

Efficacy of Germicidal Hand Wash Agents Against Transient Bacteria Inoculated onto Hands

A. Z. SHEENA¹ and M. E. STILES^{2*}

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 germicidal hand wash agents against transient bacteria (*Escherichia coli* and *Pseudomonas fluorescens*) in ground beef rubbed onto hands was determined using a hand rinse sampling technique. The reduction in *E. coli* and *P. fluorescens* counts on selective growth media and the change in count on Baird-Parker medium were used to indicate action against transient and resident bacteria, respectively. Most of the agents tested, including 4% chlorhexidine gluconate, iodophor (0.75% available iodine), Irgasan DP 300, *para*-chloro-*meta*-xylenol (PCMX) as well as the non-germicidal soap, gave marked reduction in counts of *E. coli* and *P. fluorescens* (>90% reduction), even after one 15-s wash. The hand dip treatments with iodophor (25 ppm available iodine), hypochlorite (50 ppm available chlorine) or QAC (930 ppm benzalkonium chloride) were generally less effective than hand wash treatments, especially against *P. fluorescens*. Iodophor (0.75% available iodine) and 4% chlorhexidine gluconate significantly reduced more *E. coli* on hands than the other agents.

Earlier studies by Sheena and Stiles (18,19) assessed the efficacy of a range of germicidal hand wash agents against the total microflora occurring naturally on hands. Dividing the skin microflora into transient and resident microorganisms has long been accepted (15). Transient microflora are those organisms acquired from the surroundings, which are superficially located on the skin, generally do not colonize the skin to become part of the resident microflora, and are readily removed by washing (10,11,17). Aerobic isolates identified after germicidal hand washing (18) were 85% *Staphylococcus epidermidis* and *Micrococcus* spp., indicating that the principal survivors after washing were organisms typically associated with the resident microflora of skin (16,17).

The method of application of transient microorganisms to the skin (9,13) and the species or strains of test organisms selected (2) play an important role in efficacy test-

ing. Koller et al. (8) observed differences in the level of contamination between finger tip and hand immersion contaminating techniques, but no significant difference in the efficacy of hand disinfection attributable to the method of contamination. In earlier studies, the transient microorganisms were spread on the skin (1,6,7,11,21); more recently, the natural work environment has been used to contaminate skin with a transient microflora (13,20). The natural environment approach was used in this study, with meat as the suspending agent.

Most studies have used germicidal products intended for medical use, including 70% ethyl alcohol, 70% isopropanol, alcoholic preparations of chlorhexidine, 4% chlorhexidine gluconate liquid detergent, 0.75% povidone iodine, 2% Irgasan DP 300 and 3% hexachlorophene (1,2,9,11,13). Information on the use of these agents in food handling using short exposure times is scant. In 1965, Crisley and Foter (4) reviewed antibacterial soaps for hand washing in foodservice establishments. They concluded that hand washing with non-germicidal soap was required to prevent transmission of possible pathogens from hands to foods during handling and preparation. Frequent hand washing by food handlers is considered mandatory to maintain hygienic conditions (5,14,17).

The object of this study was to evaluate and compare the efficacy of germicidal hand wash agents for control of transient microorganisms inoculated onto hands from meat.

MATERIALS AND METHODS

Two separate experiments were conducted, a 7×7 and a 5×5 Latin Square design, involving the exposure of each subject to each agent included in the experiment. The sequence in which agents were used by subjects was randomly assigned by a specified procedure (12). Each subject was exposed to all of the agents over the period of the experiment. Subjects used the assigned agent on two occasions, one 15-s exposure in the morning and two successive 15-s exposures in the afternoon. The hand washing procedure was detailed in our previous study (18). There were two testing days per week (Monday and Thursday) so that two different products were tested on each subject each week.

In the first experiment, seven agents were tested: (a) non-germicidal liquid hand soap; (b) chlorhexidine gluconate (4%) detergent solution (Hibitane, Ayerst Laboratories, Montreal) as a positive control; (c) an antibacterial gel skin cleanser containing 0.3% 2,4,4'-trichloro-2'-hydroxy

¹Department of Food Science.

