# Efficacy and Reproducibility of a Produce Wash in Killing Salmonella on the Surface of Tomatoes Assessed with a Proposed Standard Method for Produce Sanitizers

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#### ABSTRACT

The reproducibility of a method developed to evaluate point-of-use sanitizers for fresh produce was tested at three different laboratories. Mixtures of five *Salmonella* serotypes were inoculated on the surface of ripe tomatoes. After the inoculum was dry, tomatoes were placed inside a plastic bag and sprayed with sterile USP water, Dey and Engley (D/E) neutralizer broth, or a prototype Fit produce wash (PW), an alkaline solution comprised of generally recognized as safe ingredients (water, oleic acid, glycerol, ethanol, potassium hydroxide, sodium bicarbonate, citric acid, and distilled grapefruit oil), and rubbed for 30 s. The tomatoes were rinsed 10 s with 195 ml of D/E neutralizer broth (rinse solution), then combined with 20 ml of D/E neutralizer (residual wash solution) and rubbed by hand to remove residual *Salmonella*. Populations of *Salmonella* were determined for each tomato in the rinse solution and residual wash solution. Treatment with PW resulted in reductions in the number of *Salmonella* 2 to 4 logs greater than those achieved with the sterile water or D/E neutralizer broth controls. Consistent results were obtained across the three study sites, indicating reproducible results were obtained using the test method. The method used to determine the efficacy of killing or removing *Salmonella* from tomatoes in this study is suggested as a standard method for measuring the efficacy of sanitizers on tomatoes and other similar fruits and vegetables with rigid, smooth surfaces.

Infections associated with raw fruits and vegetables are not rare. A wide variety of produce has been linked by epidemiologic investigations to foodborne outbreaks for nearly a century (14, 16). The Centers for Disease Control and Prevention (CDC), however, has reported increased numbers of produce-associated outbreaks in the United States during the period of 1988 through 1992 compared to previous surveillance periods (12). Documented illnesses have been linked to bacteria, parasites, and viruses (2, 12) and have involved many types of fruits and vegetables, including tomatoes (10, 17), lettuce, alfalfa sprouts, parsley, scallions, and cantaloupe, as well as unpasteurized apple and orange juice. Factors thought to contribute to this increase include globalization of the food supply, including importation of produce from countries with lower sanitation standards; the inadvertent introduction of pathogens from new geographical areas; the development of new virulence factors by microorganisms; decreases in immunity among certain segments of the population; and changes in raw fruit and vegetable processing and eating habits (2, 11).

Microorganisms can occur on raw or minimally processed produce at populations ranging from  $10^3$  to  $10^9$  CFU/g (9, 13). Washing with tap water is a currently recommended means for reducing microbial contamination on raw fruits and vegetables. Although washing produce in water may remove some soil and other debris, it cannot be relied upon to completely remove microorganisms and may result in cross-contamination of food preparation surfaces, utensils, and other food items (1, 3, 5, 6). Treatment of raw produce with chlorinated water and other disinfectants is partially effective in removing disease-causing microorganisms from the surface of raw fruits and vegetables; however, chemical treatments cannot be relied upon to totally eliminate pathogens that occasionally occur on raw produce when used at concentrations that do not cause deterioration of sensory qualities (3).

There is a critical need for developing produce washes for consumer and related food service use. Any such wash making "germ-kill" claims must be approved and registered by the U.S. Environmental Protection Agency (EPA) prior to commercial sale. Many researchers have investigated sanitizers for their efficacy in killing pathogens on raw fruits and vegetables (3). However, methods used to evaluate these sanitizers have varied greatly, thus making comparison of results from various laboratories difficult. There are currently no standard test methods available for the EPA to evaluate, for the purpose of registration, pointof-use or home sanitizers for fresh produce. To address this situation, in September 1997, the EPA assembled a Scientific Advisory Panel to discuss the status and development of a standard method for evaluating produce sanitizers. The

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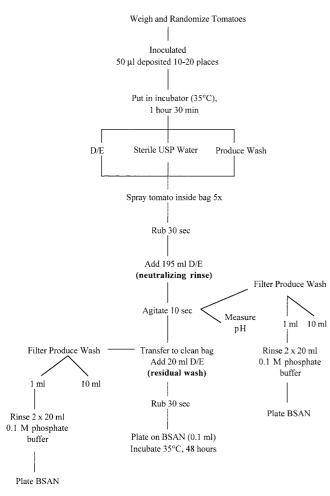


FIGURE 1. Schematic illustration of treatment and analysis methodology.

panel recommended that retail fresh produce sanitizers should be evaluated using a cocktail of at least five strains of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* originally isolated from foodborne outbreaks, with a 2-log reduction identified as a reasonable performance standard (8).

