CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

072204

The AOAC Research Institute hereby certifies the method known as:

N-Light™ *Salmonella* Risk

manufactured by

NEMIS Technologies AG
Riedhofstrasse 11
CH - 8804 Au, ZH
Switzerland

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*[SM] Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*[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.

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

Issue Date	December 17, 2024
Expiration Date	December 31, 2025

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METHOD NAME
N-Light™ *Salmonella* Risk

CATALOG NUMBER
00014

INDEPENDENT LABORATORY
Q Laboratories
1930 Radcliff Drive
Cincinnati, OH 45204 USA

APPLICABILITY OF METHOD
Analytes – *Salmonella* 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 *Salmonella* Risk method demonstrated no significant 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. in environmental surfaces (stainless steel, plastic and ceramic) after 24 h of enrichment.

ORIGINAL CERTIFICATION DATE
July 28, 2022

CERTIFICATION RENEWAL RECORD
Renewed through December 2025.

METHOD MODIFICATION RECORD
1. **February 2024 Level 3**
2. **December 2024 Level 1**

SUMMARY OF MODIFICATION
1. **Modification changes include.**
   a. **Combined lysis and AquaSaprk® tablet**
   b. **Increased size of the antibiotic tablet**
   c. **New proprietary phage cocktail**
   d. **New pre-moistening NEMIS neutralizer.**
2. **Editorial changes.**

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NONE

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	*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 1,6,14: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
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 (1)

No.	Genus	Species	Reference	Origin	Non-selective 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 diarrhoeal 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 [e]	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 [e]	Human mouth	Negative	-
32	*Yersinia*	*enterocolitica*	9610 [e]	Tissue, human	Negative	-

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

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

				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 ATCC[h] 14028	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43,0.43)
		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 ATCC 14028	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43,0.43)
		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* Enteritidis ATCC 49223	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43,0.43)
		34	20	14	0.70	(0.48,0.85)	14	0.70	(0.48,0.85)	0.00	(-0.27,0.27)
		343	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.43,0.43)
Ceramic	*Salmonella* Montevideo CIP[j] 104583	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43,0.43)
		373	20	15	0.75	(0.53,0.89)	15	0.75	(0.53,0.89)	0.00	(-0.26,0.26)
		3 700	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 vs. Alternative Confirmed (Direct Streaks to Agar Plates) (1)

				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 ATCC[h] 14028 & *C. koseri* ATCC 27028	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43,0.43)
		46 & 905	20	12	0.60	(0.39,0.78)	12	0.60	(0.39,0.78)	0.00	(-0.28,0.28)
		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 ATCC 14028 & *C. koseri* ATCC 27156	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43,0.43)
		80 & 910	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(-0.28,0.28)
		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* Enteritidis ATCC 49223	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43,0.43)
		34	20	14	0.70	(0.48,0.85)	14	0.70	(0.48,0.85)	0.00	(-0.27,0.27)
		343	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.43,0.43)
Ceramic	*Salmonella* Montevideo CIP[j] 104583	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43,0.43)
		373	20	15	0.75	(0.53,0.89)	15	0.75	(0.53,0.89)	0.00	(-0.26,0.26)
		3 700	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.

Table 5. Method Comparison Results: N-Light *Salmonella* Risk (Traditional Confirmation) vs. ISO 6579-1 (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 ATCC[h] 14028 & *C. koseri* ATCC 27028	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43,0.43)
		46 & 905	20	12	0.60	(0.39,0.78)	17	0.85	(0.64,0.95)	-0.25	(-0.48,0.03)
		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 ATCC 14028 & *C. koseri* ATCC 27156	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43,0.43)
		80 & 910	20	9	0.45	(0.26, 0.66)	8	0.40	(0.22, 0.61)	0.05	(-0.24,0.33)
		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* Enteritidis ATCC 49223	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43,0.43)
		34	20	14	0.70	(0.48,0.85)	15	0.75	(0.53,0.89)	-0.05	(-0.31,0.22)
		343	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.43,0.43)
Ceramic	*Salmonella* Montevideo CIP[j] 104583	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43,0.43)
		373	20	15	0.75	(0.53,0.89)	11	0.55	(0.34,0.74)	0.20	(-0.09,0.45)
		3 700	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.

