



# CERTIFICATION

## AOAC Research Institute *Performance Tested Methods*<sup>SM</sup>

Certificate No.  
**022104**

The AOAC Research Institute hereby certifies the method known as:

### **Environmental Scrub Sampler with Wide Spectrum Neutralizer**

manufactured by

**Neogen Corporation**  
**620 Leshar Place**  
**Lansing, Michigan 48912**  
**USA**

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*<sup>SM</sup> Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*<sup>SM</sup> certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

Bradley A. Stawick, Senior Director  
Signature for AOAC Research Institute

Issue Date  
Expiration Date

December 05, 2024  
December 31, 2026

<b>AUTHORS</b> Micki Rosauer, Wesley Thompson, Benjamin Bastin, M. Joseph Benzinger, Jr., and James Agin	<b>SUBMITTING COMPANY</b> 3M Company Food Safety Department 3M Center, Bldg 275-5W-05 St. Paul, MN 55144	<b>CURRENT SPONSOR</b> Neogen Food Safety Corporation 620 Leshler Place Lansing, MI 48912
<b>METHOD NAMES</b> Neogen® Environmental Scrub Sampler with 10 mL Wide Spectrum Neutralizer Neogen® Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer Formerly 3M™ Environmental Scrub Sampler with 10 mL Wide Spectrum Neutralizer and 3M™ Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer	<b>CATALOG NUMBERS</b> HES10WSN2G, ESS10WSN	
<b>INDEPENDENT LABORATORY</b> Q Laboratories 1930 Radcliff Drive Cincinnati, OH 45204 USA	<b>APPLICABILITY OF METHOD</b> Analytes – <i>Salmonella</i> spp. and <i>Listeria</i> spp.  Matrixes – (4" x 4") - Stainless steel, sealed concrete, plastic.  Performance claims – Performance comparable to that of Dey-Engley Neutralizing Buffer with Cellulose Sponge as outlined in the U.S. Food and Drug Administration Bacteriological Analytical Manual (BAM) Chapter 5, detection method for <i>Salmonella</i> (2020) (2) and Chapter 10, Detection of <i>Listeria monocytogenes</i> in Foods and Environmental Samples, and Enumeration of <i>Listeria monocytogenes</i> in Foods (2017) (3). In addition, as demonstrated according to ASTM E1054 – 08, Standard Test Methods for Evaluation of Antimicrobial Agents (4), the product effectively neutralizes quaternary ammonium, high acid, hydrogen/peroxyacetic acid and chlorine/bleach-based sanitizers at the concentrations tested, while being non-toxic to <i>Salmonella</i> and <i>Listeria</i> species.	
<b>ORIGINAL CERTIFICATION DATE</b> February 25, 2021	<b>CERTIFICATION RENEWAL RECORD</b> Renewed through December 2026.	
<b>METHOD MODIFICATION RECORD</b> 1. November 2021 Level 1 2. January 2024 Level 1 3. December 2024 Level 1	<b>SUMMARY OF MODIFICATION</b> 1. Editorial changes. 2. Editorial changes to rebrand method from 3M to Neogen Corporation. 3. Editorial changes.	
Under this AOAC Performance Tested Methods <sup>SM</sup> License Number, 022104 this method is distributed by: <b>NONE</b>	Under this AOAC Performance Tested Methods <sup>SM</sup> License Number, 022104 this method is distributed as: <b>NONE</b>	

**PRINCIPLE OF THE METHOD (1)**

The Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer is intended for collecting environmental monitoring surface samples in food production facilities. Pre-hydrated sampling devices are packaged in bags with a broad-spectrum neutralizing buffer. The Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer is intended for use in production and laboratory environments by professionals trained in sample collection and laboratory techniques.

The Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer is a non-specific collection method and not a stand-alone detection method. The performance of the device was evaluated by comparing recovery of target microorganisms from select environmental surfaces to that of the appropriate reference method collection method. Two microorganisms of interest in food production facilities, *Salmonella* and *Listeria* species, were tested on three environmental surfaces, stainless steel, plastic, and sealed concrete.

