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PREFACE

This report covers studies conducted from October 1991 through October 1992 on development of a waterless sanitation system to support Mobile Field Kitchens in the field. The waterless system would serve as an emergency back-up cleaning and sanitation system, in the event that hot water or a potable water supply is not available. The system can also be used to conserve water, when it is more urgently needed for cooking and drinking.

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Towellette Sanitation System For Mobile Kitchen Trailers

Introduction

Mobile and individual field feeding systems have an urgent need to develop a field sanitation system that cleans and degreases food cooking and serving utensils without water. A waterless sanitation method is also needed to clean and sanitize individual mess gear when disposable mess gear and/or a water supply are not available. The immersion heater and 32 gallon can sanitation equipment presently used (1,2), require large quantities of water, are manpower intensive, and do not allow total submersion of large pots. Because water may not be readily available in all theater and scenarios, a waterless (towellette) food service sanitation capability will give the mobile kitchen trailers (MKT) unprecedented range and independence and completely eliminate its reliance on water. The towellettes also will serve as a backup system when either hot water or a potable water supply is unavailable or is being conserved for drinking.

A 1983 Army study of several commercial wipes to sanitize individual eating utensils resulted in the selection of a "Pre-Op" wipe containing iodine (3). However, the "Pre-Op" wipe was not waterless because 10 mL of water was required to moisten it and 100 mL was required to rinse utensils between wipes; two wipes were required for each utensil. Several problems with the "Pre-Op" wipe were uncovered when field tested at Fort Devens in 1984 (4), which resulted in termination of the project. For example, too much water was used, "gear" was not clean enough to eat from, wipe was too small, "gear" was stained and had an unpleasant odor. A premoistened,

disposable towallette for personal sanitation was investigated in 1986 (5), but because the study was not completed, it has not been adopted by the military. The personal wipe was to be used in place of a shower to conserve water in an emergency and was not suitable for food contact surfaces. A more recent study demonstrated the effectiveness of a premoistened, disposable wipe for improving raw milk quality, by cleaning cow teats (6). However, the ingredients were also not suitable for food contact surfaces.

More than 100 commercial cleaning/degreasing agents were tested by the Army in search of an agent that would emulsify and remove grease from pots and pans at low temperatures (7). The failure of the prototype selected, Mikroklene^R (Ecolabs, St. Paul, MN), to cut grease at 15°C - 20°C continued the need for a cold water cleaner/degreaser for use in the field, to the present time. McCormick and Flaig (8) demonstrated that pots and pans could be successfully cleaned and sanitized at 15°C by hand scrubbing with Vesta Power solution (Calgon Vestal Laboratories, St. Louis, MO) followed by a water rinse and exposure to Syn-Cide solution (Calgon Vestal Laboratories), a quaternary ammonium sanitizing agent. This system was also successfully employed at 20°C by soldiers in the field during an exercise.(8)

The objective of this study was to develop and test a waterless sanitation system that would employ a combination of premoistened, disposable wipes incorporating detergents and sanitizers that are effective in cold water. The basic principles of cleaning and sanitizing would be observed by employing a wash cycle embodied in wipe #1, containing a detergent; a rinse cycle embodied in wipe #2, containing water; and a sanitizing cycle embodied in wipe #3, containing a sanitizer. A prewash cycle as embodied by a dry towel will be used to remove gross food residues when necessary.

MATERIALS AND METHODS

Detergent/Degreaser

Vesta Power (VP, Calgon Vestal Laboratories, St. Louis, MO) is a detergent degreaser that is authorized by the U.S. Department of Agriculture (USDA) for use in Federally inspected meat and poultry plants. A 5% aqueous solution was used. Sodium metasilicate is the active ingredient that emulsifies fat and grease.

Sanitizer

Syn-Cide Plus (SC, Calgon Vestal Laboratories, St. Louis, MO) is a quaternary ammonium (QA) disinfectant/sanitizer. It was used at 150 ppm. Syn-Cide is recommended for use in restaurants and food processing areas and is also authorized by the USDA for use in federally inspected meat and poultry plants. No water rinse is required after application and because it is a quaternary ammonium compound (QAC), a germicidal residual remains on surfaces.

