



Laboratory Services • Auditing • Consulting • Education • Research

FINAL REPORT

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CHALLENGE STUDY

VeriPure Ozonation System

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1.0 OBJECTIVE

The overall purpose of this study was to validate the ability of the VeriPure water ozonation system to remove pathogens and other microbial contaminants from food.

2.0 PROTOCOL OVERVIEW

Samples of beef steaks were obtained by Food Safety Net Services and inoculated with different concentrations of various microorganisms to determine the ability of the ozonated water to reduce the microbial population.

3.0 MATERIALS AND METHODS

3.1 Preparation of inocula

The following cultures were prepared as the challenge organisms:

- *Escherichia coli* O157:H7 (ATCC® 35150™)
- *Salmonella* cocktail (*Salmonella typhimurium* (ATCC® 14028™) and *Salmonella choleraesuis* (ATCC® 10708™))

A fresh culture of each organism was prepared from frozen stock by growing in a medium (BD Diagnostic Systems, Sparks, MD) appropriate for the organism, at the appropriate temperature for growth for 24-48 hours, under atmospheric conditions optimal for growth, i.e. aerobic, microaerophilic, or anaerobic. Each culture was passed through the appropriate broth three times, with each pass verified for purity and performance by plating onto Sheep Blood Agar and an agar appropriate for selectivity of the organism. After passing through the appropriate broth, each organism, upon reaching its optimal growth phase, was washed and resuspended in its appropriate broth.

3.2 Preparation of samples

Beef steaks were obtained by Food Safety Net Services and were subjected to a one-hour UV treatment per side to remove most, if not all, microbial background from the samples. After treatment, three separate replicate samples were plated onto Sorbitol MacConkey Agar (SMAC, BD) and incubated aerobically for 48 ± 2 hours at $35 \pm 2^\circ\text{C}$ to enumerate any remaining target background flora.

3.3 Inoculation of samples

An aliquot which contained approximately 10^8 cells/mL of each organism was surface inoculated onto the steak samples. Samples were inoculated in replicates of 6, and the amount of each organism in each sample was confirmed by enumerating according to the USDA Microbiology Laboratory Guidebook (MLG).

3.4 Determination of microbial reduction

Samples at the concentration prepared above were treated using the VeriPure ozonation water system. Three samples were neither rinsed nor immersed in ozonated water and served as the baseline control. Three samples were either rinsed or fully immersed in ozonated water for 60 seconds and 180 seconds. The average count of all three samples for each time point was reported. Results of each organism and each sample matrix were compared to the untreated controls to determine the relative pathogen reduction. Typical growth for each organism was biochemically confirmed by USDA MLG methods.

3.5 Determination of residual ozone levels during rinsing

Samples of ozonated water were taken at 0, 10, 30, 60, and 180 seconds of operation of the VeriPure system. Samples were tested in triplicate and averaged for each time point. The parts per million of ozone in each sample was assessed and compared to the amount present in the Time 0 samples to determine whether the level of ozone at each time point significantly declined over the course of rinsing.

4.0 RESULTS

4.1 Background and environmental results

Results for the uninoculated, post-UV treated samples are shown in Table 1, below.

Table 1. Background sample analyses

Sample Matrix	Sample	Salmonella	E. coli O157:H7
Steak	1	<10	<10
	2	<10	<10
	3	<10	<10

After UV treatment, no background was observed for any of the organisms of interest in the steak samples.

Results of the environmental monitoring during the treatment of test samples is shown in Table 2, including local humidity, air and water temperature, and calculated dew point during testing for each matrix.

Table 2. Environmental results

Sample Matrix	Relative Humidity	Air Temperature (°F)	Water Temperature (°F)	Dew Point (°F)
Steak	44%	71.6	78.8	53.8

Optimal performance of the VeriPure system was indicated to be at a dew point of at least 50°F. Environmental conditions created a dew point of 3.8°F above the indicated point.