**DISCUSSION OF THE VALIDATION STUDY (1)**

The Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer recovered all inclusivity organisms for both *Salmonella* and *Listeria*. The Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer was able to recover *Salmonella* spp. and *Listeria* spp. from several different environmental surfaces including: stainless steel, plastic (polystyrene), and sealed concrete. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate sampling method and the reference sampling method for all samples tested. The Wide Spectrum Neutralizer successfully neutralized a range of sanitizers including quaternary ammonium, high acid, hydrogen peroxide/peroxyacetic acid, and chlorine/bleach and was found to be non-toxic to the target organisms. The Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer was found to be a robust and stable sampling device through robustness and product consistency testing.

**Table 1. Inclusivity testing results for *Listeria* species using Wide Spectrum Neutralizer with Environmental Scrub Sampler<sup>a</sup> (1)**

Number	Strain Source	Strain ID	Genus	species	Serotype	Isolation Source	<i>Listeria</i> results
1	ATCC <sup>b</sup>	51782	<i>Listeria</i>	<i>monocytogenes</i>	3a	Cheese	positive
2	CWD <sup>c</sup>	1600	<i>Listeria</i>	<i>monocytogenes</i>	3b	Not Available	positive
3	FSL <sup>d</sup>	J1-049	<i>Listeria</i>	<i>monocytogenes</i>	3c	Not Available	positive
4	FSL	J1-129	<i>Listeria</i>	<i>monocytogenes</i>	4ab	Not Available	positive
5	ATCCC	19114	<i>Listeria</i>	<i>monocytogenes</i>	4a	Animal Tissue	positive
6	CWD	1563	<i>Listeria</i>	<i>monocytogenes</i>	4b	Lausanne, 1987	positive
7	ATCC	19116	<i>Listeria</i>	<i>monocytogenes</i>	4c	Chicken	positive
8	ATCC	19117	<i>Listeria</i>	<i>monocytogenes</i>	4d	Sheep	positive
9	ATCC	19118	<i>Listeria</i>	<i>monocytogenes</i>	4e	Chicken	positive
10	CWD	1554	<i>Listeria</i>	<i>monocytogenes</i>	1/2a	Carlisle, 1981	positive
11	ATCC	51780	<i>Listeria</i>	<i>monocytogenes</i>	1/2b	Dairy Products	positive
12	ATCC	7644	<i>Listeria</i>	<i>monocytogenes</i>	1/2c	Human Isolate	positive
13	NCTC <sup>e</sup>	10890	<i>Listeria</i>	<i>monocytogenes</i>	7	Human Feces	positive
14	NCTC	19120a	<i>Listeria</i>	<i>grayi</i>		Animal Feces	positive
15	ATCC	25401b	<i>Listeria</i>	<i>grayi</i>		Corn Stalks	positive
16	CWD	167	<i>Listeria</i>	<i>innocua</i>		Not Available	positive
17	CWD	217	<i>Listeria</i>	<i>innocua</i>		Not Available	positive
18	ATCC	19119	<i>Listeria</i>	<i>ivanovii</i>		Sheep	positive
19	ATCC	49954	<i>Listeria</i>	<i>ivanovii</i>		Food, France	positive
20	ATCC	11289	<i>Listeria</i>	<i>seeligeri</i>		Human Feces	positive
21	NCTC	11856	<i>Listeria</i>	<i>seeligeri</i>		Not Available	positive
22	ATCC	51334	<i>Listeria</i>	<i>seeligeri</i>		Intestinal Content	positive
23	ATCC	35897	<i>Listeria</i>	<i>welshimeri</i>		Not Available	positive
24	ATCC	43549	<i>Listeria</i>	<i>welshimeri</i>		Soil	positive
25	ATCC	43550	<i>Listeria</i>	<i>welshimeri</i>		Human Feces	positive

<sup>a</sup>Detection method = U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM), Chapter 10 Detection of *L. monocytogenes* in Foods and Environmental Samples, and Enumeration of *L. monocytogenes* in Foods (Revised March 2017).

