



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
072204

The AOAC Research Institute hereby certifies the method known as:

N-LightTM *Salmonella Risk*

manufactured by
NEMIS Technologies AG
Riedhofstrasse 11
8804 Au, ZH
Switzerland

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

A handwritten signature in black ink that reads 'Scott Coates'.

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date	August 5, 2022
Expiration Date	December 31, 2022

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METHOD NAME N-Light™ <i>Salmonella</i> Risk	CATALOG NUMBER 00014
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INDEPENDENT LABORATORY Q.Laboratories 1930 Radcliff Drive Cincinnati, OH 45204 USA	AOAC EXPERTS AND PEER REVIEWERS Thomas Hammack ¹ , James Agin ² , Wayne Ziemer ³ ¹ U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Maryland, USA ² Independent Consultant, Ohio, USA ³ Independent Consultant, Georgia, USA
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APPLICABILITY OF METHOD Analytes – <i>Salmonella</i> spp. Matrixes – (1" x 1" test area) - Stainless steel (AISI 304, grade 2b finish), plastic (polystyrene), and ceramic (glazed earthen) Performance claims - The N-Light <i>Salmonella</i> Risk method demonstrated no statistical difference in performance to the reference method ISO 6579-1:2017 <i>Microbiology of the Food Chain – Horizontal method for the detection, enumeration, and serotyping of Salmonella. – Part 1" (2) for the detection of Salmonella</i> spp. in environmental surfaces (stainless steel, plastic and ceramic) after 24 h of enrichment.	REFERENCE METHOD ISO 6579-1:2017 <i>Microbiology of the food chain -- Horizontal method for the detection, enumeration and serotyping of Salmonella -- Part 1: Detection of Salmonella</i> spp. (2)
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ORIGINAL CERTIFICATION DATE July 28, 2022	CERTIFICATION RENEWAL RECORD New approval 2022.
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METHOD MODIFICATION RECORD NONE	SUMMARY OF MODIFICATION NONE
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PRINCIPLE OF THE METHOD (1)

N-Light™ *Salmonella* Risk method is a qualitative test method for rapid detection of the foodborne bacterial pathogen *Salmonella* spp. The test method is suitable for use in food processing areas and equipment as part of an environmental monitoring program. The method uses AquaSpark®, a patented ultrasensitive chemiluminescent probe technology that reacts with an enzyme broadly expressed across the *Salmonella* genus. N-Light *Salmonella* Risk uses a proprietary selective culture enrichment technology, which consists of a unique enrichment broth supplemented with the antibiotics, and a bacteriophage cocktail targeting gram negative competitor species.

Following surface sampling according to ISO 18593:2017, the flocked swab is incubated in the enrichment broth, the biosafety cap is then used to permanently seal the tube, which is cultured for 24 ± 2 h the dry heat block at 37 ± 1°C. For chemiluminescent detection of *Salmonella*, the AquaSpark and lysis tablets are simultaneously released into the enrichment broth from the biosafety cap without further sample preparation. The sample is vortexed for 15 s to ensure efficient bacterial lysis and dissolution of the AquaSpark probe and incubated at 37 ± 2°C for 3 min. Subsequently, Luminescence is quantified using a NEMIS (BTL1) luminometer and a sample is considered as a presumptive positive result when the signal detected is superior to specific threshold.

DISCUSSION OF THE VALIDATION STUDY (1)

The N-Light *Salmonella* spp. kit was able to detect all the *Salmonella* strains tested during the inclusivity study including *S. bongori* and *S. enterica*. Moreover, the N-Light *Salmonella* spp. kit 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 NEMIS's isolated strains). This activity can generate a low positive signal leading to presumptive positive when grown in non-selective broth. However, NEMIS proprietary enrichment broth was able to control the growth and the non-specific signal albeit some elevated background signal for the *E. coli* strains remained. It can be assumed that within a complex food environment, there is a limited risk of false positive results depending on the sampling area and specific strains of mentioned species present. This may be deemed acceptable for a surface screening test.

Concerning the matrix study, on stainless steel with competitor micro-organism, the N-Light *Salmonella* spp. kit 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 differences between the two methods when plastic or ceramic were used as environmental surfaces.