DISCUSSION OF MODIFICATION APPROVED FEBRARY 2024 (3)

An inclusivity study showed that all *Salmonella* species and subspecies as well as more than 100 serovars of *Salmonella enterica* subsp. *enterica* are detected with the method, including serovars *S.* Typhimurium and *S.* Enteritidis. An exclusivity study showed that closely related non-*Salmonella* Gram negative species as well as numerous other bacterial species commonly occurring in food factory environments yield negative test results.

The NEMIS Technologies N-Light *Salmonella* Risk assay successfully detected *Salmonella* spp. on stainless steel environmental surfaces. When comparing results obtained from the BTL1 luminometer to the confirmation results, no false positives or false negatives were observed. Using POD analysis, no statistically significant differences were detected between the number of positive results detected by the reference method and the N-Light *Salmonella* Risk assay. Changes to the method introduced with the present level 3 modification did not change performance of the N-Light *Salmonella* Risk assay with respect to detection of *Salmonella* on stainless steel environmental surfaces compared to the previous Performance Tested Method (PTM) 072204.

The method allows the user to swab a stainless-steel environmental surface and obtain next-day results for the detection of *Salmonella*. The method is quick and simple to perform, with 10 seconds time to result for a sample. This short run time allows an analyst to process multiple samples in a short amount of time. The product manual is well written and simple enough that a technician at any level of training would be able to process samples and achieve accurate results. The BTL1 luminometer is also easy to use and displays the RLU value prominently. The instrument is compact, allowing for flexibility in process set up in any location.

Table 1: Inclusivity Panel Results (3)

No.	Species	Sub-species	Serovar	Antigenic formula/Serogroup	Source	Reference	Origin	Results
1	*S. bongori*	-	-	66:z41:-	DSM[a]	13772	Human	Positive
2	*S. bongori*	-	-	48:z35 : -	ZHAW[b]	N268-08	Environment	Positive
3	*S. bongori*	-	-	-	CCUG[c]	63587	Human feces	Positive
4	*S. enterica*	*arizonae*	-	56:z4,z23 : -	ZHAW	N09-0860	Human feces	Positive
5	*S. enterica*	*arizonae*	-	-	ZHAW	N19-181	Human feces	Positive
6	*S. enterica*	*arizonae*	-	IIIa 51:z4,z23:-	DSM	9386	Reference strain	Positive
7	*S. enterica*	*diarizonae*	-	61 : c : z35	ZHAW	N09-2338	Human feces	Positive
8	*S. enterica*	*diarizonae*	-	-	DSM	14847	Reference strain	Positive
9	*S. enterica*	*diarizonae*	-	S.III 50:5:1,5,7	Nexidia[d]	NEX-1902	Dehydrated food product	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*	-	-	DSM	9221	Reference strain	Positive
16	*S. enterica*	*indica*		VI 1,6,14,25:a:e,n,x	DSM	14848	Reference strain	Positive
17	*S. enterica*	*indica*	-	-	APHA[e]	L01098-19	-	Positive
18	*S. enterica*	*indica*	-	VI 16:z10:1,5	CIP[f]	359-82	Food	Positive
18	*S. enterica*	*indica*	-	VI 1,6 ,1 4: a : 1,5	CIP	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	CRBIP[g]	106895	Lizard	Positive
21	*S. enterica*	*salamae*	Tranoroa	II 55:k:z39	Nexidia	NEX-1258	Food	Positive
22	*S. enterica*	*salamae*	-	-	DSM	9220	Reference strain	Positive
23	*S. enterica*	*enterica*	Abaetetuba	F	Nexidia	NEX-1716	Dairy product	Positive
24	*S. enterica*	*enterica*	Adelaide	O	ZHAW	N19-976	Human feces	Positive
25	*S. enterica*	*enterica*	Agona	B	Nexidia	NEX-1639	Calf sweetbreads	Positive
26	*S. enterica*	*enterica*	Ajobo	-	ZHAW	N18-1510	-	Positive
27	*S. enterica*	*enterica*	Albany	C_3	ZHAW	N18-1907	Feed	Positive
28	*S. enterica*	*enterica*	Amsterdam	E_1	Nexidia	NEX-1767	Food isolate	Positive
29	*S. enterica*	*enterica*	Anatum	E_1	Nexidia	N1946-08	Rapeseed Sample	Positive
30	*S. enterica*	*enterica*	Aschersleben	N	Nexidia	NEX-1906	Food isolate	Positive
31	*S. enterica*	*enterica*	Bergen	X	Nexidia	NEX-1644	Food isolate	Positive
32	*S. enterica*	*enterica*	Berta	D_1	ZHAW	N19-2653	Human feces	Positive
33	*S. enterica*	*enterica*	Bijlmer	R	ZHAW	N15-2159	Human feces	Positive
34	*S. enterica*	*enterica*	Blockley	C_2	ZHAW	N18-1544	Human feces	Positive
35	*S. enterica*	*enterica*	Braenderup	C_1	Nexidia	NEX-1850	Milk	Positive
36	*S. enterica*	*enterica*	Brandenburg	B	Nexidia	NEX-1786	Cheese	Positive
37	*S. enterica*	*enterica*	Bredeney	B	Nexidia	NEX-1464	Food product	Positive
38	*S. enterica*	*enterica*	Caracas	H	Nexidia	NEX-1785	Food product	Positive
39	*S. enterica*	*enterica*	Carmel	J	ZHAW	N17-0762	Chicken	Positive
40	*S. enterica*	*enterica*	Cerro	K	ZHAW	N18-0422	-	Positive
41	*S. enterica*	*enterica*	Champaign	Q	Nexidia	NEX-1576	Food isolate	Positive
42	*S. enterica*	*enterica*	Chandans	F	Nexidia	NEX-1664	Food product	Positive
43	*S. enterica*	*enterica*	Chester	B	Nexidia	NEX-862	Duck Liver	Positive
44	*S. enterica*	*enterica*	Coeln	B	Nexidia	NEX-1106	Food isolate	Positive
45	*S. enterica*	*enterica*	Corvallis	C_2	ZHAW	N20-0386	Human feces	Positive
46	*S. enterica*	*enterica*	Cubana	G	Nexidia	NEX-1829	Food isolate	Positive

