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Research Article

MICROBIOLOGICAL METHODS

Validation of N-Light™ *Salmonella* Risk Test Kit for Detection of *Salmonella* spp. on Environmental Surfaces: AOAC *Performance Tested Method*℠ 072204

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Abstract

Background: The NEMIS N-Light™ *Salmonella* Risk method uses chemiluminescence designed for the qualitative detection of *Salmonella* spp. from environmental surface samples.
Objective: To validate the N-Light *Salmonella* Risk assay using independent and method developer validation studies according to the AOAC *Performance Tested Methods*℠ (PTM) program for the detection of *Salmonella* spp. on stainless-steel, polystyrene, and ceramic environmental surfaces.
Method: The N-Light *Salmonella* Risk assay was evaluated in a matrix study in comparison to the ISO 6579-1:2017 method ("Microbiology of the Food Chain—Horizontal Method for the Detection, Enumeration, and Serotyping of *Salmonella*—Part 1: Detection of *Salmonella* spp.") using an unpaired study design. Additional PTM studies performed were inclusivity/exclusivity, robustness, product consistency, and stability.
Results: The N-Light *Salmonella* Risk assay demonstrated a specific detection of all *Salmonella* strains tested. In the matrix study, the N-Light *Salmonella* Risk assay showed no significant differences between presumptive and confirmed results or between candidate and reference method results on the three surfaces evaluated. Data for additional PTM studies met acceptance criteria requirements.
Conclusions: The NEMIS Technologies N-Light *Salmonella* Risk assay is an effective method for the qualitative detection of *Salmonella* on stainless-steel, polystyrene, and ceramic environmental surfaces.
Highlights: The NEMIS Technologies N-Light *Salmonella* Risk assay, which is the first chemiluminescence-based detection system that uses a novel, patented dioxetane compound, allowing for easy and rapid detection of *Salmonella*.

General Information

Salmonella belong to the *Enterobacteriaceae* family and are generally motile, non-spore-forming, rod-shaped, Gram-negative bacteria. They are separated into two species, *Salmonella bongori* and *Salmonella enterica*. The nontyphoid *Salmonella* can cause salmonellosis, an infection of the gastrointestinal tract, in animals and humans. *Salmonella* have been isolated from

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productive livestock such as poultry, bovines, ovines, or swine and also from different wild animals, including deer, wild birds, and reptiles. The bacteria are usually fecal-orally transmitted to humans via contaminated food or drinking water (1).

Principle of the Method

The N-Light™ *Salmonella* assay is a qualitative test to rapidly detect *Salmonella* spp. in food processing areas and on equipment in environmental monitoring programs. The assay uses a patented ultrasensitive chemiluminescent dioxetane molecule (AquaSpark®) as a probe, which is cleaved by an esterase uniformly expressed in *Salmonella*. N-Light *Salmonella* uses a proprietary selective culture enrichment technology, which consists of a unique enrichment broth amended with antibiotics and a bacteriophage cocktail targeting Gram-negative competitor species.

Following surface sampling according to ISO 18593:2017, a swab is transferred into the enrichment broth, a biosafety cap that permanently seals the tube closed. Then the tube is incubated for 24 ± 2 h in a dry heating block at 37 ± 1°C. For detection of chemiluminescence after *Salmonella* enrichment, an AquaSpark and a lysis tablet are first simultaneously released into the enrichment broth from the biosafety cap without further sample preparation. The tube is vortexed 15 s for efficient bacterial lysis and dissolution of both tablets and incubated at 37 ± 2°C for 3 min. Subsequently, chemiluminescence is quantified using a NEMIS luminometer. A sample is considered presumptively positive if the determined relative light units (RLU) exceed a specific threshold.

Scope of Method

- **(a)** *Analyte*.—*Salmonella* spp.
- **(b)** *Matrixes*.—Stainless-steel (AISI 304, grade 2b finish), polystyrene, and ceramic (glazed earthen) 1″ × 1″ test areas.
- **(c)** *Summary of validated performance claims*.—The N-Light *Salmonella* assay demonstrated no statistical difference in performance to the reference method ISO 6579-1:2017 “Microbiology of the Food Chain—Horizontal Method for the Detection, Enumeration, and Serotyping of Salmonella—Part 1” (2) for the detection of *Salmonella* spp. on environmental surfaces (stainless steel, polystyrene, and ceramic) after 24 h of enrichment.

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).
- **(b)** *Difference of probabilities of detection (dPOD)*.—Difference of probabilities of detection is 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 (3).

Materials and Methods

Test Kit Information

- **(a)** *Kit name*.—N-Light *Salmonella* Risk.

Test Kit Components

- **(a)** *NEMIS Salmonella Enrichment Broth*.—50 tubes containing 2 mL enrichment.
- **(b)** *Selective supplement tablets and dispenser*.
- **(c)** *Flocked swabs*.
- **(d)** *Buffer water peptone solution (BPW)*.

Additional Supplies and Reagents

None.

Apparatus

- **(a)** *Dry heating block*.—Capable of maintaining 37 ± 2°C.
- **(b)** *Vortex mixer*.
- **(c)** *NEMIS Technologies BTL1 luminometer*.
- **(d)** *Serological pipet or micropipet*.—For sampling and delivering of 1–10 mL.
- **(e)** *Refrigerator*.—Capable of maintaining 2–8°C.

Cultures

- **(a)** *American Type Culture Collection (ATCC)*.—Manassas, VA, USA.
- **(b)** *Collection de l’Institut Pasteur (CIP)*.—Paris, France.
- **(c)** *Culture Collection University of Gothenburg (CCUG)*.—Goteborg, Sweden.
- **(d)** *Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ)*.—Braunschweig, Germany.
- **(e)** *Animal Plant Health Agency (APHA)*.—Addlestone, United Kingdom.
- **(f)** *NEMIS Technology Microbial Strain Collection (NEMIS)*.—Dübendorf, Switzerland.
- **(g)** *Zürcher Hochschule für Angewandte Wissenschaften (ZHAW)*.—Wädenswil, Switzerland.
- **(h)** *Robert Koch Institute (RKI and FS)*.—Berlin, Germany.
- **(i)** *Nexidia Microbial Strain Collection (NEXIDIA)*.—Dijon, France.