²Department of Foods and Nutrition and Department of Microbiology.

diphenyl ether (Irgasan DP 300); (d) germicidal liquid soap diluted to 0.25% Irgasan DP 300; (e) antiseptic liquid hand soap containing 0.65% *para*-chloro-*meta*-xylenol (PCMX) diluted to 0.325% active ingredient at the use concentration; (f) iodophor ("Tamed Iodine" Scrub) containing 0.75% available iodine; and (g) germicidal bar soap containing 1.0% trichlorocarbanilide (TCC).

In the second experiment, five agents were tested: (a') non-germicidal liquid hand soap (same as "a" above); (h) iodophor hand wash containing 0.005% available iodine; and three hand dips consisting of (i) iodophor solution containing 25 ppm available iodine, (j) sodium hypochlorite solution containing 50 ppm available chlorine, and (k) quaternary ammonium (QAC) solution containing 930 ppm benzalkonium chloride. Hand wash agents were used in 5-ml amounts, the hand dip solutions were freshly prepared in 1.5 L of deionized water.

Two bacterial strains were isolated from ground beef for use in this study: *Escherichia coli* and *Pseudomonas fluorescens*. The *E. coli* isolate was identified and confirmed by comparison with a standard strain of *E. coli* from the American Type Culture Collection (ATCC; strain 11229) and a strain (1840) previously isolated from ground beef (M.E.S.). The *P. fluorescens* isolate was similarly compared to a strain of *P. fluorescens* from the National Collection of Type Cultures (NCTC) strain 10038 and a strain (R639) from the Alberta Laboratory for Public Health, Edmonton. Cultures were carried in nutrient broth. *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. Final concentrations for *E. coli* averaged 5×10^8 CFU/ml and *P. fluorescens* averaged 1.1×10^8 CFU/ml.

A ground beef inoculum was prepared to inoculate the test organisms onto subjects' hands. Freshly prepared ground beef from a local retail store was inoculated with the *E. coli* and *P. fluorescens* test cultures to give counts of 10^6 and 10^7 CFU/g of ground beef, respectively. This necessitated a 10^{-1} dilution of the *E. coli* culture in 0.1% peptone water, whereas the *P. fluorescens* culture was inoculated without dilution. The ground beef was checked each day to determine its microbiological quality, including total aerobic plate count, coliform, *E. coli*, *P. fluorescens* and total "gram-positive cocci" counts. The levels of *E. coli* and *P. fluorescens* inocula in the ground beef were also determined. The inoculated ground beef was dispensed in two 50-g amounts in separate petri dishes to use as inocula for the fingertips.

To reduce the microbial load on hands in preparation for the experiment, subjects' rinsed their hands with 5 ml of 95% ethanol containing glycerol, and the hands were rubbed together until they were dry (3,13). The finger and thumb tips were pushed into and held in the ground beef inoculum for 5 s and the inoculum was distributed over the hands by rubbing up to the wrists until the hands were dry. The hands were washed on two separate occasions with the same agent (morning and afternoon wash). One of the hands was randomly selected for sampling for the initial count (X_0) by rinsing in 100 ml of letheen broth (LB; Difco) in a plastic bag (28.5 × 12.5 × 7.5 cm, 25 mil, Polyrama Plastics Ltd., Edmonton) using the standard hand rinse method described in our previous study (18). Hand washing and dipping were also done according to the procedures previously described (18). The sample for the first 15-s wash (X_1) was taken at the morning wash. The sample for the two successive 15-s washes (X_2) was taken at the afternoon wash and compared with the initial (X_0) count for the afternoon testing period. Subjects rinsed their hands with the glycerol in ethanol solution after the sampling procedure had been completed.

Bacteriological testing of the hand rinse samples was done by plating in duplicate onto the following Difco media; standard plate count agar (SPC), violet red bile agar (VRBA), *Pseudomonas* agar F (PAF) and Baird-Parker agar (B-P). Two sets of VRBA plates were prepared for comparison of the differential incubation temperatures. After washing hands with germicidal agents, such as chlorhexidine gluconate (4%) liquid detergent or iodophor (0.75% available iodine), injury levels between 50 and 90% were observed when the organisms were grown on selective media compared to growth on tryptic soy agar (TSA). Holding LB samples at room temperature for 1 to 2 h allowed resuscitation of injured organisms for growth on selective media. Holding LB samples for greater than 2 h resulted in growth of the microorganisms.

