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Immediate and Residual (Substantive) Efficacy of Germicidal Hand Wash Agents

A. Z. SHEENA[1] and M. E. STILES[2*]

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

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ABSTRACT

A range of commercial hand wash agents was compared against 4% chlorhexidine gluconate (Hibitane) for immediate and residual (substantive) germicidal effect in hygienic hand disinfection. Chlorhexidine gluconate (4%) liquid detergent gave an immediate and residual reduction in number of microorganisms released from finger tips after a short exposure (15-s) hand wash. An iodophor product containing 0.75% available iodine gave comparable results for the immediate reduction of microorganisms released, but it did not give a residual effect. Other products, including those containing Irgasan DP 300, *para*-chloro-*meta*-xylenol (PCMX), or low concentration iodophor (0.005% available iodine) as the active ingredient, did not give an immediate or a residual reduction in microorganisms released from finger tips.

The retention of germicidal residues on skin after washing is referred to as residual effect or substantivity. This characteristic is attributed to the physicochemical binding of the agent to the uppermost horny layer of the skin (*11*). Substantivity gives the advantage of reducing the skin microflora and preventing its colonization with pathogenic microorganisms. This effect may last for a few days depending on the type of germicide and the frequency and exclusivity of use (*4,5,14*).

At one time, hexachlorophene was one of the most common germicides in use. It relied heavily on substantivity for its germicidal efficacy (*7,20*). However, the use of hexachlorophene has decreased because of its toxicological implications, and regulations restricting its use concentration to 0.75% without medical prescription (*12,16*). Alternative agents have been introduced to replace hexachlorophene, such as chlorhexidine, which has a dramatic, immediate effect on the resident and transient microflora of the skin (*1,5,7-9*), and an excellent residual effect (*7,9,13*).

Other germicidal agents with a reported residual effect include various phenolic derivatives, such as chloroxylenol and 5-chloro-2-(2,4-dichlorophenoxy)phenol (Triclosan or Irgasan DP 300). Chloroxylenol formulated with EDTA (ethylenediaminetetraacetic acid) is highly bactericidal for skin microflora, and the effect persists for a minimum of 2 h (*3*). Irgasan DP 300 used at 2% concentration has a residual effect (*7*), but at 0.6 and 0.75% use concentrations this effect is poor (*1,7,13*).

Iodophor is widely used as a germicide for disinfection of skin of hands and operation sites (*6,9,19*). It is generally used at 0.75% available iodine (*9,13*), and it is one of the most effective germicidal agents for removal of *Staphylococcus aureus* from the skin (*10*). Iodophor markedly reduces the number of bacteria on hands after six washes over two successive days (*9*); however, it does not have a residual effect (*13,15,19,20*).

In an earlier paper (*18*), we reported the efficacy of commercial hand washes and hand washing regimes used by foodhandlers. Only iodophor (0.75% available iodine) and 4% chlorhexidine gluconate gave a significant decrease in the number of microorganisms released from hands after washing for short exposure times. The purpose of this study was to determine the immediate and residual (substantive) effect of various germicidal agents in commercial preparations, in particular, products containing Irgasan DP 300 or *para*-chloro-*meta*-xylenol (PCMX).

MATERIALS AND METHODS

This study consisted of two separate experiments, both were Latin Square designs, one a 4×4 and the other a 6×6 design. The agents in Experiment I (4×4 design) included: (A) chlorhexidine gluconate (4%) liquid detergent solution (Hibitane, Ayerst Laboratories, Montreal, Canada) as a reference agent and three products containing 2,4,4'-trichloro-2'-hydroxy diphenyl ether (Irgasan DP 300, Ciba-Geigy Ltd., Switzerland) as the active ingredient: (B) an antibacterial gel skin cleanser containing 0.3% Irgasan DP 300; (C) germicidal liquid soap containing 0.5% Irgasan DP 300; and (D) agent C diluted to 0.25% Irgasan DP 300. The agents in Experiment II (6×6 design) included: (A') the chlorhexidine gluconate (4%) reference; (E) an iodophor hand wash containing 0.005% available iodine; (F) iodophor ("Tamed Iodine" Scrub, West Chemical Products Ltd., Montreal, Canada) containing 0.75% available iodine and three products containing *para*-chloro-*meta*-xylenol (PCMX) as the active ingredient: (G) a gel containing 3% PCMX; (H) antiseptic liquid hand soap (40%) containing 0.65% PCMX; and (I) agent

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

H diluted to 0.325% PCMX. Products D and I corresponded to products J and K in our earlier study (*18*).

