



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
122002

The AOAC Research Institute hereby certifies the method known as:

N-LightTM *Listeria monocytogenes*

manufactured by
NEMIS Technologies AG
Riedhofstrasse 11
CH - 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, appearing to read "Bradley A. Stawick".

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

Issue Date
Expiration Date

February 16, 2024
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AUTHORS

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SUBMITTING COMPANY

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

METHOD NAME

N-Light™ *Listeria monocytogenes*

CATALOG NUMBERS

00009 (50 tests), 00010 (Distributor Box)
 Retired: 00005 (48 tests)

INDEPENDENT LABORATORY

Q Laboratories
 1930 Radcliff Drive
 Cincinnati, OH 45204 USA

CONTRACT LABORATORY

MODIFICATION JANUARY 2023
 Nexidia Laboratories
 15 Rue de Mayence
 21000 Dijon, France

APPLICABILITY OF METHOD

Analytes – *Listeria monocytogenes*.

Matrixes – Environmental Surface Swabs (1" x 1"): Stainless steel [AISI 304 (1.4301), grade 2b finish], plastic (polystyrene), and Ceramic (glazed earthen)

Performance claims – No significant statistical difference was detected between the N-Light *Listeria monocytogenes* assay, using the NEMIS Technologies BTL1 luminometer, and the ISO 11290-1:2017: Microbiology of the Food Chain – Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. – Part 1 (2) for detection of *L. monocytogenes* on stainless steel environmental surfaces.

ORIGINAL CERTIFICATION DATE

December 07, 2020

CERTIFICATION RENEWAL RECORD

Renewed annually through December 2024.

METHOD MODIFICATION RECORD

1. December 2021 Level 1
2. January 2023 Level 2

3. February 2024 Level 3

SUMMARY OF MODIFICATION

1. Editorial and formatting changes updated catalog numbers.
2. Modification changes include:
 - a. Changes to the AquaSpark® Tablet excipients for lower background and improved signal,
 - b. Option to use swabs with a breaking point that can be left in the enrichment tube during incubation,
 - c. Reduction in reading time of luminescence signal after tablet release reduced to 5 minutes.
3. Modification changes include:
 - a. New proprietary premoistening buffer,
 - b. Enrichment broth was optimized in terms of phage cocktail and nutrient composition.

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this method is distributed by:

NONE

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this method is distributed as:

NONE

PRINCIPLE OF THE METHOD (1)

NEMIS Technologies N-light™ *L. monocytogenes* (L. mono) assay utilizes synthetic molecules (AquaSpark™) that are designed to detect live bacteria through chemiluminescence. The mechanism of detection is the hydrolysis of the proprietary AquaSpark molecule by *L. monocytogenes* specific enzymatic cleavage (PI-PLC). This leads to the release of a dioxetane derivate which in turn leads to the emission of green light. The presence of *L. monocytogenes* is determined by the relative light unit (RLU) reading for the sample. If the sample eclipses the RLU threshold, which is set to 20,000 RLU, then the sample is deemed positive for the presence of *L. monocytogenes*. Unlike molecular methods which require a DNA purification or lysis process, the chemiluminescence detection has a simple, streamlined sampling protocol. After swabbing and incubating the enrichment for 24 h (dry bath incubator), the end user only has to dispense the AquaSpark tablet, wait 10 minutes and insert the tube containing the enrichment into the NEMIS Luminometer for detection in 10 seconds. NEMIS uses its own proprietary enrichment broth containing phage selectives which specifically reduces the background flora in particular *Enterococci*, *Staphylococci* and non-*monocytogenes Listeria* spp.

DISCUSSION OF THE ORIGINAL VALIDATION STUDY (1)

The N-Light L. mono kit was able to detect all 52 of the *L. monocytogenes* strains, representing 12 different serotypes, tested during the inclusivity study. Moreover, the N-light L. mono kit did not detect any of the 30 non-*Listeria monocytogenes* in the exclusivity strain set, which included other *Listeria* spp. The specificity of the kit was therefore validated according to the exclusivity study.

Concerning the matrix study, on stainless steel without competitive flora, the N-light L. mono method able to detect significantly more positive sample than the reference method for the low inoculation, for stainless steel and ceramics (see Table 5). For plastics on the other hand, significantly more positives were detected with the reference method. When stainless steel surface was co-inoculated with *L. monocytogenes* and *E. faecalis*, significantly more positives were detected using the N-light method compared to the reference method. It must be noted that environmental swabbing on surfaces is subject to high variation in between experimental days, therefore results need to be treated with the respective caution.

