

An extended model for transfer of micro-organisms via the hands: differences between organisms and the effect of alcohol disinfection

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SUMMARY

A model for contact transfer of micro-organisms by hand has been extended to include representatives of bacterial species responsible for a majority of hospital-acquired infections. The ability of the organisms to transfer from contaminated fabrics to hands and from hands to sterile fabrics was measured, as was their ability to survive on the skin of the hands. There were differences between the species. *Staphylococcus saprophyticus* transferred well to the hand but not as well from hand to fabric as the other species; it survived well on skin. *Pseudomonas aeruginosa*, *Klebsiella aerogenes* and *Serratia marcescens* transferred moderately well overall and also survived on the skin. These results were in contrast to those obtained with a strain of *Escherichia coli* and one of *Streptococcus pyogenes*.

The contact transfer model was used to investigate the use of small volumes of alcohol in preventing transfer via the hands. An alcohol handrub of either 0.3 ml 80% ethanol or 0.3 ml 70% isopropanol gave reductions in transfer slightly less than that of a soap and water wash. Raising the volume, and consequently the contact time, to 0.5 ml 70% isopropanol gave a 14 000-fold reduction in transfer, statistically indistinguishable from that of a thorough soap and water wash (9800-fold reduction).

INTRODUCTION

Transfer of pathogenic micro-organisms by human-to-human contact is thought to be an important route of spread in hospitals. Bacterial contamination, without apparent soiling, can be acquired during routine contact with patients and transferred to other patients. Research into the mechanism of such transfer is difficult because of the sporadic nature of hand contamination. To overcome this difficulty workers have devised laboratory-based models which involve artificial application of 'tracer' organisms. A contact transfer model (Marples & Towers, 1979) proved useful in evaluation of the relative effectiveness of some barriers to contact transfer, for instance the use of gloves or different methods of disinfection of the hands. This paper describes extension of the model to include Gram-negative bacteria and shows quantitative differences between some species in levels of transfer and survival.

The model was also used to test rapid methods of hand disinfection for the removal of transient organisms since these pose a unique problem in skin disinfection. Transient contamination is usually located only on the surface of the skin, and such contamination is readily accessible to disinfection as well as being available for efficient transfer by touch. The main methods currently used for hand decontamination are washing with either soap or disinfectant/detergent mixtures, both of which are inconvenient for repeated use and are frequently not done (Albert & Condie, 1981) or not done efficiently (Taylor, 1978*b*).

MATERIALS AND METHODS

Basic transfer model

The model is based on that of Marples & Towers (1979). The complete model involves transfer of bacteria from contaminated 'donor' fabrics to the hands and from the contaminated hands to sterile 'recipient' fabrics, the proportion transferred at each stage being obtained directly by sampling, or indirectly by inference from counts obtained at other stages (Fig. 1). Donor and recipient fabrics were 10 × 20 cm rectangles of 'J-cloth' (Johnson & Johnson Ltd) wrapped around 300 ml capacity round bottles (Winchester type, United Glass Company Ltd).

One millilitre of an overnight nutrient broth culture of the test organism was pipetted evenly on to each of two fabric-covered donor bottles. The culture was allowed to soak in and the volunteers then grasped the bottles firmly, one in either hand, for 10 s, so contaminating in a reproducible manner the palmar areas normally used for touch and manipulation. This was termed the first stage of transfer. One or both hands were then used to complete the transfer (second stage) by grasping sterile recipient fabric-covered bottles.

Fabrics were aseptically removed from the recipient bottles and placed in wide-necked jars containing 100 ml of 0.075 M phosphate buffer, pH 7.9, with 0.1 % (v/v) Triton X-100. The jars were swirled briefly and left to stand for between one and five minutes to elute the bacteria. This was sufficient to allow maximal recovery, and the species studied were not affected by this treatment. The elution fluid was diluted in quarter-strength Ringer's solution and 0.1 ml volumes inoculated on solid media. When the counts were expected to be low, portions of the liquid were filtered (Oxoid, 0.45 μm pore size) and the filters placed on solid media.

