

# Sanitizing in Dry-Processing Environments Using Isopropyl Alcohol Quaternary Ammonium Formula

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## ABSTRACT

Dry-processing environments are particularly challenging to clean and sanitize because introduced water can favor growth and establishment of pathogenic microorganisms such as *Salmonella*. Our objective was to determine the efficacy of an isopropyl alcohol quaternary ammonium (IPAQuat) formula for eliminating potential *Salmonella* contamination on food contact surfaces. Clean stainless steel coupons and conveyor belt materials used in dry-processing environments were spot inoculated in the center of coupons (5 by 5 cm) with a six-serotype composite of *Salmonella* (approximately 10 log CFU/ml), subjected to IPAQuat sanitizer treatments with exposure times of 30 s, 1 min, or 5 min, and then swabbed for enumeration of posttreatment survivors. A subset of inoculated surfaces was soiled with a breadcrumb-flour blend and allowed to sit on the laboratory bench for a minimum of 16 h before sanitation. Pretreatment *Salmonella* populations (inoculated controls, 0 s treatment) were approximately 7.0 log CFU/25 cm<sup>2</sup>, and posttreatment survivors were 1.31, 0.72, and <0.7 (detection limit) log CFU/25 cm<sup>2</sup> after sanitizer exposure for 30 s, 1 min, or 5 min, respectively, for both clean (no added soil) and soiled surfaces. Treatment with the IPAQuat formula using 30-s sanitizer exposures resulted in 5.68-log reductions, whereas >6.0-log reductions were observed for sanitizer exposures of 1 and 5 min. Because water is not introduced into the processing environment with this approach, the IPAQuat formula could have sanitation applications in dry-processing environments to eliminate potential contamination from *Salmonella* on food contact surfaces.

Numerous outbreaks of salmonellosis affecting large numbers of individuals and resulting in several deaths have been associated with low-moisture ready-to-eat products such as powdered infant formula, dried milk products, raw almonds, cereals, dry seasonings, dry pet food products, and pet treats (1–6, 10, 20). Although *Salmonella* cannot grow in dry food products with a water activity lower than 0.94, *Salmonella* is resistant to desiccation and able to survive for a long time on stainless steel under dry environmental conditions (11, 14, 20). The survival and transfer of *Salmonella* and other bacteria from contaminated surfaces or processing equipment to new foods have been widely studied. Investigations into the epidemiological and environmental patterns associated with outbreaks of salmonellosis have suggested that cross-contamination and inadequate sanitation have played major roles in the contamination of products with *Salmonella* (14, 20).

Many food processing facilities utilize detergents and sanitizers mixed with water to clean equipment; however, wet cleaning procedures in facilities that process dry

ingredients or low-moisture food products are not always appropriate. Cleaning and sanitation in dry-processing environments become particularly challenging because water introduced into systems not designed for wet cleaning can favor growth and establishment of pathogenic microorganisms such as *Salmonella* (10). Microbial niche environments can develop in cracks, crevices, pits, holes, and junctions that have accumulated food, dust, debris, and water. These areas may be hard to inspect, clean, or sanitize and can therefore harbor microorganisms and lead to inadequate control of pathogens in food processing areas (9, 10, 21).

An ideal sanitizer for a dry-processing environment should be effective for eliminating *Salmonella*, should not introduce water or excess moisture into the environment, and should dry rapidly once deposited on surfaces. The sanitizer should also be able to penetrate small cracks and crevices, thereby reaching possible niche environments. For best results, the sanitizer should have a residual antimicrobial effect against foodborne pathogens such as *Salmonella*.

A sanitizing system that utilizes an isopropyl alcohol quaternary ammonium (IPAQuat) formula is commercially available (1 Priority Biocidal, Fort Worth, TX; and Biomist, Inc., Wheeling, IL) and could reduce *Salmonella* on food contact surfaces. The sanitizing system's technology, which uses CO<sub>2</sub> as a propellant or carrier to deliver a spray of the IPAQuat formula, makes it possible to sanitize without

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adding water to food processing environments. The active IPAQuat formula contains 58.6% isopropyl alcohol and a low concentration (200 ppm) of quaternary ammonium compound (D2; 1 Priority Biocidal; and Best Sanitizers, Penn Valley, CA). Quaternary ammonium is widely used as a biocide in food processing areas to minimize bacterial growth and biofilm formation (17–19). After the alcohol evaporates, the quaternary ammonium compound leaves an antimicrobial film and residual sanitizing effect. Excessive residual ammonium buildup could lead to potential chemical contamination of a food product, so the residue must be minimized (17, 18). If the IPAQuat formula were effective, it would be applicable in dry-processing or low-moisture food environments.

