activity of all agents used in hand disinfection, they also possess

very good activity against yeasts, dermatophytes, mycobacteria and enveloped viruses (Rotter, 1999). Despite its effectiveness against these organisms, alcohols have very poor activity against bacterial spores, protozoan oocysts, and certain nonenveloped viruses (Boyce & Pittet, 2002).

The efficacy of alcohol-based hand hygiene products is affected by several factors, including alcohol species (bactericidal activity decreases in the order n-propanol > isopropanol > ethanol), duration of application and volume of alcohol (Rotter, 1999).

Alcohols evaporate quickly from the skin and do not have a sustained activity, however, re-growth of bacteria on the skin occurs slowly after use of alcohol-based hand rubs, presumably because of the sub lethal effect alcohols have on some of the skin bacteria (Lowbury et al., 1974; Lilly et al., 1979).

Hand disinfection with an alcohol-based hand rub can reduce transient bacteria by 2.6 to 6.8 log, but the effect on the resident flora is lower, with a mean reductions between 1.5 and 2.9 log (Kampf & Kramer, 2004).

The dermal tolerance is good, skin drying and irritant skin reactions may be avoided by adding suitable emollients such as glycerol, volatile silicone oils, refattening agents, and probably most importantly, rehydrating agents. In the applications discussed above, alcohols are non-toxic, they also lack any allergenic potential (Rotter, 1999).

5.3 Chlorhexidine

Chemically, chlorhexidine is a cationic bisbiguanide compound. It exists as gluconate, but also as acetate (diacetate) and as hydrochloride salts (Russell, 1986). Chlorhexidine gluconate is commonly used either at 0.5 to 0.75% in aqueous solution or in some detergent preparations or at 2 to 4% in other detergent preparations (Lowbury & Lilly, 1973; Lowbury et al., 1974). The hydrochloride is used in a powder preparation.

The antimicrobial activity of chlorhexidine is attributable to attachment to, and subsequent disruption of cytoplasmatic membranes, resulting in precipitation of cellular contents (Larson, 1995; Rotter, 1999). The antimicrobial spectrum of

chlorhexidine is broad, it has good activity against bacteria, yeasts and enveloped viruses but limited activity against mycobacteria, dermatophytes, and nonenveloped viruses.

The antimicrobial activity of chlorhexidine is only minimally affected by the presence of organic material, including blood, but its activity can be reduced by natural soaps, various inorganic anions, non-ionic surfactants, hand creams containing anionic emulsifying agents (Walsh et al., 1987; Denton, 1991; Larson, 1995).

The immediate antibacterical activity is definitely slower than that of alcohols, but the residual effect of chlorhexidine, because of its strong affinity for surfaces, is probably the best of any antiseptic available (Rotter, 1999).

A hand wash with a chlorhexidine-based soap can reduce the number of transient bacteria by 2.1 to 3 log, the effect on the resident flora is smaller, with a mean reduction between 0.4 and 2.3 log (Kampf & Kramer, 2004).

Clorhexidine is regarded as a safe antiseptic, skin irritation is usually regarded as low (Reybrouck, 1986), and there is no indication of absorption by the skin (Adler et al., 1980; Gongwer et al., 1980).

5.4 Iodine and Iodophors

Because elemental iodine causes irritation and discolouring of skin, iodophors have largely replaced iodine as the active ingredient in antiseptics. Combining iodine with various polymers increases the solubility of iodine, promotes sustained release of iodine, and reduces skin irritation. The most common polymers incorporated into iodophors are polyvinyl pyrrolidone (i. e., povidone) and ethoxylated non-ionic detergents (Anderson, 1989; Gottardi, 1991).

It is important to note that the strongest antimicrobial effect occurs with diluted rather than concentrated iodophor solutions (Davies et al., 1954; Rose & Swain, 1956).

Iodine molecules rapidly penetrate the cell wall of microorganisms and inactivate cells by forming complexes with amino acids and unsaturated fatty acids, resulting in impaired protein synthesis and alteration of cell membranes (Gottardi, 1991).

The antimicrobial spectrum of iodine preparations is wide, they have bactericidal activity, even including bacterial spores, are active against mycobacteria, viruses and fungi (Rotter, 1999).

The sustained effect is small and only short lived (Rotter et al., 1980; Koller et al., 1991).

The antimicrobial activity of iodophors can be affected by pH, temperature, exposure time, concentration of total available iodine, and the amount and type of organic and inorganic compounds present (Boyce & Pittet, 2002).

A hand disinfection with an aqueous solution of povidone-iodine (1%) can reduce the number of transient bacteria by 4.0 to 4.3 log, the effect on the resident flora is smaller, with a mean reduction of 1.9 log (Rotter, 1999).

Iodophors cause less skin irritation and fewer allergic reactions than iodine, but more irritant contact dermatitis than other antiseptics commonly used for hand hygiene (Larson et al., 1986).

5.5 Quaternary ammonium compounds

Quaternary ammonium compounds are composed of a nitrogen atom linked directly to four alkyl groups, which may vary in their structure and complexity (Merianos, 1991).

Of this large group of compounds, alkyl benzalkonium chlorides are the most widely used as antiseptics, other compounds that are used include benzethonium chloride, cetrimide, and cetylpyridinium chloride (Kramer & Wallhäusser, 1993). Quaternary ammonium compounds are seldom used alone today for skin and hand disinfection but rather are used in combination with other antiseptics such as alcohols to confer on them a sustained effect (Rotter, 1999).

The antimicrobial activity of this group of compounds likely is attributable to adsorption to the cytoplasmatic membrane, with subsequent leakage of low molecular weight cytoplasmatic constituents (Merianos, 1991).

Quaternary ammonium compounds are primarily bacteriostatic and fungistatic, although they are microbicidal especially against gram-negative bacteria (*Pseudomonas aeruginosa*) at high concentrations (Rotter, 1999). They have

relatively weak activity against mycobacteria and have greater activity against some viruses.

Their antimicrobial activity is adversely affected by the presence of organic matter, and they are incompatible with anionic detergents (Rotter, 1999).

Quaternary ammonium compounds have low allergenic and toxicity potentials.

5.6 Triclosan

Triclosan, a tri-chlorinated dioxy-diphenylether (Irgasan CH 3565 and Irgasan DP 300) is a non-ionic, colourless substance. It is contained in detergents (0.4 to 2%) and in alcohols (0.2 to 0.5%) used for hygienic and surgical hand or preoperative skin disinfection and in soaps and deodorants. It is incompatible with lecithin and some non-ionogenic detergents such as Tween 80 (Kramer & Wallhäusser, 1993).

Triclosan enters the microbial cell and affects the cytoplasmic membrane, and synthesis of RNA, fatty acids, and proteins (Jones et al., 2000).

Triclosan has good activity against vegetative bacteria (except *Pseudomonas aeruginosa*) and yeasts but limited activity against mycobacteria and dermatophytes. The activity against viruses is unknown.

A hand wash with a triclosan-based soap can reduce the number of transient flora by 2.8 log, the effect on the resident hand flora is lower, yielding a mean reduction between 0.3 and 0.8 log (Kampf & Kramer, 2004).

The dermal tolerance is rather poor. There is no indication in the literature that triclosan has a toxic, allergenic, mutagenic, or carcinogenic potential.

6 COMPLIANCE WITH HAND HYGIENE TREATMENTS

Although, hand hygiene is considered the most important measure to reduce the transmission of nosocomial pathogens from the hands of health-care workers, which are the main sources of cross-infections in health-care settings, compliance with hand hygiene treatments is still low. Average compliance with hand hygiene recommendations varies between hospital wards, among professional categories of health-care workers, and according to working conditions and has been described to vary between 16 und 81% (Pittet 2000), with an overall average of 40% (Boyce & Pittet, 2002).

Reasons reported by health-care workers for the lack of adherence with recommendations are manifold including the following: hand hygiene agents cause skin irritation and dryness; patient needs take priority over hand hygiene; sinks are inconveniently located or not available; glove use dispenses with the need for additional hand hygiene; health-care workers have inadequate knowledge of guidelines or protocols for hand hygiene; there is lack of recognition of the risk of cross-transmission of microbial pathogens; or simply forgetfulness (Pittet & Boyce, 2001).

To overcome these barriers, a new guideline for hand hygiene in health-care settings was published in 2002 by the Centers of Disease Control and Prevention (CDC), and entitled *Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force* (Boyce & Pittet, 2002). Recognizing a worldwide need to improve hand hygiene in health-care settings, the World Health Organization (WHO) published its *Guidelines on Hand Hygiene in Health Care (Advanced Draft)* in 2006 (WHO, 2006). These global guidelines reinforce the need for multidimensional strategies as the most effective approach to promote hand hygiene. Key elements include staff education and motivation, adoption of an alcohol-based hand rub as the primary method for hand hygiene, use of performance indicators, and strong commitment by all stakeholders, such as front-line staff, managers and health-care leaders, to improve hand hygiene. The recommendation for alcohol-based hand rubs is based on their broader spectrum of antimicrobial activity, faster efficacy

(Rotter, 1999), better dermal tolerance, and rather positive effect on the compliance with hand hygiene protocols compared with preparations based on other active agents such as chlorhexidine (Kampf & Kramer; 2004).

Furthermore, lack of scientific information demonstrating the definitive impact of improved hand hygiene on nosocomial infection rates has also been reported as a possible barrier to adherence with recommendations (Pittet, 2000). Several quasi-experimental clinical studies of the impact of hand hygienie treatments on the risk of nosocomial infections were published from 1977 to 1995 (Larson, 1999), and most of them showed, despite limitations, a temporal relation between improved hand hygiene practices and reduced infection rates.

However, well controlled, randomised clinical trials are difficult to carry out and a large number of patients must be included in order to obtain statistically valid results. This is the reason, why the efficacy of products for hand hygiene is usually evaluated under controlled laboratory conditions simulating practical conditions with volunteers rather than in clinical trials.

Although the initiation of hygiene measures into the operating theatre resulted in a dramatic fall of post-operative wound infections, the clinical impact and necessity of surgical hand disinfection has never been demonstrated in a randomised, controlled clinical trial (Boyce & Pittet, 2002). Therefore, it is still unknown whether the test results obtained with products for the surgical hand treatment in laboratory experiments simulating practical conditions do correlate with the frequency of surgical site infection.

7 AIMS

Since the new Centers of Disease Control and Prevention guideline on hand hygiene was published in 2002, surgical hand disinfection with alcohol-based hand rubs has become a standard worldwide to prevent the transmission of nosocomial pathogens by the hands of health-care workers (Boyce & Pittet, 2002).

A major factor for selecting a specific hand hygiene preparation is its antimicrobial efficacy. As mentioned above, in Europe the *in vivo* efficacy of preparations for surgical hand antisepsis is assessed on volunteers´ hands according to the EN 12791 standard (EN 12791, 2005). According to this European method, the antimicrobial efficacy of a product is evaluated against a standardized reference treatment which involves the application of n-propanol 60% (vol/vol) onto both hands for 3 minutes in a randomized cross-over design (test product versus reference treatment) using the split-hand model (Michaud et al., 1976). In this model, the sampling is undertaken immediately (0 hours) on one, and 3 hours after application on the other, meanwhile gloved hand. A test product shall not be significantly less effective than the reference treatment both immediately and 3 hours after application.

One aim of our research was to study the inter-laboratory reproducibility and workability of the European test standard EN 12791 in a multicenter trial by comparing the immediate antimicrobial effects of different products for surgical hand disinfection in different laboratories.

In the last decades, the recommended time for surgical hand treatment has been reduced from >10 minutes to 5 minutes, because both were found to be equally effective (Tucci et al., 1977; Galle et al., 1978). Some other authors (Hingst et al., 1992; Kappstein et al., 1993) could not find any differences between 5 and 3 minute applications and even 1.5 minutes (Kampf et al., 2005) with a very efficacious propanol-based product have been shown to be as effective as a 3 minute surgical hand disinfection with the reference alcohol of EN 12791. As these findings seemed somewhat illogical, we undertook to establish the impact of the duration of application of a surgical hand rub on the reduction of resident hand flora in a strictly homogeneous test series excluding all extraneous influences. Both, the reference of EN 12791, n-propanol 60% (vol/vol) and isopropanol 70%

(vol/vol) were used for surgical antisepsis with durations of 1, 3 or 5 minutes. Furthermore, as unfortunately no data are available for ethanol-based hand rubs at shorter durations of application, we investigated their *in vivo* efficacy when applied for 1.5 minutes.

In the European test method EN 12791, samplings for post-treatment results are taken on a single day immediately (0 hours) after disinfection and 3 hours later, accounting for immediate and sustained effects, however, no measurement of a cumulative effect is intended. By contrast, in the U.S. test method (TFM, 1994), which in fact is the ASTM test E 1115, the efficacy of 11 repetitive product applications over a 5 day period is evaluated. There, hands are sampled at three different times post application (0, 3 and 6 hours) on one day to account for immediate effects and sustained activity and on three separate days (days 1, 2, and 5) to determine if a cumulative effect had developed. Most, but not all surgeries last less than 3 hours, thus, the vast majority of surgeries are finished within a time span that is covered by the European test method, namely 3 hours. There are currently, however, no efficacy data to prove that an assignment of 3 or 6 hours is the most valid time span for establishing a sustained effect. In order to gain more information about the dynamics of bacterial recolonization on the gloved hand, the killing kinetics of different alcohol-based hand rubs were measured at various time points (0, 1, 3, and 6 hours) after disinfection with applications lasting 3 or 1.5 minutes.

According to the European test method, only the hands are treated because the highest bacterial density is found on the hands and most gloves perforate on the fingers. Thus, this mode of application does not reflect the actual mode of application as done in clinical practice, where the surgical team treats not only hands but also forearms up to the elbows. Therefore, we investigated whether the mode of application has any influence on the efficacy of the European reference disinfection procedure (n-propanol 60%, 3 minutes), a fact that might require an adaptation of the European norm. Furthermore, we studied if an alcohol-based hand rub will meet the efficacy requirements of EN 12791 in only 1.5 minutes, when applied in this modified mode onto both hands and forearms.

