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Efficacy of Germicidal Hand Wash Agents in Hygienic Hand Disinfection[1]

A. Z. SHEENA[2] and M. E. STILES[3]*

Departments of Food Science, Foods and Nutrition and Microbiology, The University of Alberta, Edmonton, Alberta, Canada T6G 2M8

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ABSTRACT

The efficacy of hygienic hand wash procedures for food handlers using germicidal soaps and hand dips was studied by measuring changes in numbers of microorganisms released from hands before and after each of two successive 15-s treatments. Both hand rinse and finger tip imprint sampling techniques were used. The experiment consisted of two (6 x 6) Latin square designs, each including a non-germicidal soap control. Of the hand dip agents, including sodium hypochlorite (50 ppm available chlorine), iodophor (25 ppm available iodine) and a quaternary ammonium compound (QAC) (930 ppm benzalkonium chloride), only the QAC gave a statistically significant decrease in the number of bacteria released when tested by the finger imprint technique. This experiment included a bar soap containing 1.0% trichlorocarbanilide which gave results equivalent to the non-germicidal soap control. Of the hand wash agents, 4% chlorhexidine gluconate and iodophor (0.75% available iodine) resulted in significant decreases in numbers of bacteria released when tested by either sampling technique. Products containing Irgasan DP 300 (0.25% active ingredient at the use concentration), tribromosalicylanilide (0.5%) and *para*-chloro-*meta*-xylenol (0.325%) were no better than the non-germicidal soap control under the conditions of this experiment. Identification of 3,591 aerobic isolates from finger imprint plates indicated that *Staphylococcus epidermidis* and *Micrococcus* spp. were the predominating organisms (85.3%) released from the hands.

Many food industries use germicidal hand washing, especially for workers handling ready-to-eat foods. These agents are used either as a hand dip, in which hands are placed in a prepared germicidal solution, or as hand washes using liquid or solid germicidal soaps. The reduction of microorganisms on skin using germicidal agents has been referred to as "degerming", and more recently, as "hygienic" hand disinfection to distinguish it from "surgical" hand disinfection (*3,29,43,46*). A concept of "virtual" disinfection was suggested by Gardner (*18*), if at least 99.9% of the microflora was removed or killed. The extent of skin disinfection depends on the method of washing. Pre-operative surgical scrubs are intended to remove both resident and transient microflora from the hands, whereas intermittent washing with germicidal soaps is expected to remove primarily the transient microflora (*3,34,42*).

Most researchers have attempted to evaluate skin germicides for surgeons and operating sites (*15,27,28,33,35*). Experiments in which hand washing was done with less thoroughness than surgical hand disinfection have been conducted on hospital staff (*10,41-43,55*). Some of these studies relate closely to needs for food hygiene, whereas studies specifically designed for food handling are limited (*9,19,22,45,51*). A review of the literature on antimicrobial hand soaps for use in food service establishments was published in 1965 (*13*). Since then, the use of hexachlorophene as a germicidal agent has been severely limited because of concerns for toxicity from dermal absorption (*44*).

Many comparative studies have been conducted on the efficacy of germicidal agents (*10,28,30,32,41,42,52*). A recurring problem is the methodology for testing product efficacy. There are no official methods for these agents comparable to the A.O.A.C. use-dilution method for sanitizer efficacy on inanimate surfaces (*2*). Multiple basin techniques to measure the rate of mechanical removal of microorganisms from the skin have been established (*12,46*). Evaluation of skin germicides has been conducted on the natural skin microflora and by applying test microorganisms to the skin (*3,34,36,56*). Recently, Ojajärvi (*42*) used contaminated gauze as a method of contaminating finger tips. Several skin sampling techniques have also been used, such as finger tip imprints onto agar plates containing germicide inactivators (*4,42,43,52,55*); rinsing techniques using rubber gloves (*1,31*), bowls (*3,23,33*) or plastic bags (*49,55*) and the rinsings plated for bacteriological analysis; adhesive tape (*58*); and by moist swabbing of the skin surface, followed by agitation of the swab in

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[2]*Department of Food Science.*
[3]*Department of Foods and Nutrition and Department of Microbiology.*

broth for plating or streaking onto an agar plate (*56*).