Safety Precautions

The following general precautions should always be followed. Clean the workstations with the disinfectant of choice (e.g., sodium hypochlorite solution, phenol solution, quaternary ammonium solution) before and after use as part of aseptic techniques. In addition to cleaning workstations, working areas should be separated for the following: media preparation, sample preparation, and pathogen detection. Gloves and other personal protective equipment should be used at all times. The NEMIS Technologies BTL1 luminometer or supplies should never be touched without wearing gloves. Never reuse kit disposables, and always change pipets and pipet tips between samples.

- **(a)** The NEMIS Technologies N-Light *Salmonella* Risk assay should be disposed of following procedures for infectious or potentially infectious products. The user should wear appropriate personal protective equipment, including (but

not limited to) protective disposable gloves, laboratory coats, and eye protection, when handling samples and kit reagents. Wash hands thoroughly after handling specimens and reagents. It is the responsibility of each laboratory to handle waste and effluents produced according to their type and degree of hazardousness and to treat and dispose them (or have them treated and disposed) in accordance with local, state, and federal regulations. Strict compliance with BSL-2 practices should be followed (3).

(b) *Salmonella* is a Biosafety Level 2 organism. Biological samples such as enrichments have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and national regulations on disposal of biological wastes. Wear appropriate protective equipment, which includes but is not limited to protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained in accordance with applicable regulatory and company/institution requirements before working with potentially infectious materials. All enrichment broths should be sterilized following confirmation.

General Preparation

(a) Use aseptic technique.
(b) Change pipet tips between samples.
(c) Do not reuse kit disposables.
(d) Clean workstations before and after use.
(e) Separate work areas for media preparation, sample preparation, and pathogen detection.

Sample Preparation

Surface areas (stainless steel, polystyrene, and ceramic) of 1″ × 1″ in size were sampled with a swab premoistened with BPW prior to sampling. After sampling, the swab is placed into a tube containing 2 mL of NEMIS *Salmonella* enrichment broth and the N-Light *Salmonella* Risk specific antibiotic tablet was dispensed into the tube. Afterwards, the tube is shaken vigorously using a vortex mixer for 15 s. The tube is closed using the cap containing the AquaSpark and Lysis tablet and then incubated at 37 ± 1°C for 24 ± 2 h in a dry heating block.

Analysis

N-Light method.—

(a) Remove tubes from the dry heat incubator.
(b) Press the button on the dispenser cap to release the AquaSpark and Lysis tablet into the enrichment tube and vortex for 15 s.
(c) Incubate in a dry heating block for 3 min at 37 ± 2°C.
(d) Read tube in the luminometer and obtain results.

Instrument loading.—

(a) Open the lid to the instrument.
(b) Load the sample tube into the instrument.
(c) Close the lid of the instrument.
(d) Press Start Run to initiate the run.
(e) Sample analysis takes 10 s, and results are displayed automatically.
(f) When the run is completed, open the lid of the luminometer and remove the sample tube.

Data analysis and interpreting results.—

(a) *Viewing results.—*
  (1) Results are displayed after sample analysis.
  (2) Sample analysis takes 10 s, and results are displayed automatically.
(b) *Interpretation.—*
  (1) 0 RLU ≤ Result < 20 000 RLU—negative sample.
  (2) 20 000 RLU ≤ Result < 50 000 RLU—“Yellow-Critical” presumptive positive.
  (3) Result > 50 000 RLU—“Red-Alert” presumptive positive.

*Confirmation.—*The N-Light *Salmonella* Risk assay test portions can be confirmed following the ISO 6579-1:2017 reference method for the detection of *Salmonella* or, alternatively, by streaking the samples onto Xylose Lysine Deoxycholate (XLD) and Brilliance™ *Salmonella* Agar (BSA) and incubating at 37 ± 1°C for 24 ± 3 h (2).

Validation Study

The N-Light *Salmonella* Risk assay was conducted under the AOAC Research Institute *Performance Tested Method*℠ (PTM) program and the *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces,* Appendix J (4). The PTM validation has two main parts: method developer studies and independent laboratory validation studies.

Method developer studies were conducted in the laboratories of Nexidia SAS and included the inclusivity/exclusivity study of the target microorganism (*Salmonella*), matrix studies for all claimed matrixes (stainless steel, polystyrene, and ceramic), product consistency, stability studies, and robustness testing.

The independent laboratory study was conducted by Q-Laboratories (Cincinnati, OH) and included the matrix study for detection of *Salmonella* on a stainless-steel surface. The reference method for the matrix study was the ISO 6579-1:2017 “Microbiology of the Food Chain—Horizontal Method for the Detection, Enumeration and Serotyping of *Salmonella*—Part 1: Detection of *Salmonella* spp.”

Results

Method Developer Studies

(a) *Methods.—*The inclusivity/exclusivity study examined the ability of the N-Light *Salmonella Risk* method to detect a variety of the claimed target strains (*Salmonella* spp.) and to distinguish those strains from closely related nontarget strains and species. One hundred and twenty-one inclusivity strains, covering the two species (*S. bongori* and *S. enterica*) and the six subspecies *S. enterica* subsp. *Enterica* (several serovars), *S. enterica* subsp. *Salamae*, *S. enterica* subsp. *Arizonae*, *S. enterica* subsp. *Diarizonae*, *S. enterica* subsp. *Houtenae*, and *S. enterica* subsp. *Indica*., were cultured in NEMIS SalM enrichment media for 24 ± 1 h at 37 ± 1°C. After incubation, the strains were tested without dilution. One replicate per strain was tested.

Exclusivity strains included 32 different non-*Salmonella*

strains, including other *Enterobacteriaceae* species strains (*Shigella* sp., *Enterobacter* sp., *Citrobacter* sp., *Escherichia* sp., *Klebsiella* sp., *Hafnia* sp., *Pantoea* sp., *Proteus* sp., and *Cronobacter* sp.). Exclusivity strains were cultivated in nonselective media, Tryptic Soy Broth (TSB), or MRS broth depending on bacterial species at conditions for optimal growth. Exclusivity cultures were not diluted prior to analysis.

Inclusivity and exclusivity cultures were blind-coded and randomized so that the analyst did not know the identity of the test samples. For each strain, codes have been randomly generated by software (Excel). Labels with the code were manually applied to each tube by Experimenter 1. Experimenter 2 conducted the N-Light *Salmonella* Risk test on the blind-coded samples. AquaSpark and lysis tablets were released from the caps to the suspension. Tubes were then mixed by vortex for 10 s. After 3 min of incubation at 37°C (dry heating block), luminescence was measured in the NEMIS luminometer (BTL1, NEMIS). Results were decoded and tabulated by strain.