Table 1: Inclusivity Panel Results (1)

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

65	<i>S. enterica</i>	<i>enterica</i>	Indiana	B	Nexidia	NEX-1402	Food product	Positive
66	<i>S. enterica</i>	<i>enterica</i>	Infantis	C ₁	Nexidia	NEX-814	Meat	Positive
67	<i>S. enterica</i>	<i>enterica</i>	Javiana	D ₁	ZHAW	N1246-08	Human feces	Positive
68	<i>S. enterica</i>	<i>enterica</i>	Johannesburg	R	ZHAW	N17-1932	Human feces	Positive
69	<i>S. enterica</i>	<i>enterica</i>	Kasenyi	P	ZHAW	N20-0227	Food	Positive
70	<i>S. enterica</i>	<i>enterica</i>	Kedougou	G ₂	Nexidia	NEX-1111	Tuna	Positive
71	<i>S. enterica</i>	<i>enterica</i>	Kentucky	C ₃	Nexidia	NEX-1617	Food isolate	Positive
72	<i>S. enterica</i>	<i>enterica</i>	Korovi	P	ZHAW	N16-0899	Feed	Positive
73	<i>S. enterica</i>	<i>enterica</i>	Kottbus	C ₂	Nexidia	NEX-1471	Food isolate	Positive
74	<i>S. enterica</i>	<i>enterica</i>	Lagos	B	Nexidia	NEX-703	Meat	Positive
75	<i>S. enterica</i>	<i>enterica</i>	Lille	C ₁	Nexidia	NEX-296	Food isolate	Positive
76	<i>S. enterica</i>	<i>enterica</i>	Litchfield	C ₂	ZHAW	N18-1222	Human feces	Positive
77	<i>S. enterica</i>	<i>enterica</i>	Livingstone	C ₁	Nexidia	NEX-1645	Food isolate	Positive
78	<i>S. enterica</i>	<i>enterica</i>	London	E ₁	Nexidia	NEX-1666	Food enrichment	Positive
79	<i>S. enterica</i>	<i>enterica</i>	Manchester	C ₂	Nexidia	NEX-1658	Food isolate	Positive
80	<i>S. enterica</i>	<i>enterica</i>	Manhattan	C ₃	Nexidia	NEX-1560	Food isolate	Positive
81	<i>S. enterica</i>	<i>enterica</i>	Mbandaka	C ₁	ZHAW	N18-1863	Human feces	Positive
82	<i>S. enterica</i>	<i>enterica</i>	Meleagridis	E ₁	Nexidia	NEX-742	Ground beef	Positive
83	<i>S. enterica</i>	<i>enterica</i>	Menston	C ₁	ZHAW	N18-1184	Human feces	Positive
84	<i>S. enterica</i>	<i>enterica</i>	Minnesota	L	ZHAW	N20-2630	Food poultry	Positive
85	<i>S. enterica</i>	<i>enterica</i>	Mississippi	G	Nexidia	NEX-1764	Food isolate	Positive
86	<i>S. enterica</i>	<i>enterica</i>	Montevideo	C ₁	Nexidia	NEX-1025	Cheese	Positive
87	<i>S. enterica</i>	<i>enterica</i>	Montevideo	C ₁	CIP	104583	Monkey	Positive
88	<i>S. enterica</i>	<i>enterica</i>	Montevideo	C ₁	Nexidia	NEX-1775	Food isolate	Positive
89	<i>S. enterica</i>	<i>enterica</i>	Muenchen	C ₂	Nexidia	NEX-1326	Food isolate	Positive
90	<i>S. enterica</i>	<i>enterica</i>	Muenster	E ₁	ZHAW	N520-08	Human feces	Positive
91	<i>S. enterica</i>	<i>enterica</i>	Napoli	D ₁	Nexidia	NEX-1863	Food isolate	Positive
92	<i>S. enterica</i>	<i>enterica</i>	Newport	C ₂	Nexidia	NEX-816	Meat	Positive