8 STUDIES

8.1 Reproducibility and workability of the European test standard EN 12791 on the effectiveness of surgical hand antiseptics: A randomized, multicenter trial

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Objectives: To evaluate the reproducibility and workability of the in vivo test model of the European test standard EN 12791 regarding the effectiveness of surgical hand antiseptics and, as a secondary objective, to evaluate the power of the model to discriminate between the effectiveness of various formulations of surgical hand antiseptics.

Design: Prospective, randomized, multicenter study with a Latin-square design.

Setting: Five laboratories at 2 universities, 2 disinfectant manufactures, and 1 private testing institution.

Participants: Twenty healthy adults in each laboratory.

Intervention: Surgical hand antisepsis was performed by scrubbing with chlorhexidine gluconate 4% detergent (CHG) or by rubbing the hands with propan-2-ol (70% by volume; Iso 70) or ethanol 85% (E 85); rubbing the hands for 3 minutes with propan-1-ol (N 60) was used as the reference disinfection procedure. We deliberately chose to use these antiseptics at the given concentrations because they were intended to cover the range of typical antiseptics submitted for approval according to the EN 12791.

Methods: In once-weekly tests, the immediate effects of the four antiseptics were established according to the method laid down in EN 12791 by assessing the release of skin flora from the fingertips as viable bacteria counts per millilitre of sampling fluids before treatment and viable bacteria counts immediately after treatment, separately for both hands, such that after four weeks each volunteer had used every formulation once.

Results: The mean log reduction (RF) for the release of bacterial skin flora (the log RF was calculated as the log count before treatment minus the log count after treatment) and corresponding standard deviations for the four hand antisepsis formulations were as follows: for CHG, 1.1 ± 0.3 colony forming units (cfu) per millilitre of sampled fluid; for Iso 70; 1.7 ± 0.3 cfu/ml; for E 85, 2.1 ± 0.3 cfu/ml; and for N 60, 2.4 ± 0.4 cfu/ml. The differences between these values proved significant ($P < .001$) by analysis of variance and in Tukey's "honestly significantly different" HSD post hoc test. Although, with regard to their immediate antibacterial activity, the same ranking

of these antiseptics was found at all laboratories, the levels of efficacy were significantly different across laboratories ($P < .001$); no statistical difference was found between left and right hands ($P > .01$). Relating the log RF values of the other three formulations to those of the reference formulation (N 60) abolished differences between laboratories ($P = .16$); in addition, the interclass correlation coefficient decreased from 9.1% to 4.5%. With 20 volunteers, a minimum difference of 0.47 log between the mean log RFs of the reference formulation and an inferior test formulation will be detected as significant at an α of .05 (one-sided) and a $1-\beta$ value of .8.

Conclusion: The test method described in EN 12791 yielded the same conclusion on the effectiveness of the tested formulations in every laboratory and proved, therefore, reproducible and workable.

INTRODUCTION

Since bacteria have been identified as a cause of surgical site infection, considerable efforts have been undertaken to keep them away from the surgical wound by mean of disinfection and sterilization procedures.[1] This applies not only to instruments and the environment of the surgical site but also to the hands of the surgical team, the bacterial flora of which can constitute a potential reservoir of pathogens. Many procedures to reduce the release of skin flora into the wound have been proposed and evaluated. This has made it necessary to assess the antimicrobial efficacy of any surgical hand disinfection regimen by both in-vitro and in-vivo tests and has stimulated interest in such assessment. Reports on such tests are numerous in the historical microbiology and surgery literature.[2]

Among the various test methods, the most notable models were those of the American surgeon P. B. Price,[3] the tests used by the Birmingham group,[2] the original method of the German Society of Hygiene and Microbiology (Deutsche Gesellschaft für Hygiene und Mikrobiologie; DGHM),[4] the split-hands model of Michaud et al.,[5] the tests of the Austrian Society for Hygiene and Microbiology[6] and the DGHM,[7] and the test established by the American Society for Testing Materials,[8] which was adopted later by the US Food and Drug Administration.[9]

Recently, the European Committee for Standardization has proposed a standard for hand disinfection, EN 12791. In July 2005, the standard was published, and it is now being enforced. In principle, to pass this test, a formulation for surgical hand disinfection shall, immediately after its application, have an antimicrobial

efficacy not significantly (P = .1) inferior to that of a reference disinfection procedure tested in parallel on the hands of 18 to 20 volunteers, who – according to a randomized cross-over design – perform both procedures in two runs at an interval of one week. If more than one product is tested, a Latin-square design must be used. The reference disinfection method includes rubbing n-propanol (60% by volume) onto both hands up to the wrists in a standardized manner for 3 minutes. The disinfection procedure with the test formulation follows the instructions of the manufacturer but shall not take longer than 5 min. It may comprise either a rubbing or a washing procedure.

In addition to the obligatory requirement mentioned above, there exists the option to demonstrate a persistent activity on the gloved hand. In this case, the reduction in the concentration of bacteria must be significantly ($P \leq .01$) greater than that achieved with the reference method. The necessary information is obtained from the other, meanwhile gloved hand, 3 hours after disinfection.

The aim of the present trial was to study reproducibility and workability of the European standard EN 1279110 in five different laboratories by assessing the immediate antimicrobial effect of four different formulations for surgical hand antisepsis, separately on left and right hands. Furthermore, we sought to evaluate the power of the model to discriminate between differently active regimens.

METHODS

Laboratories

The five participating laboratories were at two universities, two disinfectant-manufacturing firms, and one private test organization.

Test formulations

To ensure that each laboratory worked with the same test preparations, each formulation was prepared as a single batch by Bode (Hamburg Germany), a disinfectant manufacturer that also was participant in the study and distributed the formulations to the other laboratories. The following four test formulations and their concentrations were deliberately chosen because they have different strong,

immediate effects: (1) a detergent containig chlorhexidine gluconate 4% wt/wt (CHG), (2) propan-2-ol (ie, isopropanol) 70% vol/vol (Iso 70), (3) ethanol 85% vol/vol (E 85), and (4) propan-1-ol (ie, n-propanol) 60% vol/vol (N 60).

Participants

Twenty volunteers were recruited by each laboratory. They had to meet the inclusion criteria described in EN 1279110: healthy status; age of at least 18 years; not pregnant; hands with healthy skin without cuts or abrasions, with short and clean fingernails; and agreement not to use any agent with antimicrobial activity (eg, a disinfectant or antiseptic soap) on their hands for one week before and during the test phase of 4 weeks. They all gave their informed consent.

Media, Neutralizing Agent, and Soap

Media were those described in EN 12791.10 As a neutralizing agent to be added to the sampling solutions and their diluents (but not to the counting plates), a mixture was prepared of 3.0% Tween 80 (Merck), 0.3% lecithin from egg yolk (Fluka), 0.1% L-histidine hydrochloride (Merck), and 3.0% saponin (Riedel-deHaen). This mixture was used for all test formulations, including the reference formulation (N 60), though only for the post-disinfection samples.

As a soap for the preparatory hand washing to remove most of the transient flora, a sterile potassium soft soap, also produced by Bode, was used at a concentration of 20% wt/vol.

Experimental design

In each test laboratory, volunteers were allotted by a computer-generated randomization list to one of four groups, each consisting of five subjects. The four antiseptic formulations were tested in parallel, each by another group, with an interval of one week between the tests. During four-week study, every group had “rotated through” the four formulations according to a Latin square design. Hence, at the end of the trial, each group (and therefore each subject) had tested every antiseptic formulation once.

Test method

The test method was that described in EN 12791,10 but without assessing values at hour 3 after disinfection. Instead, the immediate effect of the disinfection procedures was separately studied on each hand. In short, after each patient had washed both hands with unmedicated soap, the amount of skin flora released before disinfection was assessed by rubbing and kneading the fingertips for one minute at the bottom of a Petri dish, one for each hand. Subsequently, one of the disinfection procedures was performed; the rub or wash was applied in a standardized manner. Immediately after the end of the disinfection procedure, the fingertips were sampled by the same procedure as before disinfection. From these samples and their dilutions, quantitative surface cultures were prepared and incubated at 36 °C for 18 to 24 hours and again for another 24 hours.

Statistics

Viable bacterial colony-forming units (cfu) per millilitre of sampling fluid were determined as described in EN 1279110 for the assessment of the immediate effect of the hand antisepsis formulations. The log counts before treatment were subjected to analysis of variance (ANOVA) to test for significant differences between right and left hands and between the test weeks.

The intraindividual log reduction factor (RF) values (the log RF was calculated as the log count before treatment minus the log count after treatment) were also subjected to ANOVA, with “antiseptic formulation”, “laboratory”, “hand” (left/right), and the interactions as factors, and then subjected to Tukey’s “honestly significantly different” (HSD) post-hoc test. To standardize the results, we calculated intraindividual differences between the log RFs obtained with each of the 3 test disinfectants formulations minus those of the respective values of the reference formulation (N 60), and then we used ANOVA to analyse whether there were significant differences in the mean values for the factors “antiseptic formulation”, “laboratory“, and “hand” (left/right). Furthermore, the mean log RF values for left and right hands were analysed separately with respect to the variance component introduced by the different laboratories; $P \leq .01$ was considered statistically significant. Finally, interclass correlation coefficients were computed. All tests were two-sided.

RESULTS

There were no significant differences in the log pre-treatment viable bacteria counts per millilitre of sampling fluid between left and right hands or between the four test runs. The mean log RFs achieved after a 3-minute application of the four antiseptics are depicted in Figure 1, separately for each of the five laboratories and for left and right hands. As can be seen, the lowest grand mean (ie, the mean of the mean values for all laboratories and both hands) was obtained with the CHG-containing detergent (1.1 ± 0.3 cfu/ml) followed by Iso 70 (1.7 ± 0.3 cfu/ml) and E 85 (2.1 ± 0.3 cfu/ml). The highest mean log RF was seen with N 60 (2.4 ± 0.4 cfu/ml). All of these means proved highly significantly different ($P \leq .001$) from each other. Further analysis revealed significant differences ($P < .001$) also between the mean log RFs assessed by the different laboratories but not between those of left and right hands ($P > .01$).

When, however, in an ANOVA, the individual log RFs obtained with CHG, Iso 70 and E 85 were related to those achieved with the reference formulation by calculating the intraindividual differences, as required by EN 12791, the differences for the factor “antiseptics” proved still highly significant ($P < .001$), whereas differences between the “laboratories” ($P = .16$) or between left and right “hands” ($P = .40$) did not. The mean differences with respect to the reference formulation were 1.14 log for CHG, 0.73 log for Iso 70, and 0.31 log for E 85. The variance of these differences ranged between 0.3 and 2.2, with a mean variance of 1.1.

The interclass correlation coefficient for log RFs was 0.091. This means that the differences of mean log RFs between laboratories account for 9.1% of the total variance. When, however, the differences with respect to the reference formulation were analysed, the interclass correlation coefficient was reduced to 0.045. Hence, relating the antimicrobial efficacy of a test formulation to that of the reference formulation tested in parallel further reduces differences between laboratories, such that only 4.5% of the total variance of the difference values are due to differences between laboratories.

At an alpha-level of .05, the discrimination power of the test method to detect inferiority of an antisepsis formulation, compared with the reference formulation,

amounts to a difference of 0.47 log between the two mean log RFs, with a sample size of 20 and at a statistical power of 80% (Figure 2).

DISCUSSION

This is the first multicenter trial, to our knowledge, that has studied the reproducibility and workability of the European test standard EN 12791[10] and evaluated its discriminatory power. Our results on the capacity of four formulations tested, which are known to have different activity in reducing the release of skin flora from clean hands and were assessed in five laboratories, confirm results reported previously by other investigators[11-14] and by one of us and other collegues.[15-17]

With regard to their immediate antibacterial activity, the same ranking of these antiseptics was found at each of the five laboratories. The same ranking was also seen in a pilot study in which an analogous experiment has been performed by the Vienna group (M.R., personal communication), although in quintuple. This demonstrates the ability of the test model to reproduce, at different laboratories, the ranking of the various formulations according to their effectiveness, a result that reinforces our conclusion.

This outcome of the trial with the test model is based on the fact that the efficacy of a formulation was intraindividually compared with that of the reference procedure. This procedure has several advantages: (1) with the reference formulation, an individual performance standard is set that has to be met by a test preparation; (2) every test person acts as his/her own control, thereby fulfilling statistical requirements with smaller sample sizes than needed if separate test populations are used; and (3) the test results (ie, the log RF values) from different laboratories can be standardized and therefore made comparable. The third point is clearly demonstrated by the results of ANOVA, which, when performed on log RFs, indicated that the variable “laboratories” was a significant component of the variance ($P < .001$), whereas when ANOVA was performed on the differences in the log RFs (i.e., reference formulation value minus the test formulation value) this was no longer the case ($P = .16$). This means that, with a small sample size, such as that required by the test standard EN 12791,[10] non-standardized results of

different laboratories are not comparable or, alternatively, very big sample sizes are necessary to compensate the large variability in the reduction in the viable bacterial count between individual subjects and, consequently, between test populations.

However, although the test model is shown here to be well workable, with regard to the strategy of statistical evaluation proposed by the EN 12791 test standard,10 the critical question arises of whether it is not time to replace this proceeding by the more modern and correct model of equivalence testing, which nowadays is routinely applied in clinical studies.18 Because, with regard to its immediate effect, in every evaluation of a procedure for surgical hand antisepsis the intention is the prove its equivalence with (and not its superiority over) the reference procedure, this strategy seems to be more appropriate than the comparative study model presently included in the EN 12791 standard.10 An amendment of EN 12791 with respect to this matter will undoubtedly improve the quality of the test methods used.