Preparation of Wipes

Wipes were prepared for cleaning and sanitizing by soaking the towel in the use dilution of detergent and sanitizer, or in distilled water equilibrated to 5°C and 26°C. Excess liquid was removed by allowing the towels to dry at the appropriate temperature only to the point when they no longer dripped while hanging on a line. The towels were used immediately.

Towels

Five towel types were evaluated. Four towels were 4-ply paper and one, Webril, was cloth (100% textile fiber). See Table 1. Absorbency was determined by soaking the towels in a measured volume of water, allowing the towel to drip back into the water until dripping stopped, and then measuring the volume of water remaining. Wet strength was determined by adding weights to the wet towel until they broke through. The towel was taut and carefully secured over the top of a round (11-inch diameter) stainless steel basket. See Table 2.

Test Organisms

Escherichia coli ATCC No. 11229 (American Type Culture Collection, Rockville, MD) and Staphylococcus aureus ATCC No. 6538 (9) were used as the challenge organism in foods used to soil surfaces and produce biofilms. Both organisms were cultured in trypticase soy broth (Difco, Detroit, MI) at 35°C for 24 hours. A mixed inoculum was achieved by mixing equal volumes of the two cultures. Stock cultures were carried in Plate Count agar and Cystine Trypticase agar (Difco).

Soiling Surfaces with Foods

Foods received a mixed inoculum of E. coli and S. aureus by mixing equal volumes of the two bacterial cultures to achieve approximately 10,000 bacteria per gram. Pure cultures, at the same concentration, were also added to selected foods. One mL of inoculum was blended with 100 grams of the food sample by stomaching (10) for one minute. Stainless steel pans measuring 12 inches by 12 inches were soiled by spreading 100 g of the inoculated food over the bottom, inside surface of the pan. Soiled pans were then incubated

at 35°C for 24 hours to produce a biofilm. After gross food residues were removed from the pan by scraping and/or wiping with a dry towel, the surface was air dried at 5°C or at 26°C, for 1 to 3 hours, depending on the temperature being tested. To test surfaces for the removal of fat or grease, bacon was either fried in the pan and allowed to cool, or bacon fat was melted and poured onto the bottom of the pan. Naturally soiled surfaces in food preparation areas were also tested.

Biofilms Produced by E. coli

a. Skim Milk

Biofilms were produced on stainless steel surfaces by growing E. coli in 500 mL of Skim milk (Difco) added to a 12" x 12" electric frying pan. The frying pan was presterilized by flaming three times with absolute alcohol, and covered. Following incubation for 24 hours at 35°C, plate counts of the milk were performed. The milk was poured off and the surface was air dried at 26°C for 5 minutes before it was swabbed and wiped with towellettes.

b. Phosphate Buffer Suspension

E. coli was washed off the surface of plate count agar (PCA, Difco) and adjusted turbidimetrically (Turbidimeter, Model 16800, Hach Company, Loveland, CO) to 20 million colony forming units (CFU) per mL in Butterfields phosphate buffer (10). One half mL of the suspension was spread over the bottom surface of the sterilized stainless steel frying pan and air dried at 26°C for 5 minutes.

Cleaning and Sanitizing Surfaces

Gross food residues were removed from the pans by wiping with a dry towel. The soiled surface, equilibrated to the test temperature, was then wiped with a towel moistened with Vesta Power detergent (wipe #1) until the surface appeared clean and greaseless. Residual detergent was removed by wiping the surface with a towel moistened with distilled water (wipe #2). The surface was then sanitized by wiping for 1 minute (wet contact time) with a towel moistened with Syn-Cide Plus (wipe #3). It is important to keep the surface wet for 1 minute by the application of wipe 3, in order to inactivate microorganisms remaining on the surface. All towellettes were equilibrated to the appropriate test temperature before application.