Washing procedures in both experiments were identical and followed the method previously described (*18*), with two successive 15-s exposures and the finger tip sampling technique, using separate plates for each hand. Finger tip imprints were made onto letheen agar (Difco) plates, and the inocula were spread using a sterile, glass hockey stick. All plates were incubated at 35°C for 48 h. Hands were washed three times per day on two successive days, as performed in studies by Ayliffe et al. (*1*) and Lilly and Lowbury (*7,9*). No testing was done for the following 5 d to allow any residual effect to be dissipated and for the skin microflora to become re-established.

Samples were collected before and after the first and sixth washes, before each intermediate wash, and finally, without further treatment, the morning after the final wash had been completed (Table 1). Subjects were instructed to avoid the use of any other germicidal hand wash agents during the course of the experiments. They were permitted to use non-germicidal soaps, and no control was exercised over the contamination of hands.

Data were calculated as ratios of the number of organisms released from the finger tip after washing compared to the number released before washing. Data were analyzed in three different ways using $\log_{10}$ transformed ratios in a statistical computer package for Latin Square designs (BMDP2V, Biomedical computer programs, P-series, 1979, University of California Press). The three types of analysis included: (a) immediate reduction effect based on the change in number of bacteria released during the first treatment, Y_1/Y_0 and Y_2/Y_0; (b) the reduction effect after six successive treatments over two days, Y_8/Y_7 and Y_9/Y_7; and (c) the day to day residual (cumulative) effect based on Y_5/Y_0 and Y_{10}/Y_0.

RESULTS

The mean number of microorgansims and the percentage change as a result of the germicidal hand wash treatments are shown in Table 2. The 4% chlorhexidine gluconate and iodophor (0.75% available iodine) hand washes gave a marked reduction in the number of microorganisms released from the finger tips. In most other cases, the washing procedure resulted in an increase in the number of microorganisms released, except for the low concentration iodophor (0.005% available iodine) which caused a reduction in number of microorganisms released in the sixth

TABLE 1. *Washing and sampling protocol to determine the residual efficacy of germicidal hand wash agents.*

Day	Time	Wash sequence	Treatment	Sampling
1	8 am	1st	No treatment	Y_0 - Initial count
			1st 15-s wash	Y_1 - After 1st wash
			2nd 15-s wash	Y_2 - After 2nd wash
	11 am	2nd	2 × 15-s washes	Y_3 - Before washes
	2 pm	3rd	2 × 15-s washes	Y_4 - Before washes
2	8 am	4th	2 × 15-s washes	Y_5 - Before washes
	11 am	5th	2 × 15-s washes	Y_6 - Before washes
	2 pm	6th	Before treatment	Y_7 - Before washes
			1st 15-s wash	Y_8 - After 1st wash
			2nd 15-s wash	Y_9 - After 2nd wash
3	8 am		No treatment	Y_{10} - Final sampling

TABLE 2. *Percentage mean change in number of microorganisms released from finger tips after one and six washing sequences with germicidal hand wash agents*[a].

