CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

122005

The AOAC Research Institute hereby certifies the test kit known as:

Listeria CANARY® Zephyr

manufactured by

Smiths Detection
701 E. Pratt St.
Baltimore, MD 21202
USA

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 23, 2020 – December 31, 2021). 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 28, 2020
Date

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SUBMITTING COMPANY
Smiths Detection
701 E. Pratt St.
Baltimore, MD 21202 USA

KIT NAME
Listeria CANARY® Zephyr

CATALOG NUMBERS
903-152, 903-169

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

AOAC EXPERTS AND PEER REVIEWERS
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APPLICABILITY OF METHOD
Target Organisms – *Listeria species (Listeria aquatica, L. booriae, L. cornellensis, L. fleischmannii, L. floridensis, L. innocua, L. ivanovii, L. marthii, L. monocytogenes, L. newyorkensis, L. riparia, L. seeligeri, L. welshimeri)*

Matrixes – Stainless Steel (type 304 #4 finish, 1" x 1" swab), Stainless Steel (18 GA 300 series, brush finish, NSF certified, 1" x 1" swab), plastic (HDPE, 1" x 1" swab), rubber (Silicone FDA-grade, 1" x 1" swab), and sprout irrigation water (25 mL).

Performance claims - No statistical difference was detected between the Listeria CANARY® Zephyr assay and the reference culture method in the U.S. Food and Drug Administration *Bacteriological Analytical Manual* Chapter 10 *"Detection of Listeria monocytogenes in Foods and Environmental samples, and Enumeration of Listeria monocytogenes in Foods"* (FDA/BAM Chapter 10) (2).

REFERENCE METHOD
Food and Drug Administration 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 23, 2020

CERTIFICATION RENEWAL RECORD
New Approval 2020

METHOD MODIFICATION RECORD
NONE

SUMMARY OF MODIFICATION
NONE

Under this AOAC® *Performance Tested*[SM] License Number, 122005 this method is distributed by:
NONE

Under this AOAC® *Performance Tested*[SM] License Number, 122005 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)
The Listeria CANARY® Zephyr assay is a cell-based biosensor detection platform where living cells, biosensors, express antibodies on the cell surface specific for the target. Upon target binding by the antibodies, a rapid signal transduction process within the cell is initiated, resulting in the release of intracellular calcium. The calcium release excites an intracellular calcium sensitive luminescent protein, which generates a measurable luminescent light output.
The Listeria CANARY® Zephyr assay offers a streamlined sampling process that requires no DNA purification or lysis procedure. After sampling and incubation, the end user only has to perform a few simple, easy to follow steps before sample analysis which provides a result within 60 seconds.

DISCUSSION OF THE VALIDATION STUDY (1)
The Listeria CANARY® Zephyr assay successfully detected *Listeria* spp. from environmental stainless steel swabs, siliconized rubber, and HDPE plastic (1" x 1" test area) and sprout irrigation water (25 mL). When comparing results from the assay to 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 methods and Listeria CANARY® Zephyr assay for all matrixes.
The results of the inclusivity and exclusivity evaluation demonstrated 100% agreement with expected results for the test panels and confirmed the high specificity and selectivity of the method to *Listeria* species (*Listeria aquatica, L. booriae, L. cornellensis, L. fleischmannii, L. floridensis, L. innocua, L. ivanovii, L. marthii, L. monocytogenes, L. newyorkensis, L. riparia, L. seeligeri, L. welshimeri*).
The Listeria CANARY® Zephyr assay is a simple method to perform providing accurate presumptive results in 24 hours from sample enrichment. The rapid method offers considerable cost and time savings compared to the FDA/BAM chapter 10 reference method. The user manual is simple and easy to follow while the instrument software only requires a few simple steps to operate. Sample analysis is simple enough that a technician of any training level could conduct the assay and obtain accurate results.

Table 1. Inclusivity List: *Listeria* strains (1)