<sup>b</sup>ATCC = American Type Culture Collection, Manassas, VA.

<sup>c</sup>CWD = University of Vermont Culture Collection, Burlington, VT.

<sup>d</sup>FSL = Food Safety Laboratory; Department of Food Science, Cornell University, Ithaca, NY.

<sup>e</sup>NCTC = The National Collection of Type Cultures, Salisbury, United Kingdom.

**Table 2. Exclusivity testing results for *Listeria* species using Wide Spectrum Neutralizer with Environmental Scrub Sampler<sup>a</sup> (1)**

Number	Source	Strain ID	Genus	Species	Source	<i>Listeria</i> results
1	ATCC <sup>b</sup>	7050	<i>Bacillus</i>	<i>coagulans</i>	Evaporated Milk	negative
2	ATCC	8043	<i>Enterococcus</i>	<i>hirae</i>	Not Available	negative
3	ATCC	19434	<i>Enterococcus</i>	<i>faecium</i>	Not available	negative
4	ATCC	19432	<i>Enterococcus</i>	<i>durans</i>	Not Available	negative
5	ATCC	29212	<i>Enterococcus</i>	<i>faecalis</i>	Human cerebrospinal fluid	negative
6	ATCC	6462	<i>Bacillus</i>	<i>mycoides</i>	Soil	negative
7	ATCC	11509	<i>Brochothrix</i>	<i>thermosphacta</i>	Pork Sausage	negative
8	ATCC	7468	<i>Micrococcus</i>	<i>luteus</i>	Not Available	negative
9	ATCC	6939	<i>Rhodococcus</i>	<i>equi</i>	Not available	negative
10	ATCC	29885	<i>Staphylococcus</i>	<i>warneri</i>	Not available	negative
11	ATCC	9341	<i>Kocuria</i>	<i>rhizophila</i>	Not available	negative
12	ATCC	43195	<i>Kurthia</i>	<i>gibsonii</i>	Not available	negative
13	ATCC	29247	<i>Staphylococcus</i>	<i>aureus</i>	Not available	negative
14	ATCC	12228	<i>Staphylococcus</i>	<i>epidermidis</i>	Not available	negative
15	ATCC	19615	<i>Streptococcus</i>	<i>pyogenes</i>	Pharynx of child	negative

<sup>a</sup>Detection method = U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM), Chapter 10 Detection of *L. monocytogenes* in Foods and Environmental Samples, and Enumeration of *L. monocytogenes* in Foods (Revised March 2017).

<sup>b</sup>ATCC = American Type Culture Collection, Manassas, VA.

Table 3. Inclusivity testing results for *Salmonella* species using Wide Spectrum Neutralizer with Environmental Scrub Sampler<sup>a</sup> (1)