The IPAQuat formula is effective against several pathogens. Experiments conducted using almond dust inoculated with *Salmonella* resulted in 7-log reductions when inoculated dust was treated with the IPAQuat formula (8). An independent third-party laboratory found the active formula was effective against *Escherichia coli* and *Staphylococcus aureus* with a 6.9 log CFU per carrier reduction after a 1-min sanitation treatment (15).

Preliminary research conducted in our laboratory focused on the efficacy of the IPAQuat formula for reducing *Salmonella* Enteritidis ATCC 13076 (Cornell Food Safety Laboratory, Ithaca, NY) inoculated at low and high levels onto clean (no added soil) and soiled stainless steel coupons and conveyor belt materials (polyurethane, thermoplastic polyurethane, and plastic link style) typically found in dry-processing environments (13). Although the IPAQuat formula was effective for reducing *Salmonella* Enteritidis from clean (no added soil) and soiled stainless steel coupons and belt materials, low levels of surviving organisms from soiled surfaces prompted a closer examination of the artificial soil used (i.e., flour and water solution sprayed onto the surfaces and allowed to dry). Additional experiments were conducted using a six-serotype composite of *Salmonella* isolates (from clinical, product, and environmental sources), and the soil matrix was changed to one that was more typical of a dry-processing environment (i.e., a bread crumb plus flour dry soil mixture) (13). The objective of our study was to evaluate the efficacy of the IPAQuat formula for eliminating potential *Salmonella* contamination from food contact surfaces and for its application in facilities that process dry ingredients or low-moisture food products.

## MATERIALS AND METHODS

**Experimental design.** Stainless steel coupons and belt materials were inoculated with *Salmonella* and not treated (0 s, control) or treated with a dry sweep and/or with IPAQuat formula using sanitizer exposures of 30 s, 1 min, or 5 min. Inoculated coupons and belt materials evaluated were either clean (no added soil) or had added soil. At least three samples were treated for each experiment, and each experiment was conducted three times for a total of three replications with  $n = 9$  or more.

**Culture receipt and storage.** Cultures of *Salmonella* serotypes Hartford FSL R8-5223 (peanut isolate from a project funded by International Life Sciences Institute [ILSI]), Tennessee

FSL R8-5221 (peanut isolate from a project funded by ILSI), Enteritidis FSL S5-415 (human isolate), Agona FSL S5-517 (human isolate), Newport FSL R8-4035 (isolated from a bovine farm environment), and Enteritidis FSL R8-4086 (isolated from an avian farm environment) were obtained from the Cornell Food Safety Laboratory. Cultures were streaked onto Hektoen enteric agar (HEA; BBL/Difco, BD, Sparks, MD) to check for purity. Representative colonies from the HEA purity plates were analyzed on a Vitek2 System (bioMérieux, Hazelwood, MO) to confirm *Salmonella* identification before conducting experiments.

Cultures were maintained on tryptic soy agar (TSA; BBL/Difco, BD) slants and in 10 ml of tryptic soy broth (TSB; BBL/Difco, BD), which were stored in a refrigerator set at 4°C. A separate culture was maintained frozen by transferring a single colony to a tube of TSB supplemented with 15% glycerol (Fisher Chemical, Fisher Scientific, Pittsburgh, PA), which was stored at –80°C.

**Inoculum preparation.** From the purity plates, a 10- $\mu$ l loop (Fisher Scientific) was used to transfer an isolated colony from the *Salmonella* cultures into separate 10-ml TSB tubes, which were then incubated at 35°C for 22  $\pm$  2 h. After incubation, tubes were vortexed (Pulsing Vortex Mixer, Fisher Scientific), and 0.1 ml of each culture was transferred to a fresh 10-ml tube of TSB per isolate and placed in a 35°C incubator for an additional 22  $\pm$  2 h. Following two successive transfers into TSB, cultures were stored in a refrigerator set at 4°C. Each time an experiment was to be conducted, 0.1 ml of each refrigerated TSB culture was transferred to a fresh 10-ml tube of TSB per isolate and placed in a 35°C incubator for 22  $\pm$  2 h. After incubation, 1 ml of each vortexed TSB culture tube was transferred to one premade petri plate (15 by 150 mm; BD) containing approximately 20 ml of TSA as previously described (7). This method was chosen to acclimate cells to an adherent static environment.