Survival on hands

Both hands were contaminated by grasping the donor fabrics. One hand was sampled immediately and the other after 300 s. Comparison between the two samples was used to determine whether significant loss of viable organisms had occurred. Hands were sampled using the detergent-scrub method of Williamson & Kligman (1965), as modified by Stringer & Marples (1976).

Interruption of contamination transfer

Both hands were contaminated by grasping a pair of artificially inoculated fabric-covered bottles. One hand was then used to grasp a sterile recipient fabric-covered bottle; the number of organisms transferred to this recipient fabric gave the control level of transfer. Both hands were then decontaminated and the

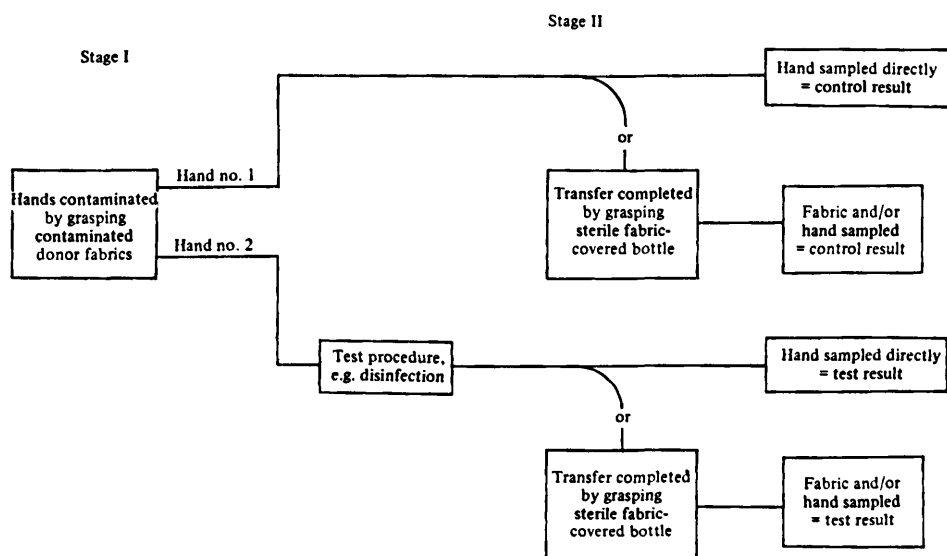


Fig. 1. Simplified scheme of the stages involved in the transfer model. The 'reduction factor' is obtained by calculation of the ratio of the control result to the test result (both as counts per cm^2) and conversion of this ratio to its logarithm (base 10).

other hand then grasped another sterile recipient fabric-covered bottle. Transfer by the second hand gave the level of reduction in transfer brought about by the decontamination procedure compared to the control. Fabrics were sampled as described above. The decontamination procedures were as follows.

(a) *Soap and water wash.* Hands were wetted under warm, running water and soaped with plain bar soap for 5 s, lathered for 5 s and then rinsed until free of lather and dried using two paper towels. The process took 50–60 s in total and was meant to represent a conscientious hand-wash.

(b) *Alcohol disinfection.* Alcohol, 0.3 ml of either 80% ethanol or 70% isopropanol, or 0.5 ml of 70% isopropanol, was pipetted into the cupped palm of one hand. Both hands were then rubbed together in a standard procedure to spread the alcohol over the palmar surfaces of the hands. The procedure took 10–15 s for the alcohol to evaporate.

Fate of organisms on decontaminated hands

A high-efficiency sampling method was used to check that the lowering of transfer after alcohol treatment was a result of disinfection rather than physical attachment to the skin. The hands of four volunteers were contaminated with *Staphylococcus saprophyticus* or *Klebsiella aerogenes* by grasping donor fabrics, disinfected with 0.5 ml of 70% isopropanol, and then the bacteria remaining on them were eluted by the scrub method of Williamson & Kligman (1965) and counted.

