J. Hyg., Camb. (1985), 94, 269–277
Printed in Great Britain

Efficacy of low-concentration iodophors for germicidal hand washing

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(*Received* 1 *October* 1984; *accepted* 3 *January* 1985)

SUMMARY

The efficacy of iodophor germicides containing different concentrations of available iodine against transient (inoculated) bacteria and the natural hand microflora was compared with chlorhexidine gluconate (2 and 4 %) liquid detergent (Hibitane), non-germicidal soap and a tap water rinse. The tap water rinse was ineffective compared with all other treatments. Only 4 % chlorhexidine gluconate liquid detergent and iodophor containing 0·75 % available iodine were significantly better than the non-germicidal soap for reduction of transient bacteria, *Escherichia coli* and *Pseudomonas fluorescens*, that had been inoculated onto hands. These agents also caused a significant reduction in the number of 'natural' micro-organisms released from hands after a standard 15 s hand wash. The low-concentration iodophor products and the product containing 2 % chlorhexidine gluconate failed to give results significantly better than the non-germicidal control soap. Baird–Parker medium and standard aerobic plate counts were highly correlated ($r = 0.82$), so that for studies of Gram-negative bacteria inoculated onto hands as a transient microflora, counts on Baird–Parker medium give a reasonable indication of the natural (residual) hand microflora.

INTRODUCTION

Iodophors are generally accepted as antibacterial agents for many purposes, including hand hygiene (Shelanski & Shelanski, 1956). Several studies have evaluated the germicidal efficacy of high-concentration (0·75 % available iodine) iodophors (Davies *et al.* 1977; Joress, 1962; Ojajärvi, 1976; Peterson, Rosenberg & Alatary, 1978; Van der Hoeven & Hinton, 1968). Earlier studies by Sheena & Stiles (1982, 1983*a,b*) indicated that among a range of commercial hand wash agents tested, using a short exposure (15 s) hand wash, the only agents that gave a significant reduction in number of micro-organisms released from hands were an iodophor product containing 0·75 % available iodine and 4 % chlorhexidine gluconate liquid detergent.

Iodophor products containing high concentrations of iodine, such as 0·75 %, create some resistance among users because of the odour of the product and colour

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https://doi.org/10.1017/S0022172400061490 Published online by Cambridge University Press

when it is applied to the skin. Low-available-iodine products have been developed (Berkelman, Holland & Anderson, 1982). One of these products was incorporated in our earlier studies (Sheena & Stiles, 1983*a,b,c*), but the results obtained for its efficacy were equivocal. In seeking a germicidal hand wash agent for effective hygiene for food handlers, the testing criteria were that the agent should reduce the number of micro-organisms released from hands after short exposure time (15 s); and that the transient bacteria inoculated onto hands should be more effectively reduced by the germicidal agent than by non-germicidal soap.

The purpose of this study was to compare the efficacy of lower-concentration iodophor products and 2% chlorhexidine gluconate liquid detergent with an iodophor product containing 0·75% available iodine (0·75% iodophor), 4% chlorhexidine gluconate liquid detergent (4% chlorhexidine) and a non-germicidal soap. The comparison was based on the ability of the agents to reduce the number of micro-organisms released from hands as a result of a short-exposure, standardized hand wash.

MATERIALS AND METHODS

Hand wash agents tested

A total of eight agents was tested: 4% and 2% chlorhexidine gluconate liquid detergents (Hibitane, Ayerst Laboratories, Montreal, Canada); and iodophor hand wash agents containing 0·75, 0·5, 0·3, 0·1, 0·01, and 0·005% available iodine (West Chemicals Ltd, Montreal, Canada and West Design Chemical Group, West Agro-Chemicals Inc., Westwood, Kansas, U.S.A.). A non-germicidal liquid soap and a tap water rinse were included as reference treatments.

Methods of testing hand wash agents

Latin square designs were used so that each subject used each agent once during the course of each experiment. Agents were randomly assigned according to the procedure specified by Myers (1972). A 5 ml aliquot of the agent was applied to wetted hands, and used in a standardized washing procedure during a 15 s exposure time, supervised by one of the researchers (A.Z.S.). The washing procedure included four different series of movements, each repeated five times: rubbing palms and fingers (including fingertips) together; followed by left palm over right dorsum; then right palm over left dorsum; and finally, palm to palm, with interlacing of the fingers. After precisely 15 s exposure, hands were rinsed under running tap water until the feeling of soapiness had been removed (*ca.* 15 to 20 s). For the tap water rinse, the standard procedure was followed under running tap water for 15 s. There were three testing days each week (alternate days).