4.2 Reduction of Salmonella in steak samples

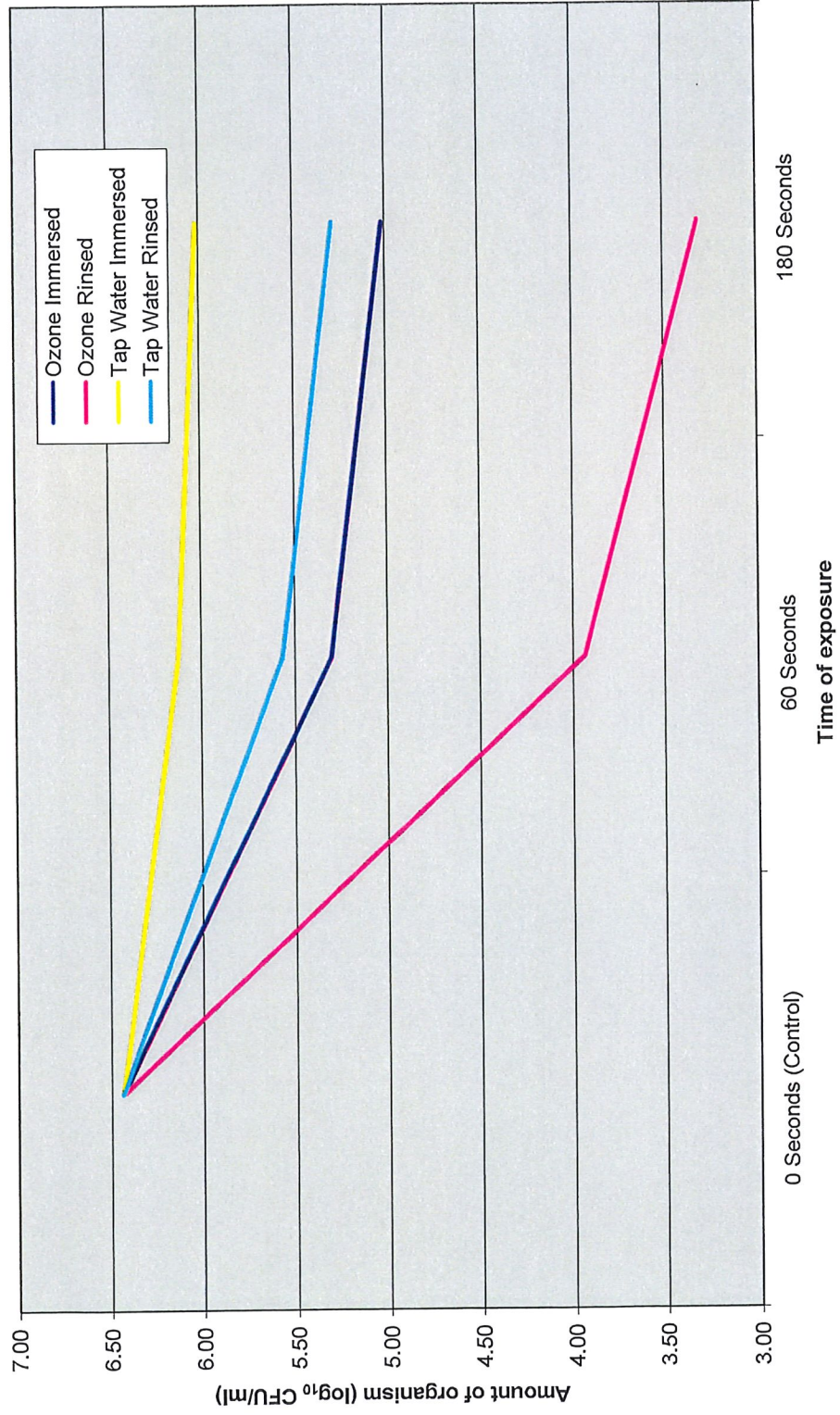
Results for *Salmonella* from the treated steak samples are shown in Table 3 below. Table 3 includes time of immersion or rinsing in both ozonated and non-ozonated (tap) water, results (in CFU/ml of rinsate) of each replicate sample for each organism, average results (in CFU/ml) of all replicates, the log₁₀ of the average, and for all times after Time 0, the observed reduction (in log₁₀ CFU/ml) for each organism.

