

Comminuted Ham Listeria Challenge Study: Effect of Treatment with Safe₂O[®]_{brand}RTE 01 or Safe₂O[®]_{brand}RTE 03 by Immersion for 30 sec and Post- treatment Incubation at 4°C

Objective:

Determine whether a 30 second treatment with Safe₂O[®]_{brand}RTE 01 or Safe₂O[®]_{brand}RTE 03 can effectively kill and inhibit *Listeria monocytogenes* outgrowth when incubated for 120 days at 4°C.

Material:

- Comminuted hams obtained from manufacturer.
- Five strains of *Listeria monocytogenes* (ATCC#: 13932, 43256, 7674, 19111 and 19115).
- Safe₂O[®]_{brand}RTE 01 was diluted one part solution with two parts water for use.
- Safe₂O[®]_{brand}RTE 03 was diluted one part solution with two parts water for use.
- Sterile water
- Sterile saline.
- Sterile peptone water.
- Sterile phosphate buffer, pH 7.38.
- Culture Media: BHI (Brain Heart Infusion Media) broth and Modified Oxford Listeria Selective Agar.

Method:

- Five strains of *Listeria monocytogenes* were cultured separately overnight in BHI broth and mixed in equal proportions. The mixture was further diluted 100,000 times with sterile saline to produce a suspension for use. Meat inoculation level was determined by removing an aliquot of the mixture, making a serial dilution and plating serial dilutions onto Modified Oxford Listeria Selective Agar plates.
- Comminuted hams were carefully unpacked and removed from the original packages onto a sterile surface inside a laminar flow bio-

safety hood, and then cut into 1.2" X 1.2" X 0.3" size pieces. Total 216 pieces.

- All pieces were placed onto a sterile surface with the apical or exterior side of the placed vertically. Pieces were exposed to a UV light for sanitation purposes. Pieces were so exposed for 15 min.
- Listeria, 20 ul, of previously diluted suspension was inoculated onto
- the skin of each piece. Following inoculation, were kept in the hood UV light off) for 30 minutes to allow bacterial attachment.
- Inoculated pieces were divided into 4 groups (T₁, T₂, C₁ and C₂), 54 pieces per group. Groups T₁, T₂ and C₁ were transferred to UV light sterilized nylon net. Group T₁ pieces were dipped in 2L Safe₂O[®]_{brand} RTE 01 for 30 seconds, group T₂ pieces were dipped in 2L Safe₂O[®]_{brand} RTE 03 for 30 seconds, group C₁ pieces were dipped in 2L sterile dH₂O for 30 seconds and group C₂ pieces were not treated.
- After the 30 sec treatment, all treated pieces were removed immediately and allowed to drain for 15 seconds.
- Each ham piece was transferred into a pre-marked vacuum pouch. Ham pieces from group C₂ pieces were directly transferred into vacuum pouches without any treatment. All pouches were kept in 4°C incubator after being vacuum sealed.
- Listeria viability was determined at one hour post-treatment and at weekly intervals thereafter.
- Microbial determination:
 - For each set time period, three pouches from each group were unpacked.
 - Five milliliters of peptone water was added into each pouch.
 - Bacteria from the surface of each ham piece were washed off by two minute hand massage of the pouch.
 - Colony forming units (CFU) per piece of ham was determined by serial dilution of an aliquot from each rinsate and plating on Modified Oxford Listeria Selective Agar plates. After plating, all plates were incubated at 37°C for about 48 hours before CFU determination.

Results:

All ham pieces were inoculated at a level of 5.66×10^2 CFU/ham piece. The results are presented in Table 1.

Table 1: Determination of the post-lethality effect and outgrowth control of a 30 second treatment with Safe₂O[®]_{brand} RTE 01 or Safe₂O[®]_{brand} RTE 03 on ham pieces inoculated with *Listeria monocytogenes* and incubated at 4°C.

Time in 4°C (weeks)	Treatment	CFU/piece	\bar{A} of CFU/piece [§]	Log Value	Log Reduction*
0	Without Any Treatment	5.00E+02	4.47E+02	2.65	
		4.60E+02			
		3.80E+02			
	Sterile dH ₂ O X 30" Dip	2.45E+02	3.43E+02	2.54	
		5.05E+02			
		2.80E+02			
	RTE 01 X 30" Dip	5.00E+00	<5.00E+00	<0.70	>1.95
		<5.00E+00			
		<5.00E+00			
	RTE 03 X 30" Dip	3.00E+00	<8.33E+00	<0.92	>1.73
		<5.00E+00			
		<5.00E+00			
1	Without Any Treatment	6.70E+02	5.47E+02	2.74	
		3.55E+02			
		6.15E+02			
	Sterile dH ₂ O X 30" Dip	1.41E+03	4.67E+03	3.67	
		4.45E+02			
		1.22E+04			
	RTE 01 X 30" Dip	1.00E+01	1.00E+01	1.00	2.67
		1.00E+01			
		1.00E+01			
	RTE 03 X 30" Dip	<5.00E+00	<5.00E+00	<0.70	>2.04
		<5.00E+00			
		<5.00E+00			