(b) *Results*.—Of the 121 specific inclusivity strains tested, 121 were detected by the N-Light method (Table 1). Of the 32 specific exclusivity strains tested, 32 were not detected by the N-Light method (Table 2). OD_{600nm} measurement of each suspension before the N-Light test confirmed that all strains have grown (data not shown). The results are shown in Table 1 and Table 2.

Matrix Study

(a) *Methods*.—The N-Light *Salmonella* method was compared to the cultural reference method for detection of *Salmonella* spp. (ISO 6579–1:2017) on environmental surfaces. Three types of environmental surfaces were tested: stainless steel [AISI 304 (1.4301), grade 2b finish], plastic (polystyrene) and ceramic (glazed earthen material). For each environmental surface, the study included five replicate test portions of uninoculated matrix, 20 replicate test portions at a low level to yield fractionally positive results, and five replicate test portions at a high level to yield consistently positive results. Fractionally positive results, those in which at least one of the methods (candidate or reference) yields 5–15 positive results out of 20 replicates examined for the low level of inoculation, are required for each matrix tested. This is an unpaired study. Separate test portions were prepared for the candidate method and reference method.

Three *Salmonella enterica* subsp. *enterica* strains were used: *S.* Typhimurium ATCC 14028 (stainless steel); *S.* Enteritidis ATCC 49223 (plastic), and *S.* Montevideo CIP 104583 (ceramic). For stainless steel, *Citrobacter koseri* ATCC 27028 was used as a competitor organism. For pure inoculum preparation, the working suspension of each strain was diluted in fresh TSB to obtain the required concentration. Bacterial concentration of inoculum was adjusted by measurement of the optical density at 600 nm and was controlled by plating the inoculum in triplicate on Tryptic Soy Agar plates (TSA) after serial decimal dilution if needed. After 24 h of incubation at 37 ± 2°C, colonies were counted on plates that presented between 15 and 300 colonies.

For the matrix study with competitive flora, a competitor organism (*C. koseri* ATCC 27028) was co-inoculated with *S.* Typhimurium ATCC 14028 on a stainless-steel surface. The competitor organism was inoculated at 10–100 times the level of the target strain. For this, a mixed culture was prepared. The working cultures of *S.* Typhimurium ATCC 14028 and *C. koseri* ATCC 27028 were diluted in fresh TSB to obtain a concentration of about twice the target concentration. The two diluted cultures were then mixed (1:1) to obtain the inoculum.

Three types of surfaces were used in the matrix study: stainless steel [AISI 304 (1.4301), grade 2b finish], rigid plastic (polystyrene), and ceramic (glazed earthen material). The three types of surfaces were supplied by NEMIS Technology and NEXIDIA. For each type of surface, nine 1″ × 1″ (6.25 cm^2) areas were defined on 4.7″ × 4.7″ plates (144 cm^2). Before using in the matrix study, plates were washed with a specific dishwashing liquid (Anios) and decontaminated by making a 15 min ethanol 70% (v/v) bath. The plates were then removed from the bath and allowed to dry for at least 1 h under the flow of a biosafety cabinet. Each 1″ × 1″ area was inoculated with 100 µL of adequate inoculum or sterile TSB. Drops were spread using a sterile loop to distribute the inoculum evenly over the surface. For drying the inoculum, environmental surfaces were placed in closed Petri dishes (245 cm × 245 cm) and under laminar flow working for 18 h. During drying, room temperature was kept at 22 ± 2°C.

For the reference method, premoistened classic swabs with BPW were used. Environmental surfaces were swabbed using firm and even pressure vertically (approximately 10 times), and then the sampler was flipped and the other side used to sample horizontally (approximately 10 times) and diagonally (approximately 10 times). Swabs were introduced in a closed tube and stored at room temperature for 2 h ± 15 min prior to analysis. Then they were introduced in tubes containing 9 mL of BPW. Tubes were mixed using a vortex for 10 s and then incubated at 37 ± 1°C for 18 h ± 2 h. After the incubation period, a 0.1 mL aliquot of the primary enrichment was transferred into 10 mL of Rappaport-Vassiliadis medium with soya (RVS), and 1.0 mL was transferred into 9 mL of Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn). The RVS broth and the MKTTn were incubated at 41.5 ± 1°C for 24 ± 3 h and at 37 ± 1°C for 24 ± 3 h, respectively. From both secondary enrichments, a loopful was streaked onto two selective agars: XLD agar plates and chromogenic BSA plates. Plates were incubated at 37 ± 1°C for 24 ± 2 h. For the confirmation step, typical colonies for each sample were selected and streaked onto TSA. Plates were incubated at 37 ± 1°C for 18–24 h. Biochemical (triple sugar iron agar test, urea agar test, and L-lysine decarboxylation medium test) and serological (Polyvalent O and H serology test) tests were performed for each presumptive sample.

For the N-Light *Salmonella* Risk method, premoistened flocked swabs with BPW were used. Environmental surfaces were swabbed using firm and even pressure vertically (approximately 10 times), and then the sampler was flipped and the other side used to sample horizontally (approximately 10 times) and diagonally (approximately 10 times). Swabs were introduced in a closed tube and stored at room temperature for 2 h ± 15 min prior to analysis. Then they were introduced into specific tubes containing 2 mL of NEMIS SalM broth (NEB). The tubes were mixed using a vortex for 10 s and incubated in a dry heating block at 37 ± 1°C for 24 ± 2 h. After the incubation period, an AquaSpark and lysis tablets were introduced in each enrichment tube and dissolved by 15 s mixing with a vortex. Tubes were incubated for 3 min at 37 ± 2°C (dry heat block) before being read in luminometer. For all test portions, before adding the AquaSpark and lysis tablet, a