The object of this study was to determine the efficacy of various hand wash and hand dip treatments to reduce the number of microorganisms released from the hands after washing, and, hence, to reduce potential contamination of foods.

MATERIALS AND METHODS

Two methods of microbiological sampling were followed: sampling by rinse solution (*49,55*) in which the hands were immersed in 100 ml of sterile letheen broth (LB; Difco), and by imprints of finger tips (*43,52*) on letheen agar (LA; Difco) plates using a 5-s contact time for thumb and finger tips. The problem of confluent growth of colonies on finger imprint plates was avoided by streaking the inocula with a sterile glass hockey stick.

The experiment was based on a Latin square design (Table 1). Two separate experiments were done simultaneously, each consisting of a 6 x 6 design. In each experiment, six agents (including a non-germicidal control) were used by each of six subjects (male and female volunteers) over a period of 6 weeks. The experiments were randomized for subjects and sequence in which agents were used. Each subject used the assigned agent once a day for two successive 15-s washes on 4 consecutive days of the week. For the remaining 3 days of the week, no treatment was applied so that any carry-over effect from one agent to another would be eliminated, as well as to allow the skin microflora to become re-established. A different agent was used by each subject, each week, according to a randomized procedure.

The two experiments allowed ten germicidal agents or washing procedures to be studied. All products were liquid, except agent F which was a germicidal bar soap. The agents were grouped based on their use as *hand dip* (in which hands were placed in 1.5 L of freshly prepared germicidal solution) or *hand wash* (using 5 ml of liquid soap). The iodophor (0.75% available iodine) and 4% chlorhexidine gluconate hand wash agents were incorporated as reference agents commonly used in surgical practice.

TABLE 1. *Latin square designs (before randomization) for germicidal hand wash experiments A and B, each containing six subjects and six agents used for six weeks.*

Experiment A

Week → / Subject	I	II	III	IV	V	VI
			Agent			
1	A[a]	B	C	D	E	F
2	B	C	D	E	F	A
3	C	D	E	F	A	B
4	D	E	F	A	B	C
5	E	F	A	B	C	D
6	F	A	B	C	D	E

Experiment B

Week → / Subject	I	II	III	IV	V	VI
			Agent			
1	G[a]	H	I	J	K	L
2	H	I	J	K	L	G
3	I	J	K	L	G	H
4	J	K	L	G	H	I
5	K	L	G	H	I	J
6	L	G	H	I	J	K

[a]Agents A and G = non-germicidal soap (control).

In the first experiment, hand dip agents and a germicidal bar soap (F) were evaluated with a non-germicidal control. The agents included: (a) non-germicidal liquid hand soap; (b) non-germicidal liquid hand soap (15 s), followed by a 15-s hand dip in sodium hypochlorite solution containing 50 ppm available chlorine; (c) sodium hypochlorite (50 ppm available chlorine); (d) quaternary ammonium (QAC) solution containing 930 ppm benzalkonium chloride; (e) iodophor solution with 25 ppm available iodine; and (f) germicidal bar soap containing 1.0% trichlorocarbanilide (TCC). In the second experiment, all the agents were used in the wash procedure. The agents included: (g) non- germicidal soap (same as "a" above); (h) iodophor "tamed iodine" scrub containing 0.75% titratable iodine (West Chemical Products Ltd., Montreal, Canada); (i) chlorhexidine gluconate (4%) solution, Hibitane (Ayerst Laboratories, Montreal, Canada); (j) germicidal liquid soap containing 0.5% 2,4,4'-trichlor-2'-hydroxy diphenyl ether (Irgasan DP 300), diluted to 0.25% active ingredient at the use concentration; (k) antiseptic liquid hand soap (40%) containing 0.65% *para*-chloro-*meta*-xylenol (PCMX), diluted to 0.325% active ingredient at the use concentration; and (l) synthetic liquid hand soap containing 0.5% tribromosalicylanilide (TBS).