Agent	Mean number (percent) survivors after each treatment[b]					
	First wash sequence			Sixth wash sequence		
	Initial	1st 15-s	2nd 15-s	Initial	1st 15-s	2nd 15-s
	Number of microorganisms × 10^1					
Experiment I						
A	84.4	8.6 (26)	1.6 (2)	7.9	0.3 (18)	0.1 (8)
B	17.0	16.2 (90)	16.1 (89)	47.6	64.1(153)	60.4(151)
C	24.5	27.0(144)	43.6(251)	25.1	16.9 (70)	17.4 (72)
D	16.8	23.6(173)	27.4(219)	17.1	17.9(278)	21.2(332)
Experiment II						
A'	13.8	5.9 (61)	1.4 (13)	3.1	0.3 (14)	0.2 (8)
E	11.7	19.9(157)	20.8(179)	13.0	7.7 (45)	9.6 (53)
F	9.4	4.8 (51)	3.5 (37)	9.0	4.8 (40)	4.1 (32)
G	14.4	23.4(140)	24.3(148)	10.1	13.1(134)	14.4(145)
H	13.4	20.4(198)	21.7(193)	11.5	10.8 (90)	19.3(122)
I	15.6	20.0(161)	21.8(206)	24.4	29.5(136)	31.3(139)

[a]Hand wash agents: A and A' = 4% chlorhexidine gluconate (Hibitane); B,C,D = Irgasan DP 300, 0.3% gel, 0.5% and 0.25% hand washes, respectively; E and F = iodophor products, 0.005% and 0.75% available iodine, respectively; G,H,I = *para*-chloro-*meta*-xylenol (PCMX), 3% gel, 0.65% and 0.325% hand washes, respectively.

[b]Mean counts and mean percentage survivors calculated from individual changes in count for each subject after the first and sixth wash sequences; first wash sequence compared to first sampling, before treatment (Y_0); sixth wash sequence compared to sampling before sixth wash (Y_7).

TABLE 3. *Summary of Duncan's multiple range test (95% confidence level) for differences among treatment means for the initial (first) hand wash sequence (Immediate Efficacy).*[a,b]

After 1st 15-s wash

Experiment I: A B C D

Experiment II: A' F G I E H

After 2nd 15-s wash

Experiment I: A B C D

Experiment II: A' F G H E I

[a]Explanation of product codes given in Table 2.
[b]Agents underlined with an unbroken line were not statistically different at the 95% confidence level.

TABLE 4. *Summary of Duncan's multiple range test (95% confidence level) for differences among treatment means for the final (sixth) hand wash sequence.*[a,b]

After 1st 15-s wash

Experiment I: A C B D

Experiment II: A' F E H G I

After 2nd 15-s wash

Experiment I: A C B D

Experiment II: A' F E H G I

[a]Explanation of product codes given in Table 2.
[b]Agents underlined with an unbroken line were not statistically different at the 95% confidence level.

washing sequence. The results of the analyses of variance indicated a highly significant effect attributable to germicidal agents ($P<0.01$). The results for the Duncan's multiple range tests for these data are shown in Tables 3 and 4. The analyses confirmed that the 4% chlorhexidine gluconate and iodophor (0.75% available iodine) products resulted in a significant reduction in the number of microorganisms released from the finger tips compared to other agents. The iodophor wash (0.005% available iodine) was not significantly better than the PCMX agents.

The day to day residual or substantive effect is indicated by the initial number of microorganisms released from the hands before treatment with the germicidal agents. This was determined initially (Y_0), before each subsequent treatment (Y_3 to Y_7) and on the third day, i.e., the morning after the sixth treatment (Y_{10}). The mean percentage number of microorganisms released from finger tips before each washing compared to the initial count (Y_0) are shown in Table 5. The only product that gave a residual effect was 4% chlorhexidine gluconate. The initial number of microorganisms released from the finger tips was always less than the initial number released (Y_0), i.e., before the first treatment. The iodophor (0.75% available iodine) gave slightly reduced initial counts. The other agents failed to achieve a general reduction in the number of organisms released from the finger tips.