Table 1. Inclusivity panel results

No.	Species	Subspecies	Serovars	Antigenic formula/Serogroup	Source	Reference	Origin	Results
1	*S. bongori*	-	–	66: z41:-	DSMZ[a]	13772	Human	Positive
2	*S. bongori*	-	–	48: z35 : -	ZHAW[b]	N268-08	Environmental	Positive
3	*S. bongori*	-	–	–	CCUG[c]	63587	Human feces	Positive
4	*S. enterica*	*arizonae*	–	–	CCUG	29867	Human feces	Positive
5	*S. enterica*	*arizonae*	–	–	CCUG	63588	Human feces	Positive
6	*S. enterica*	*arizonae*	–	44: z4, z23:-	APHA[d]	S00902-21	Reptile	Positive
7	*S. enterica*	*diarizonae*	–	61: c: z35	ZHAW	N09-2338	Human feces	Positive
8	*S. enterica*	*diarizonae*	–	50: z52: z35	CCUG	30388	Snake	Positive
9	*S. enterica*	*diarizonae*	–	S.III 50:5:1,5,7	Nexidia[e]	NEX-1902	Dehydrated food product	Positive
10	*S. enterica*	*diarizonae*			APHA	L00996-15	Chicken	Positive
11	*S. enterica*	*diarizonae*	–	50: z: z52	APHA	S00991-1	Reptile	Positive
12	*S. enterica*	*houtenae*	–	38: z4, z23 : -	ZHAW	N09-2589	Snake	Positive
13	*S. enterica*	*houtenae*	–	43: z4, z23 : -	ZHAW	N20-1583	Human feces	Positive
14	*S. enterica*	*houtenae*	–	50: z4, z23:-	CCUG	30393	Snake	Positive
15	*S. enterica*	*houtenae*	–	43: z4, z23	CCUG	30415	Snake	Positive
16	*S. enterica*	*indica*			APHA	L01098-19	Chicken	Positive
17	*S. enterica*	*indica*	–	VI 1,6 ,1 4: a : 1,5	IP[f]	359–82	Food	Positive
18	*S. enterica*	*indica*	–	VI 16: z10:1,5	IP	959/71	Human	Positive
19	*S. enterica*	*salamae*		30: l, z28: z6	ZHAW	N09-2794	Human feces	Positive
20	*S. enterica*	*salamae*	Tranoroa	1,9,12,46,27: a: z6	CIP	106895	Lizard	Positive
21	*S. enterica*	*salamae*	Tranoroa	II 55: k: z39	Nexidia	NEX-1258	Food isolate	Positive
22	*S. enterica*	*enterica*	Abaetetuba	F	Nexidia	NEX-1716	Dairy product	Positive
23	*S. enterica*	*enterica*	Adelaide	O	ZHAW	N19-976	Human feces	Positive
24	*S. enterica*	*enterica*	Adelaide	O	Nexidia	NEX-785	Food isolate	Positive
25	*S. enterica*	*enterica*	Agona	B	Nexidia	NEX-1639	Calf sweetbreads	Positive
26	*S. enterica*	*enterica*	Albany	C_3	ZHAW	N18-1907	Feed	Positive
27	*S. enterica*	*enterica*	Albany	C_3	ZHAW	N20-2523	Food poultry	Positive
28	*S. enterica*	*enterica*	Amsterdam	E_1	Nexidia	NEX-1767	Food isolate	Positive
29	*S. enterica*	*enterica*	Anatum	E_1	Nexidia	NEX-1723	Rapeseed sample	Positive
30	*S. enterica*	*enterica*	Anatum	E_1	Nexidia	NEX-1724	Canula	Positive
31	*S. enterica*	*enterica*	Aschersleben	N	Nexidia	NEX-1906	Food isolate	Positive
32	*S. enterica*	*enterica*	Bergen	X	Nexidia	NEX-1644	Food isolate	Positive
33	*S. enterica*	*enterica*	Berta	D_1	ZHAW	N19-2653	Human feces	Positive
34	*S. enterica*	*enterica*	Bijlmer	R	ZHAW	N15-2159	Human feces	Positive
35	*S. enterica*	*enterica*	Blockley	C_2	Nexidia	NEX-1574	Food enrichment	Positive
36	*S. enterica*	*enterica*	Blockley	C_2	ZHAW	N18-1544	Human feces	Positive
37	*S. enterica*	*enterica*	Braenderup	C_1	Nexidia	NEX-1850	Milk	Positive
38	*S. enterica*	*enterica*	Brandenburg	B	Nexidia	NEX-1786	Cheese	Positive
39	*S. enterica*	*enterica*	Bredeney	B	Nexidia	NEX-1464	Food product	Positive
40	*S. enterica*	*enterica*	Caracas	H	Nexidia	NEX-1785	Food product	Positive
41	*S. enterica*	*enterica*	Carmel	J	ZHAW	N17-0762	Chicken	Positive
42	*S. enterica*	*enterica*	Cerro	K	Nexidia	NEX-1657	Chick fluff	Positive
43	*S. enterica*	*enterica*	Champaign	Q	Nexidia	NEX-1576	Food isolate	Positive
44	*S. enterica*	*enterica*	Chandans	F	Nexidia	NEX-1664	Food product	Positive
45	*S. enterica*	*enterica*	Chester	B	Nexidia	NEX-862	Duck liver	Positive
46	*S. enterica*	*enterica*	Coeln	B	Nexidia	NEX-1106	Food isolate	Positive
47	*S. enterica*	*enterica*	Corvallis	C_2	ZHAW	N20-0386	Human feces	Positive
48	*S. enterica*	*enterica*	Corvallis	C_2	Nexidia	NEX-1066	Food isolate	Positive
49	*S. enterica*	*enterica*	Cubana	G	Nexidia	NEX-1829	Food isolate	Positive
50	*S. enterica*	*enterica*	Derby	B	Nexidia	NEX-700	Seafood products	Positive
51	*S. enterica*	*enterica*	Dublin	9: g, p:-	CIP[g]	110276	Bovine	Positive
52	*S. enterica*	*enterica*	Ealing	O	Nexidia	NEX-1667	Food isolate	Positive
53	*S. enterica*	*enterica*	Emek	C_3	ZHAW	N19-0283	Human feces	Positive
54	*S. enterica*	*enterica*	Enteritidis	D_1	Nexidia	NEX-1787	Cheese	Positive
55	*S. enterica*	*enterica*	Enteritidis	D_1	Nexidia	NEX-1794	Milk	Positive
56	*S. enterica*	*enterica*	Fresno	D_2	ZHAW	N17-1724	Human feces	Positive
57	*S. enterica*	*enterica*	Gateshead	D_2	ZHAW	N19-1826	Human feces	Positive
58	*S. enterica*	*enterica*	Give	E	Nexidia	NEX-1609	Food isolate	Positive
59	*S. enterica*	*enterica*	Hadar	C_3	ZHAW	N10-0099	Human feces	Positive
60	*S. enterica*	*enterica*	Havana	G	Nexidia	NEX-1152	Feed product	Positive
61	*S. enterica*	*enterica*	Heidelberg	B	Nexidia	NEX-1704	Poultry	Positive
62	*S. enterica*	*enterica*	Hofit	Q	ZHAW	N18-1113	Human feces	Positive
63	*S. enterica*	*enterica*	Hvittingfoss	I	Nexidia	NEX-1151	Food enrichment	Positive
64	*S. enterica*	*enterica*	Hvittingfoss	I	Nexidia	NEX-1467	Food isolate	Positive