Table 3. Salmonella reduction in steak samples

Exposure Time	Sample	Tap water, immersed	Ozonated water, immersed	Tap water, rinsed	Ozonated water, rinsed
0 Seconds (Control)	1	3,562,000	3,562,000	3,562,000	3,562,000
	2	2,642,000	2,642,000	2,642,000	2,642,000
	3	2,121,000	2,121,000	2,121,000	2,121,000
	Average Log ₁₀	2,775,000 6.43	2,775,000 6.43	2,775,000 6.43	2,775,000 6.43
60 Seconds	1	1,274,000	105,100	451,500	10,150
	2	1,179,000	212,000	315,600	8,900
	3	1,595,000	351,400	345,200	7,215
	Average Log ₁₀ Reduction	1,349,333 6.13 0.31 (51.38%)	222,833 5.30 1.14 (91.97%)	370,767 5.56 0.87 (86.64%)	8,755 3.94 2.50 (99.68%)
180 Seconds	1	971,500	95,000	112,800	1,270
	2	1,151,000	115,100	211,600	4,570
	3	1,007,000	101,700	297,700	1,470
	Average Log ₁₀ Reduction	1,043,167 6.02 0.42 (62.41%)	103,933 5.02 1.42 (96.25%)	207,367 5.28 1.15 (92.53%)	2,437 3.31 3.12 (99.91%)

Reduction of *Salmonella* ranged from 0.42 to 3.12 logs after 180 seconds. Results are shown graphically in Figure 1, below.

Figure 1. Reduction of *Salmonella* by ozonated and non-ozonated water



4.3 Reduction of *E. coli* O157:H7 in steak samples

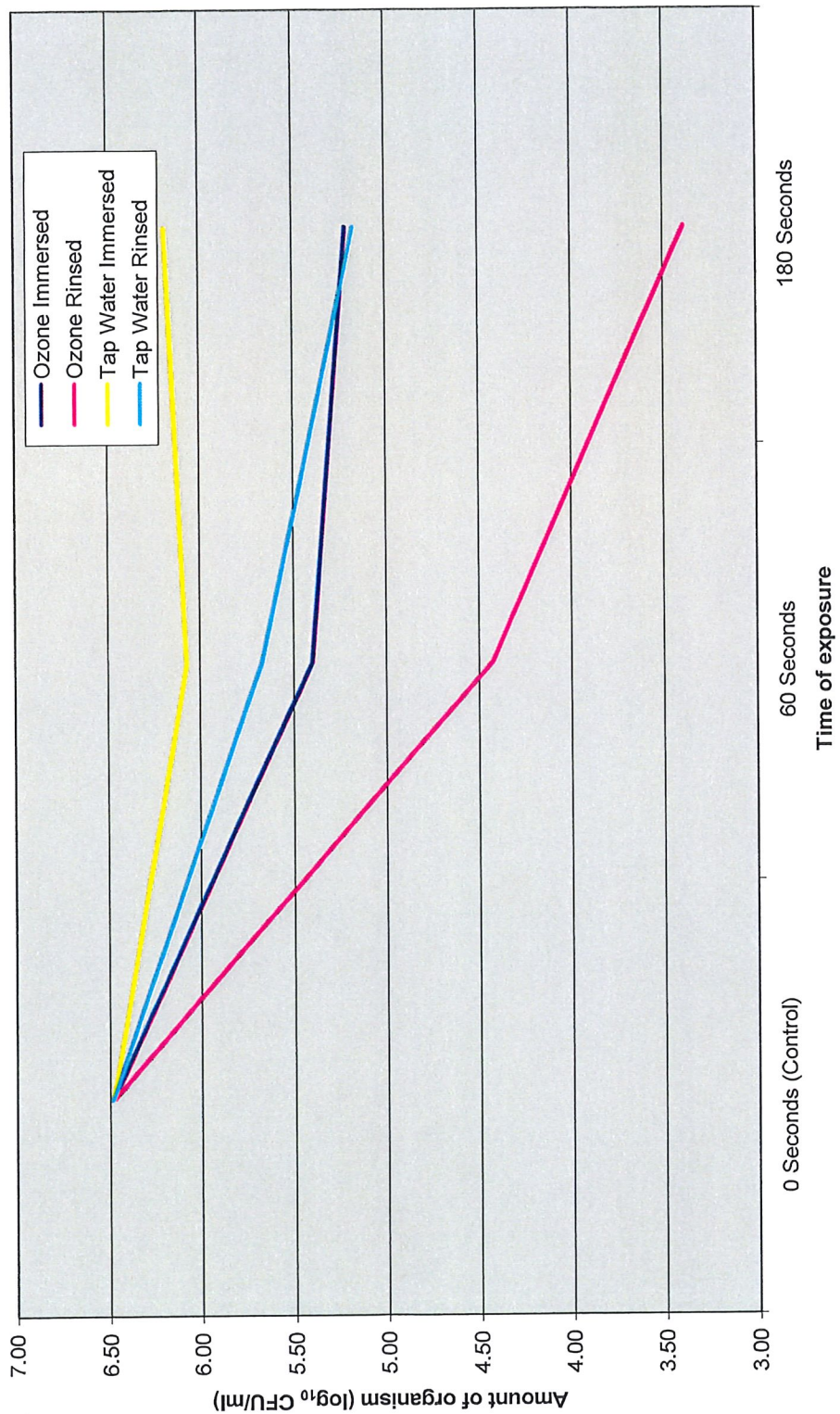
Results for *E. coli* O157:H7 from the treated steak samples are shown in Table 4 below. Table 4 includes time of immersion or rinsing in both ozonated and non-ozonated (tap) water, results (in CFU/ml of rinsate) of each replicate sample for each organism, average results (in CFU/ml) of all replicates, the log₁₀ of the average, and for all times after Time 0, the observed reduction (in log₁₀ CFU/ml) for each organism.