Enumeration Methods

Standard aerobic plate counts in PCA were performed on foods used to soil the surfaces (10). Bacteria remaining on 40 square inches of surface preceding and following the application of each wipe (towel) was determined by swabbing five, 8-square-inch areas (11) with a single swab (Millipore swab buffer unit, Bulletin AB 820, Millipore Corp., Bedford, MA 01730). The buffer contained neutralizing agents to counteract the adverse affect of any residual chlorine or quaternary ammonium compounds that may be present on surfaces after sanitation. After manually shaking the swab in the buffer for one minute, appropriate dilutions were made in Butterfields phosphate buffer. One mL of each dilution was deposited into duplicate petri plates and poured with PCA. To obtain selective counts of injured as well as noninjured cells, Baird Parker agar (Difco) was used to recover S. aureus, and trypticase soy agar (Difco) overlaid with violet red bile agar (Difco), after incubation for two hours at 35°C, was used to recover E. coli. All plates were incubated at 35°C for 48 hours.

RESULTS

Towels

The type, size, cost and source of each towel evaluated is shown in Table 1. Four of the towels were paper and one was cloth (Webril). They ranged in price from 6 cents to 13 cents each. The choice of the towel will depend on absorbency, wet strength (Table 2), and, all things being equal, the cost. The towels must be packaged and sterilized. All three wipes (detergent, water and sanitizer), supported growth of bacteria and molds on the towels when stored at 5°C and 26°C in packages sealed with and without a vacuum. Microorganisms recovered on Kimtowels and Sturdi-wipes wetted with VP, SC, and water, included Gram positive sporeforming and nonsporeforming bacilli, Gram negative bacilli, Gram positive cocci, yeast and molds.

Table 1. Specifications of Towels Evaluated for Wipes

Wipes	Mfr	Thickness	Material	Size (in)	Cost
Kimtowels ^R	Kimberly Clark	4 Ply	Paper	12 x 15	0.11
Lab Kimtowels ^R	Kimberly Clark	4 Ply	Paper	17 x 20	0.13
Kaypees ^R	Tidy Products	4 Ply	Paper	14 x 18	0.06
Sturdi-wipes ^R	Scott	4 Ply	Paper	13 x 15	0.08
Webril ^R	Kendall	a	Cloth	12 x 12	0.064

^aWebril towel is 100% textile fiber.

Table 2. Average Weight, Absorbency, and Wet Strength of Paper Towels^a

Towel	Size	Weight grams	Absorbency		Wet Strength (grams)
			mL/towel	Capacity	
Kimtowel	17" x 20"	18.19	91	5x	1764
Sturdi-wipe	13" x 13"	10.8	48	4x	1593
Kaypees	14" x 18"	10.4	47	4.5x	360
Lab Kimtowel	12" x 15"	8.1	49	6x	895
Webril	1" x 12"	3.26	17.2	5.25x	4269

^aAverage of four measurements

Detergent/Sanitizers

The properties of candidate detergent/sanitizing compounds are shown in Table 3. Vesta Power (VP) was the detergent selected for incorporation into a wipe because it demonstrated superior cleaning and degreasing properties as a liquid at temperatures as low as 15°C, when compared to 58 other cleaning agents (8). Syncide Plus (SC) was the sanitizer selected for incorporation into a wipe because of the shorter wet contact time (1 minute), it does not require a rinse following application, and a germicidal residual remains on surfaces. It was also shown to be effective as a liquid sanitizing rinse of kitchenware in the field (8). Both VP and SC are authorized by the USDA for use in federally inspected meat and poultry plants. Wipex towellettes are not approved for food contact surfaces at the present concentration of its active ingredient.