No.	Strain	Source	Origin	CANARY Results[a]
1	*Listeria aquatica*	FSL S10-1188 [b]	Running Water	+
2	*Listeria booriae*	FSL A5-0281	Dairy Processing Plant	+
3	*Listeria cornellensis*	FSL F6-0969	Water	+
4	*Listeria fleischmannii* subsp. *coloradensis*	FSL F6-1016	Grazing Pasture - Soil	+
5	*Listeria floridensis*	FSL S10-1187	Running Water	+
6	*Listeria innocua*	FSL W3-075	Food	+
7	*Listeria innocua*	F4247[d]	Food/FDA	+
8	*Listeria innocua*	F4248	Food/FDA	+
9	*Listeria innocua*	ATCC 33090	Cow brain	+
10	*Listeria innocua*	FSL C2-008	Fish Processing Plant	+
11	*Listeria innocua*	FSL X1-017	Unknown	+
12	*Listeria innocua* (hemolytic)	FSL J1-023	Unknown (donor Qualicon Inc.)	+
13	*Listeria ivanovii*	FSL C2-010	Animal	+
14	*Listeria ivanovii*	FSL C2-011	Animal	+
15	*Listeria marthii*	FSL S4-120	Pristine Environment - Forest	+
16	*Listeria marthii*	FSL S4-965	Pristine Environment -Water	+
17	*Listeria monocytogenes*	FSL- M1-004	Human sporadic case	+
18	*Listeria monocytogenes* 4b	FSL-J1-225	Human epidemic (Massachusetts, 1983)	+
19	*Listeria monocytogenes* 1/2a	FSL-J2-020	Animal, cow	+
20	*Listeria monocytogenes* 1/2a	FSL- J1-101	Hot dog	+
21	*Listeria monocytogenes* 1/2a	FSL-R2-499	Sliced turkey	+
22	*Listeria monocytogenes* 1/2a	FSL- J2-066	Animal, sheep	+
23	*Listeria monocytogenes* 1/2a	FSL-J2-063	Animal, sheep	+
24	*Listeria monocytogenes* 1/2a	FSL- J2-031	Animal, bovine	+

25	*Listeria monocytogenes* 1/2b	FSL-J2-064	Animal, cow	+
26	*Listeria monocytogenes* 1/2b	FSL-RW-502	Food epidemic, Illinois	+
27	*Listeria monocytogenes* 1/2b	FSL-J2-035	Animal, goat	+
28	*Listeria monocytogenes* 1/2c	FSL-J1-094	Human, sporadic	+
29	*Listeria monocytogenes* 3a	FSL C1-115	Human, sporadic	+
30	*Listeria monocytogenes* 3b	FSL-J1-169	Animal, goat	+
31	*Listeria monocytogenes* 3c	FSL-J1-049	Human, sporadic	+
32	*Listeria monocytogenes 4a*	ATCC 19114	Tissue, animal	+
33	*Listeria monocytogenes* 4a	FSL J1-031	Human sporadic case	+
34	*Listeria monocytogenes* 4a	FSL J1-168	Human sporadic case	+
35	*Listeria monocytogenes* 4b	FSL-N1-225	Human epidemic (US 1998-99)	+
36	*Listeria monocytogenes* 4b	FSL J1-110	Food, epidemic, Los Angeles 1985	+
37	*Listeria monocytogenes* 4b	FSL-C1-122	Human sporadic case	+
38	*Listeria monocytogenes* 4b	FSL N3-008	Food, epidemic, Halifax, 1981	+
39	*Listeria monocytogenes* 4b	FSL N3-013	Food epidemic, UK 1988-1990	+
40	*Listeria monocytogenes* 4b	FSL J1-158	Animal, goat	+
41	*Listeria monocytogenes* 4c	FSL W1-111	Unknown	+
42	*Listeria newyorkensis*	FSL M6-0635	Seafood Processing Plant	+
43	*Listeria riparia*	FSL S10-1204	Running Water	+
44	*Listeria seeligeri*	FSL H6-011	Food	+
45	*Listeria seeligeri*	FSL H6-169	Food Environment	+
46	*Listeria seeligeri*	ATCC 35967[c]	Soil	+
47	*Listeria seeligeri*	PSI 488	Unknown	+
48	*Listeria welshimeri*	FSL H6 017	Food	+
49	*Listeria welshimeri*	FSL H6-105	Food Environment	+
50	*Listeria welshimeri*	ATCC 35897	Leaf litter	+
51	*Listeria welshimeri*	PSI 494[e]	Unknown	+

[a]Result: "+" = strain detected, "-" = strain not detected;
[b]FSL- Cornell University; Ithaca, New York, United States
[c]ATCC- American Type Culture Collection; Manassas, Virginia, United States
[d]F – US Food and Drug Administration, Center for Food Safety and Nutrition College Park, Maryland, United States
[e]PSI – PathSensors, Inc; Baltimore, Maryland, United States

Table 2. Exclusivity List (1)