Bacteria

(1) *Staphylococcus saprophyticus*. CRF 31 – a pigmented novobiocin-resistant strain, enabling it to be differentiated from normal skin flora.

(2) *Pseudomonas aeruginosa*. GNSU 3245 – isolated from a urinary tract infection. Serotype 0:5d.

(3) *Escherichia coli*. NCTC 9001 – serotype 0:1.

(4) *Klebsiella aerogenes* – from clinical material. Serotype 0:21.

(5) *Serratia marcescens*. GNSU 1216 – from a urinary tract infection. Serotype 0:14.

(6) *Streptococcus pyogenes*. Group A/T6 – lacking M antigen and chosen for its reduced virulence.

All organisms except the streptococcus were grown on nutrient agar (Oxoid No. 2 C.M. 67 + 1.3 % New Zealand agar) containing 4 μ g novobiocin per ml to select for the test organism and depress the growth of the normal skin flora. Incubation was carried out aerobically at 30 °C, the plates examined and the colonies counted at 18 h and 48 h. The streptococcus required blood agar (Oxoid nutrient broth No. 2, 2.5 %; Oxoid L21 yeast extract, 0.3 %; New Zealand agar 1.3 % and Gibco defibrinated horse blood, 0.5 %) and was differentiated from normal skin flora by colonial morphology and the zones of haemolysis which it produced.

Experimental design and calculation of results

Not less than four volunteers took part in each experiment. The numbers of male and female volunteers were kept as equal as possible and right and left hands were used alternatively as controls where replicate experiments were performed.

All counts have been given and proportions calculated in terms of numbers of organisms transferred or surviving per cm² of contact area, assumed to total some 60 cm² (Marples & Towers, 1979). Because survival and transfer appear to follow logarithmic laws, mean values have been calculated as the geometric means.

Calculation of the statistical significance of the differences between the mean reductions brought about by soap and water, ethanol and isopropanol was by two-way analysis of variance. Comparison between 0.3 ml and 0.5 ml of isopropanol was performed as paired experiments and tested by the paired *t* test.

RESULTS

Table 1 shows the results for Stage I transfer – from donor fabrics to the hands; the test organisms may be placed in three groups. *Staph. saprophyticus* transferred at the highest rate to the hand even though it was present in lower numbers on the donor fabric. The four Gram-negative species all transferred to the hand at a lower rate, within a range of 0.3 %–0.5 % of the counts per cm² on the donors. *Strep. pyogenes* transferred poorly, and although the absolute numbers transferred could be increased by increasing the density of the inoculum on the donor fabric, the efficiency of transfer, measured as the proportion transferred, was decreased.

Table 2 shows the loss of viability between about 30 s and 5 min after releasing the donor fabrics. The bacteria survived well, except for *E. coli*, which decreased in numbers by over 99 %. There is a possibility that the *serratia* had also lost viability to a greater extent than the other Gram-negative species, but the variability within the data was such as to make statistical comparison uncertain.

Experiments were carried out to determine the efficiency of the second stage of

Table 1. Stage I. Transfer of bacteria from the donor fabric to the hands

Organism	Counts per cm ² of contact area*		Transfer per cm ² (%)
	on donor fabric	transferred to hands	
<i>Staph. saprophyticus</i>	4.5 × 10 ⁶	7.5 × 10 ³	1.67
<i>E. coli</i>	9.5 × 10 ⁶	4.5 × 10 ⁴	0.47
<i>Ps. aeruginosa</i>	9.0 × 10 ⁶	3.2 × 10 ⁴	0.36
<i>K. aerogenes</i>	1.8 × 10 ⁶	5.3 × 10 ³	0.29
<i>Strep. pyogenes</i>	1.7 × 10 ⁶	3.6 × 10 ²	0.021
	3.9 × 10 ⁷ †	2.7 × 10 ³	0.007
<i>Ser. marcescens</i>	4.4 × 10 ⁶	2.0 × 10 ⁴	0.46

* Geometric mean values.