A standard hand washing procedure was established, and supervised throughout the study by one of the researchers (A.Z.S.). Hands were moistened under running tap water, then washed for 15 s either by dipping in germicidal solution or by washing with hand wash agent poured onto the palm of the hand, or with the bar soap held in the hands. During this period, four different movements were each repeated five times: rubbing palms and fingers together, followed by the left palm over right dorsum, then right palm over left dorsum and finally interlacing the fingers. The washing was carried out to include the hands up to the wrist. After precisely 15 s of exposure, the hands were rinsed under running tap water until all of the feeling of soapiness had been removed (ca. 15 to 20 s). Microbiological sampling was done, and the process repeated with a second 15 s wash and microbiological sampling.

The two sampling techniques were carried out concurrently: hand rinse (X) and finger imprint (Y). In the hand rinse technique, 100 ml of letheen broth (LB; Difco) containing 35 g of 4 mm sterile glass beads were placed in a plastic bag (28.5 x 12.5 x 7.5 cm, 1.25 mil, Polyrama Plastics Ltd., Edmonton, Canada) for use as the rinse solution. In the finger imprint technique, prepoured plates of letheen agar (LA) were used. The hand to be sampled was placed in LB and rinsed in a standard manner by rubbing the glass beads over the palm of the hand 20 times. The hand was blotted on a sterile paper towel, then the finger tips were placed on a LA plate for 5 s. The hands were rinsed under running water to remove any residues of LB as well as to wet the hands for the washing procedure. The second and third samplings were done after the first and second 15-s treatments, respectively.

Because of the experimental procedure, left and right hands had to be used interchangeably. An example of the sampling protocol is shown in Table 2. Sampling from the left or the right hand was randomly assigned for each subject on day 1 of each week, then alternated on following days. Initial samples (before washing) for both hand rinse and finger imprint techniques were always taken from the same hand, thereafter, hand rinse samples were taken from the other hand and finger imprint samples were taken from the same hand as the initial sample (see Table 2).

Appropriate serial dilutions of the hand rinse samples in 99 ml of 0.1% sterile peptone water were surface streaked onto prepoured plates of standard plate count agar (SPC; Difco). Duplicate plates of each dilution were incubated at 35 C for 48 h. The finger imprint (LA) plates were immediately spread using a sterile glass hockey stick and incubated under the same conditions as described above.

A total of 3,591 cultures were isolated from the finger imprint plates for each treatment on days 1 and 3 of each week over the six week testing period. Colonies growing on the plates were selected based on the numbers and types of different bacterial colonies, similar to the procedure used for detecting coagulase-positive *Staphylococcus aureus* (*20*). Colony types with an average count of less than five, all colonies were picked; for 5-25 colonies, five were picked at random; for 25 - 99 colonies, 8 were picked; and for >99 colonies, the square root of the

TABLE 2. *Example of left and right hand sampling protocol used for four successive days of a week.*

Day	Sampling times[a]	Sampling method	
		Hand rinse	Finger imprint
1	1	L[b]	L
	2	R	L
	3	R	L
2	1	R	R
	2	L	R
	3	L	R
3	1	L	L
	2	R	L
	3	R	L
4	1	R	R
	2	L	R
	3	L	R

[a]1=before treatment, 2=after 15-s treatment, 3=after second 15-s treatment.
[b]L=left hand, R=right hand.

total number were picked. Isolates were streaked onto SPC plates and incubated at 35 C for 24 h to check their purity. A single colony was picked and inoculated onto a nutrient agar slant and subjected to the following screening tests: Gram stain, microscopic morphology, and catalase and oxidase tests. The cultures were subdivided according to the results of the four screening tests and subjected to appropriate identifying techniques based on the eighth edition of *Bergey's Manual of Determinative Bacteriology* (*11*) and Holt's shorter version of Bergey's Manual (*21*). The differentiation of the *Micrococcaceae* was based on several additional references (*5,6,26,37,50,57*). For the *Enterobacteriaceae*, Edwards and Ewing (*16*), and for the pseudomonads, King et al. (*24*) were used as additional bases for identification.

A range of standard strains of bacteria was used to check the identification procedures, including: *Staphylococcus aureus* ATCC 6538 (American Type Culture Collection, Rockville, MD), *Staphylococcus epidermidis* ATCC 14990, *Staphylococcus saprophyticus* U24423 (Alberta Laboratory of Public Health, Edmonton, Canada), *Aerococcus veridans* BC420 (Alberta Laboratory of Public Health), *Streptococcus faecalis* ATCC 7080, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 11229.