Table 1: Inclusivity Panel Results (1)

#	Organism	Serotype	Source ^a	Strain	Origin	Results
1	<i>Listeria monocytogenes</i>	1/2a	ATCC	15313	Rabbit	Positive
2	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-636	Cheese	Positive
3	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-718	Food isolate	Positive
4	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-726	Food isolate	Positive
5	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-772	Cheese	Positive
6	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-773	Cheese	Positive
7	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-789	Cheese	Positive
8	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-865	Food isolate	Positive
9	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-866	Food isolate	Positive
10	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-939	Food isolate	Positive
11	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-952	Food isolate	Positive
12	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-1492	Food isolate	Positive
13	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-1494	Food isolate	Positive
14	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-1495	Food isolate	Positive
15	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-1497	Food isolate	Positive
16	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-1498	Food isolate	Positive
17	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-1506	Food isolate	Positive
18	<i>Listeria monocytogenes</i>	1/2b	CIP	78.32 (= 105449)	Chinchilla	Positive
19	<i>Listeria monocytogenes</i>	1/2b	CIP	78.33 (= 105448)	Cerebrospinal fluid	Positive
20	<i>Listeria monocytogenes</i>	1/2b	Nexidia	NEX-626	Food isolate	Positive
21	<i>Listeria monocytogenes</i>	1/2b	Nexidia	NEX-648	Food isolate	Positive
22	<i>Listeria monocytogenes</i>	1/2b	Nexidia	NEX-720	Cheese	Positive
23	<i>Listeria monocytogenes</i>	1/2b	Nexidia	NEX-841	Food isolate	Positive
24	<i>Listeria monocytogenes</i>	1/2b	Nexidia	NEX-851	Food isolate	Positive
25	<i>Listeria monocytogenes</i>	1/2b	Nexidia	NEX-1493	Food isolate	Positive
26	<i>Listeria monocytogenes</i>	1/2b	Nexidia	NEX-870	Food isolate	Positive
27	<i>Listeria monocytogenes</i>	1/2b	Nexidia	BAA-751	-	Positive
28	<i>Listeria monocytogenes</i>	1/2c	Nexidia	NEX-777	Food isolate	Positive
29	<i>Listeria monocytogenes</i>	1/2c	Nexidia	NEX-869	Food isolate	Positive
30	<i>Listeria monocytogenes</i>	1/2c	Nexidia	NEX-868	Food isolate	Positive
31	<i>Listeria monocytogenes</i>	1/2c	ATCC	7644	Human	Positive
32	<i>Listeria monocytogenes</i>	3a	CIP	78.34	Human	Positive
33	<i>Listeria monocytogenes</i>	3a	Nexidia	NEX-627	Food isolate	Positive
34	<i>Listeria monocytogenes</i>	3a	Nexidia	NEX-1496	Food isolate	Positive
35	<i>Listeria monocytogenes</i>	3a	Nexidia	NEX-1505	Food isolate	Positive
36	<i>Listeria monocytogenes</i>	3b	CIP	78.35	Spinal fluid	Positive
37	<i>Listeria monocytogenes</i>	3b	CRBIP	13.109	Environment	Positive
38	<i>Listeria monocytogenes</i>	3c	CIP	78.36	-	Positive
39	<i>Listeria monocytogenes</i>	3c	CRBIP	13.36	Meat product	Positive
40	<i>Listeria monocytogenes</i>	4a	NCTC	5214	Brain sheep	Positive
41	<i>Listeria monocytogenes</i>	4b	CIP	78.39	Chicken	Positive
42	<i>Listeria monocytogenes</i>	4b	CRBIP	13.107	Environment	Positive
43	<i>Listeria monocytogenes</i>	4b	Nexidia	NEX-642	Cheese	Positive
44	<i>Listeria monocytogenes</i>	4b	Nexidia	NEX-754	Food isolate	Positive
45	<i>Listeria monocytogenes</i>	4b	Nexidia	NEX-929	Food isolate	Positive
46	<i>Listeria monocytogenes</i>	4b	Nexidia	NEX-1504	Food isolate	Positive
47	<i>Listeria monocytogenes</i>	4c	ATCC	19116	Chicken	Positive
48	<i>Listeria monocytogenes</i>	4d	ATCC	19118	Sheep	Positive
49	<i>Listeria monocytogenes</i>	4d	NCTC	10888	Sheep	Positive
50	<i>Listeria monocytogenes</i>	4e	CIP	78.41	Chicken	Positive
51	<i>Listeria monocytogenes</i>	7	Nexidia	NEX-843	Food isolate	Positive
52	<i>Listeria monocytogenes</i>	7	CIP	78.43	-	Positive

^aATCC: American Type Culture Collection, Manassas, VA, USA. CIP: Collection de l'Institut Pasteur, Paris, France. CRBIP: Biological Resource Center of institute Pasteur, Paris, France. NCTC: National Collection of Type Cultures, Salisbury, UK. NEXIDIA: Microbial Strain Collection, Dijon, France.

