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Research Article

MICROBIOLOGICAL METHODS

Validation of the 3M™ Environmental Scrub Sampler with Wide-Spectrum Neutralizer: AOAC *Performance Tested Method*℠ 022104

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Abstract

Background: The 3M™ Environmental Scrub Sampler with 10 mL Wide Spectrum Neutralizer is a nonspecific sampling device intended for use for environmental monitoring surface sampling.
Objective: The aim was to evaluate 3M Wide Spectrum Neutralizer using the 3M Environmental Scrub Sampler for AOAC® *Performance Tested Methods*℠ (PTM) certification.
Methods: Matrix studies, inclusivity/exclusivity, product consistency/stability, neutralization, and robustness testing were conducted for *Salmonella* and *Listeria* species. Stainless steel, sealed concrete, and plastic environmental surfaces were evaluated in the matrix study comparing the performance of the 3M™ method for sample collection to that of the U.S. Food and Drug Administration (FDA) *Bacteriological Analytical Manual* (BAM) reference methods. Four classes of sanitizers, namely quaternary ammonium, high acid, hydrogen/peroxyacetic acid and chlorine/bleach-based, were assessed in the neutralization study following ASTM E1054 - 08, *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents*. The other testing parameters followed typical PTM study design.
Results: In matrix studies the 3M sampling device demonstrated no significant differences between candidate and reference sampling method results. All inclusivity organisms were detected, and all exclusivity organisms were excluded, for both *Salmonella* and *Listeria* strains when tested by the appropriate FDA BAM detection method. Robustness, product consistency, and stability studies showed that the sampling device is not affected by lot or testing parameter differences. The Wide Spectrum Neutralizer was proven to effectively neutralize sanitizers at the concentrations tested and was itself shown to be nontoxic and did not affect target microorganism recovery.
Conclusions: The 3M Environmental Scrub Sampler with 10 mL Wide Spectrum Neutralizer is an effective, stable, robust sampling device for the recovery of *Salmonella* spp. and *Listeria* spp.
Highlight: The 3M Environmental Scrub Sampler with 10 mL Wide Spectrum Neutralizer is an acceptable sampling device for use in FDA BAM *Salmonella* and *Listeria* reference methods.

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General Information

Listeria spp. are Gram-positive, rod-shaped bacteria that are ubiquitous in soil, in water, and in several animal species intended for consumption. *Listeria monocytogenes* is of particular concern as the causative agent of listeriosis. Listeriosis is usually caused by eating food that has been contaminated with *L. monocytogenes*. It is estimated that 1600 people get listeriosis in the United States each year, resulting in about 260 deaths (1).

Salmonella is a motile, non-spore-forming, Gram-negative, rod-shaped bacterium in the family Enterobacteriaceae. Non-motile variants include S. Gallinarum and S. Pullorum. The genus *Salmonella* is divided into two species that can cause illness in humans, *Salmonella bongori* and *Salmonella enterica*, the latter being characterized as being the greatest public health concern. Every year, *Salmonella* is estimated to cause approximately 1.35 million illnesses in the United States. Of the food source cases 26 500 require hospitalization, and 420 cases lead to death (2).

Most *Salmonella* and *Listeria* infections in humans occur after consuming food that has been contaminated by fecal matter, or ingesting fluids containing *Salmonella* or *Listeria*. Contamination of food products can easily occur during the manufacturing and packaging process. *Listeria* spp. can grow at wide temperature and pH ranges and can tolerate high concentrations of sodium chloride, thereby allowing for a greater ability to contaminate food during processing. Monitoring and screening environmental surfaces within food production facilities allows for detection of possible contamination risks. Having a proper environmental monitoring plan in place allows for the ability to prevent contaminated food products from even reaching consumers (3).

A critical aspect of an environmental monitoring plan is the integrity of environmental samples tested as part of the plan. Due to required sanitization procedures for surfaces in food production facilities, sampling solutions used with environmental sampling devices must fully neutralize any residual sanitizers present in the sample so that the growth and subsequent detection of organisms collected, such as *Listeria* and *Salmonella*, are not compromised. Additionally, as the range of sanitizers available for food production facilities has expanded, neutralization of a wide range of sanitizers and compatibility with downstream diagnostic tests will greatly enhance the usefulness of the environmental sampling device as part of a successful environmental monitoring program.

Principle

The 3M Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer (3M Company, St. Paul, MN, USA) is intended for collecting environmental monitoring surface samples in food production facilities. Prehydrated sampling devices are packaged in bags with a broad-spectrum neutralizing buffer. The 3M 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 3M 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 the 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.

Scope of Method

(a) Target organisms.—*Salmonella* spp. and *Listeria* spp.
(b) *Matrixes*.—Stainless steel, sealed concrete, and plastic.
(c) *Summary of validated performance claims*.—Performance comparable to that of Dey–Engley (D/E) Neutralizing Buffer with Cellulose Sponge as outlined in the U.S. Food and Drug Administration (FDA) *Bacteriological Analytical Manual* (BAM) Chapter 5, detection method for *Salmonella* (2020) (4) and Chapter 10, "Detection of *Listeria monocytogenes* in Foods and Environmental Samples, and Enumeration of *Listeria monocytogenes* in Foods" (2017) (5). In addition, as demonstrated according to ASTM E1054 - 08, *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents* (6), the product effectively neutralizes quaternary ammonium, high acid, hydrogen/peroxyacetic acid and chlorine/bleach-based sanitizers at the concentrations tested, while being nontoxic to *Salmonella* and/or *Listeria* species.

Definitions

(a) *Probability of detection (POD)*.—The proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. POD is concentration dependent. Several POD measures can be calculated: POD_R (reference method POD), POD_C (confirmed candidate method POD), POD_{CP} (candidate method presumptive result POD), and POD_{CC} (candidate method confirmation result POD) (7).
(b) (b)*Difference of probabilities of detection (dPOD)*.— The difference between any two POD values. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Materials and Methods

Product Information

(a) *Product name*.—3M Environmental Scrub Sampler with 10 mL Wide Spectrum Neutralizer and Gloves, and 3M Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer.
(b) *Cat. Nos*.—HES10WSN2G and ESS10WSN.
(c) *Ordering information*.—https://www.3m.com/

Product Components

(a) *3M Environmental Scrub Sampler with 10 mL Wide Spectrum Neutralizer and gloves*.—One Scrub Sampler with 10 mL neutralizer and gloves.
(b) *3M Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer*.—One Scrub Sampler with 10 mL neutralizer.