2	Without Any Treatment	1.95E+03	1.91E+03	3.28	
		9.65E+02			
		2.82E+03			
	Sterile dH ₂ O X 30" Dip	4.27E+04	9.99E+04	5.00	
		8.67E+04			
		1.70E+05			
	RTE 01 X 30" Dip	1.50E+01	<1.00E+01	<1.00	>2.28
		1.00E+01			
		<5.00E+00			
RTE 03 X 30" Dip	5.00E+00	<5.00E+00	<0.70	>2.58	
	5.00E+00				
	<5.00E+00				

3	Without Any Treatment	1.15E+04	2.48E+04	4.39	
		5.47E+04			
		8.17E+03			
	Sterile dH ₂ O X 30" Dip	1.30E+04	1.77E+04	4.25	
		8.33E+03			
		3.17E+04			
	RTE 01 X 30" Dip	5.00E+00	<5.00E+00	<0.70	<3.70
		5.00E+00			
		<5.00E+00			
RTE 03 X 30" Dip	<5.00E+00	<5.00E+00	<0.70	<3.73	
	<5.00E+00				
	<5.00E+00				

4	Without Any Treatment	1.36E+05	2.37E+06	6.37	
		3.13E+04			
		6.93E+06			
	Sterile dH ₂ O X 30" Dip	4.40E+05	8.38E+06	6.92	
		4.10E+06			
		2.06E+07			
	RTE 01 X 30" Dip	1.50E+01	1.17E+01	1.07	5.31
		5.00E+00			
		1.50E+01			
RTE 03 X 30" Dip	5.00E+00	<5.00E+00	<0.70	>5.68	
	5.00E+00				
	<5.00E+00				

5	Without Any Treatment	1.21E+07	8.24E+06	6.91	
		1.97E+04			
		1.26E+07			
	Sterile dH ₂ O X 30" Dip	8.30E+07	6.67E+08	8.82	
		6.87E+08			
		1.23E+09			
	RTE 01 X 30" Dip	5.00E+00	1.33E+01	1.12	5.79
		5.00E+00			
		3.00E+01			
	RTE 03 X 30" Dip	<5.00E+00	<5.00E+00	<0.70	>6.228.60
		5.00E+00			
		<5.00E+00			

6	Without Any Treatment	9.60E+06	5.79E+06	6.76	
		5.90E+06			
		1.87E+06			
	Sterile dH ₂ O X 30" Dip	1.93E+07	1.31E+09	9.12	
		2.05E+08			
		3.70E+09			
	RTE 01 X 30" Dip	<5.00E+00	<8.33E+00	<0.92	>5.84
		1.50E+01			
		<5.00E+00			
	RTE 03 X 30" Dip	<5.00E+00	<5.00E+00	<0.70	>6.06
		<5.00E+00			
		<5.00E+00			

7	Without Any Treatment	2.85E+06	3.16E+08	8.50	
		6.30E+08			

	Sterile dH ₂ O X 30" Dip	4.87E+09	4.24E+09	9.63	
		2.72E+09			
		5.13E+09			
	RTE 01 X 30" Dip	1.50E+01	<8.33E+00	<0.92	>7.58
		<5.00E+00			
		<5.00E+00			
	RTE 03 X 30" Dip	<5.00E+00	9.50E+01	<1.96	>6.52
		<5.00E+00			
		2.75E+02			

8	Without Any Treatment	2.27E+08	1.24E+08	8.09	
		1.36E+08			
		8.07E+06			
	Sterile dH ₂ O X 30" Dip	6.00E+09	3.44E+09	9.54	
		3.60E+09			
		7.23E+08			
	RTE 01 X 30" Dip	5.00E+00	<6.67E+00	<0.82	>7.27
		1.00E+01			
		0.00E+00			
	RTE 03 X 30" Dip	<5.00E+00	<5.00E+00	<0.70	>7.39
		<5.00E+00			
		<5.00E+00			

9	Without Any Treatment	2.24E+09	1.18E+09	9.07	
		1.29E+09			
		2.73E+07			
	Sterile dH ₂ O X 30" Dip	3.77E+09	4.34E+09	9.64	
		6.17E+09			
		3.08E+09			
	RTE 01 X 30" Dip	1.00E+01	<6.67E+00	<0.82	>8.25
		5.00E+00			
		<5.00E+00			
	RTE 03 X 30" Dip	<5.00E+00	<5.00E+00	<0.70	>8.38
		<5.00E+00			
		5.00E+00			