Table 4. *E. coli* O157:H7 reduction in steak samples

Exposure Time	Sample	Tap water, immersed	Ozonated water, immersed	Tap water, rinsed	Ozonated water, rinsed
0 Seconds (Control)	1	4,493,000	4,493,000	4,493,000	4,493,000
	2	2,268,000	2,268,000	2,268,000	2,268,000
	3	2,699,000	2,699,000	2,699,000	2,699,000
	Average Log ₁₀	3,153,333 6.48	3,153,333 6.48	3,153,333 6.48	3,153,333 6.48
60 Seconds	1	1,489,000	490,000	541,700	24,800
	2	1,002,000	311,200	474,000	31,200
	3	1,125,000	103,100	395,100	25,150
	Average Log ₁₀ Reduction	1,205,333 6.07 0.40 (61.78%)	301,433 5.40 1.08 (90.44%)	470,267 5.67 0.81 (85.09%)	27,050 4.43 2.05 (99.14%)
180 Seconds	1	1,745,000	105,400	137,100	2,971
	2	1,625,000	202,300	143,000	2,650
	3	1,297,000	201,900	164,900	1,893
	Average Log ₁₀ Reduction	1,555,667 6.19 0.29 (50.67%)	169,867 5.21 1.27 (90.44%)	148,333 5.17 1.31 (95.30%)	2,505 3.39 3.09 (99.92%)

Reduction of *E. coli* O157:H7 ranged from 0.29 to 3.09 logs after 180 seconds. Results are shown graphically in Figure 2, below.

