CERTIFICATION

AOAC Research Institute
Performance Tested Methods℠

Certificate No.

121902

The AOAC Research Institute hereby certifies the method known as

InSite *Listeria*

manufactured by

Hygiena LLC
941 Avenida Acaso
Camarillo, CA USA

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*℠ 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*℠ 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, AOAC Research Institute Senior Director

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SUBMITTING COMPANY
Hygiena LLC
941 Avenida Acaso
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METHOD NAME
InSite *Listeria*

CATALOG NUMBERS
ILC050, ILC100

INDEPENDENT LABORATORY
Q Laboratories, Inc.
1400 Harrison Avenue
Cincinnati, OH 45214
USA

APPLICABILITY OF METHOD
Target organisms – *Listeria* species (*L. monocytogenes, innocua, fleischmanni, welshimeri, weihenstephanensis, ivanovii*, and *seeligeri*)

Matrixes – (4x4 in swab) – Stainless steel, ceramic, plastic

Performance claims – Performance equivalent to that of the U.S. Food and Drug Administration *Bacteriological Analytical Manual* Chapter 10 (2017): *Detection and Enumeration of Listeria monocytogenes Foods and Environmental Samples, and Enumeration of Listeria monocytogenes* reference.

REFERENCE METHOD

U.S. Food and Drug Administration (2017) *FDA Bacteriological Analytical Manual,* Chapter 10, Detection of *Listeria monocytogenes* in Foods and Environmental Samples, and Enumeration of *Listeria monocytogenes* in Foods (2)

ORIGINAL CERTIFICATION DATE
December 01, 2019

CERTIFICATION RENEWAL RECORD
Renewed through December 2026.

METHOD MODIFICATION RECORD
1. **June 2020 Level 1**
2. **December 2024 Level 1**

SUMMARY OF MODIFICATION
1. **Update catalog numbers.**
2. **Editorial changes.**

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NONE

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NONE

PRINCIPLE OF THE METHOD (1)
InSite *Listeria* media contains a proprietary mix of metabolic and selective substrates that allow the growth of *Listeria* species while restricting the growth of Gram negative and other Gram-positive bacteria. Substrates are metabolized by the selected population allowing replication. After sample collection, the device is incubated at 37 ± 1°C for 48 h. At the end of the incubation period a color change of medium from beige to black indicates a presumptive positive for presence of *Listeria* species. No color change in the media after 48 h indicates absence or presence of less than 10 CFU *Listeria*.

DISCUSSION OF THE VALIDATION STUDY (1)
The Hygiena InSite *Listeria* detected all *L. monocytogenes, innocua, innocua, fleischmanni, welshimeri, weihenstephanensis, ivanovii* and *seeligeri* tested at all dilution levels from extinction to high levels. The only species not detected was *L. grayi*.
Some Enterococci did produce a chromogenic reaction when inoculated at 10^6 and higher CFU levels. This is not the case for all strains of Enterococci.
The matrixes tested all produced equivalent recoveries at fractional levels. This was also demonstrated by Q Laboratories during independent testing.
From the plastic surfaces there was significance seen in the results. The plastic surfaces used due to impermeability were more difficult to control through drying, this may have caused some binding to the plastic.
The parameters of the test must be adhered to which are 37°C and 24 to 48 h to read initial positive but also negative.
Based on the data presented in this report, the Hygiena InSite *Listeria* is a suitable method for detection of *Listeria* on environmental surfaces.

Table 1. Inclusivity results for *Listeria* species (1)