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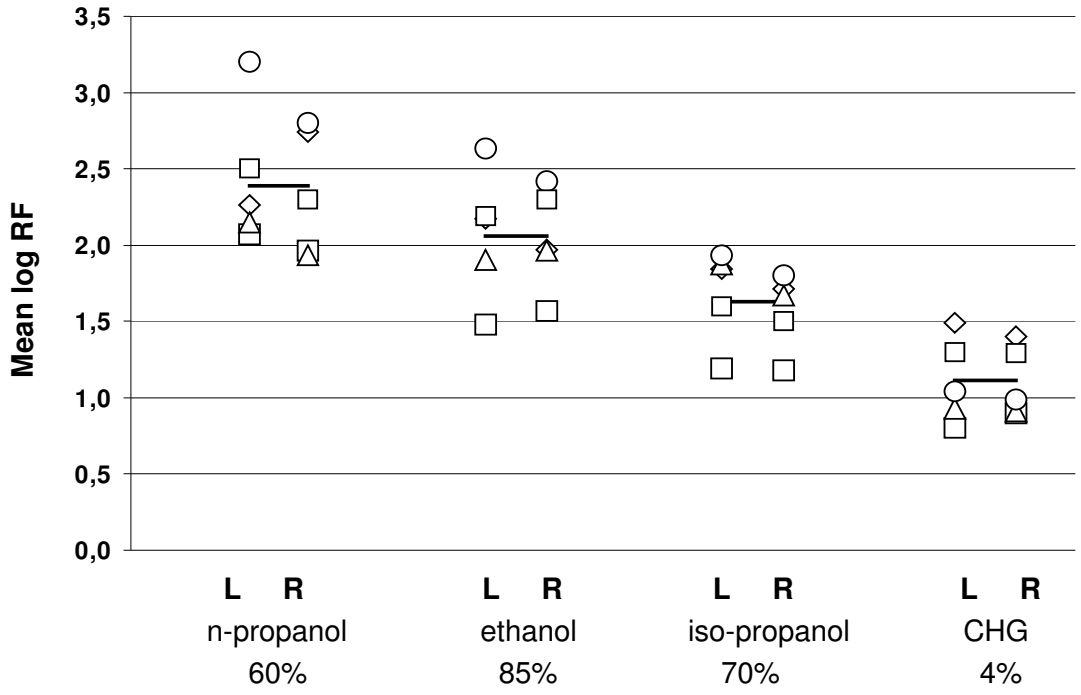

Figure 1. Immediate reduction in the rate of release of skin flora achieved by use of 4 surgical hand antisepsis formulations on left and right hands, assessed in 5 laboratories according to the EN 12791 test standard (each symbol represents 1 laboratory).

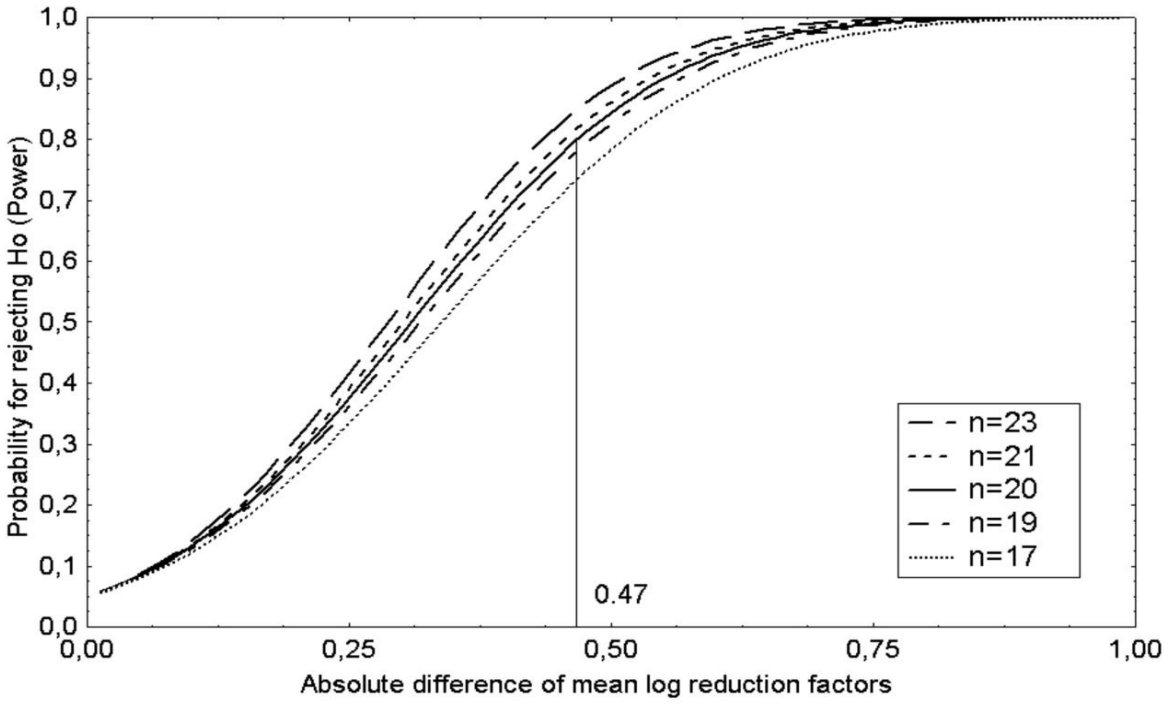

Figure 2. Power to discriminate between the mean log reduction factor achieved by the reference antisepsis formulation and that achieved by an inferior formulation tested, at an α (one-sided) of .05, and a 1-β of .8, with various sample sizes.

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8.2 Population kinetics of the skin flora on gloved hands following surgical hand disinfection with 3 propanol-based hand rubs: A prospective, randomized, double-blind trial

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***Objectives*:** To study the bacterial population kinetics on gloved hands following hand treatment with 3 optically indistinguishable alcohol-based surgical hand rubs, with and without supplements to delay bacterial regrowth, and a reference disinfection method, after 0, 1, 3, and 6 hours wearing gloves.

Design: Prospective, randomized, double-blind, balanced quasi-Greco-Latin square design.

***Setting*:** Microbiology laboratory of the Medical University Vienna, Austria.

Participants: Twenty four healthy adult volunteers without skin lesions.

Surgical hand rubs: The following hand rubs, all stained blue, were applied to the hands for 3 minutes: 1-propanol 60% vol/vol (A); 2-propanol 70% wt/wt plus chlorhexidine gluconate 0.5% wt/wt (B); 2-propanol 45% wt/wt plus 1-propanol 30% wt/wt plus mecetronium etilsulfate 0.2% wt/wt (C). As a reference formulation (R), 1-propanol 60% vol/vol, unstained, was applied for the same amount of time.

Method: In 8 once-weekly tests, 24 subjects randomly assigned to use the 4 hand rubs in groups of 6 persons each performed hand hygiene according to the method described in European Norm 12791. Every subject used one preparation at a time, the antimicrobial effect of which was evaluated at 2 sampling times. After week 8, each volunteer had tested every preparation at every pre-set sampling time. All preparations were tested in parallel.

Results: The mean pre-treatment counts of viable bacteria (in colony-forming units per milliliter) in fluid samples were not significantly different between week 1 and week 8, nor between the right and left hands (analysis of variance [ANOVA], $P > .1$). Immediately after applying the formulation (t_0), bactericidal effects of the blinded formulations A and C were equivalent to that of the reference formulation R, whereas the effect of B was questionable. The population kinetics of the flora on the hands proceeded from large and fast initial reductions of the skin flora by 2.7 log units (A), 3.1 log units (B), 3.3 log units (reference formulation), and 3.5 log units (C), to slow regrowth. However, even after 6 hours wearing gloves viable bacterial counts remained significantly ($P < .01$) below the baseline values (by 0.9 log [reference formulation], 1.1 log [A and B], and 1.5 log [C]). The slowest regrowth 1 and 3 hours after application (Δ from t_0, 0.1 log and 0.7 log respectively) was seen with

formulation C, and the slowest regrowth after 6 hours was seen with formulation B (Δ from t_0,1.6 log). These differences did, however, not reach statistical significance.

Conclusions: With respect to the rapid and dramatic antibacterial action of suitable alcohols at high concentrations and with appropriate neutralizers, the contribution of supplements to the delay of bacterial regrowth on gloved hands appears rather minor, if a product only exerts an immediate effect equivalent to that of the reference disinfection procedure described in EN 12791.

INTRODUCTION

Surgical hand disinfection has become an infection control standard worldwide.1 Its aim is the reduction of the release of resident and transient microbial flora on the hands into surgeon's gloves.2 Given an average glove perforation frequency of 18%, the immediate and persistent effects of surgical scrubs have become important quality markers for pre-operative hand preparation.3 It is, therefore, of utmost interest to identify a surgical hand rub that has maximum bactericidal efficacy on both transient and resident hand flora. Because of their wider antimicrobial spectrum,4-7 faster action,7,8 and better skin tolerability,7,9-13 alcohol-based hand rubs are often recommended1,9,14 and preferred to antimicrobial soaps containing chlorhexidine gluconate (CHG) or povidone iodine.

A definition for persistent effect with respect to hand hygiene has recently been suggested,[15] but the necessary duration of this effect remains a matter for discussion.[2,15] In the European Standard test model EN 12791, the persistent effect is measured after surgical gloves have been worn for 3 hours,[16] whereas, according to the tentative US Food and Drug Administration test method, it is assessed after 6 hours.[17] Although the duration of most surgical procedures does not exceed 3 hours,[18] there are currently no data to demonstrate whether 3 or 6 hours is most valid for measurement of persistent effect. The main aim of surgical hand disinfection is to keep the bacterial density on the hands below baseline for the duration of an operation.[15] In EN 12791, the antibacterial effect is assessed only twice (immediately after disinfection and 3 hours later). Hence, this method does not include the assessment of the effect at 6 hours. More measurements at various times would certainly be helpful for obtaining more information about the kinetics of bacterial regrowth on gloved hands.

The new Centers for Disease Control and Prevention guideline for hand hygiene recommends the use of either an antimicrobial soap or an alcohol-based hand rub supplemented with agents conferring a persistent effect for surgical hand antisepsis.[1] The agent most commonly thought to offer this persistent type of effect is CHG.[19] However, although often claimed, this effect has, to the best of our knowledge, never been adequately confirmed in a randomized, double-blind, controlled trial. Furthermore, recent data suggest that it might also be explained by the insufficiently neutralized bacteriostatic activity of CHG that is carried over with sampling fluids onto counting plates.[20] Indeed, it is well-known that effective neutralization of CHG is difficult to achieve.[7,21,22] Therefore, CHG might cause a more persistent bacteriostatic effect than would otherwise be seen with compounds that are more easily neutralized.

With the above in mind, we undertook a prospective, randomized, double-blind trial to study the immediate and persistent effects of surgical hand rubs applied for 3 minutes, with special consideration of the bacterial population kinetics on gloved hands. For this study, we measured the release of skin flora from the hands at hours 0, 1, 3 and 6 (t_o, t_1, t_3, t_6) after disinfection with 3 different alcohol-based formulations. One preparation consisted only of alcohol, the others contained either CHG or mecetronium etilsulfate (MES) as supplements, which are thought to confer a persistent effect. In addition, 1-propanol (60% vol/vol) was used for a reference formulation in the disinfection procedure described in EN 12791.

MATERIALS AND METHODS

Test preparations

The following preparations were used: (A) 1-propanol, 60% vol/vol; (B) 2-propanol (70% wt/wt) plus CHG (0.5% wt/wt) (Hibistat, Regent Medical); and (C) 2-propanol (45% wt/wt), 1-propanol (30% wt/wt) and mecetronium etilsulfate (MES) (0.2% wt/wt) (Sterillium, Bode). For blinding, the products were labeled as solutions A, B and C and stained blue so that they appeared the same. In contrast, the reference formulation, 1-propanol, was left unstained.

Volunteers

Twenty-four volunteers were included in the trial. Exclusion criteria were age of 18 years or less, pregnancy, and the presence of skin breaks such as cuts, abrasions, or other skin disorders on the hands. Nails were kept short and clean and the volunteers agreed not to ingest or use any antibacterial substance or antibacterial soap during the trial, starting one week prior to testing. All participants gave their written informed consent.

Nutrient media and non medicated soap

Media used were those described in EN 12791. Sampling and dilution solutions used tryptic soy broth (Caso broth, Merck). Counting plates used tryptic soy agar (TSA) (Caso agar, Merck).

The neutralizing agent contained in the sampling fluids and their diluents (but not in the counting plates) used for assessing post-treatment bacterial counts was a mixture of 3% Tween 80 (Merck), 3% saponin (Riedel-deHaen), 0,1% L-histidine hydrochloride (Merck) and L-cysteine (Merck). In preceding tests, this neutralizer was found to be the most active.

A dilution (20%) of non-medicated, sterile soft soap (APOCA) was used for pre-test hand cleansing.

Experimental design

A total of 8 test runs were performed, at intervals of 1 week. Twenty-four volunteers were randomly assigned by computer to use 1 of 4 hand rubs, including the reference formulation (4 groups of 6 persons), and sub-groups were randomized to use the four sampling times (0, 1, 3, and 6 hours), such that, using the split-hands model,[23] for each test run a volunteer used one preparation that was evaluated at two different sampling times. After week 8, each volunteer had tested every preparation at every pre-determined sampling time. All preparations were tested in parallel. To guarantee balance of the sequence of tests, the four groups, four preparations, and four sampling times were arranged in a quasi-Greco-Latin square under the constraint that the sampling times were not equal at both hands. The Greek letters were interpreted as applying to the left hand; the Latin letters applied to the right hand. In contrast to a true Greco-Latin square

design, however, each subject eventually tested every preparation at all four sampling times. This was accomplished by a pair of Greco-Latin squares that defined a sequence of 8 experimental conditions for each group, with two sampling times on each experimental day. Only the factor right/left hand, though balanced within each subject, was not completely factorial.