Number	Strain Source	Strain ID	Genus	species	Subspecies	Serotype	Isolation Source	<i>Salmonella</i> result
1	NCTC <sup>b</sup>	12419 <sup>a</sup>	<i>Salmonella</i>	<i>bongori</i>			Not Available	positive
2	NCTC	10946	<i>Salmonella</i>	<i>bongori</i>		Brookfield	Not Available	positive
3	ATCC <sup>c</sup>	43975 <sup>b</sup>	<i>Salmonella</i>	<i>bongori</i>			Not Available	positive
4	ATCC	13314	<i>Salmonella</i>	<i>enterica</i>	<i>arizonae</i>		Not Available	positive
5	ATCC	BAA-1577	<i>Salmonella</i>	<i>enterica</i>	<i>arizonae</i>		Not Available	positive
6	QL <sup>d</sup>	11007-4 <sup>c</sup>	<i>Salmonella</i>	<i>enterica</i>	<i>arizonae</i>		Veterinary Isolate	positive
7	QL	011414.2	<i>Salmonella</i>	<i>enterica</i>	<i>arizonae</i>		Environmental isolate	positive
8	QL	024.114	<i>Salmonella</i>	<i>enterica</i>	<i>arizonae</i>		Pet Food	positive
9	ATCC	BAA-1579	<i>Salmonella</i>	<i>enterica</i>	<i>diarizonae</i>		Not Available	positive
10	ATCC	BAA-216	<i>Salmonella</i>	<i>enterica</i>	<i>diarizonae</i>		Human blood	positive
11	ATCC	BAA-639	<i>Salmonella</i>	<i>enterica</i>	<i>diarizonae</i>		Human feces	positive
12	QL	024.516	<i>Salmonella</i>	<i>enterica</i>	<i>diarizonae</i>		Pet Food	positive
13	QL	011414.1	<i>Salmonella</i>	<i>enterica</i>	<i>diarizonae</i>		Environmental isolate	positive
14	ATCC	35640	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Abaetetuba	Creek water	positive
15	FDA <sup>e</sup>	9842 <sup>d</sup>	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Abortusequi	Not Available	positive
16	NCTC	10241	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Abortusovis	Not Available	positive
17	NCTC	6017	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Abony	Not Available	positive
18	STs <sup>f</sup>	2 <sup>e</sup>	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Adelaide	Not Available	positive
19	ATCC	51957	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Agona	Not Available	positive
20	STs	3	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Agama	Not Available	positive
21	STs	5	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Agouve	Not Available	positive
22	STs	6	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Alachua	Not Available	positive
23	STs	7	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Albany	Not Available	positive
24	ATCC	9270	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Anatum	Pork liver	positive
25	STs	11	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Arkansas	Not Available	positive
26	FDA	1206H	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Bareilly	Not Available	positive
27	STs	13	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Berta	Not Available	positive
28	STs	14	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Binza	Not Available	positive
29	STs	16	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Bovismorbificans	Not Available	positive
30	STs	18	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Brandenburg	Not Available	positive
31	NCTC	5731	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Bredeney	Not Available	positive
32	NCTC	6018	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	California	Not Available	positive
33	STs	22	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Cerro	Not Available	positive
34	ATCC	10708	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Choleraesuis	Equine isolate	positive
35	ATCC	12011	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Choleraesuis var Kunzendorf	Not Available	positive
36	STs	24	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Cubana	Not Available	positive
37	NCTC	5721	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Derby	Not Available	positive
38	STs	26	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Drypool	Not Available	positive
39	STs	27	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Dublin	Not Available	positive
40	FDA	4017H	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Eastbourne	Not Available	positive
41	ATCC	13076	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Enteritidis	Not Available	positive
42	QL	024.2	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Galiema	Environmental isolate	positive
43	STs	42	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Give	Not Available	positive
44	STs	44	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Haardt	Not Available	positive
45	ATCC	51956	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Hadar	Not Available	positive
46	STs	47	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Havana	Not Available	positive
47	ATCC	8326	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Heidelberg	Not Available	positive
48	NCTC	11304	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Indiana	Turkey	positive
49	ATCC	51741	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Infantis	Pasta	positive
50	ATCC	10721	<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i>	Javiana	Not Available	positive

<sup>a</sup> Detection method = U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM), Chapter 5, *Salmonella*.

<sup>b</sup>NCTC = The National Collection of Type Cultures, Salisbury, United Kingdom.

<sup>c</sup>ATCC = American Type Culture Collection, Manassas, VA.

<sup>d</sup>QL = Q Laboratories, Cincinnati, OH.

<sup>e</sup>FDA = Food and Drug Administration Culture Collection – Silver Spring, MD.

<sup>f</sup>STs = University of Pennsylvania – Philadelphia, PA.