Safety Precautions

The user must train its personnel in current proper methods for testing and surface sampling techniques, for example Good Laboratory Practices (GLP) (8), ISO/IEC 17025 (9), or ISO 18583:2018 (10).

To reduce the risks associated with environmental contamination:

(a) The 3M Environmental Scrub Sampler products are intended to be used for testing for microorganisms on surfaces. Surfaces may potentially contain pathogenic organisms, such as *L. monocytogenes* or *Salmonella*.
(b) Individuals should be trained in accordance with applicable regulatory and company/institution requirements before working with potentially infectious materials.
(c) All enrichment broths should be sterilized following any culture-based confirmatory steps.
(d) Strict compliance with BSL-2 (Biosafety Level 2) practices and current industry standards/local and federal regulations for disposal of contaminated waste should be followed.

To reduce the risks associated with exposure to chemicals and biohazards:

(a) Dispose of samples according to all applicable government regulations and applicable laboratory procedures. Strict compliance with BSL-2 practices should be followed.
(b) Always follow standard laboratory safety practices such as GLP (8) or ISO 17025 (9), including proper containment procedures and wearing appropriate protective apparel, disposable gloves, and eye protection while handling reagents and contaminated samples.
(c) Dispose of enriched samples and associated contaminated waste according to current industry standards/local and federal regulations for disposal of contaminated waste.

To reduce the risk associated with false-negative results resulting in the use of contaminated environmental surfaces for food or beverage products:

(a) Always reference the package label for storage instruction and expiration date.
(b) Always reference the product instruction for usage.

To reduce the risk of false-positive results due to cross-contaminated environmental surfaces for food or beverage products that may result in retesting or rejection of the food or beverage product:

(a) Do not touch the Scrub Sampler device to any unintended surface.
(b) Do not break the Scrub Sampler device while sampling.
(c) Do not reach into the Scrub Sampler device bag.

To reduce the risk of cross-contamination from reuse of the Scrub Sampler device:

(a) Do not use the same Scrub Sampler device more than once.
(b) Do not use the same Scrub Sampler device for sampling more than one surface area.
(c) Review that the bag does not have any defect that can compromise the aseptic conditions of the Scrub Sampler device.

The colors of the Scrub Sampler and stick are designed to be noticeable in case of dropping in the food production area. Consult the product Safety Data Sheet for additional information.

Sample Preparation

(a) Wearing gloves, tear off the top of the bag along the perforations.
(b) Aseptically open the bag by using the red tabs on either side of the bag. Be sure not to touch the inside or edges of the bag.
(c) Squeeze out excess solution so the device is moist but not dripping.
(d) Working from the outside of the bag, move the device up allowing the stick to protrude from the bag.
(e) Aseptically, using one hand, grasp the stick above the thumb stop and remove the device from the bag, being sure not touch the scrub sampler on the outside part of the bag.
(f) Practicing aseptic technique, press the scrub sampler device down firmly and flex the stick to ensure full contact with the sampling surface. Scour vigorously in a zigzag motion in one direction across the entire sampling surface to disrupt and/or dislodge build-up.
(g) Turn the device over to the other side, change the sampling direction by 90° and repeat the swabbing procedure in the same sampling site. Swab an area from 10 cm ×10 cm (4 inches × 4 inches) to 30 × 30 cm (12 inches ×12 inches) in size, following appropriate standards or regulatory guidance.
(h) Return the scrub sampler device back into the bag, without going beyond the thumb stop, and hold the device with one hand from the outside of the bag.
(i) Using the other hand twist the stick to separate it from the device. Allow the scrub sampler device to drop in bag. Discard the stick.
(j) Close the bag by rolling the blue wires down and folding in the ends of the wires.
(k) Following established procedures, remove any remaining neutralizing solution residue from the sampled surface.

Sample Analysis

Follow sample analysis (Enumeration or Enrichment, Detection, and Confirmation) following the detection method being used.

Validation Study

The complete validation study was conducted independently by Q Laboratories, Inc. (Cincinnati, OH) following the AOAC *Official Methods of Analysis*℠ Manual Appendix J, *Microbiology Guidelines for Methods Validation* (11).

The inclusivity/exclusivity study evaluated 25 *Listeria* strains and 15 non-*Listeria* strains with detection per the FDA BAM Chapter 10 reference method, and 50 *Salmonella* strains and 15 non-*Salmonella* strains with detection per the FDA BAM Chapter 5 reference method. For the environmental surfaces, the 3M™ Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer was evaluated following each of these detection reference methods. The neutralization study examined the neutralizing effects of the Wide Spectrum Neutralizer on four different classes of sanitizers: quaternary ammonium (e.g., Ecolab® Whisper™ V, at 800 ppm), high acid (e.g., Five Star Chemicals Star San, at 400 ppm), hydrogen/peroxyacetic acid (e.g., Ecolab® Vortexx™, at 2000 ppm), and chlorine/bleach-based (e.g., household bleach, at 100 ppm). The product consistency and stability study evaluated three lots in an accelerated stability study design using a stainless steel surface for the recovery of the target organisms *Salmonella* and *Listeria*. To evaluate the sampling device for robustness two parameters were changed: neutralizing buffer volume and hold time after sampling to evaluate the sampling device.

Inclusivity/Exclusivity

For the inclusivity/exclusivity study, all strains were pre-enriched in an appropriate broth medium. For the inclusivity evaluation, 50 *Salmonella* strains were cultured in lactose broth for 24 ± 2 h at 35°C. For *Listeria*, 15 strains were cultured in buffered *Listeria* enrichment broth with pyruvate (BLEB+p) for 4 h at 30°C. Filter sterilized selective agents were added to achieve final concentrations of 10 mg/L (acriflavine), 40 mg/L (cycloheximide), and 50 mg/L (nalidixic acid sodium salt) in the BLEB with pyruvate pre-enrichments. Incubation continued at 30°C until 24 h total enrichment time. Each inclusive pre-enrichment culture was diluted to 10^2–10^3 CFU/mL. For the exclusivity evaluation 15 non-*Salmonella* strains and 15 non-*Listeria* strains were cultured in brain heart infusion (BHI) broth for 24 ± 2 h at 35°C. Exclusivity strains were not diluted after incubation.