Table 2: Exclusivity Panel Results (1)

#	Organism	Serotype	Source ^a	Strain	Origin	Results
1	<i>Listeria innocua</i>	-	ATCC	33090	Cow brain	Negative
2	<i>Listeria innocua</i>	-	Nexidia	NEX-1849	Milk	Negative
3	<i>Listeria ivanovii</i>	-	ATCC	BAA-139	Washing water	Negative
4	<i>Listeria ivanovii</i>	-	Nexidia	NEX-923	Duck breast	Negative
5	<i>Listeria grayi</i>	-	CIP	103321	Food	Negative
6	<i>Listeria seeligeri</i>	-	CIP	100100	Soil	Negative
7	<i>Listeria seeligeri</i>	-	Nexidia	NEX-1848	Milk	Negative
8	<i>Listeria welshimerii</i>	-	CIP	81.49	Decaying vegetation	Negative
9	<i>Lactococcus lactis</i>	-	ATCC	11454	-	Negative
10	<i>Lactococcus lactis</i>	-	Nexidia	NEX-363	-	Negative
11	<i>Streptococcus thermophilus</i>	-	Nexidia	NEX-371	Food isolate	Negative
12	<i>Lactobacillus plantarum</i>	-	ATCC	14917	Pickled cabbage	Negative
13	<i>Lactobacillus plantarum</i>	-	Nexidia	NEX-EC-EnrE8	Surface	Negative
14	<i>Lactobacillus buchneri</i>	-	Nexidia	NEX-EC-E8	Surface	Negative
15	<i>Klebsiella pneumoniae</i>	-	ATCC	4352	Dairy products: cow's milk	Negative
16	<i>Enterobacter amnigenus</i>	-	Nexidia	NEX-764	Cocoa	Negative
17	<i>Cronobacter sakazakii</i>	-	ATCC	12868	-	Negative
18	<i>Escherichia coli</i>	-	ATCC	8739	Faeces	Negative
19	<i>Citrobacter braakii</i>	-	ATCC	51113	Snake	Negative
20	<i>Pantoea agglomerans</i>	-	CIP	82.100	Corn cobs	Negative
21	<i>Salmonella Typhimurium</i>	-	ATCC	14028	Chicken	Negative
22	<i>Pseudomonas aeruginosa</i>	-	ATCC	15442	Animal room water bottle	Negative
23	<i>Enterococcus avium</i>	-	ATCC	14025	-	Negative
24	<i>Enterococcus faecalis</i>	-	ATCC	51299	Peritoneal fluid	Negative
25	<i>Enterococcus hirae</i>	-	ATCC	10541	-	Negative
26	<i>Staphylococcus aureus</i>	-	Nexidia	NEX-1093	Raw milk cheese	Negative
27	<i>Staphylococcus aureus</i>	-	Nexidia	NEX-1069	Mayonnaise	Negative
28	<i>Bacillus cereus</i>	-	CIP	105151	-	Negative
29	<i>Bacillus subtilis</i>	-	ATCC	6051	-	Negative
30	<i>Clostridium perfringens</i>	-	ATCC	13124	-	Negative

^aATCC: American Type Culture Collection, Manassas, VA, USA. CIP: Collection de l'Institut Pasteur, Paris, France. CRBIP: Biological Resource Center of institute Pasteur, Paris, France. NCTC: National Collection of Type Cultures, Salisbury, UK. NEXIDIA: Microbial Strain Collection, Dijon, France.

Table 4: NEMIS Technologies N-light *L. mono* Assay: Presumptive vs. Confirmed (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	<i>Listeria monocytogenes</i> ATCC ^h BAA-751 (1/2b)	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43,0.43)
		72	20	19	0.95	(0.76,0.99)	19	0.95	(0.76,0.99)	0.00	(-0.13,0.13)
		2 200	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.43,0.43)
Stainless steel ⁱ	<i>Listeria monocytogenes</i> ATCC BAA-751 & <i>Enterococcus faecalis</i> ATCC 29212	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47,0.47)
		73 & 810	20	8	0.40	(0.22, 0.61)	8	0.40	(0.22, 0.61)	0.00	(-0.13,0.13)
		220 & 2400	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.47,0.47)
Plastic	<i>Listeria monocytogenes</i> ATCC 7644 (1/2c)	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43,0.43)
		115	20	11	0.55	(0.34,0.74)	11	0.55	(0.34,0.74)	0.00	(-0.13,0.13)
		10 000	5	4	0.8	(0.38,0.96)	4	0.80	(0.38,0.96)	0.00	(-0.45,0.45)
Ceramic	<i>Listeria monocytogenes</i> ATCC 19118 (4d)	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43,0.43)
		210	20	16	0.8	(0.58,0.92)	16	0.8	(0.58,0.92)	0.00	(-0.13,0.13)
		22 000	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.43,0.43)