**Table 4. Exclusivity testing results for *Salmonella* species using Wide Spectrum Neutralizer with Environmental Scrub Sampler <sup>a</sup> (1)**

Number	Source	Strain ID	Genus	Species	Source	<i>Salmonella</i> result
1	ATCC <sup>b</sup>	14579	<i>Bacillus</i>	<i>cereus</i>	Not Available	negative
2	ATCC	6051	<i>Bacillus</i>	<i>subtilis</i>	Not Available	negative
3	ATCC	51112	<i>Citrobacter</i>	<i>farmeri</i>	Human Feces	negative
4	ATCC	8090	<i>Citrobacter</i>	<i>freundii</i>	Not Available	negative
5	ATCC	15947	<i>Edwardsiella</i>	<i>tarda</i>	Human Feces	negative
6	ATCC	13048	<i>Klebsiella (Enterobacter)</i>	<i>aerogenes</i>	Sputum	negative
7	ATCC	23355	<i>Enterobacter</i>	<i>cloacae</i>	Not Available	negative
8	ATCC	29212	<i>Enterococcus</i>	<i>faecalis</i>	Human cerebrospinal fluid	negative
9	ATCC	25922	<i>Escherichia</i>	<i>coli</i>	Feces	negative
10	ATCC	51813	<i>Hafnia</i>	<i>alvei</i>	Milk	negative
11	ATCC	13883	<i>Klebsiella</i>	<i>pneumoniae</i>	Not Available	negative
12	ATCC	25829	<i>Morganella</i>	<i>morganii</i>	Human	negative
13	ATCC	7002	<i>Proteus</i>	<i>mirabilis</i>	Urine	negative
14	ATCC	27853	<i>Pseudomonas</i>	<i>aeruginosa</i>	Clinical Isolate	negative
15	ATCC	29930	<i>Shigella</i>	<i>sonnei</i>	Not Available	negative

<sup>a</sup>Detection method = U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM), Chapter 5, *Salmonella*.

<sup>b</sup>ATCC = American Type Culture Collection, Manassas, VA.

**Table 5. Wide Spectrum Neutralizer with Environmental Scrub Sampler, Candidate vs. Reference – POD Results (1)**

Matrix	Strain	CFU <sup>a</sup> /test area	N <sup>b</sup>	Candidate method results			Reference method <sup>f</sup> results			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				X <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Stainless Steel (4" x 4")	<i>S. Typhimurium</i> ATCC <sup>i</sup> 14028 & 10X <i>C. freundii</i> ATCC 8090	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		56	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
		230	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel with Sanitizer (4" x 4")	<i>L. monocytogenes</i> 4a ATCC 19114 & 10X <i>E. faecalis</i> ATCC 29212	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		75	20	8	0.40	0.22, 0.61	6	0.30	0.15, 0.52	0.10	-0.18, 0.36
		260	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Plastic (Polystyrene) (4" x 4")	<i>Salmonella</i> Dublin STs <sup>k</sup> 27	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		60	20	12	0.60	0.39, 0.78	10	0.50	0.30, 0.70	0.10	-0.19, 0.37
		240	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Sealed Concrete (4" x 4")	<i>Listeria innocua</i> CWD <sup>l</sup> 167	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		76	20	11	0.55	0.34, 0.74	10	0.50	0.30, 0.70	0.05	-0.24, 0.33
		280	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for the matrix in triplicate.

<sup>b</sup>N = Number of test portions.

<sup>c</sup>x = Number of positive test portions.

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials.

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials.

<sup>f</sup>Dey-Engley Neutralizing broth with cellulose sponge.

<sup>g</sup>dPOD<sub>C</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values.

<sup>h</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>i</sup>ATCC = American Type Culture Collection, Manassas, VA.

<sup>j</sup>N/A = Not applicable.

<sup>k</sup>STs = University of Pennsylvania, Philadelphia, PA.

<sup>l</sup>CWD = University of Vermont Culture Collection, Burlington, VT.