Next, 100 µL diluted inclusive pre-enrichment culture or nondiluted exclusive pre-enrichment culture was used to inoculate the 3M Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer (one device per strain). Inoculated devices were held for 2 h at room temperature (20 ± 2°C). For *Salmonella* inclusive and exclusive strains, 90 mL lactose broth was added to each device, and then incubated for 24 ± 2 h at 35°C. For *Listeria* inclusive and exclusive strains, 90 mL BLEB+p was added to each device, and then incubated for 4 h at 30°C. Selective agents were added and incubation was continued at 30°C for a total of 24–48 h.

All *Salmonella* inclusivity/exclusivity strain enrichments were struck to xylose lysine desoxycholate agar (XLD) and incubated for 24 ± 2 h at 35°C. All *Listeria* inclusivity/exclusivity strain enrichments were struck to modified Oxford agar (MOX) and incubated for 24 h at 30°C. The inclusivity and exclusivity cultures were randomized, blind-coded and then evaluated. Results are presented in Tables 1–4.

Matrix Study

The 3M Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer was evaluated following the FDA BAM Chapter 5 reference method for *Salmonella* and FDA BAM Chapter 10 for *Listeria*. The matrix study consisted of evaluating a total of 30 unpaired 4 inch × 4 inch sample replicates for each method. Within each sample set, there were 5 uninoculated samples (0 CFU/test area), 20 low-level inoculated samples (0.2–2 CFU/test area), and 5 high-level inoculated samples (2–10 CFU/test area). All samples were confirmed following either the FDA BAM Chapter 5 or Chapter 10 reference method as appropriate. Final confirmation was obtained using the Bruker MALDI Biotyper Method following AOAC *Official Method*℠ **2017.09** (12) or **2017.10** (13).

Sample Preparation

For environmental surface inoculation, a liquid culture of the target organisms was used that was specific for each surface. For plastic (polystyrene), *Salmonella* Dublin University of Pennsylvania (STs) 27 (Philadelphia, PA) was evaluated. For sealed concrete, *Listeria innocua* University of Vermont Culture

Table 1. Inclusivity testing results for *Listeria* species using 3M™ Wide Spectrum Neutralizer with 3M™ Environmental Scrub Sampler[a]

Number	Strain source	Strain ID	Genus	Species	Serotype	Isolation source	*Listeria* results
1	ATCC[b]	51782	*Listeria*	*monocytogenes*	3a	Cheese	Positive
2	CWD[c]	1600	*Listeria*	*monocytogenes*	3b	Not available	Positive
3	FSL[d]	J1-049	*Listeria*	*monocytogenes*	3c	Not available	Positive
4	FSL	J1-129	*Listeria*	*monocytogenes*	4ab	Not available	Positive
5	ATCCC	19114	*Listeria*	*monocytogenes*	4a	Animal tissue	Positive
6	CWD	1563	*Listeria*	*monocytogenes*	4b	Lausanne, 1987	Positive
7	ATCC	19116	*Listeria*	*monocytogenes*	4c	Chicken	Positive
8	ATCC	19117	*Listeria*	*monocytogenes*	4d	Sheep	Positive
9	ATCC	19118	*Listeria*	*monocytogenes*	4e	Chicken	Positive
10	CWD	1554	*Listeria*	*monocytogenes*	1/2a	Carlisle, 1981	Positive
11	ATCC	51780	*Listeria*	*monocytogenes*	1/2b	Dairy products	Positive
12	ATCC	7644	*Listeria*	*monocytogenes*	1/2c	Human isolate	Positive
13	NCTC[e]	10890	*Listeria*	*monocytogenes*	7	Human feces	Positive
14	NCTC	19120a	*Listeria*	*grayi*	—[f]	Animal feces	Positive
15	ATCC	25401b	*Listeria*	*grayi*	—	Corn stalks	Positive
16	CWD	167	*Listeria*	*innocua*	—	Not available	Positive
17	CWD	217	*Listeria*	*innocua*	—	Not available	Positive
18	ATCC	19119	*Listeria*	*ivanovii*	—	Sheep	Positive
19	ATCC	49954	*Listeria*	*ivanovii*	—	Food, France	Positive
20	ATCC	11289	*Listeria*	*seeligeri*	—	Human feces	Positive
21	NCTC	11856	*Listeria*	*seeligeri*	—	Not available	Positive
22	ATCC	51334	*Listeria*	*seeligeri*	—	Intestinal content	Positive
23	ATCC	35897	*Listeria*	*welshimeri*	—	Not available	Positive
24	ATCC	43549	*Listeria*	*welshimeri*	—	Soil	Positive
25	ATCC	43550	*Listeria*	*welshimeri*	—	Human feces	Positive

[a] Detection method = U.S. FDA-BAM, Chapter 10, "Detection *of Listeria monocytogenes* in Foods and Environmental Samples, and Enumeration of *Listeria monocytogenes* in Foods" (Revised March 2017).
[b] ATCC = American Type Culture Collection, Manassas, VA.
[c] CWD =University of Vermont Culture Collection, Burlington, VT.
[d] FSL = Food Safety Laboratory; Department of Food Science, Cornell University, Ithaca, NY.
[e] NCTC = National Collection of Type Cultures, Salisbury, United Kingdom.
[f] — = Denotes no serotype information available.

Table 2. Exclusivity testing results for *Listeria* species using 3M™ Wide Spectrum Neutralizer with 3M™ Environmental Scrub Sampler[a]

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

[a] Detection method = U.S. FDA-BAM, Chapter 10, "Detection of *L. monocytogenes* in Foods and Environmental Samples, and Enumeration of *L. monocytogenes* in Foods" (Revised March 2017).
[b] ATCC = American Type Culture Collection, Manassas, VA.