Test method

The test method was that described in EN 12791, using 24 rather than 20 volunteers and including two additional sampling times. Pre-treatment values were established by rubbing and kneading the finger-tips for 1 minute at the bottom of a Petri-dish – one for each hand – in 10 ml of tryptic soy broth without neutralizer. Subsequently, one of the surgical hand rubs was applied using the standardized rub procedure described in EN 12791: 3 ml of the hand rub was poured into the cupped hands, distributed over the skin surface up to the wrists, and vigorously rubbed into the skin. As many 3 ml-portions were applied as were necessary to keep the hands wet for a total of 3 minutes. After the end of disinfection, the fingertips were sampled according to the randomized sampling times assigned, either for immediate (t_o) post-treatment values or – after air-drying and gloving - for later post-treatment values (t_1, t_3 or t_6). At these testing times, the sampling fluids and their diluents contained neutralizer.

Quantitative surface cultures of sampling fluids and their dilutions were done on TSA. As described in EN 12791, counting plates were incubated at 36 °C; colonies were counted after 18 to 24 hours and again after incubation for a further 24 hours.

Statistics

For statistical evaluation, viable bacterial counts were processed as described in EN 12791. Bacterial reduction factors (RFs) were expressed as the difference between the log pre-test value and the log post-test value, per person and hand. RF is the ratio of pretest to posttest values (in cfu/ml) and is expressed by its decimal logarithmic value as log RF.

To ensure that intraindividual baseline conditions were comparable between left and right hands and throughout the 8 weeks of the trial, analysis of variance

(ANOVA) for repeated measurement was performed for the log pre-test values with the factors “hands” (left or right) and “weeks” (1 through 8). With α was set at 10%; the level of significance was set high in order not to overlook a potential difference that could affect the results of the tests for the different preparations. Mean log RFs derived from measurements at t_o for formulations A, B or C were compared with that of reference formulation R by means of equivalence testing applying exact 90% confidence limits according to Hodges and Lehmann[24] with a safety margin of 0.6 log. Mean RFs for formulations A, B or C derived from samples obtained at t_1, t_3 or t_6 were tested for significant differences to the corresponding means for reference formulation R, first by the non-parametric Friedmann ANOVA and subsequently in pair-wise post hoc comparisons by Wilcoxon-Wilcox-tests at P = .01 (one-sided). The log post-test values at t_6 were tested against the corresponding log pretest values by Wilcoxon matched-pairs tests.

RESULTS

As indicated by the results of ANOVA (not shown), there were neither significant differences between the mean log pre-test values for the left and right hands, nor between the log pre-test values assessed during weeks 1-8.

The results of equivalence testing (not shown) revealed that the immediate in-vivo bactericidal effects of a 3-minute application of preparations A and C were clearly equivalent to those of the reference formulation R, whereas the effect of formulation B was questionable.

As seen in the Table, at t_o the reference disinfection formulation R had reduced the bacterial release from the fingertips by 3.3 $\pm$ 1.0 log. Even 6 hours later, the mean bacterial density in the sampling fluids remained 0.9 log less than the baseline value (P < .01).

Similarly, blinded use of preparation A (n-propanol 60%) yielded comparable results at each sampling time. Preparation B (2-propanol 70% plus CHG 0.5%) was somewhat less effective, especially at t_o, though never significantly. Preparation C (2-propanol 45%, 1-propanol 30% plus MES 0.2%) proved to be the

most efficacious disinfectant at all sampling times; the differences in mean log RF values reached significance ($P \leq .01$) when compared with those of reference formulation R at t_3, and with those of formulation B at t_1 and t_3.

Six hours after disinfection, bacterial densities with all preparations were still significantly ($P < .01$) less than the baseline values.

Bacterial population kinetics on gloved hands can be described as follows: after a very large and fast reduction of bacterial release from the fingertips (mean RFs between 2.7 and 3.5 log), a slow regrowth of the skin flora is indicated by decreasing mean log RFs which, however, do not reach 0 (ie, the baseline value) even after 6 hours. The slowest regrowth at 1 and 3 hours after application was seen with formulation C (change from t_o = 0.1 log and 0.7 log, respectively). After 6 hours, the slowest regrowth was observed with formulation B (change from t_o = 1.6 log). Thus, with the preparations containing a nonvolatile active agent, a somewhat, though not significantly, slower regrowth of resident hand flora was seen, compared with the rubs A and R, which contained solely 1-propanol.

Table. Effects of 3 min surgical hand rubs with three alcohol-based preparations (A, B, C) and a reference disinfection (R) on the microbial population kinetics on gloved hands

		Mean (N = 24) log reduction factor ± SD by time after application			
	Active ingredient(s)	**Hour 0**	**Hour 1**	**Hour 3**	**Hour 6**
A	1-propanol 60%	3.1 ± 0.9	2.9 ± 1.2	2.2 ± 0.9	1.1[a] ± 1.0[b]
B	2-propanol 70% + CHG 0.5%	2.7 ± 1.2	2.3 ± 1.1[c]	1.7 ± 1.2[c]	1.1 ± 0.8[b]
C	1-propanol 30% + 2-propanol 45% + MES 0.2%	3.5 ± 1.2	3.4 ± 1.1	2.8 ± 1.3[d]	1.5[a] ± 1.0[b]
R	1-propanol 60%	3.3 ± 1.0	2.6 ± 1.3	1.8[a] ± 0.8	0.9[a] ± 0.7[b]

NOTE. CHG, chlorhexidine gluconate; MES, mecetronium etilsulfate.

[a] $P \leq .01$ vs corresponding hour 3 value.

[b] $P \leq .01$ vs baseline value.

[c] $P \leq .01$ vs formulation C.

[d] $P \leq .01$ vs reference formulation R.

DISCUSSION

For surgical hand disinfection, a fast and large immediate antimicrobial effect is desired to enable the surgical team to begin their work without delay and with clean hands. In addition, reduced bacterial release should last for the duration of an operation to help ensure that, in case of glove damage, a potential microbial inoculum in the wound is less than an infection-generating dose. Lacking epidemiological data on the magnitude of microbial reduction on the hands necessary to prevent infection - which is probably quite variable - it has arbitrarily been established in EN 12791 that the effect of a surgical scrub should at least be equivalent to a 3-minute application of hand rub containing 1-propanol 60% vol/vol.

To the best of our knowledge, this is the first prospective, randomized and double-blind trial to generate detailed information on the population kinetics of skin flora on gloved hands subsequent to surgical scrubbing with alcohol-based hand rubs with or without supplements believed to delay bacterial regrowth.

The immediate antimicrobial effect of the four preparations, as achieved with 3-minute applications, was fast and large. This is probably entirely because of the alcohols, the activity of which (up to certain limit) is positively associated with their concentration and with the type of alcohol; 1-propanol being the most effective.[19,25] Therefore, it is not surprising that preparation C (2-propanol 45%, 1-propanol 30% plus MES 0.2%), with its high total alcohol concentration of 75% (wt/wt) and 1-propanol constituting 30% of the mixture, causes the strongest reduction. This confirms the findings of other investigators who have reported that, among five surgical hand rubs, formulation C was the only one more efficacious than the reference rub.[26] In addition, it was reported recently that for hand rub C, an application time of only 1.5 minutes was still enough to exceed the efficacy of a 3-minute application of the reference disinfection formulation, which confirms the superior performance of formulation C.[27] In contrast, a 70% wt/wt 2-propanol-based rub is neither as effective as formulation C nor as 1-propanol preparations A and R at their concentration of 60% vol/vol (approximately 53% wt/wt).

Indeed, whereas the antimicrobial effects of both formulations A and C were found to be equivalent to those of reference formulation R, those of formulation B were questionable. Also, if tested in a paired fashion, the activity of formulation B was

significantly inferior to that of formulation C (Table). Hence, there exist and there have been reported significant differences in the antimicrobial efficacy of alcohol-based products.[26,27] Testing of new products is, therefore, necessary and justified, even if the active ingredients and their concentrations are known.

With regard to the effects of the hand rubs at 1, 3 and 6 hours after application, it is interesting to note that preparations B and C (containing CHG and MES, respectively) did cause some persistent effects, but in this study, they were not significant. A statement such as this must, however, be made with caution because the effects of both formulations were not compared with those of identical formulations lacking CHG or MES. This may limit the validity of conclusions about these preparations. Bacterial regrowth was slowest 1 and 3 hours after application with formulation C (containing MES), and after 6 hours with formulation B (containing CHG). Thus, it seems that MES and CHG do cause a certain persistent effect; however, it appears to be smaller than expected and reported earlier.[19] This may be explained by more effective neutralization of CHG and MES in this study. Indeed, it has been shown that in other studies, ineffective neutralization may have produced false positive efficacy data.[20]

It is noteworthy that even six hours after disinfection, the bacterial release from the fingertips was still significantly ($P < .01$) less than baseline levels, by 0.9 to 1.5 log. This signifies that bacterial regrowth on gloved hands obviously takes more than 6 hours to reach baseline levels, as long as the skin flora has been reduced substantially enough at t_o, as it was in the case with use of the alcohols alone in this study. Hence, from the results obtained with the two pure 1-propanol formulations (A and R) it may be concluded that a persistent effect may not be necessary for surgical hand rubs if they exert an immediate antimicrobial effect that is at least equivalent to that of the reference disinfection formulation described in EN 12791. Therefore, with respect to the fast and strong action of suitable alcohols at high concentrations, the contribution of supplements to delay regrowth of skin flora appears rather minor.

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8.3 Long term effect of a 1.5 minute surgical hand rub with a propanol-based hand rub on the resident hand flora

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INTRODUCTION

It has been described recently that the application of a specific propanol-based hand rub for surgical hand disinfection is equally effective according to EN 12791 in 1.5 minutes when compared with the traditionally recommended 3 minutes.[1,2] More data obtained with the test method of the US Food and Drug Administration further support the scientific basis of the 1.5 minute application for the hand rub.[3] In EN 12791, the efficacy of a procedure for surgical hand disinfection is measured immediately and 3 hours after application of the hand rub. Recent research with a 3 minute application time indicates that the bacterial density on hands remains at ~ 1 log below baseline even after 6 hours under the surgical glove.[4] But it is unknown if an equivalent effect can be achieved with a 1.5 minute application time with the same preparation.

MATERIAL AND METHODS

That is why we have measured the reduction of the resident hand bacteria for a 1.5 and 3 minute application with a hand rub based on 45% (w/w) iso-propanol, 30% (w/w) n-propanol and 0.2% (w/w) mecetronium etilsulfate (Sterillium, Bode Chemie GmbH & Co. KG, Hamburg, Germany) against the 3 minute reference treatment of EN 12791 (60% v/v n-propanol). Pre- and post-treatment values (0 and 1, 3, 6 hours after application respectively) were obtained by rubbing the finger tips in 10 ml sampling fluid. These and their diluents, which were used for

the assessment of post-treatment values, contained a mixture of 3% Tween 80 (Merck 8.22187), 3% saponin (Riedel-de-Haen, Seelze, 1.6109), 0.1% cysteine hydrochloride (Merck 1.02838) and 0.1% L-histidine hydrochloride (Merck 1.04351) for neutralization of possible residual bacteriostatic or bactericidal activity.[5]

In total, six test runs were performed at intervals of one week; 24 volunteers were randomly assigned by computer to perform one of the three disinfection procedures, including the reference procedure, (three groups of eight subjects). Using the "split-hands" model, the sampling times for the assessment of post-values were also randomly assigned to right and left hands such that, at a time, a subject used one disinfection procedure that was evaluated at two different sampling times. After the sixth week each volunteer had tested every procedure at every pre-determined sampling time. The three disinfection procedures were tested concurrently. To guarantee balance of the sequence of tests, the three groups, three procedures and four sampling times were arranged in a quasi-Greco-Latin square under the constraint that the sampling times for post-values were not equal for both hands. The Greco-letters were interpreted as applying to the left hand; the Latin ones as applying to the right hand. In contrast to a true Greco-Latin square design, however, each subject had eventually tested every procedure at all four sampling times.

RESULTS

As compared to the reference procedure R, the mean log reductions (RF) (± SD) assessed at t_o demonstrate the significantly ($P < 0.001$) higher antimicrobial efficacy of Sterillium™ (B) if used for the same application time of 180 s: 2.28 log (± 0.92) and 3.21 log (± 1.03) respectively. If used for 90 s, the efficacy of Sterillium™ is equivalent to that of the reference: 2.19 log (± 1.42) and 2.28 log (± 0.92) respectively ($P > 0.1$). The increase in the release of skin bacteria during the course of time from t_o over t_1 and t_3 to t_6 is approximately parallel (Figure 1). After 6 hours the microbial release is still significantly ($P < 0.05$) less than before disinfection by 0.74 log (A), 0.99 log (B) and 0.83 log (R).