93	<i>S. enterica</i>	<i>enterica</i>	Nima	M	Nexidia	NEX-1881	Food isolate	Positive
94	<i>S. enterica</i>	<i>enterica</i>	Oranienburg	C ₁	Nexidia	NEX-1725	Rapeseed	Positive
95	<i>S. enterica</i>	<i>enterica</i>	Orion	E ₁	Nexidia	NEX-1776	Food isolate	Positive
96	<i>S. enterica</i>	<i>enterica</i>	Ouakam	D ₂	Nexidia	NEX-837	Food isolate	Positive
97	<i>S. enterica</i>	<i>enterica</i>	Panama	D ₁	Nexidia	NEX-740	Horse steak	Positive
98	<i>S. enterica</i>	<i>enterica</i>	Plymouth	D ₂	ZHAW	N20-0792	Human feces	Positive
99	<i>S. enterica</i>	<i>enterica</i>	Poona	G	ZHAW	N19-29	Human feces	Positive
100	<i>S. enterica</i>	<i>enterica</i>	Ramatgan	N	Nexidia	NEX-311	Food isolate	Positive
101	<i>S. enterica</i>	<i>enterica</i>	Reading	B	Nexidia	NEX-919	Water	Positive
102	<i>S. enterica</i>	<i>enterica</i>	Regent	E ₁	Nexidia	NEX-1555	Food isolate	Positive
103	<i>S. enterica</i>	<i>enterica</i>	Rissen	C ₁	Nexidia	NEX-1191	Food isolate	Positive
104	<i>S. enterica</i>	<i>enterica</i>	Saintpaul	B	Nexidia	NEX-1389	Food enrichment	Positive
105	<i>S. enterica</i>	<i>enterica</i>	Sandiego	B	ZHAW	N19-1171	Human feces	Positive
106	<i>S. enterica</i>	<i>enterica</i>	Schwarzengrund	B	Nexidia	NEX-1571	Food enrichment	Positive
107	<i>S. enterica</i>	<i>enterica</i>	Senftenberg	E ₄	ZHAW	N2313-08	Human feces	Positive
108	<i>S. enterica</i>	<i>enterica</i>	Stuivenberg	E ₄	Nexidia	NEX-702	Meat	Positive
109	<i>S. enterica</i>	<i>enterica</i>	Tennessee	C ₁	Nexidia	NEX-1185	Food isolate	Positive
110	<i>S. enterica</i>	<i>enterica</i>	Thompson	C ₁	Nexidia	NEX-1569	Food product	Positive
111	<i>S. enterica</i>	<i>enterica</i>	Typhimurium	B	Nexidia	NEX-1640	Fish meal	Positive
112	<i>S. enterica</i>	<i>enterica</i>	Typhimurium	B	ATCC	14028	Clinical	Positive
113	<i>S. enterica</i>	<i>enterica</i>	Typhimurium	B	Nexidia	NEX-1742	Cheese	Positive
114	<i>S. enterica</i>	<i>enterica</i>	Umbilo	M	Nexidia	NEX-1413	Food isolate	Positive
115	<i>S. enterica</i>	<i>enterica</i>	Veneziana	F	Nexidia	NEX-780	Food isolate	Positive
116	<i>S. enterica</i>	<i>enterica</i>	Virchow	C ₁	Nexidia	NEX-1454	Food product	Positive
117	<i>S. enterica</i>	<i>enterica</i>	Virginia	C ₃	ZHAW	N18-1861	Human feces	Positive
118	<i>S. enterica</i>	<i>enterica</i>	Wandsworth	Q	ZHAW	N13-0958	Human feces	Positive
119	<i>S. enterica</i>	<i>enterica</i>	Worthington	G	Nexidia	NEX-1880	Food isolate	Positive
120	<i>S. enterica</i>	<i>enterica</i>	S.I	1, 4,[5],12:-:nonmotile	Nexidia	NEX-998	Goose viscera	Positive
121	<i>S. enterica</i>	<i>enterica</i>	S.I	1, 4,[5],12:i:-	Nexidia	NEX-1360	Food enrichment	Positive