Figure 2. Reduction of *E. coli* O157:H7 by ozonated and non-ozonated water



4.4 Measurement of residual ozone

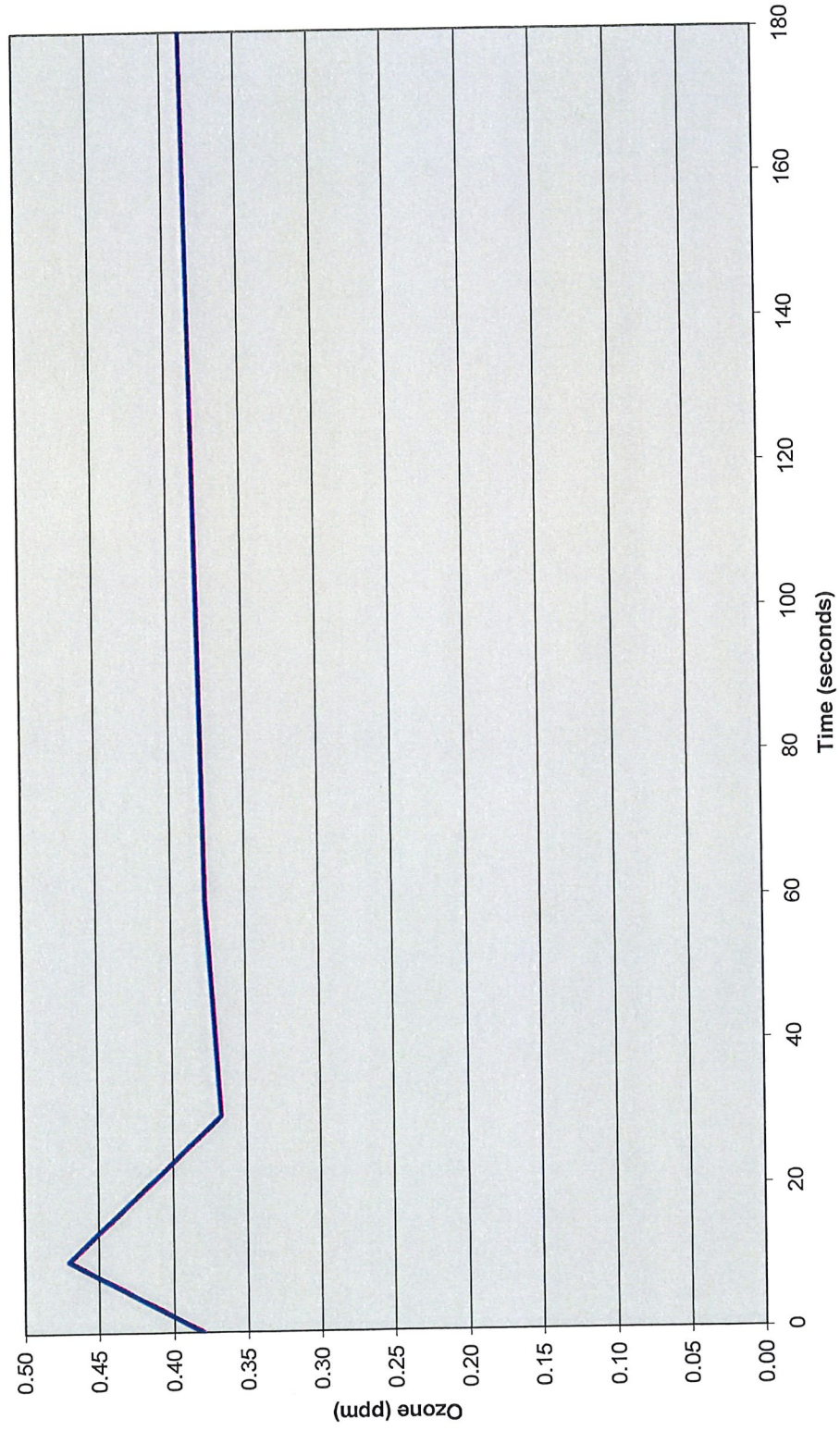
Ozone results from the ozonated rinse water analyses are shown in Table 5, including time post-ozonation, results (in ppm ozone) for each individual sample, and the average results (in ppm ozone) for all replicate samples.

Table 5. Residual ozone

Post-Ozonation Time	Sample	Ozone (ppm)
0 Seconds (Control)	1	0.36
	2	0.37
	3	0.41
	<i>Average</i>	<i>0.38</i>
10 Seconds	1	0.63
	2	0.38
	3	0.40
	<i>Average</i>	<i>0.47</i>
30 Seconds	1	0.41
	2	0.35
	3	0.34
	<i>Average</i>	<i>0.37</i>
60 Seconds	1	0.40
	2	0.37
	3	0.36
	<i>Average</i>	<i>0.38</i>
180 Seconds	1	0.36
	2	0.39
	3	0.41
	<i>Average</i>	<i>0.39</i>

Observed ozone in the water samples was relatively stable over the course of rinsing, with a spike in ozone levels seen in the 10 second samples (possibly the result of one outlying 0.63 ppm result). All other individual results ranged from 0.34 ppm to 0.41 ppm. Average ozone concentration results are shown below in Figure 3.

Figure 3. Ozone Concentration in Water Samples



5.0 CONCLUSIONS

The results of this study indicate that on steak samples the VeriPure ozonated water system is capable of reducing surface-inoculated pathogenic microorganisms from 1.27 to 1.41 logs (over 90%) after 180 seconds of immersion, nearly a log greater than the effect of non-ozonated water alone (0.29 to 0.42 logs). The greatest reduction came with constant rinsing, with observed reductions of 3.09 to 3.12 logs (over 99.9%), nearly two logs greater than the mechanical action of rinsing with tap water alone (1.15 to 1.31 logs). The greater reduction seen with the rinsed samples is likely due to the consistently high levels of ozone present in the constantly rinsed samples, as the VeriPure system continually delivered relatively high concentrations of ozone throughout the process.

6.0 REFERENCES

- Montecalvo, J. 1998. Effects of Ozonation on Surface Food Pathogen Reduction Using the Tru-Pure Ozonation System. Not Published.
- United States Department of Agriculture. 2008. USDA/FSIS Microbiology Laboratory Guidebook. Government Printing Office, Pittsburgh, PA.
- United States Food and Drug Administration. 1998. Bacteriological Analytical Manual, 8th ed. FDA, Rockville, MD.

7.0 FINAL REPORT APPROVAL

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