**Table 6. Sanitizer Neutralization per ASTM E1054 – 08 using Wide Spectrum Neutralizer with Environmental Scrub Sampler: Bleach (1)**

	<i>Listeria monocytogenes</i> (1/2a) CWD <sup>a</sup> 1554			<i>Listeria monocytogenes</i> (1/2a), CWD 1554			<i>Salmonella</i> Senftenberg ATCC <sup>b</sup> 43845			<i>Salmonella</i> Senftenberg ATCC 43845		
	Initial Time Point			Post-10 Minute Hold			Initial Time Point			Post-10 Minute Hold		
	Replicate			Replicate			Replicate			Replicate		
	A	B	C	A	B	C	A	B	C	A	B	C
Test Organism Viability <sup>c</sup> , Mean CFU/mL	37	39	42	32	35	38	51	49	54	46	50	47
Neutralizer Effectiveness <sup>d</sup> , Mean CFU/mL	34	40	38	35	36	41	40	46	45	43	51	56
Neutralizer Effectiveness Determination	Effective			Effective			Effective			Effective		
Neutralizer Effectiveness P- Value <sup>e</sup>	0.91			0.68			0.30			0.70		
Neutralizer Toxicity <sup>f</sup> , Mean CFU/mL	37	38	43	37	39	37	47	45	51	50	55	54
Neutralizer Toxicity Determination	Non-Toxic			Non-Toxic			Non-Toxic			Non-Toxic		
Neutralizer Toxicity P- Value	0.68			0.68			0.70			0.70		
Suitability Test Result (CFU/mL)	Pass			Pass			Pass			Pass		

<sup>a</sup> CWD = University of Vermont Culture Collection – Burlington, VT.

<sup>b</sup> ATCC = American Type Culture Collection - Manassas, VA.

<sup>c</sup> Referred to as Test C in ASTM E1054 – 08, Standard Test Methods for Evaluation of Antimicrobial Agents.

<sup>d</sup> Referred to as Test A in ASTM E1054 – 08, Standard Test Methods for Evaluation of Antimicrobial Agents.

<sup>e</sup> A t-test indicated no statistical significance (p> 0.05).

<sup>f</sup> Referred to as Test B in ASTM E1054 – 08, Standard Test Methods for Evaluation of Antimicrobial Agents.

**Table 7. Sanitizer Neutralization per ASTM E1054 – 08 using Wide Spectrum Neutralizer with Environmental Scrub Sampler: Star San (1)**

	<i>Listeria monocytogenes</i> (1/2a) CWD <sup>a</sup> 1554			<i>Listeria monocytogenes</i> (1/2a), CWD 1554			<i>Salmonella</i> Senftenberg ATCC <sup>b</sup> 43845			<i>Salmonella</i> Senftenberg ATCC 43845		
	Initial Time Point			Post-10 Minute Hold			Initial Time Point			Post-10 Minute Hold		
	Replicate			Replicate			Replicate			Replicate		
	A	B	C	A	B	C	A	B	C	A	B	C
Test Organism Viability <sup>c</sup> , Mean CFU/mL	37	39	42	32	35	38	51	49	54	46	50	47
Neutralizer Effectiveness <sup>d</sup> , Mean CFU/mL	33	34	38	33	35	37	44	48	51	44	53	49
Neutralizer Effectiveness Determination	Effective			Effective			Effective			Effective		
Neutralizer Effectiveness P- Value <sup>e</sup>	0.23			0.68			0.49			0.70		
Neutralizer Toxicity <sup>f</sup> , Mean CFU/mL	37	38	43	37	39	37	47	45	51	50	55	54
Neutralizer Toxicity Determination	Non-Toxic			Non-Toxic			Non-Toxic			Non-Toxic		
Neutralizer Toxicity P- Value	0.68			0.68			0.70			0.70		
Suitability Test Result (CFU/mL)	Pass			Pass			Pass			Pass		

<sup>a</sup> CWD = University of Vermont Culture Collection – Burlington, VT.

<sup>b</sup> ATCC = American Type Culture Collection - Manassas, VA.

<sup>c</sup> Referred to as Test C in ASTM E1054 – 08, Standard Test Methods for Evaluation of Antimicrobial Agents.