Recently, we conducted pilot studies that used a new laboratory testing technique based on the Scientific Advisory Panel recommendations to evaluate the efficacy of a prototype produce rinse (Fit Produce Wash; The Procter and Gamble Company, Cincinnati, Ohio) against a five-serotype cocktail of *Salmonella* inoculated onto the surface of tomatoes (4). The study reported here was designed to evaluate and validate a simplified methodology in three different laboratories.

### MATERIALS AND METHODS

This study employed a modification of a method developed by Beuchat et al. (4). Minor changes in this methodology were made to simplify the protocol. The methodology used in this experiment is summarized in Figure 1. Differences in pilot study methodology (4) and the methodology used in experiments reported here are summarized in Table 1.

**Test laboratories.** Experiments were performed using Good Laboratory Practices at a private contract laboratory (Hill Top Research, Inc., Cincinnati, Ohio), an industry laboratory (Procter and Gamble), and an academic laboratory (Department of Food Science and Technology, University of California–Davis).

**Produce evaluated.** Produce selected for evaluation were red ripe tomatoes with a weight of 100 to 350 g each that had been commercially treated with food-grade mineral oil, petrolatum, paraffin, and carnauba wax. All tomatoes were purchased from Castenillini Produce Co. (Wilder, Ky.), and identified 50-lb (22.7-kg) crates were shipped via air freight to the three study sites, where they were stored at room temperature for a maximum of 5 days before use in the experiments.

**Microorganism tested.** A mixture of five serotypes of *Salmonella* originally isolated from food or feces of infected humans or cattle was used to inoculate the tomatoes. The serotypes used and their sources were as follows: *Salmonella* Agona (alfalfa sprouts), *Salmonella* Enteritidis (patient in an egg-associated outbreak), *Salmonella* Gaminara (orange juice), *Salmonella* Montevideo (patient in a tomato-associated outbreak), and *Salmonella* Typhimurium (feces from infected cattle). All serotypes were adapted to grow in tryptic soy broth (TSB, pH 7.3; Difco Laboratories, Detroit, Mich.) supplemented with nalidixic acid (50 µg/ml; TSBN). The use of nalidixic acid–resistant cells was required to accurately monitor the fate of *Salmonella* on tomatoes relative to background microflora that might otherwise interfere with counting *Salmonella* colonies on nonselective enumeration media.

**Preparation of inocula.** Salmonella serotypes were independently cultured at  $34 \pm 3^{\circ}$ C on tryptic soy agar (TSA, pH 7.3; Difco) supplemented with 50 µg/ml of nalidixic acid (TSAN) for  $24 \pm 2$  h. Cultures were transferred twice to TSAN by sterile loop inocula at successive  $24 \pm 2$ -h intervals. Cells of each Salmonella serotype were collected by washing agar slants with 2 ml of a sterile 5% horse serum albumen (Difco) solution in sterile USP water. Equal volumes of each suspension of all five serotypes were

TABLE 1. Summary of changes in study methodology

Element	Current study	Pilot study (4)	
Produce characteristic	Waxed	Not waxed	
Drying technique	Dry in incubator at 35°C, 90 min	Dry in hood at 23°C, 1 h	
Spray technique	Five sprays per tomato to total 4.4 to 4.8 ml of PW or control solution	Spray to saturate	
Soak step	None, followed by 30-s rub	30-s hold, followed by 30-s rub	
Rinse step	Rinse with 195 ml of D/E broth, 10-s agitation	Rinse with 200 ml of distilled water, 30-s agitation	
Wash step	Wash with 20 ml of D/E broth, 30-s rub	Wash with 20 ml of 0.1% peptone, 40-s rub	

combined to create a mixture with approximately equal populations of each serotype. The inoculum was maintained at room temperature ( $22 \pm 1^{\circ}$ C) and applied to tomatoes within 1 h of preparation. If times of inoculation of the tomatoes were staggered, the inoculum was kept at 4 to 8°C and warmed to room temperature prior to inoculation. Populations (CFU/ml) of each serotype in 5% horse serum albumin, as well as in the five-serotype mixtures, were determined by surface plating samples (0.1 ml) serially diluted in sterile 0.1% peptone on TSAN. Plates were incubated at 34 ± 3°C for 48 ± 2 h before colonies were counted.