Table 3. Inclusivity testing results for *Salmonella* species using 3M™ Wide Spectrum Neutralizer with 3M™ Environmental Scrub Sampler[a]

Number	Strain Source	Strain ID	Genus	Species	Subspecies	Serotype	Isolation source	*Salmonella* result
1	NCTC[b]	12419	*Salmonella*	*bongori*			Not available	Positive
2	NCTC	10946	*Salmonella*	*bongori*		Brookfield	Not available	Positive
3	ATCC[c]	43975	*Salmonella*	*bongori*		—[g]	Not available	Positive
4	ATCC	13314	*Salmonella*	*enterica*	*arizonae*	—	Not available	Positive
5	ATCC	BAA-1577	*Salmonella*	*enterica*	*arizonae*	—	Not available	Positive
6	QL[d]	11007–4	*Salmonella*	*enterica*	*arizonae*	—	Veterinary isolate	Positive
7	QL	011414.2	*Salmonella*	*enterica*	*arizonae*	—	Environmental isolate	Positive
8	QL	024.114	*Salmonella*	*enterica*	*arizonae*	—	Pet food	Positive
9	ATCC	BAA-1579	*Salmonella*	*enterica*	*diarizonae*	—	Not available	Positive
10	ATCC	BAA-216	*Salmonella*	*enterica*	*diarizonae*	—	Human blood	Positive
11	ATCC	BAA-639	*Salmonella*	*enterica*	*diarizonae*	—	Human feces	Positive
12	QL	024.516	*Salmonella*	*enterica*	*diarizonae*	—	Pet food	Positive
13	QL	011414.1	*Salmonella*	*enterica*	*diarizonae*	—	Environmental isolate	Positive
14	ATCC	35640	*Salmonella*	*enterica*	*enterica*	Abaetetuba	Creek water	Positive
15	FDA[e]	9842	*Salmonella*	*enterica*	*enterica*	Abortusequi	Not available	Positive
16	NCTC	10241	*Salmonella*	*enterica*	*enterica*	Abortusovis	Not available	Positive
17	NCTC	6017	*Salmonella*	*enterica*	*enterica*	Abony	Not available	Positive
18	STs[f]	2	*Salmonella*	*enterica*	*enterica*	Adelaide	Not available	Positive
19	ATCC	51957	*Salmonella*	*enterica*	*enterica*	Agona	Not available	Positive
20	STs	3	*Salmonella*	*enterica*	*enterica*	Agama	Not available	Positive
21	STs	5	*Salmonella*	*enterica*	*enterica*	Agoueve	Not available	Positive
22	STs	6	*Salmonella*	*enterica*	*enterica*	Alachua	Not available	Positive
23	STs	7	*Salmonella*	*enterica*	*enterica*	Albany	Not available	Positive
24	ATCC	9270	*Salmonella*	*enterica*	*enterica*	Anatum	Pork liver	Positive
25	STs	11	*Salmonella*	*enterica*	*enterica*	Arkansas	Not available	Positive
26	FDA	1206H	*Salmonella*	*enterica*	*enterica*	Bareilly	Not available	Positive
27	STs	13	*Salmonella*	*enterica*	*enterica*	Berta	Not available	Positive
28	STs	14	*Salmonella*	*enterica*	*enterica*	Binza	Not available	Positive
29	STs	16	*Salmonella*	*enterica*	*enterica*	Bovismorbificans	Not available	Positive
30	STs	18	*Salmonella*	*enterica*	*enterica*	Brandenburg	Not available	Positive
31	NCTC	5731	*Salmonella*	*enterica*	*enterica*	Bredeney	Not available	Positive
32	NCTC	6018	*Salmonella*	*enterica*	*enterica*	California	Not available	Positive
33	STs	22	*Salmonella*	*enterica*	*enterica*	Cerro	Not available	Positive
34	ATCC	10708	*Salmonella*	*enterica*	*enterica*	Choleraesuis	Equine isolate	Positive
35	ATCC	12011	*Salmonella*	*enterica*	*enterica*	Choleraesuis var Kunzendorf	Not available	Positive
36	STs	24	*Salmonella*	*enterica*	*enterica*	Cubana	Not available	Positive

(continued)

Table 3. (continued)

Number	Strain Source	Strain ID	Genus	Species	Subspecies	Serotype	Isolation source	*Salmonella* result
37	NCTC	5721	*Salmonella*	*enterica*	*enterica*	Derby	Not available	Positive
38	STs	26	*Salmonella*	*enterica*	*enterica*	Drypool	Not available	Positive
39	STs	27	*Salmonella*	*enterica*	*enterica*	Dublin	Not available	Positive
40	FDA	4017H	*Salmonella*	*enterica*	*enterica*	Eastbourne	Not available	Positive
41	ATCC	13076	*Salmonella*	*enterica*	*enterica*	Enteritidis	Not available	Positive
42	QL	024.2	*Salmonella*	*enterica*	*enterica*	Galiema	Environmental isolate	Positive
43	STs	42	*Salmonella*	*enterica*	*enterica*	Give	Not available	Positive
44	STs	44	*Salmonella*	*enterica*	*enterica*	Haardt	Not available	Positive
45	ATCC	51956	*Salmonella*	*enterica*	*enterica*	Hadar	Not available	Positive
46	STs	47	*Salmonella*	*enterica*	*enterica*	Havana	Not available	Positive
47	ATCC	8326	*Salmonella*	*enterica*	*enterica*	Heidelberg	Not available	Positive
48	NCTC	11304	*Salmonella*	*enterica*	*enterica*	Indiana	Turkey	Positive
49	ATCC	51741	*Salmonella*	*enterica*	*enterica*	Infantis	Pasta	Positive
50	ATCC	10721	*Salmonella*	*enterica*	*enterica*	Javiana	Not available	Positive

[a] Detection method = U.S. FDA-BAM, Chapter 5, "Salmonella."
[b] NCTC = National Collection of Type Cultures, Salisbury, United Kingdom.
[c] ATCC = American Type Culture Collection, Manassas, VA.
[d] QL = Q Laboratories, Cincinnati, OH.
[e] FDA = Food and Drug Administration Culture Collection—Silver Spring, MD.
[f] STs = University of Pennsylvania—Philadelphia, PA.
[g] — = Denotes no serotype information available.

Table 4. Exclusivity testing results for *Salmonella* species using 3M™ Wide Spectrum Neutralizer with 3M™ Environmental Scrub Sampler [a]

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

[a] Detection method = U.S. FDA-BAM, Chapter 5, *Salmonella*.
[b] ATCC = American Type Culture Collection, Manassas, VA.