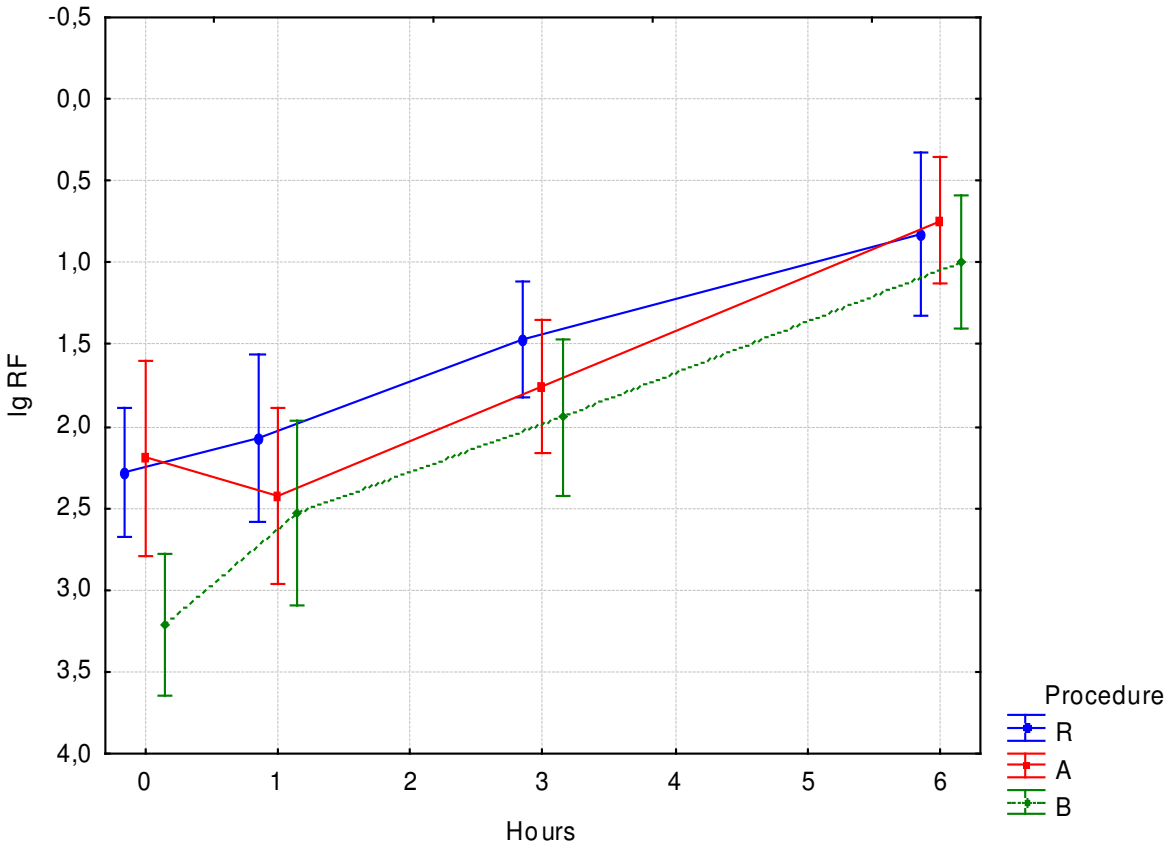

Figure 1: Mean log RFs and 95% confidence limits for Sterillium™ 90 s (A) and 180 s (B) as well as for the reference procedure (R) according to EN 12791 immediately after disinfection and population kinetics on the gloved hand during 1, 3 und 6 hours.

DISCUSSION

In accordance with the requirements of EN 12791, this antimicrobial effect of Sterillium™ would permit surgical hand disinfection for only 90 s, resulting in a microbial reduction that is equivalent to that of the reference disinfection even after 6 hours. However, from the results of this investigation it must not be concluded that a shortened duration of application of 90 s is justified for all products listed in the respective official registers of the Austrian or German Societies for Hygiene and Microbiology certifying sufficient antimicrobial efficacy of a product, or even, generally, for all alcohol-based surgical hand antiseptics. On the contrary, the suitability of each single product must be established by results of an evaluation according to EN 12791.

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8.4 Surgical hand disinfection using alcohol: the effects of alcohol type, mode and duration of application

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Summary

Due to their strong antimicrobial activity, rapid action, good dermal tolerance and ease of application, alcohol-based hand rubs are recommended for pre-operative preparation of the surgical team's hands.

Using the EN 12791 protocol, three commercial products containing either mixtures of propan-1-ol and propan-2-ol or ethanol at total alcohol concentrations (w/w) between 73% (propanols) and 78.2% (ethanol), as the main active agents, were tested with a shortened application of 1.5 min rather than the usual 3 min.

Preparation A containing 30% propan-1-ol and 45% propan-2-ol not only passed the test at this short application but even exceeded, though not significantly, the efficacy of the reference disinfection procedure in EN 12791 when applied for 3 min. Preparation B containing 45% propan-1-ol and 28% propan-2-ol fulfilled the required standard whereas the ethanol (78.2%)-based product C did not ($P < 0.1$).

This demonstrates that some, but not all, alcohol-based hand rubs pass the test even within 1.5 min, emphasising the importance of validation before a product is introduced into clinical practice.

In another series with both preparation A and 60% v/v propan-1-ol, it was demonstrated that the additional inclusion of the forearms into the disinfection procedure, not required by EN 12791 but normal practice in surgical hand disinfection, does not significantly interfere with the antimicrobial efficacy of either hand rub.

Therefore, the mode of test procedure in EN 12791 does not need specific adaptation for hand disinfection by surgical teams.

INTRODUCTION

Although the necessity of surgical hand disinfection has never been demonstrated in a randomised, controlled clinical trial it has been considered an important infection control issue since the work of Joseph Lister.[1] Its aim is the elimination of transient, and reduction of resident, microorganisms from hands of the surgical

team.[2] Nowadays, alcohol-based hand rubs are recommended for surgical hand disinfection and are preferred by some authors to preparations based on aqueous active agents such as povidone-iodine, chlorhexidine gluconate or quaternary ammonium compounds because of their better antimicrobial efficacy and good dermal tolerance.[3-5] Although in a randomised clinical trial the bacterial reduction of skin flora achievable with an alcohol-based hand rub was significantly superior to that assessed with two different hand scrubs, the clinical impact on the incidence of surgical site infections is not clear.[6]

In Europe, the in-vivo efficacy of products for surgical hand disinfection is tested according to the method described in the European Standard EN 12791 in comparison with that of a standardised reference disinfection procedure in which 60% v/v propan-1-ol is applied for 3 min.[7] The disinfection procedure with the test product must follow the instructions of the manufacturer, but the maximum length of disinfection allowed shall not take longer than 5 min.[7] However, some authors have warned against an application shorter than 2 min.[8,9]

A propanol-based hand rub has recently been described to meet the efficacy requirements of EN 12791 in only 1.5 min.[10,11] To confirm these findings, we compared the efficacy of this propanol-based hand rub at two different lengths of application, 1.5 min and 3 min (series 1).

Furthermore, as unfortunately no data are available for ethanol-based hand rubs at an application of 1.5 min we investigated the in-vivo efficacy of an ethanol-based hand rub in comparison with a propanol-based product when used only for this short length of time and in comparison with the reference disinfection with n-propanol (series 2).

Finally, as described in EN 12791, for the reference disinfection, the alcohol is rubbed only up to the wrists and therefore does not reflect the usual mode of application in clinical practice, where the surgical team not only treat their hands but also their forearms up to the elbows.[7] Whether this additional treatment of forearms negatively influences the antimicrobial effect of a surgical hand rub has never been studied before. Furthermore, it seemed necessary to investigate whether an alcohol-based hand rub that meets the efficacy requirements within only 1.5 min will also pass the test according to EN 12791 when the disinfection procedure includes the forearms. Therefore, in series 3, we investigated whether this extended mode of application has any impact on the efficacy of both the

European reference disinfection with 60% propan-1-ol and a hand rub with a commercial propanol-based product for both 1.5 and 3 min.

METHODS

Study products

The following alcohol-based hand rubs were employed:

- Product A, containing propan-1-ol, 30% w/w, propan-2-ol, 45% w/w and mecetronium ethylsulphate 0.2% (Sterillium, Bode Chemie GmbH & Co. KG, Hamburg, Germany).

- Product B, was based on propan-1-ol, 45% w/w, propan-2-ol, 28% w/w and lactic acid, 0.3% (Sensiva, Schülke & Mayr GmbH, Norderstedt, Germany).

- Product C, consisted of ethanol, 78.2% w/w and biphenyl-2-ol, 0.1% (Desderman N, Schülke & Mayr GmbH, Norderstedt, Germany).

- The reference alcohol, R, of EN 12791 was propan-1-ol, 60% v/v.

Neutraliser

Mixtures of 3.0% polysorbate 80, 3.0% saponin, 0.1% L-histidine hydrochloride and 0.1% cysteine hydrochloride or 3.0% polysorbate 80, 0.3% lecithin from egg yolk and 0.1% L-histidine hydrochloride were used for neutralisation of product A (series 1 and 3) or product B and C (series 2), respectively. In validation tests, these agents were shown to neutralise the antimicrobial effects of the disinfectants tested.

Test method and experimental design

The protocol followed the European Standard EN 12791.[7]

Twenty-one (series 1 and 2) or 20 (series 3) subjects at least 18 years old with healthy skin, without cuts or abrasions and with short and clean fingernails, were recruited. Their hands had not been treated with disinfectants or antimicrobial agents such as medicated soaps or shampoos for one week prior to, and during, the test period. Pregnant women and subjects under antibiotic therapy within the

last two weeks were excluded. Written informed consent was obtained from all volunteers.

A Latin-square design was used with three groups of seven randomly allotted subjects each (series 1 and 2) or with four groups of five subjects each (series 3), and as many experimental runs were performed as there were disinfection procedures including the reference disinfection. In every run, all disinfection procedures were employed in parallel. At the end of the whole series every subject had used each disinfection procedure once. The test runs were spaced by one week to allow re-growth of the normal skin flora.

Preparatory phase

To remove transient bacterial flora and any foreign material, a preparatory hand wash was carried out with non-medicated soap. Hands were rinsed under running tap water and dried with paper towels.

Assessment of pre-treatment values

Samples for bacterial counts were taken immediately after drying by rubbing and kneading the fingertips, including those of the thumbs, of both hands for 1 min at 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. An aliquot of 0.1 mL from each dilution was then spread on to tryptone soya agar (TSA) with sterile glass spreaders. Plates were incubated for a total of 48 h at 36 ± 1 °C before the colony-forming units (cfu) were assessed by an electronic colony counter (Fisher colony counter, Model 480, Artek Systems Corporation, Farmingdale, NY, USA).

Disinfection

Hand disinfection was performed according to the standardised reference hand-rub procedure of EN 12791. This consisted of applying and rubbing as many 3 mL portions of propan-1-ol 60% on to both hands up to the wrists as were necessary to keep the hands wet for a total of 3 min. In series 1, as many aliquots of 3 mL of product A were poured into the cupped hands and rubbed on to the skin as

necessary to keep hands wet for a total of 1.5 or 3 min. In series 2, hands were treated the same way with product B or C but only for 1.5 min. In series 3, product A was rubbed on to the hands and forearms up to the elbows for 1.5 or 3 min. As a modification of the standardised reference disinfection, R, propan-1-ol was also used with inclusion of the forearms.

Assessment of post-treatment values

To assess the immediate antimicrobial effect of the disinfection, subjects sampled their fingertips as for pre-treatment sampling but only from one randomly selected hand in 10 mL TSB plus neutraliser. The unsampled hand was covered with a sterile surgical glove for 3 h before samples were collected as described above to detect any sustained effect of the treatments.

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.

Statistics

Viable counts were processed as described in EN 12791. Pre- and post-treatment values were transformed into decimal logarithms and logarithmic intra-individual reduction factors (log RF) were calculated as the difference of log pre-treatment minus log post-treatment values separately for immediate and 3 h effects.

According to EN 12791, a hand-rub procedure (including product and mode of application) is considered sufficiently effective for surgical hand disinfection, if its immediate and 3 h effects are not significantly inferior to those obtained with the reference disinfection procedure. For products with a claim of sustained effect, the mean log RF obtained 3 h after disinfection must be significantly greater than that of the reference.

Mean log RF values of the products were tested for significant differences from the corresponding means of the reference by non-parametric tests such as Friedman analysis of variance and Wilcoxon-Wilcox tests for pair wise post-hoc comparisons. Significant differences between the means obtained with the products were supposed at levels of $P = 0.1$ (one-sided) for immediate, two-tailed $P = 0.05$ (two-sided) for 3 h and $P = 0.01$ (one-sided) for true sustained effects.

RESULTS

Comparison of 1.5 and 3 min (series 1)

The commercial product A was tested for two applications, 1.5 and 3 min (Table I).

The immediate effect of a 1.5 min application was somewhat smaller, though not significantly ($P > 0.1$), than the 3 min reference disinfection.

By contrast, when used for 3 min, product A was more effective than the reference, but also without statistical significance (two-tailed $P > 0.05$).

The mean log RFs obtained for the 3 h effects were larger, both for 1.5 and 3 min applications, though not significantly ($P > 0.01$), than the corresponding mean log RF for the reference disinfection.

No significant differences were found between the mean log RFs (immediate and 3 h effect) of product A at both applications of 1.5 min and 3 min ($P > 0.05$).

Table I. Immediate and 3 h effects of the alcohol-based hand rub A applied for 1.5 or 3 min as compared with those of the reference disinfection according to EN 12791

Product	Length of application	Mean (N = 21) log reduction ± SD	
	(min)	immediate	3 h
A	1.5	2.86 ± 1.03	1.66 ± 0.79
A	3	3.43 ± 1.28	2.16 ± 1.23
R	3	2.97 ± 0.97	1.60 ± 0.97

A: propan-1-ol, 30% w/w + propan-2-ol, 45% w/w + mecetronium etilsulphate, 0.2%.
R: reference acc. EN 12791; propan-1-ol, 60% v/v.

Comparison of propanol- and ethanol-based rubs (series 2)

The bacterial reductions obtained with products B and C, both having been tested for efficacy within 1.5 min, are shown in Table II.

The immediate effects were both smaller than that achieved with the 3 min reference disinfection. The difference from the ethanol-based product was statistically significant ($P < 0.1$).

The mean log RF values of both products for the 3 h effects were not significantly different from that of the reference.

In summary, only the short-time disinfection with product B fulfilled the efficacy requirements of EN 12791.