**Preparation of treatment solutions.** This study examined one test sanitizer solution relative to two control solutions. The test solution was a prototype Fit produce wash (PW), an alkaline solution composed of generally recognized as safe ingredients (pH 11.5) (water, oleic acid, glycerol, ethanol, potassium hydroxide, sodium bicarbonate, citric acid, and distilled grapefruit oil). The two controls were sterile USP water (pH 7.0) and Dey and Engley (D/E) neutralizer broth (pH 7.6; Difco). In each trial, seven tomatoes were subjected to the desired treatment or control (i.e., PW, USP water, or D/E neutralizer broth). Each research laboratory performed four replicate trials, for a total of 28 tomatoes subjected to each treatment and control at each laboratory.

The test sanitizer (PW) was supplied by Procter and Gamble in liquid ready-to-use form. Two production lots were used, with production lot 1 used in replicate trials 3 and 4 and production lot 2 used in replicate trials 1 and 2. D/E neutralizer broth (pH 7.6) was prepared according to the manufacturer's instructions (39 g/liter of USP deionized water) and autoclaved to sterilize. To prepare D/E neutralizer broth (pH 7.0) to be used as the 195-ml neutralizer rinse for the PW treatment, the D/E neutralizer broth also was prepared according to the manufacturer's instructions, then was adjusted to pH 7.0  $\pm$  0.2 with 0.1 M HCl and autoclaved to sterilize. The pH of each batch was confirmed postautoclaving.

**Procedure for inoculating produce.** Using a micropipetter, 50  $\mu$ l of the mixed-serotype *Salmonella* suspension containing approximately 10<sup>9</sup> CFU/ml was applied near the blossom end of each tomato within approximately a 3-cm-diameter circle, with care taken to avoid placing inoculum on the blossom scar. To prevent the inoculum from running off the side of the tomato and to facilitate drying, small, approximately equal amounts of the inoculum were applied to several spots (e.g., 10 to 25 places) on each tomato. Inoculated tomatoes were then held at  $35 \pm 2^{\circ}$ C for  $90 \pm 15$  min. Longer holding times (>3 h) reduced the population to levels too low to allow statistically valid comparison of treatments (4). The total number of *Salmonella* inoculated onto each tomato was calculated (CFU/50  $\mu$ l or CFU/tomato).

**Procedures for testing produce.** The treatment procedure was developed to simulate typical home washing habits for fresh produce, which could include a spray application followed by rubbing (e.g., 30 s), then rinsing with water (Fig. 1).

All tomatoes were treated within 2 h after the inoculum was dry. Each tomato was treated separately as follows. Upon removal from the incubator, each tomato was placed in a clean, polyethylene specimen bag (VWR Scientific, no. 11217-128, South Plainfield, N.Y.). Each tomato received one of the following spray treatments: (i) neutralizer spray (D/E neutralizer broth, pH 7.6) (control); (ii) water spray (sterile USP water) (control); or (iii) PW spray (sanitizer). All solutions were at room temperature. Using commercial 354-ml spray bottles supplied by Procter and Gamble, five sprays (4.4 to 4.8 ml per five full sprays) of each control or treatment were applied to each tomato. During spray treatment, the spray nozzle was held 10 to 15 cm from the tomato, and the

tomato was rotated to ensure coverage of the entire surface, including the stem scar area. All spraying was performed inside the bag to ensure containment of all treatment and control solutions. Each tomato was immediately rubbed while in the bag for a 30s exposure period before aseptically adding 195 ml of sterile D/ E neutralizer broth (pH 7.6 for D/E neutralizer and water control sprays and pH 7.0 for the PW treatment) to the specimen bag. The tomato was rinsed by vigorously shaking the bag by hand for 10 s (rinse step). The tomato was then aseptically transferred to a clean polyethylene specimen bag containing 20 ml of D/E neutralizer broth (pH 7.6) and rubbed for 30 s to facilitate dislodging of residual Salmonella on the tomato (residual wash step). Previous studies (4) indicated that this method was sufficient to remove most of the microorganisms remaining on the tomato. Populations recovered from skin macerates were negligible (<0.1%) compared to populations recovered in the wash water. After removal of the tomato from the specimen bag, the pH of D/E neutralizer wash (residual wash solution) was determined.