Collection (CDW) 167 (Burlington, VT) was evaluated. For stainless steel, *Salmonella* Typhimurium American Type Culture Collection (ATCC) 14028 (Manassas, VA) with competitor organism *Citrobacter freundii* ATCC 8090, and *L. monocytogenes* 4a ATCC 19114 along with competitor organism *Enterococcus faecalis* ATCC 29212 were evaluated. All cultures were propagated on tryptic soy agar (TSA) with 5% sheep blood (SBA) from a stock culture stored at –70°C. The SBA plates were incubated for 24 ± 2 h at 35 ± 1°C. A single colony was then transferred to BHI broth and incubated for 24 ± 2 h at 35 ± 1°C. The *Salmonella* and *Listeria* target cultures were then diluted in BHI broth to a low level expected to yield fractional results and a high level expected to yield all positive results. The *Citrobacter and Enterococcus* isolates were diluted in BHI broth and the stainless steel surface was inoculated at approximately 10 times the concentration of *Salmonella* and *Listeria*.

All environmental surfaces (4 inch × 4 inch test areas) were inoculated with 0.25 mL diluted inoculum and allowed to dry for 16–24 h at room temperature (20–25°C) prior to sampling. For the stainless steel surface for *Listeria*, 320 µL of Whisper V sanitizer was applied after room temperature incubation and allowed to dry for 6 h prior to sampling. For the uninoculated test portions, sterile BHI broth was used. The surfaces were sampled by using a 3M™ Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer. The surfaces were swabbed in an "N"- or "S"-shaped pattern, in four directions. To determine the inoculation level of the environmental surfaces, aliquots of each inoculum were plated onto TSA and incubated for 24 ± 2 h at 35 ± 1°C.

FDA BAM Chapter 5 Salmonella

Sponge samplers were premoistened in 10 mL D/E neutralizing broth. The surfaces were sampled by pressing one side of the sampler firmly on the surface and scoured vigorously in a zigzag pattern across the entire sampling surface. The sampler was then flipped, the direction was changed 90°, and the sampling was repeated. The sponge was then placed back in the container and submerged in the D/E broth before being stored at room temperature (20–25°C) for 2 h ± 15 min. All samples were enriched with 225 mL lactose broth, homogenized by hand massaging, and allowed to stand at room temperature (20–25°C) for 60 ± 5 min. As per the method, the pH of the enrichments was measured; all were within 6.8 ± 0.2 so no pH adjustment was necessary. Subsequently, all enrichments were incubated at 35 ± 2 °C for 24 ± 2 h.

Following incubation, 0.1 mL primary enrichment was transferred into 10 mL Rappaport Vassiliadis (RV) broth, and 1.0 mL was transferred into 10 mL tetrathionate (TT) broth. RV tubes were incubated at 42 ± 0.2 °C for 24 ± 2 h. The TT tubes were incubated at 35 ± 2 °C for 24 ± 2 h. Following incubation, a loopful of the secondary enrichments was streaked to bismuth sulfite (BS) agar, Hektoen enteric agar, and XLD, and incubated at 35 ± 2 °C for 24 ± 2 h. If no visible colonies were present after 24 h of incubation on the BS plates, they were reincubated for an additional 24 ± 2 h at 35 ± 2 °C. A minimum of two suspect colonies from each selective agar were transferred to triple sugar iron agar (TSI) and lysine iron agar (LIA) slants and incubated at 35 ± 2°C for 24 ± 2 h. Following incubation, the TSI and LIA slants were examined for typical reactions. Slants producing typical reactions were streaked to TSA and incubated for 35 ± 2°C for 18–24 h. Following incubation, isolates were serologically tested for both somatic O and flagellar H agglutination. Additionally, purified TSA isolates were identified using the Bruker MALDI Biotyper following AOAC *Official Method*^{SM} **2017.09** (12).

FDA BAM Chapter 10 Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods

Sponge samplers were premoistened in 10 mL D/E neutralizing broth. The surfaces were swabbed vertically approximately 10 times, and then the sampler was turned over and the other side was used to swab horizontally approximately 10 times and diagonally approximately 10 times. The swab was then placed back in the container and submerged in the D/E broth before being stored at room temperature (20–25°C) for 2 h ± 15 min. All samples were enriched in 225 mL ± 5 mL BLEB+p, homogenized for 2 min and incubated at 30 ± 1°C for 4 h ± 30 min. Following 4 h of incubation, selective supplements acriflavine (10 mg/L), sodium nalidixic acid (50 mg/L), and cycloheximide (40 mg/L) were added to each test portion, mixed, and incubated for the remainder of the 24 h enrichment period.

After 24 h of total incubation, the enriched samples were streaked to MOX and Brilliance^{TM} *Listeria* agar (BLA) and incubated at 35 ± 1°C for 24–48 h. The enriched samples were reincubated for an additional 24 h at 30 ± 1°C and then streaked to a second MOX agar and BLA plate, which was incubated for 24–48 h at 35 ± 1°C. All agar plates were examined for suspect colonies, and if present, at least five colonies were streaked to TSA containing 0.6% yeast extract (TSA/YE). The TSA/YE plates were incubated at 30 ± 1°C for 24–48 h and then examined for purity. Pure colonies were tested for catalase reactivity and a Gram stain was conducted. A pure *Listeria* colony was transferred to trypticase soy broth with 0.6% yeast extract (TSBYE). The TSBYE cultures were incubated at 25 ± 1°C overnight, or until the broth was turbid, indicating sufficient growth. Catalase-positive organisms were stabbed into plates of SBA and incubated at 35 ± 1°C for 24–48 h. The TSBYE tubes incubated at 25 ± 1°C were used to prepare a wet mount slide to determine the motility pattern. After incubation, the SBA plates were examined for b-hemolysis. Final confirmation was conducted using the Bruker MALDI Biotyper following AOAC *Official Method*^{SM} **2017.10**.

3M Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer

All test portions were prepared according to the protocol described previously in the *Matrix Study*, *Sample Preparation* subsection. All surfaces were sampled using the 3M Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer, and then were enriched and analyzed using either the FDA BAM Chapter 5 or Chapter 10 reference method as appropriate. All samples, regardless of presumptive results, were confirmed using the FDA BAM Chapter 5 or Chapter 10 reference method as appropriate, with final confirmation by Bruker MALDI Biotyper following AOAC *Official Method*^{SM} **2017.09** (12) and *Official Method*^{SM} **2017.10** (13).

The POD statistical analysis was used to evaluate the 3M Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer performance versus the reference method. The POD was calculated as the number of positive outcomes divided by the total number of trials. A summary of POD analyses is presented in Table 5.