(continued)

Table 1. (continued)

No.	Species	Subspecies	Serovars	Antigenic formula/Serogroup	Source	Reference	Origin	Results
65	*S. enterica*	*enterica*	Indiana	B	Nexidia	NEX-1402	Food product	Positive
66	*S. enterica*	*enterica*	Infantis	C_1	Nexidia	NEX-814	Meat	Positive
67	*S. enterica*	*enterica*	Javiana	D_1	ZHAW	N1246-08	Human feces	Positive
68	*S. enterica*	*enterica*	Johannesburg	R	ZHAW	N17-1932	Human feces	Positive
69	*S. enterica*	*enterica*	Kasenyi	P	ZHAW	N20-0227	Food	Positive
70	*S. enterica*	*enterica*	Kedougou	G_2	Nexidia	NEX-1111	Tuna	Positive
71	*S. enterica*	*enterica*	Kentucky	C_3	Nexidia	NEX-1617	Food isolate	Positive
72	*S. enterica*	*enterica*	Korovi	P	ZHAW	N16-0899	Feed	Positive
73	*S. enterica*	*enterica*	Kottbus	C_2	Nexidia	NEX-1471	Food isolate	Positive
74	*S. enterica*	*enterica*	Lagos	B	Nexidia	NEX-703	Meat	Positive
75	*S. enterica*	*enterica*	Lille	C_1	Nexidia	NEX-296	Food isolate	Positive
76	*S. enterica*	*enterica*	Litchfield	C_2	ZHAW	N18-1222	Human feces	Positive
77	*S. enterica*	*enterica*	Livingstone	C_1	Nexidia	NEX-1645	Food isolate	Positive
78	*S. enterica*	*enterica*	London	E_1	Nexidia	NEX-1666	Food enrichment	Positive
79	*S. enterica*	*enterica*	Manchester	C_2	Nexidia	NEX-1658	Food isolate	Positive
80	*S. enterica*	*enterica*	Manhattan	C_3	Nexidia	NEX-1560	Food isolate	Positive
81	*S. enterica*	*enterica*	Mbandaka	C_1	ZHAW	N18-1863	Human feces	Positive
82	*S. enterica*	*enterica*	Meleagridis	E_1	Nexidia	NEX-742	Ground beef	Positive
83	*S. enterica*	*enterica*	Menston	C_1	ZHAW	N18-1184	Human feces	Positive
84	*S. enterica*	*enterica*	Minnesota	L	ZHAW	N20-2630	Food poultry	Positive
85	*S. enterica*	*enterica*	Mississippi	G	Nexidia	NEX-1764	Food isolate	Positive
86	*S. enterica*	*enterica*	Montevideo	C_1	Nexidia	NEX-1025	Cheese	Positive
87	*S. enterica*	*enterica*	Montevideo	C_1	CIP	104583	Monkey	Positive
88	*S. enterica*	*enterica*	Montevideo	C_1	Nexidia	NEX-1775	Food isolate	Positive
89	*S. enterica*	*enterica*	Muenchen	C_2	Nexidia	NEX-1326	Food isolate	Positive
90	*S. enterica*	*enterica*	Muenster	E_1	ZHAW	N520-08	Human feces	Positive
91	*S. enterica*	*enterica*	Napoli	D_1	Nexidia	NEX-1863	Food isolate	Positive
92	*S. enterica*	*enterica*	Newport	C_2	Nexidia	NEX-816	Meat	Positive
93	*S. enterica*	*enterica*	Nima	M	Nexidia	NEX-1881	Food isolate	Positive
94	*S. enterica*	*enterica*	Oranienburg	C_1	Nexidia	NEX-1725	Rapeseed	Positive
95	*S. enterica*	*enterica*	Orion	E_1	Nexidia	NEX-1776	Food isolate	Positive
96	*S. enterica*	*enterica*	Ouakam	D_2	Nexidia	NEX-837	Food isolate	Positive
97	*S. enterica*	*enterica*	Panama	D_1	Nexidia	NEX-740	Horse steak	Positive
98	*S. enterica*	*enterica*	Plymouth	D_2	ZHAW	N20-0792	Human feces	Positive
99	*S. enterica*	*enterica*	Poona	G	ZHAW	N19-29	Human feces	Positive
100	*S. enterica*	*enterica*	Ramatgan	N	Nexidia	NEX-311	Food isolate	Positive
101	*S. enterica*	*enterica*	Reading	B	Nexidia	NEX-919	Water	Positive
102	*S. enterica*	*enterica*	Regent	E_1	Nexidia	NEX-1555	Food isolate	Positive
103	*S. enterica*	*enterica*	Rissen	C_1	Nexidia	NEX-1191	Food isolate	Positive
104	*S. enterica*	*enterica*	Saintpaul	B	Nexidia	NEX-1389	Food enrichment	Positive
105	*S. enterica*	*enterica*	Sandiego	B	ZHAW	N19-1171	Human feces	Positive
106	*S. enterica*	*enterica*	Schwarzengrund	B	Nexidia	NEX-1571	Food enrichment	Positive
107	*S. enterica*	*enterica*	Senftenberg	E_4	ZHAW	N2313-08	Human feces	Positive
108	*S. enterica*	*enterica*	Stuivenberg	E_4	Nexidia	NEX-702	Meat	Positive
109	*S. enterica*	*enterica*	Tennessee	C_1	Nexidia	NEX-1185	Food isolate	Positive
110	*S. enterica*	*enterica*	Thompson	C_1	Nexidia	NEX-1569	Food product	Positive
111	*S. enterica*	*enterica*	Typhimurium	B	Nexidia	NEX-1640	Fish meal	Positive
112	*S. enterica*	*enterica*	Typhimurium	B	ATCC	14028	Clinical	Positive
113	*S. enterica*	*enterica*	Typhimurium	B	Nexidia	NEX-1742	Cheese	Positive
114	*S. enterica*	*enterica*	Umbilo	M	Nexidia	NEX-1413	Food isolate	Positive
115	*S. enterica*	*enterica*	Veneziana	F	Nexidia	NEX-780	Food isolate	Positive
116	*S. enterica*	*enterica*	Virchow	C_1	Nexidia	NEX-1454	Food product	Positive
117	*S. enterica*	*enterica*	Virginia	C_3	ZHAW	N18-1861	Human feces	Positive
118	*S. enterica*	*enterica*	Wandsworth	Q	ZHAW	N13-0958	Human feces	Positive
119	*S. enterica*	*enterica*	Worthington	G	Nexidia	NEX-1880	Food isolate	Positive
120	*S. enterica*	*enterica*	S.I	1, 4, [5], 12:-:-nonmotile	Nexidia	NEX-998	Goose viscera	Positive
121	*S. enterica*	*enterica*	S.I	I 4, [5], 12: i:-	Nexidia	NEX-1360	Food enrichment	Positive

