Food Contact Sanitizer Screening Test: Perasan MP-2 vs *Campylobacter jejuni* and *Salmonella* Typhimurium

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Purpose

To determine whether or not the test material, peroxyacetic acid (PAA from Perasan MP-2 kills 99.999% of *Campylobacter jejuni* and *Salmonella* Typhimurium at different concentrations within 30 seconds or 60 seconds.

Methods

Test Systems:

- *Campylobacter jejuni* bacteria (ATCC 33291)
- *Salmonella* Typhimurium bacteria (ATCC 14028)

Campylobacter jejuni bacteria was cultured on Campy Cefex Agar (Hardy Diagnostics, lot number 10315) under microaerophilic conditions and incubated for 72 hours at 37 °C. A sterile loop was used to remove several bacteria colonies from the Campy Cefex Agar, which was then carefully resuspended in approximately 100 ml of sterile phosphate buffer and shaken vigorously. This bacteria / phosphate buffer solution was later used to inoculate the test materials.

Salmonella Typhimurium bacteria was cultured in nutrient broth (Sigma, St. Louis, MS, lot number 095K0035) by incubation for two days at 35 °C. The bacteria were separated from the nutrient broth by centrifugation, and carefully re-suspended in approximately 100 mL of sterile phosphate buffer, which was later used to inoculate the test materials.

Test material preparation:

Perasan MP-2: Lot 825-10-1014 analysed at 5.80% hydrogen peroxide and 15.70% PAA.

- 10 ppm PAA: 0.0634 g of Perasan MP-2 up to 1000 g Modesto City water.
- 30 ppm PAA: 0.1911 g of Perasan MP-2 up to 1000 g Modesto City water.
- 80 ppm PAA: 0.5096 g of Perasan MP-2 up to 1000 g Modesto City water.

The actual concentration of each test material was measured prior to challenge testing by using a HACH DR/700 Colorimeter and HACH 10 mL Total Chlorine pillow packets. See *Results and Discussion* below for more details.

Ninety-nine (99) mL of the diluted test material was aliquoted into a 250 mL glass beaker. The efficacy assay was performed by adding 1 mL of the appropriate test system organism and 0.1 ml of organic soil (chicken serum, Gibco, lot number 8107432) to the appropriate diluted test material. The number of *Campylobacter jejuni* bacteria and *Salmonella* Typhimurium bacteria present in each glass beaker containing the diluted test material was determined after 30 seconds and 60 seconds by duplicate plating on the Campy Cefex Agar Plates and 3M Enterobacteriaceae Petrifilms, respectively. In parallel, another 250 ml beaker was prepared as a control wherein sterile phosphate buffer dilution water was substituted for the 99 mL of test material. One (1) mL of 0.1N sodium thiosulfate was added to all challenge solutions, including controls, prior to plating to remove all peracid residuals.

The Campy Cefex Agar Plates were incubated under microaerophilic conditions for 72 hours at 37 °C. The 3M Enterobacteriaceae Petrifilms were incubated at 35 °C for 24 hours. After incubation, the plates were enumerated.

Results and Discussion

The actual concentration of each test material was measured prior to challenge testing by using a HACH DR/700 Colorimeter with a specific wavelength set for chlorine, and HACH 10 mL Total Chlorine pillow packets. The DPD Method Procedure was developed by Enviro Tech, and has been issued U.S. Patent #7,651,860. The colorimeter value was multiplied by the molecular weight ratio of chlorine to PAA (1.07), and the dilution factor of the test material. The ppm PAA of each test material can be seen in Table 1.

Table 1. Actual test material concentration.

Description	DPD Reading in ppm as Cl_2	Colorimeter Dilution	ppm PAA
Campylobacter: Nominal 10 ppm	0.94	1	10.0
Campylobacter: Nominal 30 ppm	0.32	1	34.2
Campylobacter: Nominal 80 ppm	0.78	1	83.5
Salmonella: Nominal 10 ppm	0.83	1	8.8
Salmonella: Nominal 30 ppm	0.26	1	27.8
Salmonella: Nominal 80 ppm	0.77	1	82.4

Table 2 shows the microbiological results of the test challenge materials (10 ppm, 30 ppm and 80 ppm PAA) against *Campylobacter jejuni* after 30 seconds and 60 seconds. The control (untreated) had a $\log_{10}$ 5.04 CFU/mL. The 80 ppm PAA test material had a $\log_{10}$ reduction of >5.04, corresponding to >99.999% reduction (complete kill) at 30 seconds and 60 seconds.

Table 2. *Campylobacter jejuni* in the presence of poultry serum. (control: log_{10} 5.04 CFU/mL)

Description	log10 CFU/ml remaining	log10 reduction	%reduction
Control	5.04	N/A	N/A
10 ppm PAA- 30 sec	2.70	2.34	99.534
10 ppm PAA- 60 sec	1.78	3.26	99.945
30 ppm PAA- 30 sec	1.76	3.28	99.948
30 ppm PAA- 60 sec	0.85	4.19	99.994
80 ppm PAA- 30 sec	<0.10	>5.04	>99.999
80 ppm PAA- 60 sec	<0.10	>5.04	>99.999

Table 3 shows the microbiological results of the of the test materials (10 ppm, 30 ppm and 80 ppm PAA) against *Salmonella* Typhimurium after 30 seconds and 60 seconds. The control (untreated) had a log10 5.70 CFU/ml. The 10 ppm PAA test materials had a log10 reduction of >5.70, corresponding to >99.999% reduction (complete kill) at 60 seconds but only a 59.26% reduction after 30 seconds. The 30 ppm and 80 ppm test materials provided a >99.999% reduction (complete kill) of *Salmonella* Typhimurium after 30 seconds and 60 seconds.

Table 3. *Salmonella* Typhimurium in the presence of poultry serum. (control: 5.70 CFU/mL)

Description	log10 CFU/ml remaining	log10 reduction	%reduction
Control	5.70	N/A	N/A
10 ppm PAA- 30 sec	5.32	0.39	59.26
10 ppm PAA- 60 sec	<0.10	>5.70	>99.999
30 ppm PAA- 30 sec	<0.10	>5.70	>99.999
30 ppm PAA- 60 sec	<0.10	>5.70	>99.999
80 ppm PAA- 30 sec	<0.10	>5.70	>99.999
80 ppm PAA- 60 sec	<0.10	>5.70	>99.999

Conclusions

- The purpose of this study is to determine whether or not the test material, peroxyacetic acid (PAA from Perasan MP-2) kills 99.999% of *Campylobacter jejuni* and *Salmonella* Typhimurium at different concentrations within 30 seconds or 60 seconds.

- Tables 2 and 3 show microbiological results of the test materials that had been inoculated with *Campylobacter jejuni* and *Salmonella* Typhimurium, respectively.

- The 80 ppm PAA test material had a log_{10} reduction of >5.04, corresponding to >99.999% reduction (complete kill) of *Campylobacter jejuni* at 30 seconds and 60 seconds. The 30 ppm PAA test material yielded a 4.2 log_{10} reduction in 60 seconds.

- The 30 ppm and 80 ppm test materials provided a >99.999% reduction (complete kill) of *Salmonella* Typhimurium after 30 seconds and 60 seconds.

- *Campylobacter jejuni* appears to be a more resistant organism to the exposure to PAA than Salmonella. Processors may want to consider this resistance if using low concentrations of Perasan MP-2 in poultry chiller operations (<25 ppm).