Using a separate sterile disposable L-shaped cell spreader per culture (Fisherbrand, Fisher Scientific), each culture was spread across the surface of a TSA plate and incubated for 22  $\pm$  2 h at 35°C. After incubation, 5 ml of Butterfield's buffer (3M, St. Paul, MN) was added to each TSA plate, and the lawn of culture was loosened with a sterile disposable L-shaped cell spreader (BD). Loosened cells from all isolates were collected and pooled into one sterile plastic 50-ml conical test tube (BD).

**Preparation of test surfaces.** Stainless steel coupons (type 304, 5 by 5 cm) with no. 4 finish were prepared by the machine shop at the Campbell Soup Company (Camden, NJ). Conveyor belt material ("yellow" polyurethane, Volta Belting, Pine Brook, NJ) were obtained from a dry-processing facility. The conveyor belt material was cut in the laboratory into strips (5 by 15 cm) with lines marked every 5 cm. Coupons and belt material were first cleaned with Citranox liquid-acid detergent (Fisher Scientific) and then rinsed with flowing tap water for approximately 10 min and then with by two rinses of deionized water. Cleaned coupons and belt material were placed on aluminum trays lined with absorbent blotter paper (Fisher Scientific) to dry (approximately 1 h). After drying, coupons were moved to aluminum trays lined with aluminum foil (Fisher Scientific). A second foil sheet was used to cover the tray, which was then autoclaved at 121°C for 15 min. Autoclaved coupons were left on the lab bench for 1 h to cool. Belt material was wiped until thoroughly wet with 70% alcohol prep swabs (Fisher Scientific) and rinsed with a Kimwipe (Fisher Scientific) wetted with sterile distilled water. Sanitized belt material was left on the lab bench to dry (approximately 1 h).

TABLE 1. *Salmonella* populations on stainless steel or conveyor belt material following exposure to IPAQuat treatment

Coupon type	Sample condition <sup>a</sup>	Mean (SE) <i>Salmonella</i> population (log CFU/25 cm <sup>2</sup> ) after treatment for <sup>b</sup> :			
		0 s	30 s	1 min	5 min
Stainless steel	Clean	6.93 (0.1) A a	0.75 (0.2) B b	<0.70 (0.1) A b	<0.70 (0.1) A b
	Soiled	6.72 (0.1) B a	<0.70 (0.2) B b	<0.70 (0.1) A b	<0.70 (0.1) A b
Belt material	Clean	6.99 (0.1) A a	1.31 (0.2) A b	0.72 (0.1) A c	<0.70 (0.1) A c
	Soiled	6.71 (0.1) B a	<0.70 (0.2) B b	<0.70 (0.1) A b	<0.70 (0.1) A b

<sup>a</sup> Clean indicates no added soil.

<sup>b</sup> Values are the means (standard errors) of nine measurements. Within a column, means followed by different uppercase letters are significantly different ( $P < 0.05$ ). Within a row, means followed by different lowercase letters are significantly different ( $P < 0.05$ ). The detection limit was 0.70 log CFU/25 cm<sup>2</sup>.

**Inoculation of materials.** Pooled *Salmonella* cells were diluted 10-fold using 9 ml of Butterfield's buffer to achieve approximately 10<sup>10</sup> CFU/ml. Coupons and belt material were inoculated in triplicate for each treatment time (30 s, 1 min, and 5 min) using 10 µl of the serially diluted pooled cells distributed to the center of each test surface using a repeat pipettor and tips (Rainin edp2, Mettler, Toledo, OH). Inoculated materials were loosely covered with sterile foil sheets and allowed to air dry on the lab bench overnight for 16 to 18 h (24 ± 2°C). The cell level in each inoculum was verified with serial decimal dilutions using Butterfield's buffer and pour plated onto TSA. Plates were incubated for 48 ± 2 h at 35°C, and colonies were then counted.