57	*S. enterica*	*enterica*	Derby	B	ZHAW	N2236-08	Human feces	Positive
58	*S. enterica*	*enterica*	Dublin	9:g,p:-	DSM	102345	Reference strain	Positive
59	*S. enterica*	*enterica*	Ealing	O	Nexidia	NEX-1667	Food isolate	Positive
60	*S. enterica*	*enterica*	Emek	C_3	ZHAW	N19-0283	Human feces	Positive
61	*S. enterica*	*enterica*	Enteritidis	D_1	ZHAW	N18-2081	Human feces	Positive
62	*S. enterica*	*enterica*	Enteritidis	D_1	Nexidia	NEX-1787	Cheese	Positive
63	*S. enterica*	*enterica*	Enteritidis	D_1	Nexidia	NEX-1794	Milk	Positive
64	*S. enterica*	*enterica*	Fluntern	-	ZHAW	N14-1026	Human feces	Positive
65	*S. enterica*	*enterica*	Fresno	D_2	ZHAW	N17-1724	Human feces	Positive
66	*S. enterica*	*enterica*	Gateshead	D_2	ZHAW	N19-1826	Human feces	Positive
67	*S. enterica*	*enterica*	Give	E	Nexidia	NEX-1609	Food isolate	Positive
68	*S. enterica*	*enterica*	Hadar	C_3	ZHAW	N10-0099	human feces	Positive
69	*S. enterica*	*enterica*	Havana	G	ZHAW	N16-2778	Human feces	Positive
70	*S. enterica*	*enterica*	Heidelberg	B	ZHAW	N2743-08	Human feces	Positive
71	*S. enterica*	*enterica*	Hofit	Q	ZHAW	N18-1113	Human feces	Positive
72	*S. enterica*	*enterica*	Hvittingfoss	I	Nexidia	NEX-1467	Food isolate	Positive
73	*S. enterica*	*enterica*	Indiana	B	ZHAW	N18-1741	Human feces	Positive
74	*S. enterica*	*enterica*	Infantis	C_1	ZHAW	N63-09	Human feces	Positive
75	*S. enterica*	*enterica*	Javiana	D_1	ZHAW	N2427-08	Human feces	Positive
76	*S. enterica*	*enterica*	Johannesburg	R	ZHAW	N17-1932	Human feces	Positive
78	*S. enterica*	*enterica*	Kasenyi	P	ZHAW	N20-0227	Food	Positive
79	*S. enterica*	*enterica*	Kedougou	G_2	ZHAW	N18-0040	Human feces	Positive
80	*S. enterica*	*enterica*	Kentucky	C_3	ZHAW	N54-09	Human feces	Positive
81	*S. enterica*	*enterica*	Korovi	P	ZHAW	N16-0899	Feed	Positive
82	*S. enterica*	*enterica*	Kottbus	C_2	ZHAW	N18-2064	Food isolate	Positive
83	*S. enterica*	*enterica*	Lagos	B	Nexidia	NEX-703	Meat	Positive
84	*S. enterica*	*enterica*	Lille	C_1	Nexidia	NEX-296	Food isolate	Positive
85	*S. enterica*	*enterica*	Litchfield	C_2	ZHAW	N18-1222	Human feces	Positive
86	*S. enterica*	*enterica*	Livingstone	C_1	ZHAW	N18-2015	Human feces	Positive
87	*S. enterica*	*enterica*	London	E_1	ZHAW	N18-2083	Human feces	Positive
88	*S. enterica*	*enterica*	Manchester	C_2	Nexidia	NEX-1658	Food isolate	Positive
89	*S. enterica*	*enterica*	Manhattan	C_3	ZHAW	N17-2990	Human feces	Positive
90	*S. enterica*	*enterica*	Mbandaka	C_1	ZHAW	N18-1863	Human feces	Positive