Microbiological analysis. Populations of Salmonella in the D/E rinse solution (195 ml) and the D/E residual wash solution (20 ml) were determined by spread plating samples (0.1 ml) serially diluted in D/E broth onto bismuth sulfite agar (BSA; Difco) supplemented with 50 µg/ml of nalidixic acid (BSAN). Additionally, for the PW treatment, 11 ml of the D/E rinse or residual wash solutions was filtered using a Nalgene multiport filtration unit equipped with a Gelman Sciences (Ann Arbor, Mich.) sterile single-use 0.45-µm filter (or equivalent); filters were rinsed twice with 20 ml of 0.1 M potassium phosphate buffer (pH 6.8  $\pm$  0.2) and placed onto BSAN plates. Plates were incubated at  $34 \pm 3^{\circ}$ C, for 48  $\pm$  2 h before presumptive Salmonella colonies were counted. Representative colonies were confirmed by Biolog Biochemical Analysis (Biolog Inc., Hayward, Calif.) or equivalent methodologies. Populations of Salmonella surviving the drying procedure on tomatoes were determined. In addition, the pH of the D/E neutralizer rinse was determined after the tomato was removed. Microbial enumeration was performed following standard American Public Health Association methods for the examination of water and wastewater (7).

**Determination of bioburden.** Each study site determined the number of microorganisms other than *Salmonella* on tomatoes that formed colonies on BSAN. Seven uninoculated tomatoes were analyzed using the same treatment and microbiological analysis procedures detailed above, without the spray treatment step.

Statistical analysis. Data from the three laboratories were combined and assessed using a mixed linear model analyzed with Proc Mixed in Statistical Analysis Systems Institute version 6.12 (Cary, N.C.). Two different approaches were used to assess these data. In both, the three treatments of neutralizer control, water control, and PW were considered fixed, and the four replicates and the interaction between replicates with treatment were considered random. In one approach, the study site was considered a fixed effect. In the other, the effect of study sites was considered random. The former approach is useful for looking at the efficacy of the treatments at each laboratory. The latter allows one to divide the random variability between interlaboratory and intralaboratory error. From the fixed laboratory effect model, mean  $\log_{10}$ reductions and associated 95% confidence intervals were estimated from contrasts of the water and produce spray treatments minus the neutralizer control treatment at each laboratory.

# **RESULTS AND DISCUSSION**

Antimicrobial treatment efficacy. The natural tomato bioburden on the uninoculated tomatoes failed to grow on

TABLE 2. Mea	n population of	f Salmonella recovere	d from tomatoes	after sprayir	ig with $D/E$ and $PW^a$
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		Rinse solution			Residual wash solution		
Treatment/study site	Mean log <sub>10</sub> CFU/tomato	Confidence interval (95%)	Standard error	Mean log <sub>10</sub> CFU/tomato	Confidence interval (95%)	Standard error	
D/E neutralizer control							
Lab 1	8.60	(8.10, 9.10)	0.25	6.17	(5.76, 6.59)	0.20	
Lab 2	8.30	(7.79, 8.80)	0.25	6.23	(5.81, 6.64)	0.20	
Lab 3	8.15	(7.65, 8.65)	0.25	6.01	(5.59, 6.43)	0.20	
Water control							
Lab 1	8.56	(8.06, 9.06)	0.25	6.09	(5.68, 6.51)	0.20	
Lab 2	8.30	(7.80, 8.80)	0.25	6.30	(5.89, 6.72)	0.20	
Lab 3	8.05	(7.55, 8.55)	0.25	5.76	(5.34, 6.17)	0.21	
PW							
Lab 1	3.75	(3.25, 4.25)	0.25	2.46	(2.05, 2.88)	0.20	
Lab 2	4.08	(3.58, 4.58)	0.25	3.69	(3.27, 4.11)	0.21	
Lab 3	2.82	(2.32, 3.32)	0.25	1.86	(1.44, 2.28)	0.20	

<sup>a</sup> Mean values (log<sub>10</sub> CFU/tomato) were calculated from populations detected in rinse solution and residual wash solution after treatments.