[a] DSMZ = Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany.
[b] ZHAW = Zürcher Hochschule für Angewandte Wissenschaften, Wädenswil, Switzerland.
[c] CCUG = Culture Collection University of Gothenburg, Goteborg, Sweden.
[d] APHA = Animal Plant Health Agency, Addlestone, United Kingdom.
[e] Nexidia = Nexidia Microbial Strain Collection, Dijon, France.
[f] IP = Institut Pasteur, Paris, France.
[g] CIP = Collection de l'Institut Pasteur, Paris, France.

Table 2. Exclusivity panel results

No.	Genus	Species	Reference	Origin	Nonselective broth	NEMIS SalM broth
1	*Acetobacter*	*acetii*	DSM[a] 3508	Alcohol turned to vinegar	Negative	–[b]
2	*Aeromonas*	*hydrophila*	ATCC[c] 7966	Tin of milk with a fishy odor	Negative	–
3	*Bacillus*	*cereus*	CIP[d] 78.3	Contaminant pharmaceutical preparation	Negative	–
4	*Citrobacter*	*braakii*	ATCC 51113	Snake	Negative	–
5	*Citrobacter*	*freundii*	NEX[e] 1694	Food isolate	Negative	–
6	*Citrobacter*	*koseri*	ATCC 27028	Blood culture	Negative	–
7	*Cronobacter*	*sakazakii*	CIP 57.33	Tin, dried milk	Negative	–
8	*Enterobacter*	*absuriae*	FS2[f]	Coconut water	Negative	–
9	*Enterobacter*	*cloaceae*	DSM 16657	Maize plant	Negative	–
10	*Enterococcus*	*faecalis*	ATCC 51299	Peritoneal fluid, St. Louis, MO	Negative	–
11	*Escherichia*	*coli*	CIP 54.117	Human, feces	Positive	Negative
12	*Escherichia*	*albertii*	DSM 17582	Stool from diarrheal child	Negative	–
13	*Escherichia*	*hermanii*	DSM 4560	Toe, 17-year-old female	Negative	–
14	*Hafnia*	*alvei*	ATCC 51815	Milk, Minnesota	Negative	–
15	*Klebsiella*	*oxytoca*	ATCC 51817	Milk, Minnesota	Negative	–
16	*Lactobacillus*	*sakei*	ATCC 15521	Moto, starter of sake	Negative	–
17	*Listeria*	*monocytogenes*	ATCC 19114	Tissue, animal	Negative	–
18	*Pantoea*	*agglomerans*	CIP 82.100	Corn crop, Canada	Negative	–
19	*Proteus*	*vulgaris*	ATCC 8427	Inner ear infection	Negative	–
20	*Proteus*	*mirabilis*	ATCC 7002	Urine of patient with kidney stones	Negative	–
21	*Providencia*	*alcalifaciens*	DSM 30120	Feces	Negative	–
22	*Pseudomonas*	*aeruginosa*	ATCC 9027	Outer ear infection	Negative	–
23	*Pseudomonas*	*fluorescens*	ATCC 13525	Pre-filter tanks	Negative	–
24	*Rahnella*	*aquatilis*	Probe 8.2[f]	Environment	Negative	–
25	*Serratia*	*liquefaciens*	DSM 4487	Milk; Cork, Ireland	Negative	–
26	*Serratia*	*marcescens*	CIP 53.90	Milk, Delft, The Netherlands	Negative	–
27	*Shigella*	*boydii*	RKI[g] 03/07455	Clinical	Negative	–
28	*Shigella*	*flexneri*	RKI 03/03709-1	Clinical	Negative	–
29	*Shigella*	*sonnei*	RKI 02/03828	Clinical	Negative	–
30	*Staphylococcus*	*aureus*	ATCC 6538	Human lesion	Negative	–
31	*Streptococcus*	*oralis*	102922[f]	Human mouth	Negative	–
32	*Yersinia*	*enterocolitica*	9610[f]	Tissue, human	Negative	–

[a] DSM = Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany.
[b] Negative strains were not tested in the NEMIS SalM broth.
[c] ATCC = American Type Culture Collection, Manassas, VA.
[d] CIP = Collection de l'Institut Pasteur, Paris, France.
[e] NEX = Nexidia Microbial Strain Collection, Dijon, France.
[f] NEMIS Microbial Strain collection, Dübendorf, Switzerland.
[g] RKI = Robert Koch Institute, Berlin, Germany.