Statistical analysis of the data was done using a statistical package adapted to the Latin square design (BMDP2V, Biomedical Computer Programs, P-series, 1979, University of California Press). Data were calculated as ratios based on the numbers of organisms released after the first and second 15-s treatments relative to the initial numbers released. For a normal distribution of the ratio data, log transformed ratio data were used in the analyses.

RESULTS

Initial studies were done to verify the procedures. The protocol required interchangeable use of left and right hands. Samples were taken of each hand and analyzed for differences in microbial load of the most and least frequently used hand. A student's t-test for paired data indicated no significant difference ($P>0.05$) in microorganisms released from each hand.

The need for inactivators of the germicidal agents was also studied. Possible rinse solutions for hand sampling included distilled water, normal (0.85%) saline, 0.1% peptone water, 10% nutrient broth (NB) in distilled water or normal saline, and full strength NB. When microorganisms isolated from hands were suspended in these solutions, there was no significant change in the microbial counts during 2 h at 20 C. However, only NB gave an indication of inactivating the germicidal agents. Further study showed that the chlorhexidine and Irgasan DP 300 agents were not adequately inactivated by NB.

Residues of chlorhexidine and Irgasan DP 300 released from hands after the standard wash and rinse procedure were determined. It appeared that residues equivalent to between 0.01% and 0.001% of their use concentrations were released in the rinse solution. As a result, 0.1, 0.01 and 0.001% concentrations of the use dilution of these agents were added to NB and LB. NB failed to inactivate chlorhexidine at 0.001% and Irgasan DP 300 at 0.01% of their use concentrations. In contrast, in LB there was slight inhibition of cells at 0.1% chlorhexidine, but not at 0.01%; while Irgasan CP 300 was inactivated in LB at all concentrations tested. LB and LA were therefore used as the rinse solution and growth medium for the hand rinse and finger imprint techniques, respectively.

The mean counts of the number of organisms released using the hand rinse or finger imprint sampling techniques, before and after washing with the various agents, were calculated for each agent used by six subjects for four consecutive days (24 observations per agent). These data and the percentage change in numbers of organisms released after the first and second 15-s washes are shown in Tables 3 and 4. Results for total hand sampling (hand rinse technique) generally gave counts of 10^3 per ml of LB rinse solution, representing about 10^5 organisms released from the hands, compared to the finger imprint technique that generally gave counts of 10^2 organisms on the LA plates. Correlation coefficients calculated between the two sampling methods ranged from 0.23 to 0.58, showing a weak linear relationship between the two methods.

From the data in Tables 3 and 4, it can be seen that the non-germicidal hand wash generally resulted in an increase in numbers of organisms released from the hands after the first 15-s wash, compared to a moderate decrease after the second 15-s wash. Some of the hand dip agents appeared to reduce the number of organisms released, especially when compared by the finger imprint technique, but the bar soap containing 1% trichlorocarbanilide appeared to be ineffective. Of the hand wash agents, only chlorhexidine and iodophor (agents H and I) appeared to be effective in reducing the numbers of organisms released from the hands.

Analyses of variance for the Latin square designs were done on the log transformed ratio data, representing the

TABLE 3. *Mean count and percentage change in numbers of organisms released from hands after each of two successive 15-s treatments with HAND DIP agents using two sampling techniques.*

Agents	Initial	1st wash	2nd wash
	mean count x 10^3	(percent)	
Hand rinse technique			
A. Control soap	9.0	10.5 (117)	8.1 (90)
B. Soap + hypochlorite (50 ppm)	7.2	9.5 (132)	6.2 (86)
C. Hypochlorite (50 ppm)	10.8	9.4 (87)	9.0 (83)
D. QAC (930 ppm)	9.3	8.9 (96)	5.8 (62)
E. Iodophor (25 ppm)	7.0	8.2 (117)	6.3 (90)
F. Bar soap (1.0% TCC)	5.1	6.5 (128)	5.9 (116)
	mean count x 10^2	(percent)	
Finger imprint technique			
A. Control soap	9.7	13.6 (140)	8.6 (89)
B. Soap + hypochlorite (50 ppm)	6.3	9.3 (148)	8.8 (139)
C. Hypochlorite (50 ppm)	11.8	5.2 (44)	5.8 (49)
D. QAC (930 ppm)	7.7	4.0 (52)	1.7 (22)
E. Iodophor (25 ppm)	6.3	4.6 (73)	2.6 (41)
F. Bar soap (1.0% TCC)	2.8	3.6 (129)	3.4 (121)