† A 2 ml sample of a tenfold concentrated suspension was used.

Table 2. Recovery of viable bacteria from the skin of the hands immediately after, and five minutes after, contamination

Organism	Geometric mean counts per cm ² on hands		Percentage remaining after 300 s
	Within 30 s	At 300 s	
<i>Staph. saprophyticus</i>	7.5 × 10 ³	3.2 × 10 ³	42
<i>E. coli</i>	4.5 × 10 ⁴	2.6 × 10 ²	0.59
<i>Ps. aeruginosa</i>	3.2 × 10 ⁴	1.6 × 10 ⁴	49
<i>K. aerogenes</i>	5.3 × 10 ³	2.3 × 10 ³	44
<i>Strep. pyogenes</i>	3.6 × 10 ²	2.2 × 10 ²	62
	2.7 × 10 ³ *	5.4 × 10 ²	23
<i>Ser. marcescens</i>	2.0 × 10 ⁴	3.3 × 10 ³	17

* A 2 ml sample of a tenfold concentrated suspension was used.

transfer indirectly by measuring the numbers of organisms present on the recipient fabrics and on the hands at the conclusion of the second stage of transfer. The results are shown in Table 3. The first two columns show the recovery at the end of a complete transfer, hands and recipient fabrics being sampled. The recoveries are calculated as a percentage of the count per cm² available on the donor fabrics. If it is assumed that no loss of viability occurs then the total (shown in column three) should equal the percentage transferred initially to the hands (stage 1, Table 1). In general the recovery is about 30–40% lower than for the simpler experiment but *E. coli* shows a highly reduced level of recovery, possibly as a result of the division of the inoculum between the two surfaces resulting in a more rapid drying and killing.

The efficiency of the second transfer, from hands to recipient fabrics, was estimated from these figures and is shown in the final column of Table 3. It appears that the second transfer occurs at a much higher efficiency, presumably as a result of the superficial location of the acquired contamination. *Staph. saprophyticus* transferred less readily than the Gram-negative organisms.

Table 4 shows the results of a large number of complete transfers, from fabric to fabric, using this model.

Table 3. Stage II. Transfer of bacteria from hand to recipient fabrics and overall recovery of organisms

(Recovery at end of Stage II as a percentage of the counts per cm² of donor fabric.)

Organism	Transferred to recipient fabric	Remaining on hands	Estimated total transferred from donors	Calculated transfer from hands to recipient fabrics
	(a)	(b)	(a + b)	$\left(\frac{a}{a+b}\right)$ (%)
<i>Staph. saprophyticus</i>	0.21	1.04	1.25	17
<i>E. coli</i>	0.014	0.0016	0.016	(88)*
<i>Ps. aeruginosa</i>	0.16	0.050	0.21	76
<i>K. aerogenes</i>	0.17	0.027	0.20	86

* Value suspect due to loss of viability of bacteria on hands.

Table 4. Overall transfer from donor to recipient fabrics

Organism	Geometric mean counts per cm ²		Percentage transfer from fabric to fabric
	Donors	Recipients	
<i>Staph. saprophyticus</i>	4.7×10^5	1.8×10^3	0.37
<i>E. coli</i>	6.6×10^6	1.2×10^3	0.018
<i>Ps. aeruginosa</i>	8.3×10^6	1.2×10^4	0.14
<i>K. aerogenes</i>	2.6×10^6	2.0×10^3	0.076
<i>Strep. pyogenes</i>	5.0×10^6	4.9×10^2	0.010
	$4.6 \times 10^{7*}$	2.8×10^3	0.006
<i>Ser. marcescens</i>	2.8×10^6	3.1×10^3	0.11

* A 2 ml sample of a tenfold concentrated suspension was used.