#### Preparation of clean (no added soil) and soiled surfaces.

After the inoculum was dry (2 h, 24 ± 2°C), coupons and belt material were divided onto separate sterile foil-lined trays. Coupons and belt material were labeled as clean (no added soil) or soiled. Soiled surfaces were prepared by depositing 1 g of a breadcrumb (Great Value Brand, Walmart, Bentonville, AK) and bread flour (King Arthur's, Norwich, VT) blend directly on top of the dried inoculum. The blend was first prepared by mixing 80 g of breadcrumbs with 20 g of bread flour. This blend was chosen to represent the soil one might find in a dry-processing food environment and was determined to be *Salmonella* free based on results from the BAX detection system (bioMérieux).

**Treatments using the IPAQuat formula.** The breadcrumb-flour blend (soil) was initially removed before sanitation treatments by turning the test surfaces sideways and tapping once. Treatments consisted of a dry sweep using a small handheld broom (Procter & Gamble, Cincinnati, OH) and/or an application of IPAQuat formula to inoculated surfaces using the IPAQuat-CO<sub>2</sub> sanitizing system. To use the IPAQuat-CO<sub>2</sub> sanitizer system, the valve of the system was fully opened, and the regulator knob was set at 30 lb/in<sup>2</sup>. Test materials were placed on sterile foil-lined trays on the top shelf of a lab cart and sprayed from 0.91 m away, starting on the left and working toward the right. The timer was started as a fine spray of IPAQuat formula was deposited onto the test surfaces. Sanitation treatments consisted of exposing inoculated materials to the IPAQuat formula delivered with the CO<sub>2</sub> sanitizer system for 30 s, 1 min, or 5 min. The amount of IPAQuat formula deposited on the coupons was 0.03 ± 0.01 g for a 30-s treatment, 0.05 ± 0.01 g for a 1-min treatment, and 0.07 ± 0.01 g for a 5-min treatment. Longer treatment times resulted in a higher volume of IPAQuat formula on the coupons.

**Enumeration of surviving organisms.** After sanitation, the center of the coupons and belt material were immediately swabbed

using Dacron polyester-tipped swabs and resuspended in 5-ml letheen broth tubes (3M) to neutralize the sanitizer. Before swabbing, swab tips were pressed and twisted against the side wall of the tube to wring out excess neutralizing buffer. After swabbing the surface for 5 s (30-s treatments) or 15 s (1 or 5 min treatments) as determined using a second timer, swabs were returned to letheen broth tubes to neutralize the IPAQuat formula. A shorter swabbing time was chosen for the 30-s treatment to minimize exposure to the IPAQuat formula and to enhance recovery of inoculated organisms. Swabs were vortexed for 20 s before plating to release bacteria from the swab tip. Serial 10-fold dilutions were prepared and plated using TSA pour plates to enumerate surviving organisms. Plates colonies were counted after 48 ± 2 h at 35°C.

**Counting plate colonies and making calculations.** Colonies were counted by hand using a Reichert Darkfield Quebec Colony Counter (Fisher Scientific). The number of surviving *Salmonella* cells was calculated by subtracting posttreatment survivors from pretreatment populations. Colonies were confirmed as *Salmonella* by plating on HEA.

**Statistical analyses.** Each experiment was repeated at least three times on different days, and no fewer than three coupons were used in each experiment. Microbial counts were log transformed before means and standard deviations were computed. Counts were reported as log CFU per 25 cm<sup>2</sup>.

Data were analyzed using an analysis of variance in the PROC MIXED procedures of SAS Statistical Analysis software (version 9.2, SAS Institute, Cary, NC). To analyze the data, a value of 0.7 was assigned for all counts that were below the detection limit of 0.7 log CFU/25 cm<sup>2</sup>. Fixed effects were exposure time and material; random effects were the number of replications and number of samples. Differences between mean values were considered significant at  $P < 0.05$ .