TABLE 4. *Mean count and percentage change in numbers of organisms released from hands after each of two successive 15-s treatments with HAND WASH agents using two sampling techniques.*

Agents	Initial	1st wash	2nd wash
	mean count x 10^3	(percent)	
Hand rinse technique			
G. Control soap	8.9	10.4 (117)	8.4 (94)
H. Iodophor	13.3	2.4 (43)	1.7 (30)
I. Chlorhexidine	3.7	2.2 (60)	1.8 (49)
J. Irgasan DP 300	6.8	8.2 (121)	7.4 (109)
K. PCMX[a]	8.3	7.5 (90)	5.3 (64)
L. TBS[b]	8.5	8.2 (97)	5.8 (68)
	mean count x 10^2	(percent)	
Finger imprint technique			
G. Control soap	8.1	6.7 (83)	10.5 (130)
H. Iodophor	6.4	2.1 (33)	0.6 (9)
I. Chlorhexidine	4.8	0.6 (13)	0.2 (4)
J. Irgasan DP 300	9.8	8.3 (85)	6.6 (67)
K. PCMX	11.5	8.9 (77)	10.4 (90)
L. TBS	5.9	6.8 (115)	9.1 (154)

[a] *Para*-chloro-*meta*-xylenol.
[b] Tribromosalicylanilide.

change in the number of organisms released after the two successive 15-s washes. In the hand dip experiment, using the hand rinse sampling technique, no significant difference ($P=0.59$) could be attributed to the germicidal agents. However, by the finger imprint sampling technique, agents had a significant effect ($P<0.001$). Further study of this effect using Duncan's multiple range test indicated that the difference could be attributed to the QAC dip after the first and second 15-s treatment, and the iodophor after the second treatment.

In the hand wash experiment, agents had a significant effect ($P<0.001$) by both sampling techniques. Results of the Duncan's multiple range test at the 95% confidence level are shown in Table 5. Using data for the hand rinse sampling technique, after the first 15-s exposure, the iodophor hand wash agent was significantly better than all agents except 4% chlorhexidine. However, after the second 15-s treatment, both iodophor and chlorhexidine agents gave a significant decrease in numbers of organisms released from the hands. Using data for the finger imprint sampling technique, both chlorhexidine and iodophor agents caused a significant decrease in organisms released from the hands after both the first and second treatments. Analyses for a time sequence effect were not significant, indicating that there was no cumulative or persistence (substantive) effect for these

TABLE 5. *Summary of Duncan's multiple range at the 95% confidence level for comparison among log ratio means for the hand wash agents.*[a,b]

Hand rinse technique (rank order of means)						
After first 15-s wash	H	I	J	K	L	G
	———	———				
After second 15-s wash	H	I	L	J	K	G
	———	———	———			
Finger imprint technique (rank order of means)						
After first 15-s wash	I	H	G	L	K	J
	———	———	———			
After second 15-s wash	I	H	L	J	G	K
	———	———	———			

[a]Hand wash agents: G=non-germicidal liquid hand wash (control); H=iodophor (0.75% available iodine); I=4% chlorhexidine gluconate (Hibitane); J=Irgasan DP 300 (0.25% use concentration); K=*para*-chloro-*meta*-xylenol soap (0.325% use concentration); L=tribromosalicylanilide soap (0.5% use concentration).

[b]Agents underlined with an unbroken line are not statistically different at the 95% confidence level.