Table II. Immediate and 3 h effects after short (1.5 min) application of two alcohol-based hand rubs (B and C) as compared with those of the reference disinfection according to EN 12791

Product	Length of application	Mean (N = 21) log reduction ± SD	
	(min)	immediate	3 h
B	1.5	2.74 ± 0.95	1.96 ± 0.90
C	1.5	2.36 ± 1.13*	1.69 ± 0.92
R	3	2.92 ± 0.89	1.91 ± 1.11

B: propan-1-ol, 45% w/w + propan-2-ol, 28%, w/w + lactic acid, 0.3%.
C: ethanol, 78.2% w/w + biphenyl-2-ol, 0.1%.
R: reference acc. EN 12791; propan-1-ol, 60% v/v.
*Significantly ($P < 0.1$) inferior to R.

Comparison of two modes of application (series 3)

Product A was tested in two variations, 1.5 and 3 min, but in contrast to series 1 and EN 12791, forearms were included in the application procedure (Table III).

In addition to the reference procedure of EN 12791 (excluding forearms), 60% propan-1-ol was also used in that mode of application.

The immediate effects of the reference, R, and the modified reference procedure, MR, were very similar.

An application of A during 3 min resulted in a significantly (two-tailed $P < 0.05$) stronger mean bacterial reduction compared with the corresponding one assessed with the reference disinfection.

If applied for only 1.5 min, the difference from the reference was not significant (two-tailed $P > 0.05$) and 3 h after disinfection the mean log RF was similar to that of the reference.

However, a comparison with the effect of the 3 min application of A revealed a significantly inferior effect of the short application of 1.5 min.

Table III. Immediate and 3 h effects of the alcohol-based hand rub A after application for 1.5 and 3 min with inclusion of the forearms as compared with those of the reference disinfection according to EN 12791 and with inclusion of the forearms

Product	Length of application (min)	Forearm included	Mean (N = 20) log reduction ± SD	
			immediate	3 h
A	1.5	yes	3.12 ± 1.46	1.98 ± 0.88**
A	3	yes	3.88 ± 1.03*	2.64 ± 1.09
MR	3	yes	2.90 ± 0.78	2.31 ± 0.87
R	3	no	2.86 ± 0.87	2.11 ± 0.84

A: propan-1-ol, 30% w/w + propan-2-ol, 45% w/w + mecetronium etilsulphate, 0.2%.
MR: modified reference.
R: reference acc. EN 12791; propan-1-ol, 60% v/v.
*Significantly (two-tailed $P < 0.05$) superior to R.
**Significantly ($P < 0.05$) inferior to C (3 min).

DISCUSSION

We were able to show that the propanol-based hand rub A was effective within the short application time of 1.5 min and even exceeded the efficacy of the reference

alcohol when applied for 3 min, although this was not significant in this study. The same was true for the 3 h effects.

These findings confirm previously reported data.[8,10,12] Very recently, the effect of two consecutive applications of antiseptic A on the resident hand flora was studied. First it was applied for 1.5 min. Thereafter, hands were kept gloved for 3 h and then a second application was done for either 0.5 or 1.0 or 1.5 min. The reference alcohol was always applied for 3 min. It was shown that the reference procedure produced an equally low bacterial density as the treatments with the propanol-based hand rub A. This demonstrated that by the first 1.5 min of the antiseptic procedure with formulation A, the bacterial density of resident flora had already been brought to an 'irreducible minimum', which could not be further reduced by additional applications after 3 h.[13]

Saving time without decreasing the efficacy of the surgical hand rub is of clinical relevance, because, especially in emergency surgery, a preoperative hand preparation should be as short as possible and only as long as is necessary. It is important that hands are kept wet with the disinfectant during the whole recommended length of application.[14,15]

The necessary volumes vary according to the size of hands and their and the ambient temperature: for a 3 min surgical hand rub the applied volume may reach from 6 to 12 mL, or even more.[7] In our study, 9 mL were necessary, on average, for a 3 min application, but only 6 mL for 1.5 min. Therefore, shorter durations of application require less disinfectant.

The second test series demonstrates that a propanol-based hand rub consisting of a mixture of the two propanols at high concentration can, even within 1.5 min, be at least as effective as the 3 min reference disinfection of EN 12791, whereas an ethanol-based hand rub at an equally high alcohol concentration cannot.

The antimicrobial efficacy of alcohol-based hand rubs varies considerably depending on the alcohol-species used (propan-1-ol is better than propan-2-ol and this is better than ethanol), on its concentration and on the contact time.[16,17]

For instance, hand rubs based on ethanol with weight concentrations of 85% or even 80% were indeed found to meet the requirements of EN 12791 within 2-3 min, whereas a preparation based on the lower concentration of 61% (plus 1%

chlorhexidine gluconate) failed.[18-20] However, when tested for only 1 min, even the strongest alcohol, propan-1-ol (plus 0.5% chlorhexidine), did not meet the efficacy requirements at a weight concentration of 70%.[10] Also combinations with higher alcohol content such as products containing 78.2% of ethanol (plus 0.1% biphenyl-2-ol), or an alcohol mixture of 46% ethanol plus 27% propan-2-ol (plus 1% benzyl alcohol), failed when tested for only 1 min.[10] By contrast, product B, a preparation with a comparable total alcohol content of 73%, passed the test when used for a slightly longer application, namely 1.5 min. It is probably this additional length of application of 30 s that enabled the increased effect.

Our results show that not all alcohol-based hand rubs meet the requirement of EN 12791 within this short application; the efficacy of each single product must therefore be validated.

In tests according to EN 12791, the reference disinfection procedure does not include forearms probably because the highest bacterial density is found on the hands, especially under the fingernails. [21,22] This area is regarded as the most important to be disinfected. In contrast to the mode of the reference disinfection of EN 12791, in clinical practice the surgical team will treat hands and forearms, as recommended by most national guidelines.[1,2]

To the best of our knowledge, it has not been investigated before whether the additional treatment of the forearms up to the elbows has any deleterious effect on the efficacy of the standardised reference disinfection, a fact that may need an adaptation of the norm.

We were able to show that the reference disinfection does achieve the same bacterial reduction for both immediate and 3 h effects, whether or not the forearms are being included into the disinfection procedure for the same length of time. It is not surprising that, also with this modified application (forearms included), the immediate effect of hand rub A was still found significantly (two-tailed $P < 0.05$) stronger than that of the standardised reference disinfection, R (hands only) with n-propanol. This holds true even for the short treatment of only 1.5 min immediately after disinfection where product A was more efficacious, though not significantly. In addition, the 3 h effect was also not significantly less effective than the reference. The effect 3 h after a 1.5 min treatment with product A proved significantly ($P < 0.05$) inferior to that of a 3 min treatment.

Hence, in contrast to the duration, the mode of application of an alcohol-based hand rub, in terms of inclusion of the forearms, has no significant influence on the reduction of the resident hand flora. It makes no difference to the result of a surgical hand-rub product evaluation whether the disinfectant is rubbed on to the hands only or on to the hands and forearms. It is concluded that the mode of application as described in EN 12791 needs no alteration in this respect.

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8.5 Surgical hand rub: Influence of duration of application on the immediate and 3-hour effects of n-propanol and isopropanol

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Background: The recommended duration for surgical hand treatment has been changed from 10 over 5 to 3 minutes and even shorter.

Objectives: Our objective was to study the impact of the length of surgical hand antisepsis with n-propanol 60% (vol/vol) or isopropanol 70% (vol/vol) applied for 1, 3, or 5 minutes on the reduction of resident hand flora in the setting of the microbiologic laboratory for experimental and applied testing of disinfectants and antiseptics at the Medical University Vienna, Austria, using a Latin Square design.

Methods: Our methods were according to the Austrian Guidelines for Testing Products for Surgical Hand Antisepsis. The release of bacterial hand flora of 21 subjects is assessed before and immediately after disinfection from one hand and 3 hours later from the other, meanwhile gloved, hand. Mean reduction factors (RF) are calculated.

Results: The immediate mean $\log_{10}$ RFs with n-propanol or isopropanol were 1.05, 2.03, and 2.30 and 0.74, 1.48, and 2.12, respectively, when applied for 1, 3, or 5 minutes, respectively. After 3 hours, the respective mean log10 RFs were 0.45, 1.01, and 1.60 and 0.19, 0.79, and 1.03. Thus, with increasing length of application, a highly significant trend ($P < .001$) toward higher $\log_{10}$ reductions was demonstrated. At both sampling times, n-propanol was more effective than isopropanol at the corresponding treatments. Furthermore, a highly significant ($P < .001$) association was found between the individual volunteers and the effect of the antiseptics on their hands.

Conclusion: The efficacy of surgical antisepsis is significantly associated with the length of application.

INTRODUCTION

Although the necessity for preoperative surgical hand treatment has never been proved in a carefully controlled clinical study, the reduction of the resident hand flora of the surgical team is being regarded as an important measure in the

prevention of surgical site infections for reasons being outlined and extensively referenced in the forthcoming WHO Guidelines on Hand Hygiene in Health Care.[1]

Alcohol-based hand rubs have been recommended for a long time for surgical hand antisepsis in some European countries[2] and are preferred nowadays by some authors even in North America.[3,4]

However, mode and duration of the treatment have significantly changed over time. For many years, the surgical teams have scrubbed their hands preoperatively for 10 minutes or even longer.[2,5] As a result of experimental evidence,[6,7] the recommended duration for surgical hand treatment has later been shortened to 5 minutes, a protocol still used by some surgical teams.[8] Since the publication of the European standard EN 12791[9] for testing the antimicrobial efficacy of products for surgical antisepsis in 2005, according to which a reference procedure is performed for 3 minutes concomitantly with the product under test, most teams in Europe prefer this length of application. However, the standard allows products to prove their effectiveness within up to 5 minutes.

The present study was planned and performed during the time when consultations at the European Committee for Standardization about the above-mentioned norm were still in progress. The details of the reference hand rub procedure - the results of which should serve as a yardstick for efficacy - were a special matter of discussion.

To add scientifically based arguments, we engaged in studying the killing kinetics against the resident hand flora of n-propanol and isopropanol, both of which were considered by the involved working group to be suitable candidates for a standardized reference disinfection procedure.

For this undertaking, we applied the then existing method for Testing the Efficacy of Products for Surgical Antisepsis of the Austrian Society for Hygiene, Microbiology, and Preventive Medicine (ÖGHMP).[10] Actually, together with the nearly identical test of the German Society for Hygiene and Microbiology (DGHM),[11] this method later formed the basis for the present European test standard EN 12791.

The precise aim of the present study was to establish the impact of the length of application of a surgical hand rub on the reduction of resident hand flora and to compare the immediate and after 3 hours effects of surgical hand antisepsis with

the 2 alcohols. To achieve results with high discriminatory power, great emphasis was laid on a homogenous experimental design and working with a test population of trained and always the same subjects.

MATERIALS AND METHODS

Test procedures

Study test procedures consisted of rubbing hands in a standardized manner for 1, 3, or 5 minutes with either n-propanol 60% vol/vol or isopropanol 70% vol/vol (both pro analysi grade; Merck, Darmstadt, Germany).

Volunteers

Study volunteers were 21 healthy employees of the Medical University Vienna, Austria. Participants were at least 18 years of age, without any visible lesions on their hands and with short and clean finger nails.

All of the individuals were trained in performing a standardized surgical hand rub and were available during the entire test period of 7 weeks. They agreed not to use any substance with antibacterial activity or antibacterial soap during the test period, starting 1 week prior to testing. Not included were pregnant women and subjects who were under antibiotic treatment.

Written consent was obtained from all volunteers.

Nutrient media and non medicated soap

Nutrient media consisted of Tryptic soy broth (TSB; Caso broth, 1.05459; Merck, Darmstadt, Germany) for sampling and dilution fluids;

Tryptic soy agar (TSA; Caso agar, 1.05458; Merck) for counting plates;

and non medicated soft soap (APOCA, Vienna, Austria) for the required preparatory handwash.

Experimental design

A Latin Square design was used with 7 balanced groups, each consisting of 3 subjects. For a total of 7 test runs, each one spaced from the other by an interval of 1 week, 21 subjects were randomly allotted by computer to 1 of the 7 groups (including 1 with an additional commercial product, which is here of no interest). The 7 procedures were tested in parallel, each by another group; and, after 7 weeks, each volunteer had tested every procedure once. Right and left hands were also randomized into 2 groups for the assessment of either immediate or 3-hour effects but without regard of dominant or non dominant hand.

Test method

The method was that described in the respective guideline of ÖGHMP for testing products for surgical hand antisepsis, a precursor of today's European Standard EN 12791.[9,10]

After a preparatory hand wash with 5 mL of non medicated soap for 2 minutes and drying with paper towels, pre-treatment values were established.

For this purpose, the fingertips including those of the thumbs of both hands were rubbed and kneaded for 1 minute at the bottom of a Petri dish - 1 for each hand - each containing 10 mL of TSB. Subsequently, one of the disinfection procedures was performed according to a standardized application procedure as described in the guideline. Portions of 3 mL of the respective alcohol were poured into the cupped dry hands and vigorously rubbed onto both hands up to the wrists, and as many portions were applied as was necessary to keep hands wet for the preset length of surgical hand rub of 1, 3, or 5 minutes.

Immediately after disinfection, the fingertips of 1 (randomly selected) hand were sampled as described above for the pre-treatment values. The other, meanwhile gloved, hand was sampled after 3 hours for the 3-hour post treatment values.

From all sampling fluids and their dilutions, quantitative surface cultures were prepared by spreading volumes of 0.1 mL on TSA with sterile glass spatulas. Counting plates were incubated at 36°C ± 1°C for a total of 48 hours, and colony forming units (cfu) were counted by means of an electronic colony counter (Fisher colony counter, Model 480; Artek Systems Corporation, Farmingdale, NY).

Allowing for the dilutions of the sampling fluids, the number of cfu per millilitre was calculated.