The X_0 LB samples were plated immediately onto agar media; the X_1 and X_2 LB samples were held 1.5 h at 20°C for recovery of any injured

cells. Initial studies indicated that this treatment of samples reduced the level of injury for growth on selective media. X_0 samples were diluted 1:10 with 0.1% peptone water for pour plating with VRBA and overlaid with 5 ml of the VRBA medium. X_1 and X_2 samples were pour plated on VRBA without dilution. Prepared plates of SPC, PAF and B-P were surface streaked with 0.1-ml portions of samples. The two sets of VRBA plates were incubated separately at 35 and 45°C for 24 h. SPC and B-P plates were incubated at 35°C for 48 h, and PAF plates were incubated at 20°C for 72 h.

Data were calculated as ratios of the number of organisms released from hands after washing compared to the number released before washing. Mean counts and mean percentage change in number of bacteria released from hands were based on individual changes in count for each subject. The data were analyzed 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).

RESULTS

The microbiological techniques allowed five microbial parameters to be monitored, including transient and resident microflora. The SPC count was intended to determine the total transient and resident flora. The VRBA counts determined the efficacy of the agents against *E. coli* (incubated at 45°C) and total coliform-type bacteria (incubated at 35°C). The correlation between VRBA counts at 45 and 35°C was $r > 0.98$, indicating that the transient *E. coli* strain inoculated onto hands predominated the VRBA counts at both temperatures. Only data for VRBA at 35°C are presented. PAF counts were used to indicate the efficacy of the agents against *P. fluorescens*. The *P. fluorescens* strain grew as distinctive fluorescent yellow colonies on PAF medium. Only typical colony types on the PAF medium were included in the presumptive *P. fluorescens* count. Initially, typical colonies were isolated from the PAF plates and confirmed as *P. fluorescens* by comparison with reference strains. B-P medium was used to monitor *Micrococcaceae*-type organisms released from the hands, which were considered to represent typical resident microflora as well as organisms possibly acquired from the ground beef. A summary of the probabilities of a significant effect attributable to agents is shown in Table 1.

Data for the percentage mean reduction in total count of transient and resident flora on hands monitored using SPC counts at 35°C are shown in Table 2. The reduction in number of bacteria released from hands as a result of one and two successive 15-s washes was not impressive. All products gave reduced counts as a result of the treatments, implicating the transient flora in this measure. Only three agents (4% chlorhexidine gluconate and the iodophor products containing 0.75 or 0.005% available iodine) achieved 80% or slightly greater reduction in count with one 15-s wash. Non-germicidal soap achieved a 75% reduction. After two successive 15-s washes, most agents (including the non-germicidal soap) achieved virtually 80% reduction in SPC counts. Only the 4% chlorhexidine gluconate and Irgasan DP 300 (0.25%) washes gave better than 90% reduction. In Experiment I there was a significant effect attributable to agents (see Table 1).

Duncan's multiple range tests for differences among treatment means are shown in Table 3. On SPC medium,

TABLE 1. Summary of probabilities (*P*) of a significant effect attributable to agents as a result of Latin Square design analyses of variance.

Medium ^a	Experiment I		Experiment II	
	After 1 × 15-s wash	After 2 × 15-s washes	After 1 × 15-s wash	After 2 × 15-s washes
SPC	0.0415 ^b	0.0468 ^b	0.4218	0.4946
VRBA (35)	0.0239 ^b	0.0290 ^b	0.1612	0.3188
VRBA (45)	0.0450 ^b	0.0061 ^c	0.5113	0.5211
PAF	0.1743	0.3336	0.9876	0.0246 ^b
B-P	0.3382	0.4651	0.2412	0.0534 ^b

^aMicrobiological media: SPC, standard plate count agar; VRBA, violet red bile agar; PAF, *Pseudomonas* agar F; B-P, Baird-Parker agar.

^bSignificant at the 95% confidence level ($P < 0.05$).