TABLE 6. *Frequency of different screening test groups (gram stain, morphology, catalase and oxidase tests) of microorganisms isolated from finger imprint samples from hands.*

Group	Screening tests[a]	Frequency					
		Total		Before wash		After wash	
		No.	%	No.	%	No.	%
I	1 1 1 2	3062	85.3	1547	79.9	1515	91.5
II	1 1 2 2	46	1.3	34	1.8	12	0.7
III	1 2 1 2	91	2.5	64	3.3	27	1.6
IV	2 2 1 1	38	1.1	28	1.4	10	0.6
V	2 2 1 2	60	1.7	43	2.2	17	1.0
Others		90	2.5	74	3.9	16	1.0
No growth		204	5.6	145	7.5	59	3.6

[a]Numerical codes refer, in sequence, to gram stain 1=positive, 2=negative; cell morphology 1=coccus, 2=rod; catalase and oxidase tests 1=positive, 2=negative.

agents with this protocal over the 4-day testing period.

A total of 3,591 isolates was selected from finger imprint plates before and after the two 15-s treatments on days 1 and 3 for each week of the study. The types of organisms isolated based on the four screening tests are shown in Table 6. There were five main groups (I-V) that could be identified. Group I, which comprised gram-positive coccus-shaped, catalase-positive, oxidase-negative organisms, predominated. Group I organisms accounted for 85.3% of all isolates; 79.9% of the 1,935 isolates from plates of finger imprint samples before hand washing and 91.5% of 1,656 isolates from plates after the two 15-s hand washes. The other groups (II-V) of microorganisms each represented less than 3.0% of the total isolates, but these groups of organisms were generally present in slightly greater concentration before than after washing with germicidal agents.

Since the gram-positive coccus-shaped, catalase-positive, oxidase-negative (group I) microorganisms were such a large group, a randomized sample of 264 (8.6%) of these isolates, representing each sub-group based on colony size and appearance, was subjected to further identification. Based on the identification criteria: oxidative and fermentative reactions on glucose and mannitol, motility, novobiocin sensitivity and resistance, hydrogen peroxide formation and coagulase production, these isolates were identified as shown in Table 7. A total of 220 (83.3%) of these isolates were *S. epidermidis* and 44 (16.7%) were *Micrococcus* spp. No *S. aureus, S. saprophyticus* or *Aerococcus* spp. were detected among these isolates.

TABLE 7. *Differentiation of* Micrococcaceae (*gram-positive cocci, catalase-positive, oxidase-negative*) (*based on* 5,6,21,50, 57).

	Glucose		Mannitol		Novobiocin[a]	Hydrogen peroxide	Coagulase
	Ox.	Ferm.	Ox.	Ferm.			
S. aureus	+	+	+	+	S	-	+
S. epidermidis	+	+	-	-	S	-	-
S. saprophyticus	+	weak	+	-	R	-	-
Micrococcus spp.	V[b]	-	V	-	V	-	-
Aerococcus spp.	+	+	V	-		+	-

[a]S=sensitive to 0.6 μg novobiocin/ml; R=growth in the presence of 1.6 μg novobiocin/ml (25).

[b]5 =variable positive or negative reaction.

The 46 group II isolates were grown on 5% sheep blood agar plates and inoculated into nutrient broth for incubation at 45 C. The results indicated that these organisms were non-group D, gamma-hemolytic *Streptococcus* spp. The 91 group III isolates were checked for spore production and motility, and were identified as 50% *Bacillus* spp. and 50% *Corynebacterium* spp.

The group IV isolates were oxidase-positive, gram-negative rods. Using oxidative and fermentative reactions on glucose and motility and growth in nutrient broth at 42 C as differentiating tests, the 38 isolates were identified as *Moraxella* spp. (44%), *Flavobacterium* spp. (28%) and *Pseudomonas* spp. (28%). The group V isolates were oxidase-negative, gram-negative rods. They were also identified using oxidative and fermentative reactions on glucose and motility tests and, in the case of possible *Enterobacteriaceae*, by the BBL Minitek technique (Becton Dickinson Canada, Mississauga, Ontario). These 60 isolates were identified as *Acinetobacter* spp. (70%), *Pseudomonas* spp. (15%) and *Enterobacteriaceae* (15%) which included one *Escherichia coli* isolate and four *Klebsiella pneumoniae* isolates.

The incidence of isolates that failed to grow (Table 6) at time of identification was attributed to the length of time that they were stored on nutrient agar under refrigeration. However, this did not affect the results for the screening tests because they were done at the time that the isolate was originally taken from the plating medium.