No.	*Listeria* species	Serogroup	Source	Origin	InSite *Listeria* Result[a]
1	*L. monocytogenes*		ATCC[b] 15313	Unknown	+
2	*L. monocytogenes*	4b	ATCC 19115	Human	+
3	*L. monocytogenes*	1/2a	ATCC 19111	Poultry, England	+
4	*L. monocytogenes*		ATCC 35152	Guinea pig, England	+
5	*L. monocytogenes*	4e	ATCC 19118	Chicken, England	+
6	*L. monocytogenes*		ATCC 4428	Rabbit	+
7	*L. monocytogenes*		ATCC 43250	ATCC 35152	+
8	*L. monocytogenes*		ATCC 43256	Mexican-style Cheese	+
9	*L. monocytogenes*		ATCC 51414	Raw milk	+
10	*L. monocytogenes*		ATCC 43249	ATCC 35152	+
11	*L. monocytogenes*		ATCC 700301	Scott A strain	+
12	*L. monocytogenes*		ATCC 43251	ATCC 35152	+
13	*L. monocytogenes*		ATCC 700302	Scott A Strain	+
14	*L. monocytogenes*		ATCC 7648	Unknown	+
15	*L. monocytogenes*		ATCC 49592	Derived from CAP strain	+
16	*L. monocytogenes*		ATCC 49593	Derived from S-437	+
17	*L. monocytogenes*	2	ATCC 19112	Spinal fluid of Scottish man	+
18	*L. monocytogenes*		ATCC 7646	Sheep	+
19	*L. monocytogenes*		ATCC 53979	Unknown	+
20	*L. monocytogenes*		ATCC 7644	Human	+
21	*L. monocytogenes*		ATCC BAA-751	Unknown	+
22	*L. monocytogenes*		ATCC SLR2249	Unknown	+
23	*L. monocytogenes*	4b	ATCC 13932	Spinal fluid meningitis	+
24	*L. monocytogenes*	7	NCTC[c]10890	Unknown	+
25	*L. monocytogenes*	1/2a	ATCC BAA-2660	Unknown	+
26	*L. monocytogenes*	1/2a	ATCC 51775	Dairy products, Belgium	+
27	*L. monocytogenes*	4a	ATCC 19114	Animal tissue	+
28	*L. monocytogenes*	1/2b	ATCC 51780	Dairy Product- Cheese Belgium	+
29	*L. monocytogenes*	4c	ATCC 19116	Chicken, England	+
30	*L. monocytogenes*	1/2a	ATCC 51773	Dairy products, Belgium	+
31	*L. monocytogenes*	1/2b	ATCC BAA-2658		+
32	*L. monocytogenes*	1/2a	ATCC BAA-2659	Unknown	+
33	*L. monocytogenes*	1/2a	ATCC BAA-2657	Unknown	+
34	*L. monocytogenes*	3a	ATCC 51782	Dairy products, Belgium	+
35	*L. monocytogenes*	4b	ATCC 51776	Dairy products, Belgium	+
36	*L. monocytogenes*	4b	ATCC 51777	Dairy products, Belgium	+
37	*L. monocytogenes*	1/2a	ATCC 51774	Blood-human Brussels Belgium	+
38	*L. monocytogenes*	4d	ATCC 19117	Sheep, USA	+
39	*L. monocytogenes*	1/2c	ATCC 51779	Dairy products, Belgium	+
40	*L. monocytogenes*	4b	ATCC 51778	Dairy products, Belgium	+
41	*L. monocytogenes*		L10890-Q[d]	Unknown	+
42	*L. monocytogenes*	3c	L2933-Q	Cooked Chicken	+
43	*L. monocytogenes*	3a	L3007c-Q	Unknown	+
44	*L. monocytogenes*	3b	L3408a-Q	Chicken liver pâté	+
45	*L. monocytogenes*	3b	L3624-Q	Cooked turkey	+
46	*L. monocytogenes*	3a	L3751-Q	Cooked turkey	+
47	*L. monocytogenes*	3c	L4072-Q	Cooked chicken	+
48	*L. monocytogenes*	3b	L4152a-Q	Chicken	+
49	*L. monocytogenes*	3b	L4157-Q	Pepper quiche	+
50	*L. monocytogenes*	1/2c	L4292-Q	Cooked meat	+
51	*L. monocytogenes*	1/2c	L4339a-Q	Ice cream	+
52	*L. monocytogenes*	3b	L4374a-Q	Pâté	+
53	*L. monocytogenes*	3a	L4385a-Q	Pâté	+
54	*L. monocytogenes*	3c	L4395-Q	Unknown	+
55	*L. monocytogenes*	1/2c	L4505a-Q	Hard boiled eggs	+
56	*L. monocytogenes*	3c	L4662a-Q	Sandwich	+
57	*Listeria innocua*		ATCC 51742	Cabbage	+
58	*Listeria innocua*		ATCC 49595	Derived from ATCC 33090	+
59	*Listeria innocua*	6b	ATCC 33091	Feces of pregnant woman	+
60	*Listeria innocua*		ATCC BAA-349	Unknown	+
61	*Listeria innocua*	6a	ATCC BAA-680	Cheese, Morocco	+
62	*Listeria innocua*	6b	ATCC 46547	Bovine brain	+
63	*Listeria innocua*	6a	ATCC 11288	Cow brain	+
64	*Listeria innocua*	6a	ATCC 33090	Cow brain	+
65	*Listeria innocua*		L807-Q	Chopped pork and ham	+
66	*Listeria innocua*		L811-Q	Ham cured shoulder	+
67	*Listeria innocua*		LDP1-Q	Unknown	+