<sup>d</sup> Referred to as Test A in ASTM E1054 – 08, Standard Test Methods for Evaluation of Antimicrobial Agents.

<sup>e</sup> A t-test indicated no statistical significance (p> 0.05).

<sup>f</sup> Referred to as Test B in ASTM E1054 – 08, Standard Test Methods for Evaluation of Antimicrobial Agents.

**Table 8. Sanitizer Neutralization per ASTM E1054 – 08 using Wide Spectrum Neutralizer with Environmental Scrub Sampler: Vortex (1)**

	<i>Listeria monocytogenes</i> (1/2a) CWD <sup>a</sup> 1554			<i>Listeria monocytogenes</i> (1/2a), CWD 1554			<i>Salmonella</i> Senftenberg ATCC <sup>b</sup> 43845			<i>Salmonella</i> Senftenberg ATCC 43845		
	Initial Time Point			Post-10 Minute Hold			Initial Time Point			Post-10 Minute Hold		
	Replicate			Replicate			Replicate			Replicate		
	A	B	C	A	B	C	A	B	C	A	B	C
Test Organism Viability <sup>c</sup> , Mean CFU/mL	37	39	42	32	35	38	51	49	54	46	50	47
Neutralizer Effectiveness <sup>d</sup> , Mean CFU/mL	33	35	39	35	32	41	46	53	49	43	49	52
Neutralizer Effectiveness Determination	Effective			Effective			Effective			Effective		
Neutralizer Effectiveness P- Value <sup>e</sup>	0.52						0.66					
Neutralizer Toxicity <sup>f</sup> , Mean CFU/mL	37	38	43	37	39	37	47	45	51	50	55	54
Neutralizer Toxicity Determination	Non-Toxic			Non-Toxic			Non-Toxic			Non-Toxic		
Neutralizer Toxicity P- Value	0.68						0.70					
Suitability Test Result (CFU/mL)	Pass			Pass			Pass			Pass		

<sup>a</sup> CWD = University of Vermont Culture Collection – Burlington, VT.

<sup>b</sup> ATCC = American Type Culture Collection - Manassas, VA.

<sup>c</sup> Referred to as Test C in ASTM E1054 – 08, Standard Test Methods for Evaluation of Antimicrobial Agents.

<sup>d</sup> Referred to as Test A in ASTM E1054 – 08, Standard Test Methods for Evaluation of Antimicrobial Agents.

<sup>e</sup> A t-test indicated no statistical significance (p> 0.05).

<sup>f</sup> Referred to as Test B in ASTM E1054 – 08, Standard Test Methods for Evaluation of Antimicrobial Agents.

**Table 9. Sanitizer Neutralization per ASTM E1054 – 08 using Wide Spectrum Neutralizer with Environmental Scrub Sampler: Whisper V (1)**

	<i>Listeria monocytogenes</i> (1/2a) CWD <sup>a</sup> 1554			<i>Listeria monocytogenes</i> (1/2a), CWD 1554			<i>Salmonella</i> Senftenberg ATCC <sup>b</sup> 43845			<i>Salmonella</i> Senftenberg ATCC 43845		
	Initial Time Point			Post-10 Minute Hold			Initial Time Point			Post-10 Minute Hold		
	Replicate			Replicate			Replicate			Replicate		
	A	B	C	A	B	C	A	B	C	A	B	C
Test Organism Viability <sup>c</sup> , Mean CFU/mL	37	39	42	32	35	38	51	49	54	46	50	47
Neutralizer Effectiveness <sup>d</sup> , Mean CFU/mL	40	43	48	36	41	40	50	47	54	46	53	59
Neutralizer Effectiveness Determination	Effective			Effective			Effective			Effective		
Neutralizer Effectiveness P- Value <sup>e</sup>	0.08						0.42					
Neutralizer Toxicity <sup>f</sup> , Mean CFU/mL	37	38	43	37	39	37	47	45	51	50	55	54
Neutralizer Toxicity Determination	Non-Toxic			Non-Toxic			Non-Toxic			Non-Toxic		
Neutralizer Toxicity P- Value	0.68						0.70					
Suitability Test Result (CFU/mL)	Pass			Pass			Pass			Pass		

<sup>a</sup> CWD = University of Vermont Culture Collection – Burlington, VT.