^aDSMZ = Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany.

^bZHAW = Zürcher Hochschule für Angewandte Wissenschaften, Wädenswil, Switzerland.

^cCCUG = Culture Collection University of Gothenburg, Goteborg, Sweden.

^dAPHA = Animal Plant Health Agency, Addlestone, United Kingdom.

^eNexidia = Nexidia Microbial Strain Collection, Dijon, France.

^fIP = Institut Pasteur, Paris, France.

^gCIP = Collection de l'Institut Pasteur, Paris, France.

Table 2: Exclusivity Panel Results (1)

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

^aDSM = Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany.

^b- = Negative strains were not tested in the NEMIS SaIM broth.

^cATCC = American Type Culture Collection, Manassas, VA.

^dCIP = Collection de l'Institut Pasteur, Paris, France.

^eNEX = Nexidia Microbial Strain Collection, Dijon, France.

^f = NEMIS Microbial Strain collection, Dübendorf, Switzerland.

^gRKI: Robert Koch Institute, Berlin, Germany.

Table 3. N-Light Salmonella Risk: Presumptive vs. confirmed (traditional with secondary enrichments) (1)

Matrix	Strain	cfu/test area ^a	N ^b	Candidate method presumptive			Candidate method confirmed				
				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)
	& C. koseri ATCC 27028	600 & 8 140	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)
	& C. koseri ATCC 27156	450 & 3 200	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)
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	3 700	5	3	0.60	(0.23,0.88)	3	0.60	(0.23,0.88)	0.00	(-0.46,0.46)

^acfu/Test area determined by plating the inoculum in duplicate.

^bN = Number of test portions.

^cx = Number of positive test portions.

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^hATCC = American Type Culture Collection, Manassas, VA, USA.

ⁱPerformed by AOAC qualified independent laboratory Q Laboratories, Cincinnati, OH, USA.

^jCIP = Collection de l'Institut Pasteur, Paris, France.

Table 4. N-Light *Salmonella* Risk: Presumptive vs. Alternative Confirmed (Direct Streaks to Agar Plates) (1)

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.	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43,0.43)
	Typhimurium 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)
	& <i>C. koseri</i> ATCC 27028	600 & 8 140	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.	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43,0.43)
	Typhimurium 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)
	& <i>C. koseri</i> ATCC 27156	450 & 3 200	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43,0.43)
Plastic	<i>Salmonella</i>	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43,0.43)
	Enteritidis ATCC 49223	34 343	20 5	14 5	0.70 1.00	(0.48,0.85) (0.57,1.00)	14 5	0.70 1.00	(0.48,0.85) (0.57,1.00)	0.00	(-0.27,0.27) (-0.43,0.43)
	<i>Salmonella</i>	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43,0.43)
Ceramic	Montevideo CIP ^j 104583	373 3 700	20 5	15 3	0.75 0.60	(0.53,0.89) (0.23,0.88)	15 3	0.75 0.60	(0.53,0.89) (0.23,0.88)	0.00	(-0.26,0.26) (-0.46,0.46)

^acfu/Test area determined by plating the inoculum in triplicate.

^bN = Number of test portions.

^cx = Number of positive test portions.

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

^g95% 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

ⁱPerformed by AOAC qualified independent laboratory Q Laboratories, Cincinnati, OH, USA.

^jCIP = Collection de l'Institut Pasteur, Paris, France.

Table 5. Method Comparison Results: N-Light *Salmonella* Risk (Traditional Confirmation) vs. ISO 6579-1 (1)

Matrix	Strain	cfu/test area ^a	N ^b	Candidate method confirmed			ISO 6579-1			dPOD _{CP} ^f	95% CI ^g
				x ^c	POD _C ^d	95% CI	x	POD _R ^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)	17	0.85	(0.64,0.95)	-0.25	(-0.48,0.03)
	& <i>C. koseri</i> ATCC 27028	600 & 8 140	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)	8	0.40	(0.22, 0.61)	0.05	(-0.24,0.33)
	& <i>C. koseri</i> ATCC 27156	450 & 3 200	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43,0.43)
Plastic	<i>Salmonella</i>	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43,0.43)
	Enteritidis ATCC 49223	34 343	20 5	14 5	0.70 1.00	(0.48,0.85) (0.57,1.00)	15 5	0.75 1.00	(0.53,0.89) (0.57,1.00)	-0.05 0.00	(-0.31,0.22) (-0.43,0.43)
	<i>Salmonella</i>	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43,0.43)
Ceramic	Montevideo CIP ^j 104583	373 3 700	20 5	15 3	0.75 0.60	(0.53,0.89) (0.23,0.88)	11 3	0.55 0.60	(0.34,0.74) (0.23,0.88)	0.20 0.00	(-0.09,0.45) (-0.46,0.46)

^acfu/Test area determined by plating the inoculum in triplicate.

^bN = Number of test portions.

^cx = Number of positive test portions.

^dPOD_C = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.

^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials.

^fdPOD_C = Difference between the candidate method and reference method POD values.

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^hATCC = American Type Culture Collection, Manassas, VA, USA.

ⁱPerformed by independent AOAC certified laboratory Q Laboratories, Cincinnati, OH, USA.

^jCIP = Collection de l'Institut Pasteur, Paris, France.

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