Neutralization

The neutralizing capacity of the 3M Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer was evaluated against four different classes of sanitizers: quaternary ammonium (e.g., Ecolab Whisper V 800 ppm), high acid (e.g., Five Star San 400 ppm), hydrogen/peroxyacetic acid (e.g., Ecolab Vortexx 2000 ppm), and chlorine/bleach-based (e.g., household bleach 100 ppm). The neutralizer effectiveness and toxicity were evaluated according to ASTM E1054 - 08, *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents*, using *S.* Senftenberg ATCC 43845 and *L. monocytogenes* (1/2a) CWD 1554. *S.* Senftenberg was cultured in lactose broth at 35 ± 1°C for 24 ± 2 h and diluted to approximately 10^4 CFU/mL. *L. monocytogenes* was cultured in BLEB+p at 30 ± 1°C for 24–48 h and diluted to approximately 10^4 CFU/mL.

Neutralizer effectiveness was evaluated by adding 100 µL of each strain diluted to 10^4 CFU/mL (final concentration 30–100 CFU/plate) to a 3M Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer. A 1 mL volume of a 1:50 dilution of sanitizer was added and massaged by hand. An initial enumeration, within 1 min, was conducted, with another enumeration after a 10 min hold. Each enumeration consisted of three replicates.

Neutralizer toxicity was evaluated by adding 100 µL of each strain to a 3M Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer. A 1 mL volume of phosphate-buffered saline (PBS) was added and massaged by hand. An initial enumeration, within 1 min, was conducted, with another enumeration after a 10 min hold. Each enumeration consisted of three replicates.

Table 5. 3M™ Wide Spectrum Neutralizer with 3M™ Environmental Scrub Sampler, candidate versus reference—POD results

Matrix	Strain	CFU[a]/test area	Candidate method results				Reference method results[f]				
			N[b]	x[c]	POD_{CP}[d]	95% CI	x	POD_{CC}[e]	95% CI	$dPOD_{CP}$[g]	95% CI[h]
Stainless steel (4 inch × 4 inch)	S. Typhimurium ATCC[i] 14028 & 10X *C. freundii* ATCC 8090	NA[j]	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 inch × 4 inch)	*L. monocytogenes* 4a ATCC 19114 & 10X E. faecalis ATCC 29212	NA	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 inch × 4 inch)	*Salmonella* Dublin STs[k] 27	NA	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 inch × 4 inch)	*Listeria innocua* CWD[l] 167	NA	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

[a] CFU/test area = Results of the CFU/test area were determined by plating the inoculum for the matrix in triplicate.
[b] N = Number of test portions.
[c] x = Number of positive test portions.
[d] POD_C = Candidate method confirmed positive outcomes divided by the total number of trials.
[e] POD_R = Reference method confirmed positive outcomes divided by the total number of trials.
[f] Dey–Engley neutralizing broth with cellulose sponge.
[g] $dPOD_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values.
[h] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[i] ATCC = American Type Culture Collection, Manassas, VA.
[j] NA = Not applicable.
[k] STs = University of Pennsylvania, Philadelphia, PA.
[l] CWD = University of Vermont Culture Collection, Burlington, VT.

An organism viability and test material control were conducted alongside the neutralization study. For the organism viability control, each organism was diluted to approximately 10^2 CFU/mL and 1 mL transferred to 9 mL PBS. An initial enumeration, within 1 min, was conducted, with another enumeration after a 10 min hold. For the test material control each organism was diluted with the sanitizer product to approximately 10^2 CFU/mL and allowed to sit for a 10 min hold time. Each control consisted of three replicates. The test material control replicates for each sanitizer did not produce any growth. The neutralization data and the analysis of variance (ANOVA) statistical analysis for each sanitizer are presented in Tables 6–9.

Product Consistency and Stability Study

For product stability and lot-to-lot consistency, an accelerated stability of the shelf life was conducted as kits could not be selected from different time points in the real-time shelf life. *S.* Newport ATCC 6962 was cultured in lactose broth at 35 ± 1°C for 24 ± 2 h and diluted in 0.1% peptone water so that the target strain was at a level to yield fractional positives. Ten 4 inch × 4 inch stainless steel test areas were inoculated per lot. *C. freundii* ATCC 8090, a closely related non-*Salmonella* strain, was cultured in BHI for 24 h at 37°C and not diluted before testing. Ten 4 inch × 4 inch stainless steel test areas were inoculated per lot. After sampling with the 3M™ Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer, all samples were evaluated using the FDA BAM Chapter 5 detection method. *L. monocytogenes* (1/2 b) ATCC 51780 was cultured in BLEB+p at 30°C for 24–48 h and diluted in 0.1% peptone water so that the target strain was at a level to yield fractional positives. Ten 4 inch × 4 inch stainless steel test areas were inoculated per lot. *E. faecalis* ATCC 29212 a closely related non-*Listeria* strain was cultured in BHI for 24 h at 37°C and not diluted before testing. Ten 4 inch ×4 inch stainless steel test areas were inoculated per lot. After sampling with the 3M Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer, all samples were evaluated using the FDA BAM Chapter 10 detection method. A summary of the study outline and product information are displayed in Table 10 for the stability evaluation. A detailed summary of results and the POD analyses are displayed in Table 11. Overall, there was no significant difference in the results.

Robustness

For the robustness study, two testing parameters were changed to evaluate a total of seven testing combinations, with the seventh combination being the nominal conditions following the product instructions. The two testing parameters that were changed included the hold time after sampling prior to enrichment (0, 48, and 96 h), and neutralizing buffer volume (9, 10, and 11 mL). Ten replicates of each testing combination were evaluated.

S. Enteritidis ATCC 13076 was cultured in lactose broth at 35 ± 1°C for 24 ± 2 h and diluted to approximately 10^4 CFU/mL. Next, 100 µL diluted pre-enrichment culture was used to inoculate the 3M Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer. Inoculated devices were held for 2 h at room temperature (20 ± 2°C). After each combination's required hold time 1 mL buffer was spread-plated onto XLD agar and incubated for 24 h at 35°C and the colonies were counted and recorded.