68	*Listeria fleischmannii*		L24998-Q	Hard Cheese	+
69	*Listeria fleischmannii*		L250003-Q	Cheese ripening cellar	+
70	*Listeria welshimeri*	6b	ATCC 43549	Unknown	+
71	*Listeria welshimeri*	6b	ATCC 35897	Decaying plant matter	+
72	*Listeria welshimeri*	6a	ATCC 43548	Unknown	+
73	*Listeria welshimeri*	1/2b	ATCC 43550	Soil	+
74	*Listeria welshimeri*	6a	ATCC 43551	Human feces	+
75	*Listeria welshimeri*		L50-Q	Unknown	+
76	*Listeria welshimeri*		L8080-11-Q	Unknown	+
77	*Listeria welshimeri*		L1925-Q	Chicken	+
78	*Listeria welshimeri*		L2161a-Q	Sausage	+
79	*Listeria welshimeri*		L2652-Q	Salami	+
80	*Listeria welshimeri*		L2816-Q	Raw chicken	+
81	*Listeria welshimeri*		L4329t-Q	Food	+
82	*Listeria welshimeri*		L4329w-Q	Food	+
83	*Listeria welshimeri*		L4334a-Q	Smoked mackerel	+
84	*Listeria welshimeri*		6VL4-VS-Q	Unknown	+
85	*Listeria weihenstephanensis*		24698-Q	Unknown	+
86	*Listeria weihenstephanensis*		24699-Q	Unknown	+
87	*Listeria ivanovii*	5	ATCC 19119	Bulgarian sheep	+
88	*Listeria ivanovii*		ATCC BAA-753	Quality control strain	+
89	*Listeria ivanovii*		ATCC 700402	Quality control strain	+
90	*Listeria ivanovii*		ATCC 49953	Goat, Belgium	+
91	*Listeria ivanovii*		ATCC BAA-139	Washing water, Switzerland	+
92	*Listeria seeligeri*		L2837-Q	Crab pate	+
93	*Listeria seeligeri*		L2900-Q	Cooked chicken	+
94	*Listeria seeligeri*		ATCC 35967	Soil, Germany	+
95	*Listeria grayi*		ATCC 25401	Standing corn stalks and leaves	-
96	*Listeria grayi*		ATCC 19120	Animal feces, Denmark	-
97	*Listeria grayi*		ATCC 700545	Unknown	--
98	*Listeria grayi*		ATCC 25403	Corn stalks and leaves, USA	-

[a]InSite chromogenic color change result.
[b]ATCC: American Type Culture Collection, Manassas, VA.
[c]NCTC: National Collection of Type Cultures, Porton Down, Salisbury, UK.
[d]Lxxxx-Q:DPOnt Qualicon Culture Collection, Wilmington, DE.

Table 2. Exclusivity results for non-*Listeria* strains tested (1)