^aCFU/Test area determined by plating the inoculum in duplicate (shown as average)

^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

ⁱ Performed by independent AOAC certified laboratory Q Laboratories, Cincinnati, OH, USA

Table 5: Method Comparison Results: NEMIS Technologies *L. mono* Assay vs. ISO 11290-1 (1)

Matrix	Strain	CFU/Test Area ^a	N ^b	Candidate method results (confirmed)			ISO 11290-1 results (confirmed)			dPOD ^c	95% CI ^g
				x ^c	POD ^c ^d	95% CI	x	POD ^R ^e	95% CI		
Stainless steel	<i>Listeria monocytogenes</i>	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47,0.47)
	ATCC ^h BAA-751	72	20	19	0.95	(0.76,0.99)	15	0.75	(0.53,0.89)	0.20	(-0.03, 0.42)
	(1/2b)	2 200	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.47,0.47)
Stainless steel ⁱ	<i>Listeria monocytogenes</i>	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43, 0.43)
	ATCC BAA-751 & <i>Enterococcus faecalis</i>	73 & 810	20	8	0.4	(0.22, 0.61)	6	0.30	(0.15, 0.52)	0.10	(-0.18, 0.36)
	ATCC 29212	220 & 2400	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.43, 0.43)
Plastic	<i>Listeria monocytogenes</i>	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47,0.47)
	ATCC 7644 (1/2c)	115	20	11	0.55	(0.34,0.74)	14	0.70	(0.48,0.85)	-0.15	(-0.41,0.14)
		10 000	5	4	0.80	(0.38,0.96)	5	1.00	(0.57,1.00)	-0.20	(-0.62,0.28)
Ceramic	<i>Listeria monocytogenes</i>	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47,0.47)
	ATCC 19118 (4d)	210	20	16	0.80	(0.58,0.92)	12	0.60	(0.39,0.78)	0.20	(-0.08,0.44)
		22 000	5	5	1.00	(0.57,1.00)	4	0.8	(0.38,0.96)	0.20	(-0.28,0.62)

^aCFU/Test area determined by plating the inoculum in triplicate (shown as average)

^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.

^h ATCC = American Type Culture Collection, Manassas, VA, USA

ⁱ Performed by independent AOAC certified laboratory Q Laboratories, Cincinnati, OH, USA

DISCUSSION OF MODIFICATION APPROVED JANUARY 2023 (3)

Since the original validation (Certificate No. 122002 – December 11th 2020), the N-Light *Listeria monocytogenes* method for the detection of *L. monocytogenes* was modified to improve the detection on surfaces. The N-light *L. monocytogenes* kit was able to detect all the *L. monocytogenes* strains, covering serotypes 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 4e and 7, during the inclusivity study. Moreover, the N-Light *L. monocytogenes* kit did not detect any of the exclusivity strains, which included non-*monocytogenes* *Listeria* species in the exclusivity strain set.

In the matrix study on stainless steel with competitor micro-organism (*E. faecalis*), the N-Light *L. monocytogenes* method detected significantly more positive test portions in fractional results compared to the ISO 11290-1.

During the robustness study, changes to incubation time, time of incubation between tablet release and measurement, and method for swab agitation did not show any statistical differences in terms of assay performance. However, the time of incubation between tablet release and signal measurement may affect the signal values and will be underlined in the method kit instructions.

The accelerated stability study demonstrated that shelf-life exposure does not change the POD in a statistically significant manner for the detection of positive signals from *L. monocytogenes*. However, after 24 days of storage at 37°C, a significant increase of the signal was observed for the competitive micro-organism. Therefore, a shelf life of longer than 6 months will need to be proven by real time measurements at the recommended storage temperature (2-8°C).

Table 1: Inclusivity Panel Results (3)

No.	Organism	Serotype	Source ^a	Strain	Origin	Results
1	<i>Listeria monocytogenes</i>	1/2a	ATCC	15313	Rabbit	Positive
2	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-636	Cheese	Positive
3	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-772	Cheese	Positive
4	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-773	Cheese	Positive
5	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-789	Cheese	Positive
6	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-866	Food isolate	Positive
7	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-952	Food isolate	Positive
8	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-1495	Food isolate	Positive
9	<i>Listeria monocytogenes</i>	1/2b	CIP	78.32 (= 105449)	Chinchilla	Positive
10	<i>Listeria monocytogenes</i>	1/2b	CIP	78.33 (= 105448)	Cerebrospinal fluid	Positive
11	<i>Listeria monocytogenes</i>	1/2b	Nexidia	NEX-648	Food isolate	Positive
12	<i>Listeria monocytogenes</i>	1/2b	Nexidia	NEX-720	Cheese	Positive
13	<i>Listeria monocytogenes</i>	1/2b	Nexidia	NEX-1493	Food isolate	Positive
14	<i>Listeria monocytogenes</i>	1/2c	Nexidia	NEX-869	Food isolate	Positive
15	<i>Listeria monocytogenes</i>	1/2c	ATCC	7644	Human	Positive
16	<i>Listeria monocytogenes</i>	3a	CIP	78.34	Human	Positive
17	<i>Listeria monocytogenes</i>	3b	CIP	78.35	Spinal fluid	Positive
18	<i>Listeria monocytogenes</i>	3b	CRBIP	13.109	Environment	Positive
19	<i>Listeria monocytogenes</i>	3c	CRBIP	13.36	Meat product	Positive
20	<i>Listeria monocytogenes</i>	4a	NCTC	5214	Brain sheep	Positive
21	<i>Listeria monocytogenes</i>	4b	CIP	78.39	Chicken	Positive