Data analysis

For statistical evaluation, all pre- and post-disinfection values were expressed as decadic logarithms (log_{10}), and the logarithmic reduction factor (log_{10} RF) was calculated as the intraindividual difference between the log_{10} pre-treatment minus the log_{10} post treatment value of the same hand.

Mean log_{10} RFs were tested for significant differences by the nonparametric Friedman analysis of variance and subsequently by post hoc comparisons applying 1-sided Wilcoxon-Wilcox tests at P = .01.

Differences between the 2 alcohol species were tested for each of the 3 durations of application by 2-sided Wilcoxon-Wilcox tests (two-tailed P = .05). Trends were analysed by Page tests (P = .01) as a measure for monotonous association between log10 reduction and length of application.

Finally, the strength of association between the results from the various procedures with the individual subjects was determined by Kendall's coefficient of concordance with an agreed level of significance of P = .01.

RESULTS

Table 1 shows the mean log_{10} bacterial reductions immediately after disinfection with n-propanol 60% vol/vol and isopropanol 70% vol/vol and from there it is evident that both alcohols cause increasing bacterial reductions the longer they are applied to the hands. This trend is highly significant (P < .001).

Between 1 and 3 minutes as well as 1 and 5 minutes, the increase of the mean log_{10} reductions is significant (P < .01) with both alcohols but not between 3 and 5 minutes.

The above-mentioned results are exactly mirrored 3 hours after disinfection, although with lower reductions indicating regrowth of the bacterial flora on the gloved hands (Table 2).

N-propanol 60% was always more effective than isopropanol 70% for both the immediate and after 3 hours effects, but the differences between the means were not statistically significant (two-tailed P > .01).

Furthermore, a highly significant (P < .001) association (not shown) between the individual subjects and the bacterial reductions from their hands was found. This means that, on average, the antiseptic effect on the hands of some subjects was - above chance - always smaller than on those of others regardless of the length of application and of the alcohol tested.

Table 1. Influence of the length of surgical hand antisepsis with n-propanol 60% (vol/vol) and isopropanol 70% (vol/vol) on the *immediate* effects

Alcohol and conc.	**Mean (N = 21) log Reduction (Standard Deviation)**						**Trend**
	Length of Application (min)						
% (vol/vol)	1	1 vs 3	3	3 vs 5	5	5 vs 1	1 ⇨ 3 ⇨ 5
n-propanol 60	1.05 (0.65)	*	2.03 (0.99)	n.s.	2.30 (1.30)	*	< 0.001
isopropanol 70	0.74 (0.65)	*	1.48 (0.88)	n.s.	2.12 (0.96)	*	< 0.001

n.s., Not significant (P > .01).
*Significant (P ≤ .01).

Table 2. Influence of the length of surgical hand antisepsis with n-propanol 60% (vol/vol) and isopropanol 70% (vol/vol) on the *3-hours* effects

Alcohol and conc.	Mean (N = 21) log Reduction (Standard Deviation)						Trend
	Length of Application (min)						
% (vol/vol)	1	1 vs 3	3	3 vs 5	5	5 vs 1	1 ⇨ 3 ⇨ 5
n-propanol 60	0.45 (0.49)	*	1.01 (0.52)	n.s.	1.60 (0.99)	*	< 0.001
isopropanol 70	0.19 (0.59)	*	0.79 (0.56)	n.s.	1.03 (0.67)	*	< 0.001

n.s., Not significant (P > .01).
*Significant (P ≤ .01).

DISCUSSION

In our study, we were able to show that the antimicrobial efficacy of a surgical hand treatment with n-propanol 60% or isopropanol 70% is significantly associated with the duration of treatment (P < .001).

However, controversial results are reported in the literature. Hingst et al compared 3- and 5-minute applications of different products and, depending on the formula of the active agent, no significant differences were found in bacterial reduction between the 2 lengths of treatment: a 3-minute rub with 60% n-propanol proved as effective as a 5-minute application.[12] In another study, 2 procedures, a 2-minute application of 60% n-propanol and a 3-minute application of 70% isopropanol (plus 0.5% chlorhexidine gluconate), were as effective as a 5-minute application of 60% n-propanol.[13] Babb et al found a 2-minute application of 70% isopropanol only marginally more effective than a 30-second application.[14] Another investigator studied the effect of the duration of scrubbing with Betadine liquid soap (povidone iodine) for 3, 5, and 10 minutes. With the 10-minute scrub, the reductions were somewhat greater than with the 3- and 5-minute scrubs but not significantly, and no correlation was observed between the duration of scrubbing and bacterial

reduction.[15] A comparison of different lengths of surgical hand treatment with 0.5% chlorhexidine gluconate in 70% ethanol and 0.5% chlorhexidine diacetate in water demonstrated reductions of hand flora, which, after 30 seconds, were nearly as strong as after a 2-minute treatment.[16]

The reasons for the discrepant results may be different; however, it seems plausible that differences in the effect of the duration of the surgical hand rub can only be detected in an experimental test design with high discriminatory power and a test population with always the same subjects as used in this investigation. With n-propanol 60%, immediate effects of mean bacterial reductions ranging between 2.3 and 2.9 $\log_{10}$ have been described for both 3- and 5-minute treatments.[17] The after 3 hours effects on gloved hands have been found to range between 1.6 and 1.8 $\log_{10}$.[17] With isopropanol, the efficacy is somewhat smaller. In the present study, comparable reductions were observed. Even stronger effects with bacterial reductions of 3.2 to 3.9 $\log_{10}$ can be achieved by increasing the concentrations of these alcohols of up to 75% wt/wt.[18,19] It is conceivable, therefore, that new analogues formulations that are now commercially available in Europe will pass the test according to EN 12791 even in 90 seconds.[18-20]

Further analysis of our results reveals that the numerical increase in the antimicrobial activity of the 2 tested alcohols was larger between 1 and 3 minutes than it was between the 3- and 5-minute applications. This can be explained by the initially fast and strong bactericidal activity of alcohols fading asymptotically as time increases. Therefore, surgical hand rub protocols with alcohol-based formulations taking longer than 5 minutes are not worth the effort.

At comparable lengths of application, n-propanol 60% proved always more effective than isopropanol 70%, although, in this study, not significantly, which confirms our earlier reported results.[21] However, these findings are not surprising because this ranking of the alcohol types according to their bactericidal activity is well-known.[17]

With the 2 alcohols, our subjects produced highly concordant ($P < .001$) bacterial reductions at the 3 different durations of application. This means that, during the 7 experimental runs, the individual reductions found for every subject were - within the population of test persons - more often at the same or at a similar rank than to be expected by chance. This finding underlines the importance of testing a product for surgical hand antisepsis in parallel with a reference treatment and, hereby, to

compare the effects of both procedures intraindividually as an internal control. Indeed, this is allowed for by the test model in the actual European Standard EN 12791.[9] According to this norm, the efficacy of a treatment with a product under test shall not be inferior to that of the reference immediately after the end of application. Furthermore, if there is an additional claim for sustained activity on the gloved hand, the product must prove its significantly ($P \leq .01$) stronger efficacy as compared with the reference 3 hours after disinfection.

In conclusion, the results of this investigation demonstrate that, at least in a well-controlled in vivo laboratory model, a highly significant association of the duration of surgical hand antisepsis and its antimicrobial efficacy can be detected. Therefore, hand treatments of too short a duration will cause only poor reductions of the hand flora, whereas treatments lasting too long are an uneconomic use of time because they are not followed by a linear increase in effect and rather undesired adverse effects on dermal tolerance may occur. However, because the efficacy of an alcohol-based hand rub depends not only on the length of application but also on the alcohol type (n-propanol being more efficacious than isopropanol) and its concentration (the higher the more effective), every product must be tested before it is introduced into clinical practice by a standardized procedure as is for instance described in the European test standard EN 12791.

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9 SUMMARY

Surgical hand disinfection is an accepted infection control standard worldwide with the aim of eliminating the transient and reducing the resident flora on the hands of the surgical team to a minimum in case of noticed or unnoticed surgical glove perforation (Boyce & Pittet, 2002).

Before a product for surgical hand disinfection is introduced into clinical practice, the evaluation of its antimicrobial efficacy is of great importance. In Europe, the *in vivo* efficacy of such products is usually tested according to the European norm EN 12791 (EN 12791, 2005) on the hands of volunteers in comparison to the efficacy of a standardized reference disinfection procedure with n-propanol 60% (vol/vol) for 3 minutes in a cross-over-design. Hereby, the bacterial reduction is measured immediately after disinfection ("immediate effect") on one hand and, for the assessment of a possible sustained effect, after 3 hours ("3 hours effect") on the other, mean-while gloved hand. According the requirements of EN 12791, a test product shall not be significantly less efficacious than the reference procedure both immediately and 3 hours after application.

Our aim was to evaluate the reproducibility and workability of this *in vivo* test method in a prospective, randomized, multicenter trial in five laboratories by assessing the immediate antimicrobial effects of four different surgical hand preparations (chlorhexidine gluconate 4% (wt/wt) soap; ethanol 85% (vol/vol); isopropanol 70% (vol/vol) and n-propanol 60% (vol/vol) as reference), separately on left and right hands.

Although, with regard to their immediate antibacterial activity, the same ranking of the four preparations (chlorhexidine gluconate < isopropanol < ethanol < n-propanol) was found at all five laboratories, the levels of efficacy were significantly different across laboratories ($P < 0.001$). No statistical difference, however, was found between left and right hands ($P > 0.01$). In our study, the test method described in EN 12791 led to the same conclusion on the efficacy of the tested preparations in each of the five laboratories and proved, therefore, reproducible and workable.

By surgical hand disinfection the release of resident bacterial flora from the hands is intended to be reduced for the duration of an operation to an amount that, in case of perforation of the surgical glove, the bacterial inoculum introduced into the surgical wound remains below an infection generating dose. Since more than 100 years hands of the surgical team have therefore been treated pre-operatively with antiseptics. But surgical hand antisepsis underwent changes in the past as mode and duration of the surgical hand treatment have been significantly altered. Today, a fast and strong immediate antimicrobial effect of surgical hand antisepsis is desired to enable the surgical team to begin their work without delay and with "safe" hands. Lacking suitable antiseptics, this was not possible in former times although strong efforts were undertaken with changing success to shorten the procedures that often took 15 minutes or more. After all, the recommended durations decreased from >10 over 5 to 3 minutes (Tucci et al., 1977; Hingst et al., 1992), and even a 1.5 minute treatment with a very efficacious propanol-based product (Kampf et al., 2005) has been shown to be suitable for surgical hand disinfection according to EN 12791.

To confirm these findings, we studied the killing kinetics of the European reference alcohol, n-propanol 60% (vol/vol), and isopropanol 70% (vol/vol) against the resident skin flora on the hands within 1, 3 and 5 minutes. Furthermore, we investigated the *in vivo* efficacies of various alcohol-based hand rubs (containing either mixtures of n-propanol and isopropanol or ethanol alone at total alcohol concentrations (wt/wt) between 73% (propanols) and 78.2% (ethanol), when applied for 1.5 minutes in comparison to the reference disinfection of EN 12791.

It was shown that there exists a highly significant trend ($P < 0.001$) toward higher log reductions with increasing duration of application. Thus, the efficacy of surgical antiseptics is significantly associated with the duration of their application. N-propanol was more effective than isopropanol at the corresponding treatments at both sampling times (0 hours and 3 hours). Furthermore, a highly significant ($P < 0.001$) association was found between the individual volunteers and the effect of the surgical hand rubs on their hands. Our results further demonstrated that some, mainly propanol-containing, but not all, mainly ethanol-containing, alcohol-based hand rubs pass the test of EN 12791 even within 1.5 minutes, emphasising the importance of validation of each single product, for instance according to EN 12791, before it is introduced into clinical practice.

According to the method of EN 12791, samplings are performed only at two different time points after application (0 and 3 hours). By contrast, in the U.S. test method (TFM, 1994), hands are sampled at times 0, 3 and 6 hours to account for immediate effects and sustained activity of various length. Although most surgeries last less than 3 hours and the vast majority of surgeries are performed in a span of time covered by EN 12791, there are currently no efficacy data to justify that 3 or 6 hours is the most valid duration of a sustained effect for surgical hand antisepsis.

With the above in mind, we studied the immediate and sustained effects, with special consideration of the bacterial population kinetics on gloved hands, of various alcohol-based surgical hand rubs applied for 3 minutes and 1.5 minutes and measured the release of skin flora from the hands at various time points (0, 1, 3, and 6 hours) after disinfection. One hand rub consisted only of alcohol, the others contained either chlorhexidine gluconate or mecetronium etilsulfate as supplements, which are thought to confer a persistent effect.

The population kinetics of all tested hand rubs proceeded from strong and fast initial reductions of the skin flora to a slow re-growth which, even with alcohol alone, remained significantly ($P < 0.01$) below baseline even after 6 hours under the gloves. Consequently, the contribution of supplements to delay bacterial re-growth on gloved hands appears rather minor, if only a product exerts an immediate effect as strong as that of the reference disinfection procedure described in EN 12791. This is, because re-growth of the resident skin flora is slow even without the presence of bacteriostatic supplements. As the majority of all surgical interventions takes less than 3 hours the sampling time for sustained effects as required by EN 12791 can be regarded as clinically relevant.

In tests according to EN 12791, only hands are treated with the disinfectant, probably because the highest bacterial density is found on the hands, especially under the fingernails, and most gloves are perforated on the fingers. By contrast, in clinical practice the surgical team will treat both hands AND forearms as recommended of various national guidelines (Boyce & Pittet, 2002; Labadie et al., 2002).

Therefore, we investigated whether this extended mode of application has any impact on the efficacy of the European reference disinfection procedure (n-

propanol 60%, 3 minutes), a fact that might require an adaptation of the European norm. Furthermore, we studied if an alcohol-based hand rub will meet the efficacy requirements of EN 12791 in only 1.5 minutes, when applied in that modified mode onto both, hands and forearms.