## RESULTS

Mean *Salmonella* populations attached to clean (no added soil) stainless steel coupons and belt material before sanitation treatments ranged from 6.93 to 6.99 ± 0.1 log CFU/25 cm<sup>2</sup> (Table 1). Pretreatment *Salmonella* populations attached to soiled materials were lower ( $P < 0.05$ ) at 6.71 to 6.72 ± 0.1 log CFU/25 cm<sup>2</sup> (Table 1). Sanitation treatments applied to inoculated clean (no added soil) coupons resulted in a 5.93 ± 0.2-log reduction after a 30 s of sanitizer exposure. After 1- or 5-min sanitation treatments, populations were 0.71 ± 0.1 log CFU/25 cm<sup>2</sup> and below the level of detection (0.70 ± 0.1 log CFU/25

cm<sup>2</sup>), respectively, representing a 6.25 to 6.26 ± 0.1-log reduction.

Sanitation treatments applied to soiled coupons resulted in 6.02 ± 0.2-log reductions after sanitizer exposures of 30 s, 1 min, and 5 min. *Salmonella* populations were below the level of detection (0.70 ± 0.2 log CFU/25 cm<sup>2</sup>) after a 30 s of treatment using the IPAQuat formula. Sanitation treatments applied to soiled belt material yielded results similar to those observed for soiled coupons. *Salmonella* populations were below the level of detection (0.70 ± 0.2 log CFU/25 cm<sup>2</sup>) after 30 s of treatment and resulted in 6.02 ± 0.2-log reductions. Overall, the duration of sanitizer exposure did not affect (*P* > 0.05) inactivation of attached *Salmonella* for most of the coupons (Table 1).

The level of survivors (1.31 ± 0.2 log CFU/25 cm<sup>2</sup>) recovered from clean (no added soil) belt material after a 30-s sanitation treatment was higher (*P* < 0.05) than that for all other materials and conditions (clean and soiled) studied. A 30-s treatment using the IPAQuat formula resulted in a 5.68 ± 0.2-log reduction under the conditions of this study (Table 1). Treatments of 1 or 5 min using the IPAQuat formula resulted in 6.0 ± 0.1-log reductions (Table 1). Dry sweeping of coupons recovered populations of 6.81 ± 0.1 log CFU/25 cm<sup>2</sup> and did not substantially (≤0.03 log CFU/25 cm<sup>2</sup>) reduce the *Salmonella* spot inoculated onto the coupons.

## DISCUSSION

In previous studies, the IPAQuat formula was effective for reducing microbial populations when applied to inoculated bench tops (12) or added to inoculated almond dust (8). The results of the present study confirm these findings (8, 12).

A reported 7-log reduction was found when the IPAQuat formula was added to almond dust samples mixed with *Salmonella* broth cultures (8). Sanitizer efficacy studies are often conducted using planktonic cells in broth cultures, but dried cells may not react the same way. False assumptions could be made, especially if cells have an intact glycocalyx (16). The present study was conducted using dried cells that were attached to stainless steel or conveyor belt material typically found in dry-processing environments.

Although approximately 5-log reductions in *Salmonella* were obtained after a 30-s treatment with the IPAQuat formula, low levels of *Salmonella* were recovered from swabbed sample surfaces after this treatment. Because salmonellosis has been associated with low levels of *Salmonella* in low-moisture products (<1 CFU/g) (10), low surviving levels of *Salmonella* would not be acceptable. A sanitation treatment of at least 1 min using the IPAQuat formula resulted in 6-log reductions of *Salmonella*. Most samples taken from surfaces treated for 1 min were below the detection limit (0.70 log CFU/25 cm<sup>2</sup>), and all swab results from surfaces treated for 5 min were below the detection limit. Under the conditions of this study, dry sweeping was not effective for eliminating *Salmonella* from test materials, although removing

organic debris before sanitation treatments would be necessary.

Because salmonellae have been reported to survive in dry environments for long periods and to survive on stainless steel for several days (11, 14, 20), effective cleaning and sanitation is important to ensure safe food production and to eliminate the potential for products to become cross-contaminated from the processing environment. The IPAQuat formula could have sanitation applications in dry-processing environments or facilities that process low-moisture products. Because water is not introduced into the processing environment with this formula, the risk of spreading *Salmonella* in the facility and onto food products is reduced. After the alcohol in the IPAQuat formula evaporates, the residual antimicrobial film left by the quaternary ammonium compound would help to minimize bacterial growth and biofilm formation (17–19).

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