No.	Genus species	Source	Origin	InSite Result[a]
1	*Enterococcus durans*	ATCC[b] 11576	Dairy products	+[c]
2	*Enterococcus faecalis*	ATCC 51299	Peritoneal fluid	+[d]
3	*Enterococcus faecalis*	ATCC 33186	Unknown	-
4	*Enterococcus faecalis*	ATCC 700802	Unknown	-
5	*Enterococcus faecalis*	ATCC 51575	Unknown	-
6	*Enterococcus faecalis*	ATCC 49533	Unknown	-
7	*Escherichia coli*	ATCC 8739	Feces	-
8	*Salmonella enterica* subsp. *enterica*	ATCC 49223	Guinea pig, Exeter, England	-
9	*Salmonella enterica* subsp. *enterica*	ATCC 9207	Pork liver Chicago, IL	-
10	*Bacillus spizizenii*	ATCC 6633	Unknown	-
11	*Staphylococcus aureus*	ATCC 6538	Human lesion	-
12	*Pseudomonas aeruginosa*	ATCC 9027	Outer ear infection	-
13	*Enterococcus gallinarum*	ATCC 49573	Chicken intestine	-
14	*Citrobacter braakii*	ATCC 51113	Snake, France	-
15	*Rhodococcus wratislaviensis*	ATCC 51786	Soil	-
16	*Diplococcus pneumoniae*	ATCC 4951	Unknown	
17	*Micrococcus luteus*	ATCC 4698	Unknown	-
18	*Staphylococcus epidermidis*	ATCC 51625	Blood- human	-
19	*Klebsiella oxytoca*	ATCC 43086	Unknown	-
20	*Candida glabrata*	ATCC 15545	Young woman fata septicemia	-
21	*Saccharomyces cerevisiae*	ATCC 77533	Unknown	-
22	*Salmonella* Paratyphi A	ATCC 9150	Unknown	-
23	*Salmonella* Typhimurium	ATCC 13311	Food poisoning, human feces	-
24	*Pseudomonas putida*	ATCC 49128	Clinical isolate	-
25	*Pseudomonas protegens*	ATCC 17386	Tryptophan- enriched water, Berkeley, CA	-
26	*Bacillus subtilis* subsp. *thruingiensis*	ATCC 1092	Animal tissue	-
27	*Bacillus cereus*	ATCC 33019	Powdered milk-base infant formulae, Colombia	-
28	*Bacillus subtilis* subsp. *subtilis*	ATCC 6051	Unknown	-
29	*Klebsiella pneumoniae*	ATCC 10034	Unknown	-
30	*Candida albicans*	ATCC 10231	Man with bronchomycosis	-
31	*Enterobacter cloacae* subsp. *cloacae*	ATCC 10347	Spinal fluid	-
32	*Citrobacter freundii*	NCTC[e] 9750	Unknown	-
33	*Citrobacter koseri*	ATCC 27156	Unknown	-
34	*Carnobacterium gallinarum*	4063-Q[f]	Unknown	-
35	*Lactobacillus curvatus*	7332-Q	Unknown	-
36	*Lactobacillus lactis*	659-Q	Unknown	-
37	*Salmonella kentucky*	2628-Q	Unknown	-
38	*Staphylococcus capitis*	1111-Q	Unknown	-
39	*Staphylococcus sciuri*	1113-Q	Unknown	-
40	*Staphylococcus warneri*	1105-Q	Unknown	-
41	*Staphylococcus xylosus*	1107-Q	Unknown	-
42	*Staphylococcus xylosus*	1112-Q	Unknown	-
43	*Streptococcus bovis*	692-Q	Unknown	-
44	*Streptococcus mutans*	3992-Q	Unknown	-
45	*Streptococcus salivarius*	3993-Q	Unknown	-
46	*Streptococcus sangunis*	3994-Q	Unknown	-
47	*Streptococcus thermophilus*	691-Q	Unknown	-
48	*Streptococcus thermophilus*	11498-Q	Unknown	-

[a]InSite chromogenic color change result.

[b]ATCC: American Type Culture Collection, Manassas, VA.

[c]+ Detected at a level of >410,000 CFU.

[d]+ Detected at a level of >1,000,000 CFU.

[e]NCTC: National Collection of Type Cultures, Porton Down, Salisbury, UK.

[f]xxxx-Q: DuPont Qualicon Culture Collection, Wilminton, DE

Table 3. InSite *Listeria* Results: Presumptive vs. Confirmed (1)

				InSite *Listeria* presumptive			InSite *Listeria* confirmed				
Matrix	Strain	Inoculation level[a]	N[b]	x[c]	POD_{CP}[d]	95% CI	x	POD_{CC}[e]	95% CI	$dPOD_{CP}$[f]	95% CI[g]
Stainless steel	*L. monocytogenes* (ATCC[h] 53979, 4a) 4a/10X *E. faecalis* (ATCC 49452)	0, 1163	5	0	0	(0.00, 0.43)	0	0	(0.00, 0.43)	0	(-0.47, 0.47)
		70, 1163	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0	(-0.13, 0.13)
		$7.0x10^6$, $1.2x10^8$	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0	(-0.47, 0.47)
Stainless steel[i]	*L. monocytogenes* 4a (CWD[j] 1620)/10X *E. faecalis* (ATCC 29212)	0, $3.2x10^3$	5	0	0	(0.00, 0.43)	0	0	(0.00, 0.43)	0	(-0.47, 0.47)
		$1.4x10^2$, $3.2x10^3$	20	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0	(-0.13, 0.13)
		$4.0x10^2$, $2.8x10^4$	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0	(-0.47, 0.47)
Stainless steel	*L. innocua* (ATCC 11288)	0	5	0	0	(0.00, 0.43)	0	0	(0.00, 0.43)	0	(-0.47, 0.47)
		1375	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0	(-0.13, 0.13)
		$4.8x10^6$	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0	(-0.47, 0.47)
Ceramic	*L. monocytogenes* 1/2a (ATCC 19111)	0	5	0	0	(0.00, 0.43)	0	0	(0.00, 0.43)	0	(-0.47, 0.47)
		812	20	15	0.75	(0.53, 0.88)	15	0.75	(0.53, 0.88)	0	(-0.13, 0.13)
		4.35×10^6	5	5	1.00	(0.00, 0.43)	5	1.00	(0.00, 0.43)	0	(-0.47, 0.47)
Plastic	*L. monocytogenes* 4b (ATCC 19115)	0	5	0	0	(0.00, 0.43)	0	0	(0.00, 0.43)	0	(-0.47, 0.47)
		6675	20	20	1.00	(0.84, 1.00)	20	1.00	(0.84, 1.00)	0	(-0.13, 0.13)
		2.9×10^6	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0	(-0.47, 0.47)