Analyses of variance were done on the $\log_{10}$ transformed ratio data for 24 h (Y_5/Y_0) and 48 h (Y_{10}/Y_0) after treatment was initiated. Levels of significance were $P<0.05$ for Experiment I, comparing Irgasan DP 300 products to 4% chlorhexidine gluconate, and $P=0.001$ for Experiment II, comparing PCMX and iodophor products to 4% chlorhexidine gluconate. Differences among the means were not as significant as might be expected. Duncan's multiple range test for differences among treatment means are shown in Table 6. In Experiment I, there were no significantly different products at 24 h, and only 4% chlorhexidine gluconate and 0.25% Irgasan DP 300 dif-

TABLE 5. *Percentage mean change in number of microorganisms released from finger tips before each hand wash treatment and the day after the final treatment (Residual Efficacy).*[a]

	Treatment					
Agent	2nd Y_3/Y_0	3rd Y_4/Y_0	4th Y_5/Y_0	5th Y_6/Y_0	6th Y_7/Y_0	Next day Y_{10}/Y_0
Experiment I						
A	36[b]	43	38	14	10	20
B	133	96	189	161	252	170
C	245	199	112	74	205	164
D	156	203	164	87	94	304
Experiment II						
A'	89	73	63	45	35	74
E	108	119	134	132	132	138
F	73	94	84	84	92	108
G	179	142	151	144	144	115
H	227	172	176	238	151	193
I	131	141	141	168	131	144

[a]See Table 2 for agent codes.
[b]Percent microorganisms released before each treatment compared to initial count (Y_0).

TABLE 6. *Summary of Duncan's multiple range test (95% confidence level) for differences among treatment means 24 and 48 h after the initial wash sequence (Residual Efficiacy).*[a,b]

After 24 h (before 4th wash Y_5/Y_0)

Experiment I: A C D B

Experiment II: A' F G E I H

After 48 h (morning after sixth treatment Y_{10}/Y_0)

Experiment I: A C B D

Experiment II: A' G F E I H

[a]Explanation of product codes given in Table 2.
[b]Agents underlined with an unbroken line were not statistically different at the 95% confidence level.

fered significantly at 48 h. In Experiment II, 4% chlorhexidine gluconate gave a significant reduction in number of microorganisms released compared to the low concentration (0.325 and 0.65%) PCMX products for both the 24- and 48-h tests. The results observed for 3% PCMX gel and the iodophor products were not significantly different from those for 4% chlorhexidine gluconate.

DISCUSSION

This study enabled an assessment of both the short- and long-term (residual) efficacy of these germicidal hand wash agents. The results for the immediate effect were in agreement with the results of our previous study (*18*), in which only 4% chlorhexidine gluconate and iodophor containing 0.75% available iodine gave a significant reduction in the number of microorganisms released from finger tips after washing for a short exposure time.

In this study, the higher concentrations of Irgasan DP 300 and PCMX products and the gel-based formulations failed to give a significant reduction in the number of microorganisms released from finger tips. This indicates that the poor results that we reported for Irgasan DP 300 and PCMX under our testing conditions (*18*) were not attributable to the lower use concentrations selected and were probably not a problem with formulation.

The experimental protocol involving six hand wash treatments over two successive days has been used by other researchers (*1,7,9*) to assess the efficacy of repeated use of germicidal hand wash agents. For chlorhexidine gluconate, a residual effect was observed which agreed with other reports (*1,7,9,19*). The iodophor (0.75% available iodine) reduced the number of microorganisms released from hands, but the effect was not significantly different and a residual effect was not indicated. The low concentration iodophor (0.005% available iodine) deserves further attention. Although it did not result in a significant reduction in number of microorganisms released from the hands on initial usage, after the sixth use a significant effect was observed. For the Irgasan DP 300 products, no residual effect was noted. However, literature references indicated a residual effect for this agent under various conditions of use and concentration (*2,3,7,17*).

The only agents that could be considered effective in reducing the number of microorganisms released from finger tips under these conditions of hygienic hand disinfection using short exposure times were the 4% chlorhexidine gluconate and iodophor (0.75% available iodine) products. The low concentration iodophor (0.005% available iodine) showed some promise and warrants further study because of the practical disadvantages of higher concentration iodophors, namely color and odor. This study indicated that alternative agents for hand washing with short exposure times are more likely to be found among the iodophor and chlorhexidine gluconate products than the Irgasan DP 300 or PCMX products.

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