Alcohol disinfection

Log reductions ranged from 2.53 for the effect of 0.3 ml of 80% ethanol on *Staph. saprophyticus* – representing a 340-fold reduction in transfer – to 4.55 for the effect of soap and water on *Ser. marcescens* – representing a 35000-fold reduction (Table 5a). Variability within each species and treatment group was such that differences between treatments could rarely be shown to be significant. Only when the results for all species shown in Table 5a were combined did the difference between the treatment means become statistically significant, showing that soap and water was more effective in reducing transfer than was 0.3 ml of either alcohol. If the results with the different disinfection methods were combined (Column 4, Table 5a), analysis showed that the organisms differed in their susceptibility. Inspection of the data indicated that this was due to differences between *Staph. saprophyticus* and the other organisms, transfer of the staphylococcus being less affected by the disinfection procedures. It was frequently not possible to recover viable *Strep. pyogenes* A/T6 from recipient fabrics after hand disinfection had been carried out, making calculation of mean log disinfection levels difficult. The results have been calculated as a log (x+1) transformation. The geometric mean log reductions were: for soap and water, 4.58 logs reduction; for 0.3 ml 80% ethanol, 4.90 logs reduction; and for 0.3 ml 70% isopropanol, 4.63 log reduction, i.e. all 38000-fold reductions, or greater.

Table 5a. Effect of different hand treatments on transfer of bacteria from donor to recipient fabrics

Organism	Mean* logarithmic reductions in transfer			Mean value for all treatments on same species
	Soap and water	0.3 ml 80% ethanol	0.3 ml 70% isopropanol	
<i>Staph. saprophyticus</i>	2.98	2.53	2.61	2.71
<i>Ps. aeruginosa</i>	4.32	3.66	3.23	3.73
<i>K. aerogenes</i>	4.09	4.20	3.76	4.02
<i>Ser. marcescens</i>	4.55 ^a	3.61 ^b	3.97	4.04
Mean value for same treatment on all species	3.99 ^x	3.50 ^y	3.20 ^y	3.56

* Mean of eight experiments.

Value ^a differs from ^b and value ^x from ^y ($P < 0.05$).

Table 5b. Analysis of variance within data used for Table 1a

		d.f.	Mean square	F ratio
Total sum of squares	79.53	95	—	—
Column sum of squares	38.42	11	3.492	7.13
Treatments (T)	6.40	2	3.203	6.55*
Organisms (O)	28.21	3	9.403	19.22*
T × O	3.79	6	0.632	1.29
Error sum of squares	41.11	84	0.489	—

* ($P < 0.01$).

d.f., Degrees of freedom.

Table 6. Effect of 0.3 ml and 0.5 ml 70% isopropanol on transfer of bacteria

Organism	Mean* logarithmic reductions in transfer		P value
	0.3 ml 70% isopropanol	0.5 ml 70% isopropanol	
<i>Staph. saprophyticus</i>	3.08	3.80	< 0.05
<i>Ps. aeruginosa</i>	3.51	4.21	< 0.05
<i>K. aerogenes</i>	3.37	4.19	< 0.05
<i>Ser. marcescens</i>	3.97	4.44	N.S.
Mean value for same treatment on all species	3.48	4.16	< 0.05

* Mean of eight experiments.

N.S., Not significant.

Effectiveness of 0.5 ml compared with 0.3 ml of 70% isopropanol (Table 6)

The larger volume of isopropanol gave higher mean levels of disinfection with each of the species tested, though the differences was not statistically significant for the experiments conducted with the serratia. Overall, 0.5 ml of 70% isopropanol was as effective as the soap and water wash used in the previous experiments.