22	<i>Listeria monocytogenes</i>	4b	CRBIP	13.107	Environment	Positive
23	<i>Listeria monocytogenes</i>	4b	Nexidia	NEX-642	Cheese	Positive
24	<i>Listeria monocytogenes</i>	4c	ATCC	19116	Chicken	Positive
25	<i>Listeria monocytogenes</i>	4d	ATCC	19118	Sheep	Positive
26	<i>Listeria monocytogenes</i>	4e	CIP	78.41	Chicken	Positive
27	<i>Listeria monocytogenes</i>	7	Nexidia	NEX-843	Food isolate	Positive

^aAmerican Type Culture Collection (ATCC), Manassas, VA, USA; Collection de l'Institut Pasteur (CIP), Paris, France; National Collection of Type Cultures (NCTC), Salisbury, United Kingdom; Nexidia Microbial Strain Collection (NEXIDIA), Dijon, France.

Table 2: Exclusivity Panel Results (3)

No.	Organism	Source ^a	Strain	Origin	Results
1	<i>Listeria innocua</i>	ATCC	33090	Cow brain	Negative
2	<i>Listeria innocua</i>	Nexidia	NEX-1849	Milk	Negative
3	<i>Listeria ivanovii</i>	ATCC	BAA-139	Washing water	Negative
4	<i>Listeria ivanovii</i>	Nexidia	NEX-923	Duck breast	Negative
5	<i>Listeria grayi</i>	CIP	103321	Food	Negative
6	<i>Listeria seeligeri</i>	CIP	100100	Soil	Negative
7	<i>Listeria seeligeri</i>	Nexidia	NEX-1848	Milk	Negative
8	<i>Listeria welshimerii</i>	CIP	81.49	Decaying vegetation	Negative
9	<i>Klebsiella pneumoniae</i>	ATCC	4352	Dairy products: cow's milk	Negative
10	<i>Enterobacter amnigenus</i>	Nexidia	NEX-764	Cocoa	Negative
11	<i>Escherichia coli</i>	ATCC	8739	Feces	Negative
12	<i>Citrobacter braakii</i>	ATCC	51113	Snake	Negative
13	<i>Pantoea agglomerans</i>	CIP	82.100	Corn crops	Negative
14	<i>Salmonella Typhimurium</i>	ATCC	14028	Chicken	Negative
15	<i>Enterococcus faecalis</i>	ATCC	51299	Peritoneal fluid	Negative

^aAmerican Type Culture Collection (ATCC), Manassas, VA, USA; Nexidia Microbial Strain Collection (NEXIDIA), Dijon, France; Collection de l'Institut Pasteur (CIP), Paris, France.

Table 3. N-Light *Listeria monocytogenes*: Presumptive vs. Confirmed (with enrichments in Fraser-Demi and Fraser) (3)

Matrix	Strain	cfu/Test area ^a	Candidate method presumptive				Candidate method confirmed				
			N ^b	x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI	dPOD _{CP} ^f	95% CI ^g
Stainless steel	<i>L. monocytogenes</i>	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47,0.47)
	ATCC BAA-751 & <i>E. faecalis</i>	17 & 637	20	15	0.75	(0.53,0.89)	16	0.80	(0.58,0.92)	-0.05	(-0.21,0.11)
	ATCC 29212	140 & 8 630	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.47,0.47)

^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.

^h ATCC = American Type Culture Collection, Manassas, VA, USA.

Table 4. N-Light *Listeria monocytogenes*: Presumptive vs. Alternative Confirmed (Direct Streaking onto Agar Plates) (3)

Matrix	Strain	cfu/Test area ^a	Candidate method presumptive				Candidate method confirmed				
			N ^b	x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI	dPOD _{CP} ^f	95% CI ^g
Stainless steel	<i>L. monocytogenes</i>	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47,0.47)
	ATCC BAA-751 & <i>E. faecalis</i>	17 & 637	20	15	0.75	(0.53,0.89)	16	0.80	(0.58,0.92)	-0.05	(-0.21,0.11)
	ATCC 29212	140 & 8 630	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.47,0.47)

^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.