Hands were sampled before (X_0) and after (X_1) the 15 s hand wash treatment. One hand was randomly selected for sampling before treatment. The hand was immersed in 100 ml letheen broth (Difco) in a plastic bag (28·5 × 12·5 × 7·5 cm, 1·25 mil, Polyrama Plastics Ltd, Edmonton, Canada), containing 35 g of 4 mm sterile glass beads. The selected hand was rinsed in a standard manner by rubbing the glass beads twenty times over the palm of the hand. Hands were rinsed under running tap water to remove residues of letheen broth. After washing and rinsing the hands, the hand which had not previously been sampled was rinsed in letheen broth to obtain the sample after treatment (X_1).

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Efficacy against inoculated (transient) bacteria

Hands were contaminated with *Escherichia coli* and *Pseudomonas fluorescens* strains that had previously been isolated from ground beef. The test organisms were grown in nutrient broth (Difco). *E. coli* was incubated at 35°C for 18 h and *P. fluorescens* was incubated at 20 °C for 30 h, for use in the experiments. The test organisms were inoculated into ground beef to give 10^6 and 10^7 colony forming units (c.f.u.) per g for the two organisms, respectively. The experiment was repeated using increased concentrations of test organisms in the ground beef at 10^7 and 10^8 c.f.u./g, respectively. The microbial quality of the ground beef was monitored each day, including: total aerobic plate count on Standard Plate Count agar (SPC, Difco); coliform bacteria and presumptive *E. coli* on Violet Red Bile agar (VRB, Difco) incubated at 35 and 45 °C, respectively; *P. fluorescens* count on Pseudomonas agar F(PAF, Difco); and micrococcaceae-type (shiny black) colonies on Baird–Parker medium (B–P, Difco). The inoculated ground beef was dispensed in two 50 g amounts in plastic petri dishes, and used as the inoculum for the fingertips.

Subjects first rinsed their hands with 5 ml of 95% ethanol containing 1% glycerol. Hands were rubbed together until dry. Finger and thumb tips were pushed into, and held in the ground beef inoculum for 5 s. The inoculum was distributed over the hands by rubbing, up to the wrists, until the hands were dry. Then the sampling and washing procedures were done. After completing the sampling procedure, hands were again rinsed with the glycerol in ethanol solution.

The X_0 letheen broth samples were plated without delay; the X_1 samples were plated after they had been held at 20 °C for 1·5 h, for resuscitation of cells from possible injury. Appropriate dilutions of the letheen broth rinses were prepared in 0·1% peptone water to give 30–300 c.f.u./plate. The dilutions were pour plated on VRB and overlayered with 5 ml of VRB medium before incubation at 45 °C for 24 h to determine the *E. coli* count. Samples were surface plated onto pre-poured plates of PAF for the *P. fluorescens* count and onto B–P for the micrococcaceae-type count. Plates were incubated at 20 °C for 72 h (PAF) and at 35 °C for 48 h (B–P).

Efficacy against natural skin microflora

The procedure was the same as that used for the transient microflora study, without the inoculation of hands or use of the glycerol in ethanol rinses. Subjects with 'socially' clean hands were sampled by the standard rinsing technique immediately before (X_0) and after (X_1) the hand wash treatments. Letheen broth samples were plated in duplicate onto SPC and B–P, using pour- and surface-plating techniques, respectively. The plates were incubated at 35 °C for 48 h before being counted.

Statistical analysis

An analysis of variance was done on $\log_{10}$ reduction or change ratios (X_1/X_0) (Rotter, Mittermayer & Kundi, 1974) using a computerized statistical package for Latin square designs (BMDP2V, *Biomedical Computer Programs*, P-series, 1979, University of California Press). The significance of differences among mean $\log_{10}$ ratios was assessed using Duncan's multiple range test.