DISCUSSION

This evaluation of germicidal hand dips and hand washes for use by food handlers was based on the ability of the agents to reduce the number of bacteria released from the hands after washing, hence reducing the potential for food contamination with bacteria from this source. The two methods for assessing the bacteria released could have different practical implications. Although the hand rinse sampling technique is generally considered to be more reliable, it might be argued that in many cases the finger tips are more significant to food contamination than the complete hand. The finger imprint method is often used for field studies because it is time saving and conducive to collaboration of workers (*52*); however, it is generally considered to be less reliable than other sampling techniques (*4,42,43,55*). In our studies, the finger imprint data gave more favorable efficacy data than the hand rinse data.

It has been suggested that germicidal hand wash agents should be evaluated by in-use tests, which closely resemble practical conditions (*41*). An important aspect of practical conditions for this study was the time of exposure to germicidal agents. Short exposure times have been reported to range from 10 to 30 s (*23*). Many hospital-oriented studies have used 30 s to 2 min exposure times, some have used 15 s (*42*). For this study, two successive 15-s washes were used because a 15-s wash was considered more likely to be achieved in practice. The results indicated that the second 15-s wash, included to indicate whether a total 30-s wash should be recommended, did not have a marked influence on the efficacy of the germicidal agents.

The correlation between the results for the two testing techniques in this study were relatively poor, representing a weak linear relationship between the two tests. Other workers (*4*) reported "fairly good correlation" between finger imprint and hand rinse techniques, but the correlation coefficients were not given. From this study it appears that the two sampling techniques are measuring different microbial parameters. However, there are possible differences in exposure times of the different areas of the hand in the hand wash experiment, as opposed to the hand dip experiment. In hand washing, a 5 ml portion of germicidal agent was applied to the palm of the hand and timing was begun. The agent was then spread progressively over the hand, commencing with the finger tips. Furthermore, the hand wash technique only used four different movements within the 15-s wash time and represented a practical type of hand wash that might be expected of food handlers. Other procedures have been more exhaustive, including rotational rubbing of the dorsum and fingers in the palm of the hand (*3*). Nonetheless, similar opinions of the value of agents have been reported by different workers using different techniques (*32*).

The results were initially analyzed on the basis of the log number of bacteria released from the hands. The significant differences between subjects and the same individual from day to day made such analyses meaningless. Similar results have been reported by other workers (*23*) and, hence, analyses were based on the 15-s and total of two treatments (30 s) "reduction factor" as used by Lilly et al. (*30*) and described by Rotter et al. (*48*).

Some agents, notably the non-germicidal soap, resulted in higher counts after the first 15-s wash than before washing. Similar increases in count have been reported for non-germicidal soaps (*3,9,30*), whereas others reported moderate decreases in counts after one and six applications (*27,28*). Bar soap has been cited as effective for control of transient microorganisms (*34*), so that little added value may be expected from germicidal hand washes for 30 s. In our studies some agents produced a significant reduction in the numbers of microorganisms released from the hands after the first and second 15-s exposures.

The hand dips used in the first experiment were generally unsatisfactory because they failed to give a reduction in numbers of bacteria released compared to non-germicidal soap. The only exception to this was the QAC hand dip. The positive electrical charge of the QAC on skin is considered to account for the retention of bacteria on the cutaneous surface (*8*). The use of QAC (1.2% benzalkonium chloride) was discontinued in another study (*10*) because of skin irritation and poor reduction of microbial counts. The iodophor hand dip with 25 ppm available iodine gave poor reduction of microbial counts compared to the iodophor hand wash containing 0.75% titratable iodine.

The bar soap containing 1/ trichlorocarbanilide (TCC) was included in these studies because it was the only form in which this germicide was commercially available. Results with this agent were disappointing, resulting in overall increases in numbers of bacteria released from the hands. A similar result was observed (*7*), but a change in flora from *S. epidermidis* to *Acinetobacter* and *Micrococcus* was reported. Successful studies have been reported with 1% and 2% concentrations of TCC (*47,58*). These concentrations of TCC were reported to give results similar to those for the same concentrations of hexachlorophene (*23*).