91	*S. enterica*	*enterica*	Meleagridis	E_1	Nexidia	NEX-742	Ground beef	Positive
92	*S. enterica*	*enterica*	Menston	C_1	ZHAW	N18-1184	Human feces	Positive
93	*S. enterica*	*enterica*	Minnesota	L	ZHAW	N20-2630	Food poultry	Positive
94	*S. enterica*	*enterica*	Mississippi	G	ZHAW	N19-1634	Human feces	Positive
95	*S. enterica*	*enterica*	Montevideo	C_1	ATCC	8387	Reference strain	Positive
96	*S. enterica*	*enterica*	Muenchen	C_2	Nexidia	NEX-1326	Food isolate	Positive
97	*S. enterica*	*enterica*	Muenster	E_1	ZHAW	N520-08	Human feces	Positive
98	*S. enterica*	*enterica*	Napoli	D_1	ZHAW	N18-2031	Human feces	Positive
99	*S. enterica*	*enterica*	Newport	C_2	ZHAW	N2932-08	Human feces	Positive
100	*S. enterica*	*enterica*	Nima	M	Nexidia	NEX-1881	Food isolate	Positive
101	*S. enterica*	*enterica*	Oranienburg	C_1	Nexidia	NEX-1725	Rapeseed	Positive
102	*S. enterica*	*enterica*	Orion	E_1	Nexidia	NEX-1776	Food isolate	Positive
103	*S. enterica*	*enterica*	Ouakam	D_2	Nexidia	NEX-837	Food isolate	Positive
104	*S. enterica*	*enterica*	Panama	D_1	Nexidia	NEX-740	Horse steak	Positive
105	*S. enterica*	*enterica*	Plymouth	D_2	ZHAW	N20-0792	Human feces	Positive
106	*S. enterica*	*enterica*	Poona	G	ZHAW	N19-29	Human feces	Positive
107	*S. enterica*	*enterica*	Ramatgan	N	Nexidia	NEX-311	Food isolate	Positive
108	*S. enterica*	*enterica*	Reading	B	Nexidia	NEX-919	Water	Positive
109	*S. enterica*	*enterica*	Regent	E_1	Nexidia	NEX-1555	Food isolate	Positive
110	*S. enterica*	*enterica*	Rissen	C_1	ZHAW	N18-2034	Human feces	Positive
111	*S. enterica*	*enterica*	Saintpaul	B	ZHAW	N18-1764	Human feces	Positive
112	*S. enterica*	*enterica*	Sandiego	B	ZHAW	N19-1171	Human feces	Positive
113	*S. enterica*	*enterica*	Schwarzengrund	B	Nexidia	NEX-1571	Food enrichment	Positive
114	*S. enterica*	*enterica*	Senftenberg	E_4	DSM	10062	Reference strain	Positive
115	*S. enterica*	*enterica*	Stanley	-	ZHAW	N29-07	Human feces	Positive
116	*S. enterica*	*enterica*	Stuivenberg	E_4	Nexidia	NEX-702	Meat	Positive
117	*S. enterica*	*enterica*	Tennessee	C_1	ZHAW	N18-2117	Human feces	Positive
118	*S. enterica*	*enterica*	Thompson	C_1	Nexidia	NEX-1569	Food product	Positive
119	*S. enterica*	*enterica*	Typhimurium	B	Nexidia	NEX-1640	Fish meal	Positive
120	*S. enterica*	*enterica*	Typhimurium	B	ATCC[h]	14028	Clinical	Positive
121	*S. enterica*	*enterica*	Typhimurium	B	Nexidia	NEX-1742	Cheese	Positive
122	*S. enterica*	*enterica*	Veneziana	F	ZHAW	N18-2078	Human feces	Positive