Table 3. N-Light *Salmonella* Risk: Presumptive versus confirmed (traditional with secondary enrichments)

				Candidate method presumptive			Candidate method confirmed				
Matrix	Strain	cfu/Test area[a]	N[b]	x[c]	POD_{CP}[d]	95% CI	x	POD_{CC}[e]	95% CI	$dPOD_{CP}$[f]	95% CI[g]
Stainless steel	S. Typhimurium	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(−0.43, 0.43)
	ATCC[h] 14028	46 & 905	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(−0.28, 0.28)
	and *C. koseri* ATCC 27028	600 & 8140	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(−0.43, 0.43)
Stainless steel[i]	S. Typhimurium	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(−0.43, 0.43)
	ATCC 14028	80 & 910	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(−0.28, 0.28)
	and *C. koseri* ATCC 27156	450 & 3200	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(−0.43, 0.43)
Plastic	*Salmonella*	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(−0.43, 0.43)
	Enteritidis	34	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(−0.27, 0.27)
	ATCC 49223	343	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(−0.43, 0.43)

(continued)

Table 3. (continued)

Matrix	Strain	cfu/Test area[a]	N[b]	Candidate method presumptive			Candidate method confirmed			dPOD$_{CP}$[f]	95% CI[g]
				x[c]	POD$_{CP}$[d]	95% CI	x	POD$_{CC}$[e]	95% CI		
Ceramic	*Salmonella*	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(−0.43, 0.43)
	Montevideo	373	20	15	0.75	(0.53, 0.89)	15	0.75	(0.53, 0.89)	0.00	(−0.26, 0.26)
	CIP[j] 104583	3700	5	3	0.60	(0.23, 0.88)	3	0.60	(0.23, 0.88)	0.00	(−0.46, 0.46)

[a] cfu/Test area determined by plating the inoculum in duplicate.
[b] N = Number of test potions.
[c] x = Number of positive test portions.
[d] POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[e] POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[f] dPOD$_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h] ATCC = American Type Culture Collection, Manassas, VA, USA.
[i] Performed by AOAC qualified independent laboratory Q Laboratories, Cincinnati, OH, USA.
[j] CIP = Collection de l'Institut Pasteur, Paris, France.

Table 4. N-Light *Salmonella* Risk: Presumptive versus alternative confirmed (direct streaks to agar plates)

Matrix	Strain	cfu/Test area[a]	N[b]	Candidate method presumptive			Candidate method confirmed			dPOD$_{CP}$[f]	95% CI[g]
				x[c]	POD$_{CP}$[d]	95% CI	x	POD$_{CC}$[e]	95% CI		
Stainless steel	S. Typhimurium	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(−0.43, 0.43)
	ATCC[h] 14028	46 & 905	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(−0.28, 0.28)
	and C. *koseri*	600 & 8140	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(−0.43, 0.43)
	ATCC 27028										
Stainless steel[i]	S. Typhimurium	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(−0.43, 0.43)
	ATCC 14028	80 & 910	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(−0.28, 0.28)
	and C. *koseri*	450 & 3200	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(−0.43, 0.43)
	ATCC 27156										
Plastic	*Salmonella*	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(−0.43, 0.43)
	Enteritidis	34	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(−0.27, 0.27)
	ATCC 49223	343	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(−0.43, 0.43)
Ceramic	*Salmonella*	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(−0.43, 0.43)
	Montevideo	373	20	15	0.75	(0.53, 0.89)	15	0.75	(0.53, 0.89)	0.00	(−0.26, 0.26)
	CIP[j] 104583	3700	5	3	0.60	(0.23, 0.88)	3	0.60	(0.23, 0.88)	0.00	(−0.46, 0.46)

[a] cfu/Test area determined by plating the inoculum in triplicate.
[b] N = Number of test potions.
[c] x = Number of positive test portions.
[d] POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[e] POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[f] dPOD$_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h] ATCC = American Type Culture Collection, Manassas, VA, USA.
[i] Performed by AOAC qualified independent laboratory Q Laboratories, Cincinnati, OH, USA.
[j] CIP = Collection de l'Institut Pasteur, Paris, France.

0.1 mL aliquot of enrichment was transferred to 9 mL of BPW and incubated at 37°C for 18 ± 2 h. After incubation, secondary enrichments (RVS and MKKTn) and confirmation were performed according to ISO 6579:2017. Additionally, from the NEMIS sample tube, a 10 µL aliquot of enrichment was streaked onto XLD Agar and chromogenic BSA plates, and plates were incubated at 37 ± 1°C for 24 ± 2 h. Then, the confirmation steps were carried out as described in reference method.

(b) *Results*.—For each surface type, PODs with 95% CIs were calculated for the candidate method's presumptive and confirmed results and the reference method's results for each contamination level. dPODs were determined between the candidate method's presumptive and confirmed results, as well as between the candidate method's confirmed results and the reference method's results. No differences were observed between the candidate method's presumptive and confirmed results using the reference method confirmation procedure or the NEMIS recommended alternative confirmation procedure (Tables 3 and 4). Small differences were observed between the candidate method's confirmed results and the reference method's

Table 5. Method comparison results: N-Light Salmonella Risk (traditional confirmation) versus ISO 6579-1

				Candidate method confirmed			ISO 6579-1				
Matrix	Strain	cfu/Test area[a]	N[b]	x[c]	POD_C[d]	95% CI	x	POD_R[e]	95% CI	$dPOD_{CP}$[f]	95% CI[g]
Stainless steel	*S.* Typhimurium	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(−0.43, 0.43)
	ATCC[h] 14028	46 & 905	20	12	0.60	(0.39, 0.78)	17	0.85	(0.64, 0.95)	−0.25	(−0.48, 0.03)
	and *C. koseri*	600 & 8140	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(−0.43, 0.43)
	ATCC 27028										
Stainless steel[i]	*S.* Typhimurium	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(−0.43, 0.43)
	ATCC 14028	80 & 910	20	9	0.45	(0.26, 0.66)	8	0.40	(0.22, 0.61)	0.05	(−0.24, 0.33)
	and *C. koseri*	450 & 3200	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(−0.43, 0.43)
	ATCC 27156										
Plastic	*Salmonella*	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(−0.43, 0.43)
	Enteritidis	34	20	14	0.70	(0.48, 0.85)	15	0.75	(0.53, 0.89)	−0.05	(−0.31, 0.22)
	ATCC 49223	343	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(−0.43, 0.43)
Ceramic	*Salmonella*	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(−0.43, 0.43)
	Montevideo	373	20	15	0.75	(0.53, 0.89)	11	0.55	(0.34, 0.74)	0.20	(−0.09, 0.45)
	CIP[j] 104583	3700	5	3	0.60	(0.23, 0.88)	3	0.60	(0.23, 0.88)	0.00	(−0.46, 0.46)

[a] cfu/Test area determined by plating the inoculum in triplicate.
[b] N = Number of test potions.
[c] x = Number of positive test portions.
[d] POD_C = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.
[e] POD_R = Reference method confirmed positive outcomes divided by the total number of trials.
[f] $dPOD_C$ = Difference between the candidate method and reference method POD values.
[g] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h] ATCC = American Type Culture Collection, Manassas, VA, USA.
[i] Performed by independent AOAC certified laboratory Q Laboratories, Cincinnati, OH, USA.
[j] CIP = Collection de l'Institut Pasteur, Paris, France.

results, which are not unexpected because of the unpaired study design. However, no statistically significant differences were evident (Table 5).