10	Without Any Treatment	2.87E+08	2.62E+09	9.42	
		4.00E+09			
		3.57E+09			
	Sterile dH ₂ O X 30" Dip	6.30E+09	3.93E+09	9.59	
		2.10E+09			
		3.40E+09			
	RTE 01 X 30" Dip	<5.00E+00	<6.67E+00	0.82	<8.89
		1.00E+01			
		5.00E+00			
	RTE 03 X 30" Dip	<5.00E+00	<5.00E+00	<0.70	>8.72
		<5.00E+00			
		<5.00E+00			

11	Without Any Treatment	3.40E+09	9.39E+09	9.97	
		1.30E+09			
		N/A			
	Sterile dH ₂ O X 30" Dip	4.40E+09	4.93E+09	9.69	
		5.53E+09			
		4.87E+09			
	RTE 01 X 30" Dip	5.00E+00	5.00E+00	0.70	8.67
		5.00E+00			
		5.00E+00			
	RTE 03 X 30" Dip	<5.00E+00	<5.00E+00	<0.70	<8.67
		<5.00E+00			
		<5.00E+00			

12	Without Any Treatment	2.73E+09	9.40E+08	8.97	
		5.33E+07			
		3.33E+07			
	Sterile dH ₂ O X 30" Dip	3.06E+09	3.15E+09	9.50	
		2.55E+09			
		3.83E+09			
	RTE 01 X 30" Dip	<5.00E+00	<5.00E+00	<0.70	8.27
		<5.00E+00			
		5.00E+00			
	RTE 03 X 30" Dip	<5.00E+00	<5.00E+00	<0.70	>8.27
		<5.00E+00			
		<5.00E+00			

13	Without Any Treatment	1.47E+09	2.08E+09	9.32	
		2.68E+09			
		2.09E+09			
	Sterile dH ₂ O X 30" Dip	3.15E+09	3.12E+09	9.49	
		2.94E+09			
		3.26E+09			
	RTE 01 X 30" Dip	5.00E+00	<5.00E+00	0.70	>8.62
		<5.00E+00			
		<5.00E+00			
	RTE 03 X 30" Dip	<5.00E+00	<5.00E+00	<0.70	>8.62
		<5.00E+00			
		<5.00E+00			

14	Without Any Treatment	1.99E+09	7.72E+09	9.89	
		1.90E+10			
		2.13E+09			
	Sterile dH ₂ O X 30" Dip	2.40E+09	1.86E+09	9.27	
		2.52E+09			
		6.47E+08			
	RTE 01 X 30" Dip	1.00E+01	<6.67E+00	0.82	>8.48
		5.00E+00			
		<5.00E+00			
	RTE 03 X 30" Dip	<5.00E+00	<5.00E+00	<0.70	>8.60
		<5.00E+00			
		<5.00E+00			

15	Without Any Treatment	2.81E+09	2.81E+09		
		8.67E+08			
		1.68E+09			
	Sterile dH ₂ O X 30" Dip	3.77E+09	1.90E+09		
		2.83E+09			
		4.00E+09			
	RTE 01 X 30" Dip	<5.00E+00	<5.00+00	<0.70	>8.55
		<5.00E+00			
		<5.00E+00			
	RTE 03 X 30" Dip	<5.00E+00	<5.00E+00	<0.70	>8.55
		<5.00E+00			
		<5.00E+00			

16	Without Any Treatment	2.53E+09	2.45E+09		
		1.82E+09			
		3.00E+09			
	Sterile dH ₂ O X 30" Dip	3.63E+09	2.66E+09		
		2.83E+09			
		4.00E+09			
	RTE 01 X 30" Dip	<5.00E+00	<5.00E+00	<0.70	>8.69
		<5.00E+00			
		<5.00E+00			
	RTE 03 X 30" Dip	<5.00E+00	<5.00E+00	<0.70	>8.62
		<5.00E+00			
		<5.00E+00			

17	Without Any Treatment	1.78E+09	1.96E+09	9.29	
		2.27E+09			
		1.82E+09			
	Sterile dH ₂ O X 30" Dip	1.81E+09	2.52+09	9.40	
		2.34E+09			
		3.37E+09			
	RTE 01 X 30" Dip	<5.00E+00	<5.00E+00	<0.70	>8.59
		<5.00E+00			
		<5.00E+00			
	RTE 03 X 30" Dip	<5.00E+00	<5.00E+00	<0.70	>8.59
		<5.00E+00			
		<5.00E+00			

§ **Detection limit 5 CFU/piece.**

***Relative to Group C₂.**

Conclusions:

As can be seen from Table 1, treatment with Safe₂O[®]_{brand} RTE 01 and Safe₂O[®]_{brand} RTE 03 were effective in preventing *Listeria* outgrowth over the study period. In addition, both treatments caused a post-lethality effect in excess of 2 logs.

Based on FSIS Directive 10,240.4, a treatment with either Safe₂O[®]_{brand} RTE 01 or Safe₂O[®]_{brand} RTE 03 would effectively allow the manufacturer to establish an Alternative 1 status for ham products treated as prescribed above.