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Table 1. *Efficacy of germicidal hand wash agents against* Escherichia coli *and* Pseudomonas fluorescens *artificially inoculated onto hands from ground meat**

	E. coli			*P. fluorescens*		
	Initial count	Final*† count	Reduction (%)	Initial count	Final count	Reduction (%)
Agent	(mean count × 10^3)			(mean count × 10^3)		
A Control soap	5·0	0·2	(96·3)	3·0	0·1	(96·5)
B Chlorhexidine (4 %)	6·0	0·08	(98·9)	4·0	0·04	(99·1)
C Chlorhexidine (2 %)	6·0	0·2	(97·0)	3·6	0·1	(97·1)
D Iodophor (0·75 %)	6·6	0·05	(99·2)	3·4	0·02	(99·5)
E Iodophor (0·5 %)	5·1	0·1	(97·9)	3·2	0·06	(97·8)
F Iodophor (0·3 %)	6·3	0·2	(97·5)	3·5	0·1	(97·4)
G Iodophor (0·1 %)	6·2	0·1	(97·9)	3·3	0·1	(97·2)
H Iodophor (0·01 %)	6·3	0·2	(97·3)	4·1	0·07	(97·8)
I Tap water	4·6	1·0	(78·4)	3·0	0·9	(71·3)

* Mean counts and mean percentage reduction in number of bacteria inoculated onto hands calculated from individual changes in count for each subject.

† Final count is obtained after a 15 s wash.

Table 2. *Summary of Duncan's multiple range test* (95 % *confidence level*) *for differences among treatment means* (*based on counts on VRB and PAF media*)*†

	Rank order of treatment means
E. coli	D B E G F H C A I
P. fluorescens	D B E H F G C A I

* For key to product codes see Table 1.

† Agents underlined with an unbroken line are not statistically different.

Chemical test

The available iodine concentration of the iodophor products was determined by titration of available iodine against standardized sodium thiosulphate solution (A.O.A.C., 1975).

RESULTS

The iodophor germicides generally contained a slight excess of available iodine. The actual available-iodine concentrations were 0·78, 0·56, 0·32, 0·13 and 0·01 %, compared with the manufacturer's listed concentrations of 0·75, 0·5, 0·3, 0·1 and 0·005 % available iodine, respectively. Only the product with the lowest available iodine content differed markedly between actual and manufacturer's available-iodine concentration. This was necessitated by the shelf stability of this product. This product is referred to as 0·01 % available iodine, unless a 1 : 1 dilution was made with distilled water, then it is referred to as '0·005 %' available iodine.

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Table 3. *Change in residual micrococcaceae-type bacteria released from hands after use of germicidal hand wash agents, measured by growth on Baird–Parker medium**

	Hands inoculated with transient flora			Uninoculated, socially clean hands		
	Initial count	Final† count	Change (%)	Initial count	Final count	Change (%)
Agent	(mean count × 10^2)			(mean count × 10^2)		
A Control soap	1·9	2·9	(167)	1·1	1·4	(136)
B Chlorhexidine (4%)	2·1	1·1	(47)	1·2	0·5	(49)
C Chlorhexidine (2%)	2·4	1·9	(83)	1·0	0·5	(78)
D Iodophor (0·75%)	1·3	0·9	(72)	1·2	0·5	(47)
E Iodophor (0·5%)	1·5	1·1	(71)	1·1	0·4	(59)
F Iodophor (0·3%)	2·2	1·4	(59)	1·3	1·0	(81)
G Iodophor (0·1%)	1·3	1·8	(139)	1·1	0·9	(74)
H Iodophor (0·01%)	1·9	1·8	(93)	1·3	1·0	(89)
I Tap water	1·0	2·3	(234)	2·3	1·9	(98)

* Mean counts and mean percentage change in number of bacteria released from hands are based on individual changes in counts for each subject.

† Final count is obtained after a 15 s wash.

Efficacy against inoculated (transient) bacteria

The reduction of *E. coli* and *P. fluorescens* on hands as a result of 15 s exposure to the hand wash agents is shown in Table 1. The two experiments were done with different levels of bacteria inoculated onto hands, so that the second experiment represents a more sensitive test. All of the agents, including the non-germicidal soap, resulted in mean percentage reductions in *E. coli* and *P. fluorescens* greater than 95%. Only the 15 s tap water rinse failed to achieve this level of reduction of the transient bacteria on hands. Analyses of variance of the $\log_{10}$ transformed reduction ratios indicated a significant effect ($P < 0{\cdot}001$) attributable to agents.