L. monocytogenes (4 b) CWD 1563 was cultured in BLEB+p at 30°C for 24–48 h and diluted to approximately 10^4 CFU/mL. Next, 100 µL diluted pre-enrichment culture was used to inoculate the 3M Environmental Scrub Sampler Stick with 10 mL Wide Spectrum

Table 6. Sanitizer Neutralization per ASTM E1054 - 08 using 3M™ Wide Spectrum Neutralizer with 3M™ Environmental Scrub Sampler: bleach

	Listeria monocytogenes (1/2a)			*Listeria monocytogenes*			*Salmonella* Senftenberg			*Salmonella* Senftenberg		
	CWD[a] 1554			(1/2a), CWD 1554			ATCC[b] 43845			ATCC 43845		
	Initial time point			Post 10 min hold			Initial time point			Post 10 min hold		
	Replicate			Replicate			Replicate			Replicate		
	A	B	C	A	B	C	A	B	C	A	B	C
Test organism viability, mean CFU/mL[c]	37	39	42	32	35	38	51	49	54	46	50	47
Neutralizer effectiveness, mean CFU/mL[d]	34	40	38	35	36	41	40	46	45	43	51	56
Neutralizer effectiveness determination		Effective			Effective			Effective			Effective	
Neutralizer effectiveness *P*-value[e]			0.91						0.30			
Neutralizer toxicity, mean CFU/mL[f]	37	38	43	37	39	37	47	45	51	50	55	54
Neutralizer toxicity determination		Nontoxic			Nontoxic			Nontoxic			Nontoxic	
Neutralizer toxicity *P*-value			0.68						0.70			
Suitability test result (CFU/mL)		Pass			Pass			Pass			Pass	

[a] CWD = University of Vermont Culture Collection, Burlington, VT.
[b] ATCC = American Type Culture Collection, Manassas, VA.
[c] Referred to as Test C in ASTM E1054 - 08, *Standard Test Methods for Evaluation of Antimicrobial Agents*.
[d] Referred to as Test A in ASTM E1054 - 08, *Standard Test Methods for Evaluation of Antimicrobial Agents*.
[e] A *t*-test indicated no statistical significance ($P > 0.05$).
[f] 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 3M™ Wide Spectrum Neutralizer with 3M™ Environmental Scrub Sampler: Star San

	Listeria monocytogenes (1/2a)			*Listeria monocytogenes*			*Salmonella* Senftenberg			*Salmonella* Senftenberg		
	CWD[a] 1554			(1/2a), CWD 1554			ATCC[b] 43845			ATCC 43845		
	Initial time point			Post 10 min hold			Initial time point			Post 10 min hold		
	Replicate			Replicate			Replicate			Replicate		
	A	B	C	A	B	C	A	B	C	A	B	C
Test organism viability, mean CFU/mL[c]	37	39	42	32	35	38	51	49	54	46	50	47
Neutralizer effectiveness, mean CFU/mL[d]	33	34	38	33	35	37	44	48	51	44	53	49
Neutralizer effectiveness determination		Effective			Effective			Effective			Effective	
Neutralizer effectiveness *P*- value[e]			0.23						0.49			
Neutralizer toxicity, mean CFU/mL[f]	37	38	43	37	39	37	47	45	51	50	55	54
Neutralizer toxicity determination		Nontoxic			Nontoxic			Nontoxic			Nontoxic	
Neutralizer toxicity *P*-value			0.68						0.70			
Suitability test result (CFU/mL)		Pass			Pass			Pass			Pass	

[a] CWD = University of Vermont Culture Collection, Burlington, VT.
[b] ATCC = American Type Culture Collection, Manassas, VA.
[c] Referred to as Test C in ASTM E1054 - 08, *Standard Test Methods for Evaluation of Antimicrobial Agents*.
[d] Referred to as Test A in ASTM E1054 - 08, *Standard Test Methods for Evaluation of Antimicrobial Agents*.
[e] A *t*-test indicated no statistical significance ($P > 0.05$).
[f] 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 3M™ Wide Spectrum Neutralizer with 3M™ Environmental Scrub Sampler: Vortexx

	Listeria monocytogenes (1/2a)			*Listeria monocytogenes*			*Salmonella* Senftenberg			*Salmonella* Senftenberg		
	CWD[a] 1554			(1/2a), CWD 1554			ATCC[b] 43845			ATCC 43845		
	Initial time point			Post 10 min hold			Initial time point			Post 10 min hold		
	Replicate			Replicate			Replicate			Replicate		
	A	B	C	A	B	C	A	B	C	A	B	C
Test organism viability, mean CFU/mL[c]	37	39	42	32	35	38	51	49	54	46	50	47
Neutralizer effectiveness, mean CFU/mL[d]	33	35	39	35	32	41	46	53	49	43	49	52
Neutralizer effectiveness determination		Effective			Effective			Effective			Effective	
Neutralizer effectiveness *P*- value[e]			0.52						0.66			
Neutralizer toxicity, mean CFU/mL[f]	37	38	43	37	39	37	47	45	51	50	55	54
Neutralizer toxicity determination		Nontoxic			Nontoxic			Nontoxic			Nontoxic	
Neutralizer toxicity *P*-value			0.68						0.70			
Suitability test result (CFU/mL)		Pass			Pass			Pass			Pass	

[a] CWD = University of Vermont Culture Collection, Burlington, VT.
[b] ATCC = American Type Culture Collection, Manassas, VA.
[c] Referred to as Test C in ASTM E1054 - 08, *Standard Test Methods for Evaluation of Antimicrobial Agents*.
[d] Referred to as Test A in ASTM E1054 - 08, *Standard Test Methods for Evaluation of Antimicrobial Agents*.
[e] A *t*-test indicated no statistical significance ($P > 0.05$).
[f] 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 3M™ Wide Spectrum Neutralizer with 3M™ Environmental Scrub Sampler: Whisper V

	Listeria monocytogenes (1/2a)			*Listeria monocytogenes*			*Salmonella* Senftenberg			*Salmonella* Senftenberg		
	CWD[a] 1554			(1/2a), CWD 1554			ATCC[b] 43845			ATCC 43845		
	Initial time point			Post 10 min hold			Initial time point			Post 10 min hold		
	Replicate			Replicate			Replicate			Replicate		
	A	B	C	A	B	C	A	B	C	A	B	C
Test organism viability, mean CFU/mL[c]	37	39	42	32	35	38	51	49	54	46	50	47
Neutralizer effectiveness, mean Cfu/mL[d]	40	43	48	36	41	40	50	47	54	46	53	59
Neutralizer effectiveness determination		Effective			Effective			Effective			Effective	
Neutralizer effectiveness *P*-value[e]			0.08						0.42			
Neutralizer toxicity, mean CFU/mL[f]	37	38	43	37	39	37	47	45	51	50	55	54
Neutralizer toxicity determination		Nontoxic			Nontoxic			Nontoxic			Nontoxic	
Neutralizer toxicity *P*-value			0.68						0.70			
Suitability test result (CFU/ml)		Pass			Pass			Pass			Pass	