Fate of organisms on decontaminated hands

When *Staph saprophyticus* was used, the mean number of organisms found on the hands after disinfection with 0.5 ml of 70% isopropanol was 0.6 organisms per cm². In the absence of disinfection the figure was 7.5×10^3 per cm² – a reduction of 4.10 log units (i.e. 10000-fold reduction). With *K. aerogenes*, 0.18 organisms were recovered per cm² after disinfection, compared with 5.3×10^3 per cm² without disinfection – a reduction of 4.47 log units. Both reductions are similar in magnitude to those obtained for the effect of 0.5 ml of isopropanol on the transfer from fabric to fabric (Table 6).

DISCUSSION

The method of application of test bacteria in this model produces an inoculum on the hand which is not visible, dries quickly and is superficially located, as transient contaminants are believed to be. The method is reasonably reproducible, but it is necessary to use a large number of volunteers and replicate experiments if statistically valid comparisons are sought. The numbers of organisms transferred to the hands are similar to the upper values found by researchers studying naturally acquired contamination. For instance, Salzman, Clark & Klemm (1967) found 7% of hospital personnel to have greater than 10^4 'coliform' organisms on their hands. Knittle, Eitzman & Baer (1975) reported 9.3% of hand cultures obtained from 13 nurses in an Intensive Care Nursery as containing more than 10^4 colony counts of Gram-negative organisms.

The relevance to rates of transfer occurring in hospital is unknown and the many ways in which contamination may be acquired make it impossible to model by a single procedure. Skin to skin contact is likely to be of greater importance than fabric to skin, at least where nursing procedures are concerned. The great difference between the low proportion transferred in Stage I and the very high proportion transferred in Stage II requires further investigation. The high rates of transfer from hand to fabric (Stage II) are similar to the results obtained by Marples & Towers (1979) when they used a broth culture pipetted directly on to the hands before transfer took place.

The reproducible difference between *Staph. saprophyticus* and the Gram-negative organisms suggests that this organism may have an affinity for skin not shared by the other species tested. It transferred well to the hands and less well away from them. *Staph. saprophyticus* has been used previously in similar experiments to represent *Staph. aureus* because it was thought comparable in its response to physical agents (Marples & Towers, 1979).

E. coli and *Strep. pyogenes* did not transfer well overall. *E. coli* was picked up well from the donor fabric but did not survive well on skin. The second stage of transfer usually took only about 15 s to carry out, compared with the 300 s over which loss of viability was measured, but it is possible that once the inoculum was divided between the hands and the recipient fabric the further loss of moisture increased the rate of death. This result confirms what other workers have found – that *E. coli* is not typical of the Gram-negative rods in ability to survive on the skin and in the environment (Hart, Gibson & Buckles, 1981). The streptococcus transferred very poorly to the hands. The reason for this is not

known. Recovery of this organism from the fabric was about fourfold lower than for the other species (results not shown) which may be due to preferential binding to the fabric. Increasing the density of the bacteria did not produce a proportional increase in transfer to the hands but raised the count sufficiently to enable their use in tests of hand disinfection. The strain used was, for reasons of safety, a laboratory variant chosen because it had lost its virulence antigen. It may differ considerably from wild-type streptococci and it cannot be concluded from our results that streptococci would fail to be transferred by hand in hospital wards.

Amongst the remaining species tested, *Ps. aeruginosa*, *Kl. aerogenes* and *Ser. marcescens*, only the differences in rates of overall transfer of the pseudomonad and the klebsiella were statistically significant, though it is unclear whether these differences are large enough to represent a difference in the rates of transfer of infection. The model represents transfer from, and survival on, apparently unsoiled surfaces. Transfer and survival would differ if the bacteria were present as part of gross, e.g. direct faecal, contamination of the hands.

Alcohol disinfection

Previous tests measuring disinfection of recently acquired flora have used large inocula, usually in nutrient broth suspension, applied to the hands (Dineen, 1978; Aly & Maibach, 1980; Rotter, Koller & Wewalka, 1980) or as drops of suspension rubbed into the fingertips (Ayliffe, Babb & Quoraishe, 1978). This method is based on a model (Marples & Towers, 1979) that leaves a thin, even and fast-drying layer of bacteria, physically similar to that occurring on microbiologically contaminated hands that are not grossly soiled.