^cSignificant at the 99% confidence level ($P < 0.01$).

after one 15-s wash, only 4% chlorhexidine gluconate and iodophor (0.75% available iodine) showed a significantly greater decrease in count compared to the Irgasan DP 300 wash and the TCC bar soap. After two successive 15-s washes, only PCMX gave a significantly poorer result than the chlorhexidine and iodophor (0.75% available iodine) products. The rest of the agents, including the non-germicidal soap, were not significantly different from these two agents.

Results of the efficacy of the germicidal agents against *E. coli* are shown in Table 4. All agents, including the non-germicidal soap, gave greater than 90% reduction in count. Only 4% chlorhexidine gluconate achieved 99% re-

duction after two successive 15-s washes. A significant effect was attributed to agents in Experiment I (see Table 1). Duncan's multiple range tests for differences among treatment means (see Table 3) revealed that, after one 15-s wash, the iodophor (0.75% available iodine) gave a significantly greater reduction than all other agents, except 4% chlorhexidine gluconate. After the two 15-s wash sequences, significant differences were observed. However, 4% chlorhexidine gluconate was not significantly more effective against *E. coli* than iodophor (0.75% available iodine), 0.3% Irgasan DP 300 gel and PCMX (0.325%) antiseptic hand soap. These agents, except PCMX, gave a significantly greater reduction of *E. coli* compared to the non-germicidal soap.

Data for *P. fluorescens* are shown in Table 5. Results similar to those for *E. coli* were observed, except that the TCC bar soap (one 15-s wash) and dip methods of hand washing only gave 80 to 90% reduction in *P. fluorescens* count, whereas other agents gave greater than 90% reduction. The only significant effect attributable to agents in Experiment II was against *P. fluorescens* (see Table 1). Duncan's multiple range tests on these data indicated a significantly greater decrease in *P. fluorescens* count attributable to the iodophor wash (0.005% available iodine) and non-germicidal soap compared to iodophor and hypochlorite dips (Table 3).

Changes in the "resident" microflora measured on B-P medium are shown in Table 6. These changes showed a marked difference to the trends observed for the "transient" microflora (Tables 2, 4 and 5). The non-germicidal soap caused a marked increase in the number of microor-

TABLE 2. Reduction in total number of bacteria (counts on SPC) released from hands as a result of one or two successive 15-s hand wash treatments.

Agent ^a	Initial count	After		Initial count	After 2 × 15-s washes
		1 × 15-s wash	[Mean count × 10 ² (percent)] ^b		
<i>Experiment I</i>					
A. Control soap	11.0	2.9 (75)		10.0	2.1 (79)
B. Chlorhexidine	11.0	1.8 (85)		9.4	0.3 (96)
C. Irgasan gel	7.6	2.7 (66)		8.5	1.6 (80)
D. Irgasan wash	9.9	4.3 (58)		9.4	1.0 (90)
E. PCMX	8.1	3.2 (65)		7.8	2.4 (74)
F. Iodophor	11.0	1.8 (81)		8.2	0.7 (88)
G. TCC	7.4	3.0 (56)		8.6	1.8 (80)
<i>Experiment II</i>					
A'. Control soap	8.5	3.5 (48)		11.0	1.6 (82)
H. Iodophor wash	12.0	2.5 (80)		9.9	0.9 (84)
I. Iodophor dip	12.0	3.4 (66)		11.0	1.8 (84)
J. Hypochlorite dip	8.7	3.4 (62)		13.0	2.4 (81)
K. QAC dip	9.9	3.3 (67)		10.0	2.6 (76)

^aHand wash agents: A and A', non-germicidal liquid hand wash; B, 4% chlorhexidine gluconate (Hibitane); C, Irgasan DP 300 (0.3% gel); D, Irgasan DP 300 (0.25%) hand wash; E, *Para*-chloro-*meta*-xylenol (PCMX) (0.325%) hand wash; F, Iodophor (0.75% available iodine); G, 1% Trichlorocarbanilide (TCC) bar soap; H, Iodophor hand wash (0.005% available iodine); I, Iodophor hand dip containing 25 ppm available iodine; J, sodium hypochlorite dip containing 50 ppm available chlorine; K, Quaternary ammonium (QAC) dip containing 930 ppm benzalkonium chloride.

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

TABLE 3. Summary of Duncan's multiple range test (95% confidence level) for differences among treatment means on different microbiological media^{a,b,c}.