Many studies of germicidal agents have been designed to determine immediate and persistent (substantive) efficacy of the agents (*23,27,28,32,41*) by testing for bacterial reduction after one wash and after six successive washes, usually over a 2-day test period. In this study, the "immediate" efficacy was studied. However, the occurrence of a substantive effect over the 4-day test period was checked, but no substantivity was observed with any of the agents. Hexachlorophene used exclusively (*12,31*), and other agents including 4% chlorhexidine gluconate (*27,32,41*) and Irgasan DP 300 (*28*), have been reported to give cumulative effects.

Hexachlorophene was excluded from our studies because its use has been discontinued by many manufacturers, and Canadian regulations limit its use to

0.75% without medical prescription (*38*). Chlorhexidine was included as a medical reference agent, and iodophor was marketed both for medical use and as a germicidal hand wash agent for foodhandlers. These were the only products that resulted in a significant decrease in the numbers of bacteria released from hands in the hand wash experiment. Other researchers have reported even greater reductions in microbial contamination of hands with one application of 4% chlorhexidine gluconate (*4,30,32,42,52*) than we observed; however, some marked differences have been noted between different types of chlorhexidine preparations (*27,32,35,41*), Iodophor was reported to give 50 to 70% reduction of microflora on the hands (*32,41*) with poor cumulative action (*35*), yet up to 90% reduction after 6 successive treatments (*32*). In our studies, the iodophor hand wash gave 60 to 70% reduction of the bacteria released after 15-s exposure by the two techniques. None of these agents achieved levels of "virtual disinfection", i.e., 99.9% reduction in the number of bacteria released from the hands.

The other agents, including *para*-chloro-*meta*-xylenol (PCMX), Irgasan DP 300 and tribromosalicylanilide gave poor germicidal results in this experiment. PCMX is a well-known antiseptic agent, but there have been conflicting reports about its efficacy (*14*), especially related to formulation and in-use concentration. Irgasan DP 300 at a use concentration of 0.25% in this study gave poor results. Use of 0.6% of this agent was reported to be poor in reducing numbers of bacteria on the hands, and not much better than ordinary soaps (*41*), whereas 0.75% use concentration gave 20.9% reduction after one application and 56.2% after six applications (*28*). A concentration of 2% Irgasan DP 300 was reported to be necessary to achieve comparable results with those observed using 3% hexachlorophene or 4% chlorhexidine gluconate (*28*).

The predominating microorganisms isolated and identified in these studies were *S. epidermidis* and *Micrococcus* spp., typical of resident bacteria on skin that are harmless commensals (*17,40,53,54*). Anaerobic *Corynebacterium acnes*, which has been shown to predominate on skin of some individuals (*17*), would not be detected by our methods. The subjects selected for these studies were laboratory workers and foodhandlers; however, the foodhandlers were not working in a commercial food operation and did not have high levels of transient bacteria such as those described by Seligmann and Rosenbluth (*51*). The transient-type bacteria identified among the isolates were reduced after washing which is in agreement with other reports (*4,55*). The incidence of enteric-type organisms (coliforms and faecal *E. coli*) on hands has been associated with meat and foodhandling (*9,51*) and on hands of nurses in nurseries (*55*), but they are readily removed by hand washing, even with soap and water (*9*).

From the standpoint of reduction of microorganisms released from hands, many of the germicidal agents tested in this study were no better than non-germicidal soap. Notable exceptions to this were the hand wash agents containing chlorhexidine gluconate and iodophor. The former is not a product that would find ready acceptance in the food industry in its present form, however, the latter is marketed for both medical and food industry use. Microbiological studies indicated that most bacteria identified were resident rather than transient-type skin microorganisms. It is generally considered that transients may not act like resident (normal) skin flora in the washing process and that transient microorganisms represent the major concern in cross-contamination (*42*). It has been suggested that soap and water might be adequate for general hand washing and that germicidal agents would only be required for aseptic procedures (*4*). Our results, based on the desirability of reducing contamination of foods with organisms from hands, did not support this attitude. However, a study specifically related to the control of transient-type bacteria on hands would add to this information on the efficacy of germicidal hand wash agents for hygienic hand disinfection.

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