Table 3. Properties of Candidate Detergent and Sanitizers

Agent	Type	Mfr	Conc	Active Ingrid	Wet Contact Time
Vesta Power	Detergent	Calgon	3-5%	SMS ^a	Clean
Syn-Cide Plus	Sanitizer	Calgon	150 ppm	QA ^b	1 Min
Mandate	Sanitizer	Klenzade	1300 ppm	Fatty Acids	2 Min
K-San	Sanitizer	Klenzade	100 ppm	QA+Acid	2 Min
Sani-Cloth	Sanitizer	Nice-Pak	5-8%	QA+Alcohol	5 Min
Wipex ^c	Sanitizer	Winfield	10%	QA	5 Min

^aSMS = Sodium Metasilicate

^bQA = Quaternary Ammonium

^cWipex does not yet have FDA approval for food contact surfaces

Bactericidal Efficacy of Wipes

A stainless steel frying pan was contaminated with 0.5 mL of a phosphate buffered suspension of *E. coli* and then was cleaned and sanitized with 3 wipes applied in sequence (Table 4). Before applying the wipes, the *E. coli* counts ranged from 594 to 2196 CFU per 40 square inches. The VP wipe (wipe #1) reduced the counts to zero, so there were no counts obtained after application of wipes 2 (water) and 3 (sanitizer). Apparently *E. coli* was not firmly attached in the absence of a suitable substrate.

Table 4. Bactericidal efficacy of wipes on stainless steel frying pan surface

Wipes	E. coli CFU ^a
	Per 40 square inches
None	594 648 2196
Vesta Power	0
Water	0
Syn-Cide Plus	0

^aColony Forming Units - Three repetitions

Table 5 shows the reduction of microbial contaminants from naturally soiled surfaces in a bakery and a military kitchen. Reductions on stainless steel surfaces were greater than on the wood surface. As expected, wood is more difficult to clean and sanitize than stainless steel, because of cracks and crevices and the porous nature of wood. However, the wiping regimen effectively reduced indigenous counts by more than 96 percent after application of the SC wipe. Indigenous counts on stainless steel were reduced by more than 98% to 100%.

Table 5. Bactericidal efficacy of wipes on countertops in food preparation areas^a

Wipe	Percent Reduction on Countertops ^b	
	Wood	Stainless Steel
None	0	0
Vesta Power	>75	78 - >96
Syn-Cide Plus	>96	>98 - 100

^aNatick bakery and Headquarters Company dining hall kitchen

^b40 square inches of counter top (5 x 8 sq. in. areas swabbed)

Table 6 shows the efficacy of the three wipes, applied in sequence, on the reduction of bacteria in biofilms produced on stainless steel (frying pan). The biofilms were produced by mixed and pure cultures of E. coli and S. aureus in various foods and skim milk. The bacteria grew in the food to more than 10^9 organisms per gram. After removal of gross food residues, the soiled pan and the towellettes moistened with the detergent, water and sanitizer were equilibrated to 5°C and 26°C for one to three hours. The surfaces were then swabbed for bacterial recovery before and after application of each wipe. After application of all three wipes, bacterial reduction was greatest at 26°C and exceeded five logs (99.999%). A five log reduction was also achieved at 5°C except in biofilms produced in beef stew and corn beef hash, in which reductions were 99.98 and 99.99%, respectively. Wiping the surfaces with VP, followed by wiping with a towellette containing water, was effective in cleaning and reducing bacterial counts at both temperatures. The effectiveness of wipes #1 and #2 in cleaning the surfaces undoubtedly contributed to the successful reductions by the sanitizing (SC) wipe #3. While there appeared to be a temperature effect, which was not unexpected, the difference in percent reduction at the two temperatures was minimal and may be due, in part, to the effort and "elbow grease" expended.

Table 6. Towellette Removal of Biofilms Produced on Stainless Steel Surfaces by *E. coli* and *S. aureus* in Selected Foods.

Food	Organism	Average percent bacterial reduction by wipes ^a			
		5°C		26°C	
		Wipes 1&2	Wipes 3	Wipes 1&2	Wipes 3
Beef stew	Mixed ^b	81.7	99.98	98.4	100.0
Chicken ala king	Mixed	99.92	99.999	99.98	99.9994
Chicken stew	Mixed	99.7	100.0	99.78	99.999
Corn beef hash	Mixed	99.75	99.99	99.999	100.0
Escal. potatoes	Mixed	99.99	100.0	99.997	99.9993
Pork chow mein	<i>E. coli</i>	99.98	99.99999	99.99995	99.99995
	<i>S. aureus</i>	99.59	99.999	—	—
	Mixed	99.97	99.9993	99.987	100.00
Tuna and noodles	Mixed	99.97	99.9998	99.999	100.0
Skim milk	<i>E. coli</i>	—	—	99.9984	99.99996

^aWipe #1 contained Vesta Power detergent; wipe #2 contained deionized water; wipe #3 contained Syn-Cide sanitizer.