Differences among reduction ratio means, determined using Duncan's multiple range test at the 95% confidence level, are summarized in Table 2. Some minor differences were apparent between experiments; however, all agents resulted in a significantly better reduction of *E. coli* and *P. fluorescens* counts than the tap water rinse. The greatest decrease in *E. coli* and *P. fluorescens* counts was observed with the 4% chlorhexidine and 0·75% iodophor products. These two agents were generally significantly better than other agents in reducing the number of the test organisms released from hands after the 15 s wash.

Efficacy against natural (resident) skin microflora

The change in micrococcaceae-type bacteria released from hands before and after washing for 15 s, and the mean percentage change in number of micro-organisms released, are shown in Table 3. The tap water rinse, non-germicidal soap and the lower-concentration iodophor products generally resulted in an increased number of bacteria released from hands after the 15 s wash. An analysis of variance of the $\log_{10}$ transformed change ratios indicated a significant effect ($P < 0{\cdot}01$) attributable

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Table 4. *Log_{10} mean ratios and standard deviations for the effect of germicidal hand wash agents used by 28 subjects, measured by growth on Baird–Parker medium*

Agent	Log_{10} mean X_1/X_0	Standard deviation
A Control soap	0·17	0·27
B Chlorhexidine (4 %)	−0·33	0·29
C Chlorhexidine (2 %)	−0·19	0·48
D Iodophor (0·75 %)	−0·30	0·46
E Iodophor (0·5 %)	−0·21	0·43
F Iodophor (0·3 %)	−0·17	0·35
G Iodophor (0·1 %)	0·05	0·33
H Iodophor (0·01 %)	0·002	0·33
I Tap water	0·15	0·31

to agents. Differences among change ratio means were determined using Duncan's multiple range test. The differences were less distinct than those observed for transient bacteria. However, 4 % chlorhexidine was significantly better than the tap water rinse, non-germicidal soap and iodophor product containing 0·1 % available iodine, but compared with other germicidal agents it did not give a significantly better reduction in numbers of bacteria released from hands.

The study of the efficacy of the germicidal agents against natural (incidental transient and resident) microflora of hands was repeated using subjects without specifically inoculated (transient) bacteria on their hands. Hand rinse samples were plated onto SPC and B–P to measure the change in number of bacteria released from hands before and after germicidal hand washing. The similarity of the results on B–P medium may be seen from the data in Table 3. A significant correlation exists between counts on SPC and B–P ($r = 0·82$). The 0·75 % iodophor and 4 % chlorhexidine products were not significantly different from each other. Their use resulted in a significantly lower number of bacteria released from hands after the 15 s hand wash when compared with all agents, except the iodophor product containing 0·5 % available iodine. Both 4 % chlorhexidine and 0·75 % iodophor resulted in a decrease in number of micro-organisms released from hands for all subjects. The 0·5 % iodophor gave a reduction in count for 8 out of the 10 subjects.

The lack of a clear-cut distinction between agents against the micrococcaceae-type bacteria on hands was attributed to the variance of the data. The $\log_{10}$ mean ratios and standard deviations for 28 subjects participating in the experiments are shown in Table 4. The non-germicidal soap resulted in 26 out of 28 subjects with an increased, or no change in, number of micro-organisms released from hands after treatment, whereas the 4 % chlorhexidine resulted in 25, and 0·75 % iodophor resulted in 19, out of the 28 subjects with a decreased number of micro-organisms released from hands after washing. Other agents which showed an overall decrease in number of bacteria released from hands had larger standard deviations than the control soap or 4 % chlorhexidine, which could be attributed to considerable variation in results between subjects participating in the experiments.

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DISCUSSION

Most studies of the effectiveness of germicidal agents for hand washing are based on the control of the natural (incidental transient and resident) skin microflora. Ojajärvi (1980) emphasized the importance of the transient microflora in transmission of cross infection. In the present study, an attempt was made to study both the resident microflora and a transient microflora consisting of *E. coli* and *P. fluorescens* inoculated onto hands from ground beef. These bacteria were chosen because the presence of *E. coli* is used as an indicator of possible enteric pathogens in food, and *P. fluorescens* represents potential spoilage bacteria.