Experiment I	
(i) SPC	
After 1 × 5-s wash	F B <u>A C E D G</u>
After 2 × 15-s washes	B F <u>D A C G E</u>
(ii) VRBA	
After 1 × 15-s wash	F <u>B E G D A C</u>
After 2 × 15-s washes	B F C <u>E D G A</u>
Experiment II	
(i) PAF	
After 2 × 15-s washes	H A' <u>K I J</u>
(ii) B-P	
After 2 × 15-s washes	K J <u>H I A'</u>

^aFor key to product codes see Table 2.

^bMicrobiological media: SPC, standard plate count agar; VRBA, violet red bile agar; PAF, Pseudomonas agar F; B-P, Baird-Parker agar.

^cAgents underlined with an unbroken line are not statistically different at the 95% confidence level.

organisms released from hands after one or two successive 15-s washes. This applied also to most of the germicidal washes or dips except 4% chlorhexidine gluconate, iodophor (0.75% available iodine) and the QAC dip. QAC dip treatment gave a significant reduction in number of microorganisms released from hands compared to iodophor dip and non-germicidal soap.

DISCUSSION

Results of this study on transient bacteria inoculated

onto hands from meats contrasts markedly with our earlier results for the total hand microflora (18,19). However, the previous studies involved primarily the resident microflora. Both test organisms included in this study were gram-negative bacteria originally isolated from meat. *E. coli* was selected because of its role as an indicator organism and as a possible indicator of what might happen to related pathogenic bacteria, such as *Salmonella* (14). *P. fluorescens* was selected to indicate activity against spoilage-type bacteria. This particular strain was selected because of its pigmentation on PAF that made it easy to detect in mixed culture.

These transient bacteria from meats inoculated onto hands were markedly reduced by the short-exposure washes used in this study. Effective reduction or elimination of transient microflora by non-germicidal soap has been widely reported (2,11,13,20); however, more recent studies with agents such as alcohol, povidone-iodine and chlorhexidine have given better results than non-germicidal soaps (3,6,9,13). Our results confirmed the improved action of 4% chlorhexidine gluconate and iodophor (0.75% available iodine) against *E. coli* compared to non-germicidal soap.

In our studies on resident microflora (18,19), the efficacy of germicidal products containing Irgasan DP 300 or para-chloro-meta-xyleneol (PCMX) for short-exposure hand washing was not satisfactory. However, against the transient bacteria in this study, these agents were far more effective. Trichlorocarbanilide (TCC) bar soap and hand dips gave the least effective results against transient bacteria. Results of this and our earlier study (18) favor hand washing over hand dipping techniques both for the bacteriological results as well as practical control.

The use of Baird-Parker (B-P) agar to monitor resident flora was justified by the similarity of these results compared to those obtained on the non-selective medium used in our previous study (18). Coagulase-negative

TABLE 4. Efficacy of germicidal hand wash agents against *Escherichia coli* artificially inoculated onto hands from ground meat (based on count on VRBA incubated at 35°C)^a.

Agents	Initial count	After		
		1 × 15-s wash [Mean count × 10 ² (percent)]	2 × 15-s washes	
Experiment I				
A. Control soap	8.4	0.6 (94)	6.0	0.4 (95)
B. Chlorhexidine	7.9	0.2 (97)	6.5	0.1 (99)
C. Irgasan gel	6.3	0.3 (92)	11.0	0.2 (98)
D. Irgasan wash	6.8	0.4 (93)	5.7	0.2 (97)
E. PCMX	5.0	0.2 (95)	3.4	0.1 (98)
F. Iodophor	6.3	0.1 (98)	3.6	0.1 (98)
G. TCC	6.1	0.3 (94)	7.8	0.2 (97)
Experiment II				
A'. Control soap	7.2	0.3 (95)	8.8	0.2 (97)
H. Iodophor wash	9.7	0.4 (96)	5.8	0.1 (98)
I. Iodophor dip	11.0	0.5 (96)	8.4	0.2 (97)
J. Hypochlorite dip	5.7	0.4 (93)	10.0	0.4 (96)
K. QAC dip	8.9	0.3 (97)	8.2	0.4 (97)

^aSee footnotes of Table 2.