^bCultures of *E. coli* and *S. aureus* were mixed in equal volumes and added to the food that was spread over the surface of stainless steel pans. The soiled pans were then incubated at 35°C for 24 hours.

DISCUSSION

Removal and penetration of biofilms on surfaces represent the worst possible challenge for cleaners and sanitizers. Biofilms were deliberately produced on stainless steel surfaces in this study by growing *E. coli* and *S. aureus* in thermostabilized military rations to very high numbers. These biofilms are formed by the attachment of microorganisms to surfaces and the

accumulation of layers of fat, protein, polysaccharides, and other materials produced by microorganisms, as well as food debris (12,13,15,16). Because biofilms are persistent and very difficult to remove from surfaces, they are a problem for the food industry since they can provide attached pathogens protection against chemical sanitizers (12). Consequently, failure to remove the biofilm with a detergent could result in contamination of a food process by the attached organisms even though equipment surfaces were flushed with a sanitizer before production. Attached cells are known to be more resistant to chemicals (12).

To remove and inactivate adherent organisms on a surface, sanitizing agents must be preceded by effective cleaners (13,14,15,16). None of the chemical cleaners used by Krysiniski et al. (13) removed attached organisms from polyester/polyurethane chips when used alone, but when followed by a sanitizer, effective reductions were achieved. In a study of a clean-in-place system, removal of attached cells was most effective when a detergent preceded the sanitizer (15). This may be explained by the fact that detergents contain surfactants which act by reducing surface tension, thereby suspending and removing greasy soils, which enables the sanitizers to inactivate organisms that remain behind. For these reasons, a single sanitizing wipe containing an iodophore, as used by previous investigators (3,4), was not as effective as the combination of wipes used in this study. Furthermore, iodophores were among the least effective sanitizers used in a recent study to remove attached cells on surfaces (13).

Because the surface of bacteria is negatively charged and hydrophilic, quaternary ammonium compounds, such as those found in Syn-Cide, absorb to it, penetrate the cell wall, and rupture the cytoplasmic membrane, killing the cell (12,17). However, this action may not occur if cells are protected by a

biofilm that prevents penetration of the sanitizer into the cell. The mechanism for the effectiveness of the towellette sanitation system presented in this report may be due to the disruption and removal of the protective biofilm from both surfaces and cells by rubbing with wipe #1, containing VP detergent. Vesta Power contains sodium metasilicate, which mixes with and emulsifies fats and grease, allowing the sanitizer in wipe #3 to penetrate and remove the adherent cells that remained behind. Adherent cells may also be more sensitive to sanitizers after removal from the surface by the detergent (12). This three-wipe system reduces bacterial counts from 99.999% to 100%.

CONCLUSIONS

The towellette or waterless sanitation system devised and tested is feasible and was effective in sanitizing stainless steel surfaces. Towellettes will provide Mobile Kitchens with a back-up system for cleaning serving utensils, individual mess gear, small equipment and table tops in the event that a suitable water supply is not available. The towels (wipes) must be properly packaged to prevent drying during long-term storage and they must be sterilized to prevent growth of bacteria and molds while stored.

RECOMMENDATIONS

This study should be continued with the following specific goals as recommendations:

- Test the wipes under field conditions in an exercise with mobile field kitchens.
- Determine packaging and sterilization system for the wipes containing the reagents specified.
- Sterilize the wipes by irradiation to prevent possible thermal inactivation of reagents.
- Perform microbiological studies to verify the efficacy of the sterilization process. Locate a commercial source of the wipes.

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