[a]Inoculation level = CFU applied to each 4" x 4" surface area (test portion). Inoculation levels for *Listeria* are presented first, then *Enterococcus*.
[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]American Type Culture Collection, Manassas, VA.
[i]Matrix tested in the independent laboratory, Q Laboratories, Cincinnati, OH.
[j]University of Vermont Culture Collection, Burlington, VT.

Table 4. Method Comparison Results: InSite *Listeria* vs. BAM Ch. 10 (1)

				InSite *Listeria* results			BAM Ch. 10 results				
Matrix	Strain	Inoculation level[a]	N[b]	x[c]	POD_C[d]	95% CI	x	POD_R[e]	95% CI	$dPOD_C$[f]	95% CI[g]
Stainless steel	*L. monocytogenes* (ATCC[h] 53979, 4a) 4a/10X *E. faecalis* (ATCC 49452)	0, 1163	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0	(-0.43, 0.43)
		70, 1163	20	12	0.60	(0.39, 0.78)	7	0.35	(0.18, 0.57)	0.25	(-0.05, 0.49)
		$7.0x10^6$, $1.2x10^8$	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0	(-0.43, 0.43)
Stainless steel[i]	*L. monocytogenes* 4a (CWD[j] 1620)/10X *E. faecalis* (ATCC 29212)	0, $3.2x10^3$	5	0	0	(0.00, 0.43)	0	0	(0.00, 0.43)	0	(-0.43, 0.43)
		140, $3.2x10^3$	20	10	0.50	(0.30, 0.70)	11	0.55	(0.34, 0.74)	-0.05	(-0.33, 0.24)
		400, $2.8x10^4$	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0	(-0.43, 0.43)
Stainless steel	*L. innocua* (ATCC 11288)	0	5	0	0	(0.00, 0.43)	0	0	(0.00, 0.43)	0	(-0.43, 0.43)
		1375	20	12	0.60	(0.39, 0.78)	14	0.70	(0.48, 0.85)	-0.10	(-0.36, 0.18)
		$4.8x10^6$	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0	(-0.43, 0.43)
Ceramic	*L. monocytogenes* 1/2a (ATCC 19111)	0	5	0	0	(0.00, 0.43)	0	0	(0.00, 0.43)	0	(-0.43, 0.43)
		812	20	15	0.75	(0.53, 0.88)	16	0.80	(0.58, 0.92)	-0.05	(-0.30, 0.21)
		4.35×10^6	5	5	1.00	(0.00, 0.43)	5	1.00	(0.56, 1.00)	0	(-0.43, 0.43)
Plastic	*L. monocytogenes* 4b (ATCC 19115)	0	5	0	0	(0.00, 0.43)	0	0	(0.00, 0.43)	0	(-0.43,0.43)
		6675	20	20	1.00	(0.84, 1.00)	9	0.45	(0.26, 0.66)	0.55	(0.29, 0.74)
		2.9×10^6	5	5	1.00	(0.57, 1.00)	5	1.00	(0.56, 1.00)	0	(-0.43, 0.43)

[a]Inoculation level = CFU applied to each 4" x 4" surface area (test portion). Inoculation levels for *Listeria* are presented first, then *Enterococcus*.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]POD_C = Candidate method presumptive positive outcomes confirmed positive.
[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]American Type Culture Collection, Manassas, VA.
[i]Matrix tested in the independent laboratory, Q Laboratories, Cincinnati, OH.
[j]University of Vermont Culture Collection, Burlington, VT.

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