It was demonstrated that the mode of application of an alcohol-based hand rub, in terms of the inclusion of forearms, has no significant influence on the reduction of the resident hand flora. It makes no difference to the result of a surgical hand disinfectant evaluation whether the disinfectant is rubbed only on to the hands or onto hands AND forearms. Hence, the mode of application as described in EN 12791 needs no alteration in this respect.

10 ZUSAMMENFASSUNG

Die chirurgische Händedesinfektion ist zu einem weltweiten Standard der Infektionsprävention geworden, mit dem Ziel die transiente Handflora zu eliminieren und die Abgabe residenter Handflora auf ein Minimum zu reduzieren, um das Risiko postoperativer Wundinfektionen nach bemerkter oder unbemerkter Perforation der OP-Handschuhe zu minimieren (Boyce & Pittet, 2002).

Bevor ein Produkt für die chirurgische Handantisepsis in die klinische Praxis übernommen wird, ist eine Evaluierung seiner antibakteriellen Wirkung von großer Bedeutung. In Europa wird jene gemäß der Europäischen Norm EN 12791 an den Händen von Probanden im Vergleich zu einem Referenzverfahren (n-Propanol 60% (v/v) für 3 Minuten) im Überkreuzdesign untersucht (EN 12791, 2005). Dabei werden an einer Hand ein „Sofort-Effekt“ (Sofortwirkung) unmittelbar nach der Desinfektion und ein „3-Stunden-Effekt“ (Langzeitwirkung) an der anderen, zwischenzeitlich behandschuhten Hand gemessen. Ein chirurgisches Händedesinfektionsmittel darf weder in der Sofort- noch in der Langzeitwirkung signifikant schlechter sein als das Referenzverfahren, um den Wirksamkeits-anforderungen der EN 12791 zu entsprechen.

Ein Ziel der vorliegenden Arbeit war die Evaluierung der Machbarkeit und der Reproduzierbarkeit der Europäischen Testmethode EN 12791. Dazu wurde in einer prospektiven, randomisierten Multicenterstudie in fünf verschiedenen Laboratorien die unmittelbare antibakterielle Wirkung („Sofort-Effekt“) von vier verschiedenen Präparaten (Chlorhexidingluconat-Seife 4% (w/w); Ethanol 85% (v/v); Isopropanol 70% (v/v) und n-Propanol 60% (v/v) als Referenzalkohol) separat an jeweils beiden Händen untersucht.

In allen fünf Laboratorien wurde für die unmittelbare Wirkung die gleiche Rangordnung der vier Präparate nachgewiesen (Chlorhexidingluconat < Isopropanol < Ethanol < n-Propanol), wobei die erhobene Wirksamkeit in den Laboratorien allerdings signifikant unterschiedlich ($P < 0.001$) war. Zwischen linken und rechten Händen wurden dagegen keine statistischen Unterschiede festgestellt ($P > 0.01$). In unseren Versuchen führte die in EN 12791 beschriebene

Testmethode in allen fünf Laboratorien zu den gleichen Schlussfolgerungen und erwies sich daher als machbar und reproduzierbar.

Durch die chirurgische Händedesinfektion soll die Abgabe hauteigener Bakterien von den Händen für die Dauer einer Operation so weit verringert werden, dass im Falle einer OP-Handschuhperforation das in die Wunde eingebrachte mikrobielle Inokulum unter der infektionserzeugenden Dosis bleibt. Seit mehr als 100 Jahren werden daher die Hände des Chirurgen prä-oerativ mit Antiseptika behandelt, wobei sich Art und Dauer der Behandlung in dieser Zeit sehr verändert haben. Heute wird eine starke und rasche antibakterielle Sofortwirkung gefordert, sodass das OP-Team seine Arbeit mit „sicheren Händen“ und ohne Zeitverzögerung beginnen kann. Das war mangels geeigneter Antiseptika früher allerdings nicht möglich und eine Verkürzung der damals oft 15 Minuten übersteigenden Prozeduren wurde mit wechselndem Erfolg angestrebt. Immerhin gelang es mit der Zeit, die empfohlene Behandlungsdauer von >10 auf 5 und weiter auf 3 Minuten zu verkürzen (Tucci et al., 1977; Hingst et al., 1992) und 2005 wurde für ein Präparat auf Propanolbasis erstmalig eine Anwendung von 1.5 Minuten als gleich wirksam wie die 3-minütige Referenzdesinfektion der heute in Europa eingeführten Prüfnorm EN 12791 beschrieben (Kampf et al. 2005).

Um diese Befunde zu bekräftigen, haben wir die Wirkkinetik von n-Propanol 60% (v/v), dem Referenzalkohol der EN 12791, und von Isopropanol 70% (v/v) auf die residente Handflora bei Behandlungsdauern von 1, 3 und 5 Minuten geprüft. Außerdem untersuchten wir die *in vivo* Wirksamkeiten verschiedener alkoholischer Händedesinfektionsmittel (Mischungen von n- und Isopropanol oder Ethanol mit Alkoholkonzentrationen (w/w) zwischen 73% (Propanole) und 78% (Ethanol) bei einer verkürzten Desinfektionsdauer von 1.5 Minuten im Vergleich zum Referenzdesinfektionsverfahren der Europäischen Norm.

Es konnte gezeigt werden, dass die antibakterielle Wirksamkeit der beiden Alkohole, n-Propanol 60% (v/v) und Isopropanol 70% (v/v), signifikant mit der Dauer der Händedesinfektion korreliert ($P < 0.001$). N-Propanol war in allen drei Behandlungen sowohl in seinem Sofort-, als auch in seinem 3-Stunden-Effekt wirksamer als Isopropanol. Außerdem wurde eine hochsignifikante Assoziation zwischen den einzelnen Versuchspersonen und den Effekten der Alkohole auf deren Händen nachgewiesen ($P < 0.001$). Unsere Ergebnisse zeigten zudem,

dass die Anforderungen von EN 12791 bei einer verkürzten Behandlungsdauer der Hände von nur 1.5 Minuten nicht von allen alkoholischen Händedesinfektionsmitteln, nämlich hauptsächlich solchen auf Ethanolbasis, erfüllt werden. Dieser Umstand unterstreicht wiederum die Wichtigkeit des Wirksamkeitsnachweises jedes einzelnen Präparates für die chirurgische Händedesinfektion, beispielsweise gemäß der in der Europäischen Norm beschriebenen Testmethode.

Während in Europa entsprechend der Europäischen Norm EN 12791, die Wirksamkeit eines chirurgischen Händedesinfektionsmittels sofort (0 Stunden) und 3 Stunden nach der Behandlung beurteilt wird, werden in Nordamerika gemäß der U.S. Testmethode (TFM, 1994) Untersuchungen zu drei verschiedenen Zeitpunkten (0, 3 und 6 Stunden) nach der Desinfektion durchgeführt. Leider gibt es zur Zeit keine Wirksamkeitsdaten, die 3 oder 6 Stunden als die „richtige“ Zeitspanne für einen Langzeiteffekt eines chirurgischen Händedesinfektionsmittels rechtfertigen würden.

Daher untersuchten wir die Populationskinetik der residenten Handflora an der behandschuhten Hand unter Einwirkung verschiedener alkoholischer Händedesinfektionsmittel zu verschiedenen Zeitpunkten (0, 1, 3 und 6 Stunden) nach Applikationen von 3 und 1.5 Minuten. Ein Desinfektionsmittel enthielt lediglich Alkohol, die anderen zusätzlich Supplemente von Chlorhexidingluconat oder Mecetroniumetilsulfat, von denen angenommen wird, dass sie eine Langzeitwirkung unter dem OP-Handschuh bewirken.

Es konnte gezeigt werden, dass die getesteten Händedesinfektionsmittel sowohl in 3, als auch in 1.5 Minuten die Anforderungen von EN 12791 erfüllten und einen derart starken Soforteffekt ausübten, dass selbst bei Verwendung von Alkohol allein die hauteigene Flora der desinfizierten Hand auch nach 6 Stunden immer noch signifikant unter dem Ausgangsniveau lag. Eine Supplementierung von alkoholischen Händeantiseptika mit Wirkstoffen, die eine antibakterielle Langzeitwirkung unter dem Handschuh vermitteln, erscheint daher unnötig, wenn nur die unmittelbare Sofortwirkung des Desinfektionsmittels stark genug ist. Die Hautflora wird nämlich auch ohne bakteriostatisch wirkende Supplemente nur langsam restituiert. Da die Mehrzahl der gängigen chirurgischen Eingriffe nicht

länger als 3 Stunden dauert, kann daher die in EN 12791 angegebene Zeit als klinisch relevant betrachtet werden.

Entsprechend dem in der Europäischen Norm EN 12791 beschriebenen Standarddesinfektionsverfahren, werden lediglich die Hände mit dem Desinfektionsmittel behandelt und zwar deshalb, weil an den Händen, und dort speziell unter den Fingernägeln, die Bakteriendichte am höchsten ist und OP-Handschuhe überwiegend an den Fingerspitzen perforiert werden. Diese Anwendung entspricht allerdings nicht den Gegebenheiten in der klinischen Praxis, wo entsprechend den Empfehlungen diverser nationaler Leitlinien die Hände UND die Unterarme desinfiziert werden (Boyce & Pittet, 2002; Labadie et al., 2002).

Daher untersuchten wir, ob diese erweiterte Behandlungsart einen Einfluss auf die antibakterielle Wirksamkeit des Europäischen Referenzverfahrens (n-Propanol 60% (v/v), 3 Minuten) hat, ein Umstand, der eine entsprechende Adaptierung der Europäischen Testmethode verlangen würde. Außerdem sollte untersucht werden, ob eine verkürzte, 1.5-minütige Applikation eines alkoholischen Händedesinfektionsmittels die Wirksamkeitsanforderung der Norm auch dann noch erfüllt, wenn es in der eben beschriebenen, modifizierten Art unter Einschluss der Unterarme angewendet wird.

Es konnte gezeigt werden, dass im Gegensatz zur Behandlungsdauer, die Art der Applikation eines Händedesinfektionsmittels, konkret die zusätzliche Desinfektion der Unterarme, keinen signifikanten Einfluss auf die Reduktion der residenten Händeflora hat. Für das Ergebnis der Wirksamkeitsprüfung eines chirurgischen Händedesinfektionsmittels ist es daher unerheblich, ob das Desinfektionsmittel auf den Händen allein oder auf den Händen UND Unterarmen geprüft wird. Eine, der klinischen Praxis entsprechende Adaptierung des in der Europäischen Testmethode EN 12791 beschriebenen Standarddesinfektionsverfahrens ist demnach nicht notwendig.

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12 APPENDIX

12.1 Curriculum vitae

24. 09. 1971	BORN IN VIENNA/ AUSTRIA AS MIRANDA LHOTSKY
1981 - 1985	BUNDESGYMNASIUM XVIII, VIENNA/ AUSTRIA
1985 - 1989	BUNDESREALGYMNASIUM XVIII, VIENNA/ AUSTRIA
1989 - 1997	STUDY OF FOOD SCIENCE AND BIOTECHNOLOGY, UNIVERSITY OF NATURAL RESOURCES AND APPLIED LIFE SCIENCES, VIENNA
20. 10. 1997	GRADUATION AS DIPLOMINGENIEUR (EQUIV. MASTERS)
SINCE 1997	ASSISTANT AT THE CLINICAL INSTITUTE OF HYGIENE AND MEDICAL MICROBIOLOGY, MEDICAL UNIVERSITY, VIENNA

- COOPTED BOARD MEMBER OF THE AUSTRIAN SOCIETY OF HYGIENE, MICROBIOLOGY AND PREVENTIVE MEDICINE (ÖGHMP)
- MEMBER OF THE EXPERTISE COMMITTEE OF THE AUSTRIAN SOCIETY OF HYGIENE, MICROBIOLOGY AND PREVENTIVE MEDICINE
- ADVISORY BOARD MEMBER OF THE AUSTRIAN SOCIETY FOR STERILE SUPPLY (ÖGSV)
- MEMBER OF DIFFERENT COMMITTEES IN THE AUSTRIAN STANDARDS INSTITUTE

ATTENDANCE AT NATIONAL AND INTERNATIONAL SYMPOSIA ON HYGIENE:

26. 05. - 28. 05. 1998	26. ANNUAL CONFERENCE OF THE ÖGHMP, MILLSTATT/ AUSTRIA*
01. 06. - 02. 06. 1999	15. DOSCH-SYMPOSIUM, ST. WOLFGANG/ AUSTRIA*
23. 05. - 25. 05. 2000	27. ANNUAL CONFERENCE OF THE ÖGHMP, GOLDEGG/ AUSTRIA*
02. 03. 2001	BAXTER SYMPOSIUM, DIALYSEFACHTAGUNG, ULM/ GERMANY*
29. 05. - 30. 05. 2001	16. DOSCH-SYMPOSIUM, ST. WOLFGANG/ AUSTRIA*
02. 06. - 04. 06. 2003	17. DOSCH-SYMPOSIUM, KITZBÜHEL/ AUSTRIA*
30. 05. - 01. 06. 2005	18. DOSCH-SYMPOSIUM , GOLDEGG/ AUSTRIA*
23. 06. 2005	KISS-BASEL, "SURGICAL HAND DISINFECTION", BASEL/ SWITZERLAND
27. 10. 2005	KISS-BASEL, „NOSOKOMIALE INFEKTIONEN DURCH LUFT UND WASSER", BASEL/ SWITZERLAND*
03. 05. - 05. 05. 2007	WFHSS, BADEN/ AUSTRIA
26. 05. - 29. 05. 2008	31. ANNUAL CONFERENCE OF THE ÖGHMP, BAD ISCHL/ AUSTRIA*

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