123	*S. enterica*	*enterica*	Virchow	C_1	ZHAW	N61-09	Human feces	Positive
124	*S. enterica*	*enterica*	Virginia	C_3	ZHAW	N18-1861	Human feces	Positive
125	*S. enterica*	*enterica*	Wandsworth	Q	ZHAW	N13-0958	Human feces	Positive
126	*S. enterica*	*enterica*	Worthington	G	Nexidia	NEX-1880	Food isolate	Positive

[a] DSM = DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany
[b] ZHAW = Microbial strain collection of the Laboratory for Food Microbiology, University of Applied Sciences Zurich (ZHAW), Switzerland
[c] CCUG = Culture Collection University of Gothenburg, Gothenburg, Sweden
[d] Nexidia = Microbial strain collection Nexidia SAS, Dijon, France
[e] APHA = Microbial strain collection of the Animal and Plant Health Agency, Surrey, United Kingdom
[f] CIP = Collection de l'Institut Pasteur, Paris, 303 France
[g] CRBIP = Biological Resource Center of Institute Pasteur, Paris, France
[h] ATCC = American Type Culture Collection, Manassas, VA, USA

Table 2: Exclusivity Panel Results (3)

No.	Genus	Species	Reference	Origin	Non-selective broth
1	*Aeromonas*	*hydrophila*	ZHAW[a] HER 1209	Sewage effluent	Negative
2	*Aeromonas*	*media*	NEMIS[b] LU-SM 36C	Egg factory	Negative
3	*Bacillus*	*licheniformis*	NEMIS BC-LM 29.12	Chocolate factory	Negative
4	*Citrobacter*	*braakii*	ATCC[c] 51113	Snake	Negative
5	*Citrobacter*	*freundii*	Nexidia[d] NEX-1694	Food isolate	Negative
6	*Citrobacter*	*koseri*	ATCC 27028	Blood culture	Negative
7	*Cronobacter*	*dublinensis*	DSM[e] 18797	Mild powder	Negative
8	*Enterobacter*	*absuriae*	ZHAW 2449	Drinking water	Negative
9	*Enterobacter*	*cloaceae*	DSM 16657	Maize plant	Negative
10	*Enterococcus*	*faecalis*	ATCC 51299	Peritoneal fluid, St. Louis, MO	Negative
11	*Escherichia*	*alvei*	NEMIS LU-SM 129A	Egg factory	Negative
12	*Escherichia*	*coli*	CIP[f] 54.117	Human, feces	Negative
13	*Escherichia*	*fergusoni*	NEMIS LS-SM49A	Egg factory	Negative
14	*Escherichia*	*vulneris*	NEMIS BC-SM87	Chocolate factory	Negative
15	*Klebsiella*	*oxytoca*	ATCC 43863	Unknown	Negative
16	*Lactobacillus*	*lactis*	NEMIS Mi-LM-17.1	Meat factory	Negative
17	*Listeria*	*monocytogenes*	ATCC 19114	Tissue, animal	Negative
18	*Pantoea*	*agglomerans*	ZHAW SA-ME07	Celery	Negative
19	*Proteus*	*mirabilis*	ATCC 29906	Unknown	Negative
20	*Proteus*	*vulgaris*	NEMIS MH-LM 54.2	Cheese smear water	Negative
21	*Providencia*	*vermicola*	NEMIS LU-SM 74E	Egg factory	Negative
22	*Pseudomonas*	*aeruginosa*	NEMIS MI-LM 16.1	Meat factory	Negative
23	*Pseudomonas*	*fluorescens*	NEMIS MI-LM 1.2	Meat factory	Negative
24	*Rahnella*	*aquatilis*	ZHAW SA-ME02	Carrot	Negative
25	*Serratia*	*marcescens*	DSM 30122	Pasteurized milk	Negative
26	*Serratia*	*ureilytica*	NEMIS LU-SM 37A	Egg factory	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*	NEMIS MA-LM 3.2	Meat factory	Negative
31	*Streptococcus*	*parauberis*	NEMIS DY-LM 140.3	Salmon factory	Negative
32	*Yersinia*	*enterocolitica*	ATCC 23715	Human blood	Negative