Independent Laboratory Studies

(a) *Methods*.—The study was conducted by the independent laboratory. The N-Light *Salmonella* Risk method was compared to the ISO 6579-1:2017 reference method using 30 unpaired sample replicates each. Within each sample set, there were five uninoculated samples, 20 low-level inoculated samples, and five high-level inoculated samples following an unpaired study design. After sampling, swabs were incubated at 37 ± 1°C for 24 ± 2 h before being analyzed by the NEMIS Technologies BTL1 luminometer. The reference method swabs were evaluated at 34–38°C after 18 ± 2 h of enrichment. Regardless of the presumptive results for the method comparison, all samples were culturally confirmed following ISO 6579-1:2017 (selective enrichment through colony confirmation). In addition, candidate method enriched samples were confirmed using an alternative approach by streaking 10 µL from each enriched portion directly to XLD and a chromogenic agar (BSA) and incubated at 37 ± 1°C for 24 ± 3 h. Final confirmation for all samples was obtained by Bruker MALDI Biotyper following AOAC *Official Method of Analysis*[SM] **2017.10** (5).

For stainless-steel surface inoculation, a liquid culture of *S.* Typhimurium ATCC 14028 and *C. koseri* ATCC 27156, which acted as the competitor organism, was used for inoculation. Both cultures were propagated on Tryptic Soy Agar with 5% Sheep Blood (SBA) from a stock culture stored at −70°C. The SBA was incubated for 24 ± 2 h at 35 ± 1°C. A single colony was transferred to Brain Heart Infusion (BHI) broth and incubated for 24 ± 2 h at 35 ± 1°C.

The *S.* Typhimurium culture was diluted in BHI broth to a low level expected to yield fractional results and a high level expected to yield all positive results. The *C. koseri* culture was diluted in BHI broth to 10 times the concentration of the target organism on a stainless-steel surface. 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.

A stainless-steel surface (1″ × 1″ test area) was inoculated with 0.1 mL of the diluted inoculum and allowed to dry for 16–24 h at room temperature (18–25°C) prior to sampling. For the noninoculated test portions, sterile BHI broth was used. The surfaces were sampled by premoistening a swab in BPW. 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. Swabs were allowed to sit at room temperature for 2 h ± 15 min prior to analysis.

For the reference method, swabs were premoistened in 1 mL of BPW. Surfaces were swabbed vertically approximately 10 times, and then the sampler was turned over and the surface was swabbed horizontally approximately 10 times and diagonally approximately 10 times. Swabs were stored at room temperature (20–25°C) for 2 h ± 15 min. After 2 h, swabs were placed into a test tube containing 9 mL of BPW and incubated at 34–38°C for 18 ± 2 h. At 18 h, 0.1 mL of the primary

enrichment was transferred into 10 mL of RVS, and 1.0 mL was transferred into 10 mL of MKTTn. RVS tubes were incubated at 41.5 ± 1°C for 24 ± 3 h, and MKTTn tubes were incubated at 37 ± 1°C for 24 ± 3 h. After incubation, RVS and MKTTn broths were streaked onto XLD and BSA. Plates were incubated at 37 ± 1°C for 24 ± 3 h.

Plates were examined for suspect colonies, and, if present, one typical colony from each agar was selected and streaked onto a nonselective agar. Plates were incubated at 34–38°C for 24 ± 3 h. Polyvalent O and H serology tests were performed. Final confirmation was conducted using the Bruker MALDI Biotyper following AOAC Method **2017.09.**

For the N-Light *Salmonella* Risk method, stainless-steel surface test areas were sampled as described previously. After incubation, all test portions were processed using the NEMIS Technologies BTL1 luminometer. Regardless of presumptive results, all enriched portions went through the ISO 6579-1:2017 reference method confirmation process (transfer to selective enrichment and plating) and an alternative confirmation process (direct streak onto XLD and BSA). Final confirmed results were obtained by serological agglutination (poly O and poly H) and the Bruker MALDI Biotyper following AOAC Method **2017.09**.

(b) *Results.*—The N-Light *Salmonella* method successfully detected *Salmonella* on stainless-steel environmental surfaces. When comparing results obtained from the BTL1 luminometer to the confirmed results, no false positives or false negatives were observed. Using POD analysis (Least Cost Formulations, Ltd., AOAC Binary Data Interlaboratory Study Workbook Version 5.1, Virginia Beach, VA), no statistically significant differences were observed between the number of positive samples detected by the reference method and the NEMIS Technologies *Salmonella* assay (Tables 3–5).

Discussion

The N-Light *Salmonella* assay was able to detect all the *Salmonella* strains tested during the inclusivity study, including *S. bongori* and *S. enterica*. Moreover, it did not detect 32 strains of non-*Salmonella* in which closely related species such as *E. coli* and *Citrobacter* were tested. The specificity of the kit was therefore validated according to the inclusivity/exclusivity study. However, during the method developer study, some *Enterobacteriaceae* strains were able to exhibit an enzymatic activity used by the N-Light assay. These included strains of *E. coli* (DSM 1576 and ATCC 35218), *Klebsiella oxytoca* (ATCC 13182), and *Citrobacter freundii* (two of NEMIS's isolated strains). This activity generates a low positive signal, leading to presumptive positive results when bacteria are grown in nonselective broth. However, NEMIS proprietary enrichment broth controlled the growth of these bacteria and reduced the unspecific signal. It can be assumed that within a complex food environment there is a limited risk of false positive results depending on the sampling area. This may be acceptable for a surface screening test.

Concerning the matrix study, on stainless steel with a competitor microorganism, the N-Light *Salmonella* assay did not shown differences in fractional results compared to the ISO 6579-1 during method developer and independent laboratory studies. In the same way, there is no significant difference between the two methods when plastic or ceramic were used as environmental surfaces.

Conclusions

The data from these studies support the product claim that the NEMIS Technologies N-Light *Salmonella* Risk assay can detect *Salmonella* spp. from environmental surfaces (stainless steel, plastic, and ceramic) when using the BTL1 luminometer. The results obtained by the POD analysis of the method comparison study demonstrated that there were no statistically significant differences between the number of positive samples detected by the candidate and the ISO 6579-1:2017 methods for the three environmental surfaces.

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