The disinfection results show that washing with soap and water was marginally superior to rubbing the hands with 0.3 ml of ethanol or isopropanol. Soap and water produced, on average, a 10000-fold reduction in transfer, 0.3 ml of ethanol a 3200-fold and isopropanol a 1400-fold reduction. The efficiency of the rub with isopropanol was increased by use of 0.5 ml instead of 0.3 ml. It then had the same efficiency in transfer interruption as soap and water.

Analysis suggested that much of the variation was due to differences in susceptibility of the different species, *Staph. saprophyticus* appearing to be generally more 'resistant' to the disinfection procedures than were the Gram-negative species. The strain of streptococcus was so sensitive to the procedures that often no viable organisms could be recovered. This complicated the calculations but suggested reductions greater than 4.5 logs – the limit of detection in our experiments. As the streptococcus was a laboratory variant, its reaction to the disinfection procedures may not be typical.

The volume of alcohol used was a compromise between convenience and effectiveness. Larger volumes of alcohol took longer to dry but allowed greater contact time between alcohol and bacteria. Some volunteers found it difficult to give adequate coverage of the hands when using 0.3 ml volumes. Increasing the volume to 0.5 ml resulted in a fivefold decrease in transfer with only a 5 s increase in contact time. The interruption of transfer brought about by 0.5 ml isopropanol was statistically indistinguishable from the reduction caused by the soap and water wash but took about one-third the time. The mechanism of action of the alcohol appeared to be due to killing and not simply by physical prevention of transfer. The concentrations of ethanol and isopropanol used were those found

to be optimal for skin disinfection by Rotter, Koller & Kundi (1977). There was no evidence of a difference between the two types of alcohol at the concentrations used.

In contrast to the surgical scrub, which is concerned with reducing the total number of bacteria on the hand, hand hygiene is exclusively directed at recently acquired flora. The data confirm that handwashing is an effective method of removing recently acquired bacteria, though the experimental handwash, taking 50–60 s including drying, was far more thorough than those observed in hospitals. Taylor (1978*b*) recorded mean washing times for various grades of hospital staff as being between 20 and 22 s. Hand washing with soap and water, or the modified use of a surgical scrub, is frequently not done after patient contact (Albert & Condie, 1981) and is often ineffectively performed (Taylor, 1978*a, b*). Handwashing after patient contact has been recommended (Steere & Mallison, 1975) but is found in practice to be too inconvenient and time-consuming if performed conscientiously. One study has shown an increased level of bacteria on nurses' hands resulting from frequent handwashing with disinfectant/detergent mixtures (Ojajarvi, Makela & Rantasalo, 1977). From the time a handwash is started the individual normally remains at or near the sink until the hands have been dried. Alcohols have the advantage that they can be applied to the hands after contact with one patient and evaporated by rubbing whilst moving on to the next task or patient. Alcohols have the reputation of defatting and drying the skin. This occurs when large volumes are used and dissolved lipids are lost along with the excess fluid. If a small volume, such as 0.5 ml, of alcohol is applied and rubbed to dryness without any run-off then dissolved lipid will be redeposited on the same areas of skin. The alcohol treatment was acceptable to all our volunteers. Addition of a humectant, such as glycerol, may improve the general condition of the hands and should not lead to any accumulation of a sticky residue if a low concentration is used (0.5% or less). Glycerol has been shown to reduce the shedding of skin scales, contaminated with resident flora, from the hands (Meers & Yeo, 1978). A small volume of alcohol is a rapid, convenient and acceptable method of prevention of transfer of recently acquired bacterial contamination on the hands of hospital staff. Consideration should be given to its use as a practicable alternative to handwashing after patient contact where soiling of the hands is not apparent.

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