BSAN (<2 CFU/tomato). Populations of viable Salmonella recovered from rinse and residual wash solutions are listed in Table 2 for inoculated tomatoes. The CFU/ml of rinse solution represents the mean number of viable Salmonella cells removed from the sprayed tomato during the rinse step and thus potentially available to cross-contaminate other food or food preparation surfaces in a food service or home use situation. The CFU/ml of residual wash solution represents the population of viable cells remaining on the surface of the tomato after spraying and rinsing that might be ingested by the consumer. Both rinse and residual wash solutions from tomatoes treated with PW contained significantly lower numbers (at least 2 log<sub>10</sub> CFU lower) of Salmonella than the control solutions from tomatoes sprayed with water or D/E neutralizer broth. Mean populations detected in rinse and residual wash solutions from tomatoes

treated with PW ranged from 2.82 to 4.08  $\log_{10}$  CFU/tomato and from 2.46 to 3.69  $\log_{10}$  CFU/tomato, respectively, across the three study sites. Comparable mean counts for the neutralizer broth control spray ranged from 8.15 to 8.60  $\log_{10}$  CFU/tomato (rinse) and from 6.01 to 6.23  $\log_{10}$  CFU/ tomato (residual wash), respectively.

The pH of the rinse solution was measured after the tomato was removed. The pH of the rinse solution was approximately 7.4 to 7.6 for water and D/E neutralizer controls and 7.7 to 7.8 for the PW treatment.

Results of the efficacy of PW spray and water treatments compared to neutralizer spray treatment are shown in Table 3. Also shown is the efficacy of PW spray treatment compared to the water spray. The D/E neutralizer and water control spray were virtually identical, with mean  $\log_{10}$  reductions (water versus neutralizer) ranging from

TABLE 3. Reduction in populations of Salmonella on tomatoes treated with water and PW compared to D/E neutralizer control and
on tomatoes treated with PW compared to water control <sup>a</sup>

		Rinse solution	Residual wash solution			
Treatment/study site	Mean log <sub>10</sub> reduction (CFU/tomato)	Confidence interval (95%)	Standard error	Mean log <sub>10</sub> reduction (CFU/tomato)	Confidence interval (95%)	Standard error
Water versus D/E neutralizer						
Lab 1	0.04	(-0.65, 0.72)	0.33	0.08	(-0.40, 0.56)	0.23
Lab 2	0.00	(-0.69, 0.69)	0.33	-0.07	(-0.56, 0.41)	0.23
Lab 3	0.11	(-0.58, 0.79)	0.33	0.25	(-0.23, 0.74)	0.24
PW versus D/E neutralizer						
Lab 1	4.85	(4.16, 5.53)	0.33	3.71	(3.23, 4.20)	0.23
Lab 2	4.22	(3.53, 4.91)	0.33	2.54	(2.05, 3.03)	0.24
Lab 3	5.33	(4.64, 6.02)	0.33	4.15	(3.67, 4.63)	0.23
PW versus water						
Lab 1	4.81	(4.12, 5.49)	0.33	3.63	(3.15, 4.12)	0.23
Lab 2	4.22	(3.53, 4.91)	0.33	2.61	(2.13, 3.10)	0.24
Lab 3	5.22	(4.54, 5.91)	0.33	3.90	(3.41, 4.38)	0.24

<sup>a</sup> Mean values (log<sub>10</sub> CFU/tomato) were calculated from populations detected in rinse solution and residual wash solution after treatments.

TABLE 4. Estimated covariance parameters for random effects of replicate and study site

	Rinse	solution	Residual wash solution		
Covariance parameter	Estimate	Ratio (%)	Estimate	Ratio (%)	
Study site	0.05	6	0.06	7	
Study site $\times$ Treatment	0.00	0	0.09	10	
Study site $ imes$ Replicate	0.03	4	0.07	8	
Study site $\times$ Treatment $\times$ Replicate	0.21	28	0.06	7	
Total interlaboratory variability	0.29	38	0.28	32	
Replicate	0.00	0	0.00	0	
Replicate $\times$ Treatment	0.00	0	0.00	0	
Residual	0.48	62	0.61	68	
Total intralaboratory variability	0.48	62	0.61	68	

0.00 to 0.11 log<sub>10</sub> CFU/tomato (rinse) to −0.07 to 0.25 log<sub>10</sub> CFU/tomato (residual wash). The produce spray treatment was significantly more lethal to *Salmonella* (>2-log reduction) relative to the neutralizer control, with mean log<sub>10</sub> reductions of 4.22 to 5.33 log<sub>10</sub> CFU/tomato (rinse) and 2.54 to 4.15 log<sub>10</sub> CFU/tomato (residual wash). More importantly, the lower limit of the 95% confidence interval for the rinse means was ≥3.53 log<sub>10</sub> CFU/tomato; the lower limit of the 95% confidence spray treatment was also compared to water spray treatment, with a mean difference of 4.22 to 5.22 log<sub>10</sub> CFU/tomato (rinse) and 2.61 to 3.90 log<sub>10</sub> CFU/tomato (residual wash).