Table 5. Method Comparison Results: N-Light *Listeria monocytogenes* (Traditional Confirmation) vs. ISO 11290-1:2017 (3)

Matrix	Strain	cfu/Test		Candidate Method Confirmed			ISO 11290-1:2017			dPOD _{CP} ^f	95% CI ^g
		Area ^a	N ^b	x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI		
Stainless steel	<i>L. monocytogenes</i>	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43,0.43)
	ATCC ^h BAA-751	17 & 637	20	15	0.75	(0.53,0.89)	6	0.30	(0.15,0.52)	0.45	(0.14,0.66)
	<i>E. faecalis</i> ATCC 29212	140 & 8 630	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.43,0.43)

^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.

DISCUSSION OF MODIFICATION APPROVED FEBRUARY 2024 (4)

An inclusivity/exclusivity study showed that all major serotypes of *L. monocytogenes* are detected with the method while non-*L. monocytogenes* *Listeria* species and numerous other bacterial species commonly occurring in food factory environments yield negative test results.

The NEMIS Technologies N-Light *Listeria monocytogenes* assay successfully detected *L. monocytogenes* 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 observed between the number of positive samples detected by the reference method and the N-Light *Listeria monocytogenes* assay. The method allows the user to swab a surface and obtain next-day results for the presence of *L. monocytogenes*.

The method is quick and easy to perform with the instrument, only taking 10 seconds to provide a 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 follow it and achieve accurate results. The BTL1 luminometer is also easy to use and displays the RLU value prominently. The instrument is very small and takes up little bench space, allowing for flexibility in process set up.

Table 1: Inclusivity Panel Results (4)

No.	Organism	Serotype	Source	Strain	Origin	Results
1	<i>Listeria monocytogenes</i>	1/2a	ATCC ^a	15313	Rabbit	Positive
2	<i>Listeria monocytogenes</i>	1/2a	Nexidia ^b	NEX-YAe	Cheese	Positive
3	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-718	Food isolate	Positive
4	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-726	Food isolate	Positive
5	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-772	Cheese	Positive
6	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-773	Cheese	Positive
7	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-789	Cheese	Positive
8	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-865	Food isolate	Positive
9	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-866	Food isolate	Positive
10	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-939	Food isolate	Positive
11	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-952	Food isolate	Positive
12	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-1492	Food isolate	Positive
13	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-1494	Food isolate	Positive
14	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-1495	Food isolate	Positive
15	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-1497	Food isolate	Positive
16	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-1498	Food isolate	Positive
17	<i>Listeria monocytogenes</i>	1/2a	Nexidia	NEX-1506	Food isolate	Positive
18	<i>Listeria monocytogenes</i>	1/2b	CIP ^c	78.32 (= 105449)	Chinchilla	Positive
19	<i>Listeria monocytogenes</i>	1/2b	CIP	78.33 (= 105448)	Cerebrospinal fluid	Positive
20	<i>Listeria monocytogenes</i>	1/2b	Nexidia	NEX-626	Food isolate	Positive
21	<i>Listeria monocytogenes</i>	1/2b	Nexidia	NEX-648	Food isolate	Positive
22	<i>Listeria monocytogenes</i>	1/2b	Nexidia	NEX-720	Cheese	Positive
23	<i>Listeria monocytogenes</i>	1/2b	Nexidia	NEX-841	Food isolate	Positive
24	<i>Listeria monocytogenes</i>	1/2b	Nexidia	NEX-851	Food isolate	Positive
25	<i>Listeria monocytogenes</i>	1/2b	Nexidia	NEX-1493	Food isolate	Positive
26	<i>Listeria monocytogenes</i>	1/2b	Nexidia	NEX-870	Food isolate	Positive
27	<i>Listeria monocytogenes</i>	1/2b	Nexidia	BAA-751	Not available	Positive
28	<i>Listeria monocytogenes</i>	1/2c	Nexidia	NEX-777	Food isolate	Positive
29	<i>Listeria monocytogenes</i>	1/2c	Nexidia	NEX-869	Food isolate	Positive
30	<i>Listeria monocytogenes</i>	1/2c	Nexidia	NEX-868	Food isolate	Positive
31	<i>Listeria monocytogenes</i>	1/2c	ATCC	7644	Human	Positive
32	<i>Listeria monocytogenes</i>	3a	CIP	78.34	Human	Positive
33	<i>Listeria monocytogenes</i>	3a	Nexidia	NEX-627	Food isolate	Positive
34	<i>Listeria monocytogenes</i>	3a	Nexidia	NEX-1496	Food isolate	Positive
35	<i>Listeria monocytogenes</i>	3a	Nexidia	NEX-1505	Food isolate	Positive
36	<i>Listeria monocytogenes</i>	3b	CIP	78.35	Spinal fluid	Positive