[a] CWD = University of Vermont Culture Collection, Burlington, VT.
[b] ATCC = American Type Culture Collection, Manassas, VA.
[c] Referred to as Test C in ASTM E1054 - 08, *Standard Test Methods for Evaluation of Antimicrobial Agents*.
[d] Referred to as Test A in ASTM E1054 - 08, *Standard Test Methods for Evaluation of Antimicrobial Agents*.
[e] A *t*-test indicated no statistical significance ($P > 0.05$).
[f] Referred to as Test B in ASTM E1054- 08, *Standard Test Methods for Evaluation of Antimicrobial Agents*.

Neutralizer. Inoculated devices were held for 2 h at room temperature (20 ± 2°C). After each combination's required hold time 1 mL buffer was spread-plated onto MOX agar and incubated for 24 h at 35°C and the colonies were counted and recorded.

After counting, samples were decoded, and the mean and standard deviation for each combination was calculated. An ANOVA was carried out to determine if the means were significantly different between the combinations separately for each target strain. Data demonstrated that small changes in testing parameters did not impact the performance of the sampling device. The study parameters, data summary, and ANOVA results for each target analyte and treatment combination are presented in Tables 12–16.

Results

As per criteria outlined in Appendix J of the *Official Methods of Analysis*℠ Manual, fractional positive results were obtained in the matrix study for all surfaces using the 3M Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer.

For the inclusivity study, all 25 *Listeria* strains and 50 *Salmonella* strains tested were recovered. For the exclusivity study, all 15 *Listeria* exclusivity strains and 15 *Salmonella* exclusivity strains were correctly excluded. The neutralization study show that the Wide Spectrum Neutralizer is a nontoxic and effective neutralizer. The product consistency and stability study proved the 3M Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer is a stable sampling device. The robustness study showed the 3M Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer is a robust sampling device and that variations of buffer volume and hold time have no effect on level of recovery.

The POD analysis between the 3M Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer and the reference sampling method in the matrix study indicated that there was no significant difference at the 5% level between the number of positive results by the methods. The POD analysis between 3M Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer presumptive and confirmed results indicated that there was no significant difference at the 5% level for the confirmation procedure.

Table 10. 3M™ Wide Spectrum Neutralizer with 3M™ Environmental Scrub Sampler: product stability and lot-to-lot outline and information

Storage type	Storage temperature	Time points (from date of production)
Accelerated	Variable; 2–8°C, 25 + 1 °C, 45 + 1 °C	0 months, 6 months, 12 days
Lot information		
Lot 1	WSN HS01 Lot 323 42–0029-9161–2	
Lot 2	WSN HS01 Lot 324 42–0029-9162–0	
Lot 3	WSN HS01 Lot 510 42–0029-9163–8	

Discussion

The 3M Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer recovered all inclusivity organisms for both *Salmonella* and *Listeria*. The 3M Environmental Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer was able to recover *Salmonella* spp. and *Listeria* spp. from several different environmental surfaces, namely 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, namely quaternary ammonium, high acid, hydrogen peroxide/peroxyacetic acid, and chlorine/bleach, and was found to be nontoxic to the target organisms. The 3M 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.

Conclusions

The data from these studies, within their statistical uncertainty, support the product claims of the 3M Scrub Sampler Stick with 10 mL Wide Spectrum Neutralizer for stainless steel, plastic (polystyrene), and sealed concrete environmental surfaces. Also, the data support the product claims of ability to neutralize a wide range of sanitizers. The results obtained by the POD analysis of the environmental surfaces study demonstrated

Table 12. 3M™ Wide Spectrum Neutralizer with 3M™ Environmental Scrub Sampler: robustness experimental design

Treatment combination	Hold time, h	Neutralizing buffer vol., mL
1	0	9
2	0	11
3	48	9
4	48	11
5	96	9
6	96	11
7 (normal condition)	0	10

Table 11. 3M™ Wide Spectrum Neutralizer with 3M™ Environmental Scrub Sampler: stability and lot-to-lot inoculated test portions—POD results

Time point, months	Target	*N*	*x*	POD	95% CI
0	*Listeria monocytogenes* (1/2b) ATCC[a] 51780	10	7	0.70	0.40, 0.89
	Salmonella Newport ATCC 6962	10	6	0.60	0.31, 0.83
6	*Listeria monocytogenes* (1/2b) ATCC 51780	10	6	0.60	0.31, 0.83
	Salmonella Newport ATCC 6962	10	7	0.70	0.40, 0.89
12	*Listeria monocytogenes* (1/2b) ATCC 51780	10	6	0.60	0.31, 0.83
	Salmonella Newport ATCC 6962	10	5	0.50	0.24, 0.76

[a] ATCC = American Type Culture Collection—Manassas, VA.

Table 13. 3M™ Wide Spectrum Neutralizer with 3M™ Environmental Scrub Sampler: robustness *Listeria* data

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. 3M™ Wide Spectrum Neutralizer with 3M™ Environmental Scrub Sampler: robustness test portions—*Listeria* ANOVA results[a]

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	—[b]	—	—
Total	1000.286	69	—	—	—	—

[a] Single factor ANOVA.
[b] — = Not applicable.

Table 15. 3M™ Wide Spectrum Neutralizer with 3M™ Environmental Scrub Sampler: robustness *Salmonella* data

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. 3M™ Wide Spectrum Neutralizer with 3M™ Environmental Scrub Sampler: robustness test portions—*Salmonella* ANOVA results[a]

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	—[b]	—	—
Total	952.3429	69	—	—	—	—

[a] Single-factor ANOVA.
[b] — = Not applicable.

that there were no statistically significant differences between the number of positive samples detected by the candidate and the reference sampling methods for all samples tested for all matrixed evaluated.

Conflict of Interest

None declared.

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