**Interlaboratory reproducibility.** Results were consistent across the three laboratories, with the largest difference between highest and lowest values being obtained using PW spray (1.26 and 1.83  $\log_{10}$  CFU/tomato for rinse solution and residual wash solution, respectively) (Table 2). The greater variability in PW spray data is attributed in part to variability in amount and composition of organic material, wax, and soil on the surface of tomatoes and its impact on rate of killing *Salmonella* during the 30-s treatment period. The neutralizer (0.45 and 0.22  $\log_{10}$  CFU/tomato for rinse solution and residual wash solution, respectively) and water (0.51 and 0.54  $\log_{10}$  CFU/tomato for rinse solution and residual activity, and their mean  $\log_{10}$  counts appear more consistent across laboratories.

Reproducibility is further supported by the narrow range of mean  $\log_{10}$  reductions resulting from produce spray and water spray treatments relative to the neutralizer and differences in mean  $\log_{10}$  reduction between PW spray and water spray treatments (Table 3). For each laboratory, the lower limits of the 95% confidence intervals (mean log reduction neutralizer – mean log reduction PW) exceeded 2.0-log<sub>10</sub> reductions for both rinse and residual solutions. Additionally, confidence intervals were relatively narrow (<1.38 log<sub>10</sub> CFU/tomato).

The components of variability seen in the rinse and residual wash steps are outlined in Table 4. Analysis shows that 38% (rinse step) and 32% (residual wash step) of the variability are attributable to laboratory differences, with the remainder attributed to differences between replicates

and tomatoes. Tilt and Hamilton (15) reviewed the literature on repeatability and reproducibility of germicide tests. Although our results are not directly comparable, one can use their results to estimate percentages of study variances that can be attributed to differences in laboratories. The percentages of variability we observed are below the average of those calculated from their paper (49%). Thus, with most of the variability attributed to the conduct of the experiment at each study site, reproducibility across laboratories is further supported.

Technical rationale for proposed standard testing methodology. This study expanded the testing of a proposed standard method that was previously developed and verified in pilot studies (4). The proposed methodology was designed to be consistent with EPA Scientific Advisory Panel recommendations and to maximize the likelihood that similar efficacy results could reasonably be expected to be achieved by consumers and food service personnel. In the experiments reported here, several minor procedural modifications were made in the methodology used in pilot studies in order to simplify and standardize the testing methods without compromising sensitivity or reproducibility (Table 1). A key change was the use of commercially waxed tomatoes instead of nonwaxed tomatoes, to simulate worstcase conditions that could be encountered by consumers. Waxes and oils are often applied to produce to reduce water loss and improve appearance at the point of sale and could represent a significant organic load that could reduce the antimicrobial efficacy of any sanitizing procedure. Limited studies indicated that recoveries of Salmonella and efficacies of water and PW were similar for waxed and unwaxed tomatoes (data not shown). Other protocol differences included alterations in the technique used to dry the inoculated tomatoes, standardization in the amount of PW spray treatment or control solution applied to the inoculated tomatoes, elimination of a 30-s soak period before rubbing tomatoes, use of 195 ml of D/E neutralizer broth (with 10s agitation) rather than 200 ml of distilled water (with 30s agitation) in the rinse step, and a wash with D/E broth (30-s rub) rather than peptone water (40-s rub) in the residual wash step.

In conclusion, results of this study provide additional information on the efficacy of a prototype produce wash in treating tomatoes inoculated with a five-serotype *Salmo-nella* inoculum. When compared to control sprays (sterile water and D/E neutralizer broth), treatment for 30 s with PW resulted in a 2- to 4-log<sub>10</sub> reduction of *Salmonella* population over that achieved using USP water.

This study also confirms the reproducibility of similar methodology previously evaluated in a pilot study (4). Results were consistent across three different study sites, further demonstrating the reproducibility of results obtained using a proposed standard method. This reproducibility strengthens our recommendation that this method be adopted as a "standard" spray sanitation method for use by the EPA and academic and industrial researchers for determining the efficacy of fresh produce sanitizers in killing pathogens on tomatoes and similar produce items. Modifications of the method could be made for application to other produce items where spraying or rubbing is not a typical usage technique.

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