37	<i>Listeria monocytogenes</i>	3b	CRBIP ^d	13.109	Environment	Positive
38	<i>Listeria monocytogenes</i>	3c	CIP	78.36	Not available	Positive
39	<i>Listeria monocytogenes</i>	3c	CRBIP	13.36	Meat product	Positive
40	<i>Listeria monocytogenes</i>	4a	NCTC	5214	Brain sheep	Positive
41	<i>Listeria monocytogenes</i>	4b	CIP	78.39	Chicken	Positive
42	<i>Listeria monocytogenes</i>	4b	CRBIP	13.107	Environment	Positive
43	<i>Listeria monocytogenes</i>	4b	Nexidia	NEX-642	Cheese	Positive
44	<i>Listeria monocytogenes</i>	4b	Nexidia	NEX-754	Food isolate	Positive
45	<i>Listeria monocytogenes</i>	4b	Nexidia	NEX-929	Food isolate	Positive
46	<i>Listeria monocytogenes</i>	4b	Nexidia	NEX-1504	Food isolate	Positive
47	<i>Listeria monocytogenes</i>	4c	ATCC	19116	Chicken	Positive
48	<i>Listeria monocytogenes</i>	4d	ATCC	19118	Sheep	Positive
49	<i>Listeria monocytogenes</i>	4d	NCTC	10888	Sheep	Positive
50	<i>Listeria monocytogenes</i>	4e	CIP	78.41	Chicken	Positive
51	<i>Listeria monocytogenes</i>	7	Nexidia	NEX-843	Food isolate	Positive
52	<i>Listeria monocytogenes</i>	7	CIP	78.43	Not available	Positive

^a ATCC: American Type Culture Collection, Manassas, VA, USA.

^b CIP: Collection de l'Institut Pasteur, Paris, 303 France.

^c CRBIP: Biological Resource Center of institute Pasteur, Paris, France.

^d NCTC: National Collection of 304 Type Cultures, Salisbury, UK.

^e Nexidia: Microbial Strain Collection Nexidia SAS, Dijon, France.

Table 2: Exclusivity Panel Results (4)

No.	Organism	Source	Strain	Origin	Results	N-Light broth
1	<i>Listeria innocua</i>	ATCC ^a	33090	Cow brain	Negative	Not tested
2	<i>Listeria innocua</i>	Nexidia ^b	NEX-1849	Milk	Negative	Not tested
3	<i>Listeria ivanovii</i>	ATCC	BAA-139	Washing water	Negative	Not tested
4	<i>Listeria ivanovii</i>	Nexidia	NEX-923	Duck breast	Negative	Not tested
5	<i>Listeria grayi</i>	CIP ^c	103321	Food	Negative	Not tested
6	<i>Listeria seeligeri</i>	CIP	100100	Soil	Negative	Not tested
7	<i>Listeria seeligeri</i>	Nexidia	NEX-1848	Milk	Negative	Not tested
8	<i>Listeria welshimerii</i>	CIP	81.49	Decaying vegetation	Negative	Not tested
9	<i>Listeria weihenstephanensis</i>	DSM ^d	24699	Hydrophyte in pond	Negative	Not tested
10	<i>Listeria fleischmanii</i>	DSM	25003	Cheese ripening cellar	Negative	Not tested
11	<i>Lactococcus lactis</i>	ATCC	11454	Reference strain	Negative	Not tested
12	<i>Lactococcus lactis</i>	Nexidia	NEX-363	Food	Negative	Not tested
13	<i>Streptococcus thermophilus</i>	Nexidia	NEX-371	Food isolate	Negative	Not tested
14	<i>Lactobacillus plantarum</i>	ATCC	14917	Pickled cabbage	Negative	Not tested
15	<i>Lactobacillus plantarum</i>	Nexidia	NEX-EC-EnrE8	Surface	Negative	Not tested
16	<i>Lactobacillus buchneri</i>	Nexidia	NEX-EC-E8	Surface	Negative	Not tested
17	<i>Leuconoctoc mesenteroides</i>	NEMIS	KL-LM 18.1	Salad factory	Negative	Not tested
18	<i>Klebsiella pneumoniae</i>	ATCC	4352	Dairy products: cow's milk	Negative	Not tested
19	<i>Aerococcus viridans</i>	NEMIS ^e	MA-LM 191.1	Meat factory	Negative	Not tested
20	<i>Micrococcus yunnanensis</i>	NEMIS	OS-LM 206.2	Meat factory	Negative	Not tested
21	<i>Enterobacter amnigenus</i>	Nexidia	NEX-764	Cocoa	Negative	Not tested
22	<i>Enterobacter cloacae</i>	DSM	30060	Minced meat	Negative	Not tested
23	<i>Cronobacter sakazakii</i>	ATCC	12868	Reference strain	Negative	Not tested
24	<i>Escherichia coli</i>	ATCC	8739	Faeces	Negative	Not tested
25	<i>Citrobacter braakii</i>	ATCC	51113	Snake	Negative	Not tested
26	<i>Pantoea agglomerans</i>	CIP	82.100	Corn crops	Negative	Not tested
27	<i>Salmonella Typhimurium</i>	ATCC	14028	Chicken	Negative	Not tested
28	<i>Pseudomonas aeruginosa</i>	ATCC	15442	Animal room water bottle	Negative	Not tested
29	<i>Providencia vermicola</i>	NEMIS	LU-SM 74E	Egg factory	Negative	Not tested
30	<i>Morganella morganii</i>	NEMIS	LU-SM 74E	Egg factory	Negative	Not tested
31	<i>Enterococcus avium</i>	ATCC	14025	Human feces	Negative	Not tested
32	<i>Enterococcus faecalis</i>	ATCC	51299	Peritoneal fluid	Positive	Negative
33	<i>Enterococcus hirae</i>	ATCC	10541	Reference strain	Positive	Negative
34	<i>Staphylococcus aureus</i>	Nexidia	NEX-1093	Raw milk cheese	Negative	Not tested
35	<i>Staphylococcus aureus</i>	Nexidia	NEX-1069	Mayonnaise	Negative	Not tested
36	<i>Bacillus cereus</i>	CIP	105151	Reference strain	Negative	Not tested
37	<i>Bacillus licheniformis</i>	NEMIS	MH-LM 128.2	Cheese dairy	Negative	Not tested
38	<i>Bacillus subtilis</i>	ATCC	6051	Reference strain	Negative	Not tested
39	<i>Clostridium perfringens</i>	ATCC	13124	Reference strain	Negative	Not tested