[a] ZHAW = Microbial strain collection of the Laboratory for Food Microbiology, University of Applied Sciences Zurich (ZHAW), Switzerland.
[b] NEMIS =Microbial strain collection NEMIS Technologies AG, Au (ZH), Switzerland.
[c] ATCC = American Type Culture Collection, Manassas, VA, USA.
[d] Nexidia = Microbial strain collection Nexidia SAS, Dijon, France.
[e] DSM = DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany.
[f] CIP = Collection de l'Institut Pasteur, Paris, 303 France.
[g] RKI = Microbial strain collection of the Robert Koch Institute, Berlin, Germany.

Table 3. NEMIS N-Light *Salmonella* Risk (candidate method) vs. ISO 6579-1:2017 (reference method) – POD Results (3)

Matrix	Strain	cfu[a]/Test area	N[b]	x[c]	Candidate PODc[d]	95% CI	X	Reference PODR[e]	95% CI	dPODc[f]	95% CI[g]
Stainless steel (1" x 1")	*S.* Typhimurium ATCC[h] 14028	N/A[i]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		90 & 1100	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.050	-0.24, 0.33
	& *C. koseri* ATCC 27156	500 & 6100	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 each matrix in triplicate.
[b]N = Number of test portions.
[c]x = Number of positive test portions.
[d]PODC = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.
[e]PODR = Reference method positive outcomes divided by the total number of trials.
[f]dPODC= Difference between the confirmed candidate method result and reference 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]N/A = Not applicable.

Table 4. NEMIS N-Light *Salmonella* Risk (candidate method) presumptive vs. confirmed per ISO 6579-1:2017 procedure – POD Results (3)

Matrix	Strain	cfu[a]/ Test area	N[b]	x[c]	Candidate presumptive POD_{CP}[d]	95% CI	X	Candidate confirmed POD_{CC}[e]	95% CI	$dPOD_{CP}$[f]	95% CI[g]
Stainless steel (1" x 1")	*S.* Typhimurium ATCC[h] 14028	N/A[i]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	&	90 & 1100	20	9	0.45	0.29, 0.66	9	0.45	0.29, 0.66	0.00	-0.13, 0.13
	C. koseri ATCC 27156	500 & 6100	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

[a]cfu/Test area = Results of the cfu/Test area were determined by plating the inoculum for each matrix in triplicate.
[b]N = Number of test portions.
[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 candidate method presumptive and 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]N/A = Not applicable.

Table 5. NEMIS N-Light *Salmonella* Risk (candidate method) presumptive vs. confirmed per alternative N-Light *Salmonella* procedure – POD Results (3)

Matrix	Strain	cfu[a]/ Test area	N[b]	x[c]	Candidate presumptive POD_{CP}[d]	95% CI	X	Candidate confirmed POD_{CC}[e]	95% CI	$dPOD_{CP}$[f]	95% CI[g]
Stainless Steel (1" x 1")	*S.* Typhimurium ATCC[h] 14028	N/A[i]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
	&	90 & 1100	9	9	0.45	0.29, 0.66	9	0.45	0.29, 0.66	0.00	-0.13, 0.13
	C. koseri ATCC 27156	500 & 6100	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

[a]cfu/Test area = Results of the cfu/test area were determined by plating the inoculum for each matrix in triplicate.
[b]N = Number of test portions.
[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 confirmed 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]N/A = Not applicable.

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