CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

122002

The AOAC Research Institute hereby certifies the method known as:

N-Light™ *L. monocytogenes*

manufactured by

NEMIS Technologies AG
Überlandstrasse 109
8600 Dübendorf
Switzerland

This method has been evaluated in the AOAC® *Performance Tested Methods*SM Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC® Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*SM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above-mentioned method for a period of one calendar year from the date of this certificate (December 29, 2021 – December 31, 2022). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

December 29, 2021
Date

2275 Research Blvd., Ste. 300, Rockville, Maryland, USA Telephone: +1-301-924-7077 Fax: +1-301-924-7089
Internet e-mail: aoacri@aoac.org * World Wide Web Site: http://www.aoac.org

METHOD AUTHORS
Delphine Larose, Nicolas Desroches, Lukas Reinau, Lars Fieseler, Mario Hupfeld

SUBMITTING COMPANY
NEMIS Technologies AG
Überlandstrasse 109
8600 Dübendorf
Switzerland

METHOD NAME
N-light™ *L. monocytogenes*

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

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

AOAC EXPERTS AND PEER REVIEWERS
Yi Chen[1], Catharine Carlin[2], Salvatore Parisi[3]
[1] U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD, USA
[2] Mérieux NutriSciences, Crete, IL, USA
[3] Al Balqa' Applied University, Sicily, Italy

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 - The N-light™ *L. monocytogenes* method performance is comparable to the reference method 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* for the detection of *L. monocytogenes* in environmental surfaces (stainless steel, plastic and ceramic) after enrichment [3].

REFERENCE METHODS
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: Detection method. Geneva, Switzerland (3)

ORIGINAL CERTIFICATION DATE
December 07, 2020

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2022.

METHOD MODIFICATION RECORD
1. **December 2021 Level 1**

SUMMARY OF MODIFICATION
1. **Editorial and formatting changes, updated catalog numbers.**

Under this AOAC® *Performance Tested*℠ License Number, 122002 this method is distributed by:
NONE

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

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

[a]**ATCC:** 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	*Listeria monocytogenes* 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]	*Listeria monocytogenes* ATCC BAA-751 &*Enterococcus faecalis* 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	*Listeria monocytogenes* 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	*Listeria monocytogenes* 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)

[a]CFU/Test area determined by plating the inoculum in duplicate (shown as average)
[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 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$_C$[f]	95% CI[g]
				x[c]	POD$_C$[d]	95% CI	x	POD$_R$[e]	95% CI		
Stainless steel	*Listeria monocytogenes* ATCC[h] BAA-751 (1/2b)	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47,0.47)
		72	20	19	0.95	(0.76,0.99)	15	0.75	(0.53,0.89)	0.20	(-0.03, 0.42)
		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]	*Listeria monocytogenes* ATCC BAA-751 &*Enterococcus faecalis* ATCC 29212	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.43, 0.43)
		73 & 810	20	8	0.4	(0.22, 0.61)	6	0.30	(0.15, 0.52)	0.10	(-0.18, 0.36)
		220 & 2400	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.43, 0.43)
Plastic	*Listeria monocytogenes* ATCC 7644 (1/2c)	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47,0.47)
		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	*Listeria monocytogenes* ATCC 19118 (4d)	0	5	0	0.00	(0.00,0.43)	0	0.00	(0.00,0.43)	0.00	(-0.47,0.47)
		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)

[a]CFU/Test area determined by plating the inoculum in triplicate (shown as average)
[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

REFERENCES CITED

1. Larose, D., Desroches, N., Reinau, L., Fieseler, L., and Hupfeld, M., Validation of N-Light™ *Listeria, L. monocytogenes* for the Detection of *Listeria monocytogenes on* environmental surfaces, AOAC® *Performance Tested℠* certification number 122002.
2. 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: Detection method. Geneva, Switzerland