^a ATCC: American Type Culture Collection, Manassas, VA, USA. CIP: Collection de l'Institut Pasteur, Paris, 303 France.

^b Nexidia: Microbial Strain Collection Nexidia SAS, Dijon, France.

^c CIP: Collection de l'Institut Pasteur, Paris, 303 France.

^d DSM: DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany.

^e NEMIS: Microbial Strain Collection NEMIS AG, Au, Switzerland.

Table 3. N-Light *Listeria monocytogenes* vs. Reference (ISO 11290-1:2017) Method – POD Results (4)

Matrix	Strain	cfu ^a / Test Area	N ^b	Candidate			Reference			dPOD _c ^f	95% CI ^g
				x ^c	POD _c ^d	95% CI	x	POD _r ^e	95% CI		
Stainless Steel (1" x 1")	<i>L. monocytogenes</i> ATCC ^h BAA-751 & <i>E. faecalis</i> ATCC 29212	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		70 & 820	20	9	0.450	0.26, 0.66	6	0.30	0.15, 0.52	0.15	-0.14, 0.41
		450 & 5500	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^acfu/Test area = Results of the cfu/Test area were determined by plating the inoculum for each matrix 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 confirmed candidate method result and reference 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.

ⁱN/A = Not applicable.

Table 4. N-Light *Listeria monocytogenes* presumptive vs. confirmed ISO 11290-1:2017 – POD results (4)

Matrix	Strain	cfu ^a / Test Area	N ^b	Candidate presumptive			Candidate confirmed			dPOD _{cp} ^f	95% CI ^g
				x ^c	POD _{cp} ^d	95% CI	X	POD _{cc} ^e	95% CI		
Stainless Steel (1" x 1")	<i>L. monocytogenes</i> ATCC ^h BAA-751 & <i>E. faecalis</i> ATCC 29212	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		70 & 820	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		450 & 5500	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^acfu/Test area = Results of the cfu/Test area were determined by plating the inoculum for each matrix 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 confirmed candidate method p0resumptive 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.

ⁱN/A = Not applicable.

Table 5. N-Light *Listeria monocytogenes* presumptive vs. confirmed per alternative N-Light *Listeria monocytogenes* procedure – POD results (4)

Matrix	Strain	cfu ^a / Test Area	N ^b	Candidate presumptive			Candidate confirmed			dPOD _{cp} ^f	95% CI ^g
				x ^c	POD _{cp} ^d	95% CI	X	POD _{cc} ^e	95% CI		
Stainless Steel (1" x 1")	<i>L. monocytogenes</i> ATCC ^h BAA-751 & <i>E. faecalis</i> ATCC 29212	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		70 & 820	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		450 & 5500	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^acfu/Test area = Results of the cfu/Test area were determined by plating the inoculum for each matrix in triplicate.

^bN = Number of test portions.

^cx = Number of positive test portions.

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

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

^fdPOD_{cp} = Difference between the confirmed candidate method result and reference 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.

ⁱN/A = Not applicable.

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