<sup>b</sup> ATCC = American Type Culture Collection - Manassas, VA.

<sup>c</sup> Referred to as Test C in ASTM E1054 – 08, Standard Test Methods for Evaluation of Antimicrobial Agents.

<sup>d</sup> Referred to as Test A in ASTM E1054 – 08, Standard Test Methods for Evaluation of Antimicrobial Agents.

<sup>e</sup> A t-test indicated no statistical significance (p> 0.05).

<sup>f</sup> Referred to as Test B in ASTM E1054 – 08, Standard Test Methods for Evaluation of Antimicrobial Agents.

**Table 12. Wide Spectrum Neutralizer with Environmental Scrub Sampler: Robustness Experimental Design (1)**

Treatment Combination	Hold Time <sup>a</sup> (Hours)	Neutralizing Buffer Volume (mL)
1	0	9
2	0	11
3	48	9
4	48	11
5	96	9
6	96	11
7 (Normal Condition)	0	10

<sup>a</sup>Samplers were held at 4-8°C before processing, with the exception of the 0 hour time point.

**Table 13. Wide Spectrum Neutralizer with Environmental Scrub Sampler: Robustness *Listeria* Data (1)**

Combination	Replicates (CFU/mL)									
	A	B	C	D	E	F	G	H	I	J
1	82	90	94	86	84	91	85	93	90	94
2	85	93	94	87	86	87	89	92	88	90
3	84	78	89	92	93	87	88	81	82	86
4	88	89	94	82	86	85	84	93	91	87
5	91	90	90	87	93	89	91	88	95	87
6	87	98	90	88	86	91	87	89	92	92
7	93	88	94	84	87	94	88	87	86	89

**Table 14. Wide Spectrum Neutralizer with Environmental Scrub Sampler: Robustness Test Portions– *Listeria* ANOVA Results<sup>a</sup> (1)**

Groups	Count	Sum	Average	Variance		
Row 1	10	889	88.9	18.98889		
Row 2	10	891	89.1	9.433333		
Row 3	10	860	86	23.11111		
Row 4	10	879	87.9	15.21111		
Row 5	10	901	90.1	6.544444		
Row 6	10	900	90	12.44444		
Row 7	10	890	89	12.22222		
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	118.6857	6	19.78095	1.413566	0.223507	2.246408
Within Groups	881.6	63	13.99365			
Total	1000.286	69				

**Table 15. Wide Spectrum Neutralizer with Environmental Scrub Sampler: Robustness *Salmonella* Data (1)**

Combination	Replicates (CFU/mL)									
	A	B	C	D	E	F	G	H	I	J
1	84	82	86	81	86	88	86	84	82	80
2	85	78	83	84	81	78	80	83	81	86
3	84	86	88	86	83	84	78	82	81	85
4	85	87	81	78	83	84	86	79	84	86
5	86	81	89	85	76	84	89	90	88	85
6	81	84	84	89	86	81	90	88	92	94
7	85	86	82	80	89	83	88	87	82	94

**Table 16. Wide Spectrum Neutralizer with Environmental Scrub Sampler: Robustness Test Portions– *Salmonella* ANOVA Results<sup>a</sup> (1)**

Groups	Count	Sum	Average	Variance		
Row 1	10	839	83.9	6.766667		
Row 2	10	819	81.9	7.655556		
Row 3	10	837	83.7	8.233333		
Row 4	10	833	83.3	9.344444		
Row 5	10	853	85.3	18.23333		
Row 6	10	869	86.9	19.87778		
Row 7	10	856	85.6	17.15556		
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	166.9429	6	27.82381	2.231856	0.051372	2.246408
Within Groups	785.4	63	12.46667			
Total	952.3429	69				

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