TABLE 5. Efficacy of germicidal hand wash agents against *Pseudomonas fluorescens* artificially inoculated onto hands from ground meat (based on count on PAF)^a.

Agent	Initial count	After 1 × 15-s wash	Initial count	After 2 × 15-s washes
[Mean count × 10 ² (percent)]				
Experiment I				
A. Control soap	4.3	0.2 (96)	3.4	0.1 (98)
B. Chlorhexidine	3.2	<0.1 (98)	2.5	<0.1 (98)
C. Irgasan gel	2.4	0.2 (93)	3.3	0.2 (95)
D. Irgasan wash	2.9	0.2 (95)	3.7	0.2 (93)
E. PCMX	2.9	0.1 (97)	2.6	<0.1 (99)
F. Iodophor	2.6	0.1 (96)	2.5	<0.1 (99)
G. TCC	2.7	0.4 (88)	3.2	0.2 (92)
Experiment II				
A'. Control soap	3.5	0.2 (93)	3.6	0.2 (96)
H. Iodophor wash	4.1	0.2 (95)	3.1	0.1 (96)
I. Iodophor dip	3.9	0.3 (90)	3.8	0.5 (83)
J. Hypochlorite dip	3.9	0.6 (87)	4.2	0.8 (80)
K. QAC dip	3.5	0.5 (88)	4.4	0.5 (90)

^aSee footnotes of Table 2.

TABLE 6. Mean count and percentage change in residual-type (*Micrococcaceae*) bacteria released from hands after use of germicidal hand wash agents measured by growth on Baird-Parker agar (based on count on B-P)^a.

Agent	Initial count	After 1 × 15-s wash	Initial count	After 2 × 15-s washes
[Mean count × 10 ² (percent)]				
Experiment I				
A. Control soap	0.9	1.2 (291)	0.7	0.4 (113)
B. Chlorhexidine	1.5	0.5 (83)	0.2	<0.1 (57)
C. Irgasan gel	0.6	0.4 (88)	0.2	0.3 (119)
D. Irgasan wash	0.4	0.6 (246)	0.1	0.3 (192)
E. PCMX	1.1	2.4 (275)	0.6	0.7 (160)
F. Iodophor	2.1	1.4 (83)	0.5	0.2 (74)
G. TCC	1.9	2.6 (134)	0.4	0.9 (158)
Experiment II				
A'. Control soap	0.9	2.3 (313)	0.2	0.7 (263)
H. Iodophor wash	0.7	1.5 (226)	0.4	0.7 (155)
I. Iodophor dip	1.3	1.5 (137)	0.1	0.4 (242)
J. Hypochlorite dip	1.6	1.7 (246)	0.7	0.4 (234)
K. QAC dip	1.5	1.9 (84)	0.7	0.2 (68)

^aSee footnotes of Table 2.

staphylococci are part of the resident microflora of skin (15,17). The preferential activity of QAC's against gram-positive bacteria might account for the favorable result of the QAC dip against "resident" flora. This confirmed our previous observation of the efficacy of a QAC dip (18).

Coliform bacteria and associated enteric pathogens are generally absent from skin, except in some special studies of food handlers (14). *Staphylococcus aureus* is generally associated with the nasal cavity (22) but it may be carried on skin as part of the transient microflora (11). The incidence of coagulase-positive staphylococci is greater among meat handlers, with a tendency for these organisms to become part of the resident skin microflora (16,17). This weighs in favor of the selection of germicidal hand washes as opposed to non-germicidal soaps for hygienic hand dis-

infection of food handlers. Bacteriologically, 4% chlorhexidine gluconate and iodophor (0.75% available iodine) remain the agents of choice for their better action against resident and transient skin bacteria.

The 4% chlorhexidine gluconate product (Hibitane) was selected as a reference agent for this study because of its use in medical practice. It is probably unsuitable for food handlers in this formulation. However, our studies with 4% chlorhexidine gluconate detergent solution confirmed its marked residual (substantive) effect (19) and a need for special care in neutralizing its antibacterial activity for efficacy testing (18). Iodophor (0.75% available iodine) and equivalent products with relatively high concentrations of iodine are used in medical practice and by food handlers. There is resistance to the latter products because of color

and odor, hence the low iodine products, such as product H (0.005% available iodine), warrant further study.

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