When transient bacteria are inoculated onto hands in relatively large numbers, the resident microflora cannot be enumerated by conventional techniques. As a result, the resident microflora was estimated on Baird–Parker medium, which is generally used for selective growth of *Staphylococcus aureus*. This was done because many resident skin micro-organisms that grow aerobically are non-pathogenic. micrococcaceae-type bacteria that should grow on Baird–Parker medium (Noble & Somerville, 1974; Sheena & Stiles, 1982; Stiles & Ng, 1981).

A washing time of 15 s was used in this study, because it was considered more likely to represent hand washing practice by food handlers than the longer 30 s washing time used in many hospital studies (Ayliffe, Babb & Quoraishi, 1978; Lilly & Lowbury, 1978; Ojajärvi, 1976). Ojajärvi (1980) used a 15 s wash in a study of hand hygiene of nurses, based on the observation that 15 s was more representative of hand washing times in ward practice. A water rinse was included in this study because some food handlers use a brief water rinse instead of a soap wash. The water rinse was ineffective against transient bacteria, and generally caused an increased number of resident-type bacteria to be released from hands after the rinse.

The repeated 9 × 9 Latin square experiment gave useful confirmation of the data. Reductions in *E. coli* and *P. fluorescens* counts and changes in number of 'resident' micro-organisms released from hands after washing were consistent with earlier observations (Sheena & Stiles, 1983*b*). Chlorhexidine gluconate (4 %) liquid detergent (Hibitane) and iodophor hand washing containing 0·75 % available iodine (Prepodyne or 'Tamed Iodine Scrub') were shown to be effective against the transient bacteria inoculated onto hands (Sheena & Stiles, 1983*b*), and they reduced the number of bacteria released from hands after short exposure washing (Sheena & Stiles, 1982). They were the only agents tested that met the criteria established for this study.

Dilute preparations of iodophor solutions have been shown to be more effective than high (10 %) concentrations against certain micro-organisms in *in vitro* studies (Berkelman *et al.* 1982). New iodine preparations with low available-iodine contents, down to 0·01 % available iodine, have been patented (U.S. Patent, 1981) for various uses, including germicidal hand wash agents. A range of iodophor products with available iodine concentrations from 0·75 to 0·01 % was included in this study to determine their *in vivo* efficacy under these experimental conditions. The 2 % chlorhexidine gluconate liquid detergent was also included as a possible alternative to 4 % chlorhexidine or the iodophor products.

All agents markedly reduced the transient bacteria inoculated onto hands. Only

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4 % chlorhexidine and 0·75 % iodophor gave reductions significantly greater than the non-germicidal soap. Under the conditions of this study, intermediate and low-concentration iodophor products, as well as the 2 % chlorhexidine gluconate product, are of questionable value when compared with non-germicidal soap. The differentiation of these products, therefore, could rely on their action against the resident microflora. Analyses based on the counts on Baird–Parker medium as a measure of the resident microflora were not clear. The large variance in the data made it difficult to distinguish differences between products. Furthermore, the use of B–P medium to assess the efficacy against the 'resident' microflora is a compromise necessitated by the conditions of the experiment.

The experiment comparing agents against the natural hand microflora, using a standard aerobic plate count (SPC) and B–P to monitor changes in number of micro-organisms released from hands, also revealed considerable variability in the data between subjects. However, the study confirmed that only the 4 % chlorhexidine and 0·75 % iodophor products significantly reduced the number of 'natural' micro-organisms released from hands, and that the 0·5 % iodophor product gave an intermediate effect. Under the conditions of these experiments, therefore, the only germicidal agents that effectively reduced the transient microflora inoculated on to hands and the natural microflora of hands, during a short-exposure hand wash, were the 4 % chlorhexidine gluconate liquid detergent and the iodophor product containing 0·75 % available iodine. Additional studies on the effectiveness of these agents will be done under practical conditions of food handling.

The authors thank their colleagues who served as volunteers for these studies. The study was supported by funds from an Agriculture Canada research contract.

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