No.	Organism	Source	Origin	CANARY Result[a]
1	*Bacillus cereus*	ATCC 49064[b]	Meat Loaf	-
2	*Bacillus mycoides*	ATCC 6462	Soil	-
3	*Bacillus subtilis*	ATCC 6633	Environmental Isolate	-
4	*Brochothirix thermosphactra*	ATCC 11509	Pork Sausage	-
5	*Candida auris*	CDC B11903[c]	Clinical	-
6	*Candida parapsilosis*	ATCC 28475	Skin	-
7	*Carnobacterium gallinarum*	ATCC 49517	Ice Slush around Chicken Carcasses	-
8	*Citrobacter freundii*	ATCC 43864	NA	-
9	*Enterococcus faecium*	ATCC 19434	Unknown	-
10	*Erysipelothrix rhusiopathiae*	ATCC 19414	Spleen, Pig	-
11	*Escherichia coli*	ATCC 43888	Human Feces	-
12	*Escherichia fergusonii*	ATCC 35469	Feces, Human	-
13	*Klebsiella aerogenes*	ATCC 13048	Sputum	-
14	*Klebsiella pneumoniae*	ATCC 13882	NA	-
15	*Kurthia gibsonii*	ATCC 43195	NA	-
16	*Lactobacillus acidophilus*	ATCC 4356	Human	-
17	*Lactobacillus brevis*	ATCC 14869	Feces, Human	-
18	*Lactobacillus casei*	ATCC 393	Dairy Products	-
19	*Lactobacillus delbrueckii* subsp. *lactis*	ATCC 12315	Dairy Products	-
20	*Lactobacillus plantarum* subsp. *plantarum*	ATCC 14917	Pickled Cabbage	-
21	*Leuconostoc mesenteroides* subsp. *mesenteroides*	ATCC 8293	Fermenting Olives	-
22	*Listeria grayi*	ATCC 25400	Corn Stalk	-
23	*Listeria grayi*	ATCC 25402	Corn Stalk	-
24	*Listeria grayi*	ATCC 25403	Corn Stalk	-
25	*Micrococcus luteus*	ATCC 4698	NA	-
26	*Propionibacterium freudenreichii* subsp. *freudenreichii*	ATCC 6207	Swiss Cheese	-
27	*Proteus vulgaris*	ATCC 49132	Clinical Isolate	-
28	*Pseudomonas aeruginosa*	ATCC 10145	NA	-
29	*Rhodococcus equi*	ATCC 6939	Lung Abscess	-
30	*Salmonella* Cholerasuis	ATCC 7001	NA	-
31	*Salmonella* Typhimurium	ATCC 15277	NA	-
32	*Staphylococcus schleiferi* subsp. *schleiferi*	ATCC 43808	Jugular Catheter	-
33	*Streptococcus faecalis*	ATCC 29212	Urine	-
34	*Streptococcus salivarius* subsp. *thermophilus*	ATCC 19258	Pasteurized Milk	-

[a]Result: "+" = strain detected, "-" = strain not detected.
[b]ATCC- American Type Culture Collection; Manassas, Virginia, United States
[c]CDC – Centers for Disease Control and Prevention; Atlanta, Georgia, United States

Table 3. Listeria CANARY® Zephyr Assay: Presumptive vs. Confirmed (1)

Matrix	Strain	MPN/Test Portion; CFU/Test Area[a]	N[b]	Listeria CANARY Zephyr Assay Presumptive			Listeria CANARY Zephyr Assay Confirmed			dPOD$_{CP}$[f]	95% CI[g]
				x[c]	POD$_{CP}$[d]	95% CI	x	POD$_{CC}$[e]	95% CI		
Sprout irrigation water (25 mL)	*L. monocytogenes* ATCC 19114 & E. faecalis ATCC 29212	-	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.66 (0.42, 1.04)	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(-0.28, 0.28)
		2.52 (1.26, 5.02)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Sprout irrigation water (25 mL)[j]	*L. monocytogenes* ATCC 19115	-	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.47, 0.47)
		0.62 (0.37, 0.94)	20	10	0.5	0.30, 0.70	10	0.5	0.30, 0.70	0	(-0.13, 0.13)
		2.10 (1.13, 4.99)	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	(-0.47, 0.47)
Stainless steel swab (1" x 1")	*L. monocytogenes* FSL-J2-064 & *E. faecalis* ATCC 2912	0/100	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		2.5/100	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(-0.28, 0.28)
		10/100	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Stainless steel swab (1" x 1")[j]	*L. monocytogenes* ATCC BAA-751 & E. faecalis *ATCC 29212*	0/100	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.47, 0.47)
		47/500	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	(-0.13, 0.13)
		180/2200	5	5	1.00	0.57, 1.00	5	1.00	(0.57, 1.00)	0.00	(-0.47, 0.47)
Silicone Rubber Swab (1" x 1")	*L. fleischmannii* FSL F6-1016	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		2.8	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		10	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
HDPE Swab (1" x 1")	*L. innocua* ATCC 33090	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		110	20	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0.00	(-0.28, 0.28)
		200	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.
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.

Table 4. Method Comparison Results: Listeria CANARY® Zephyr Assay vs. BAM Ch. 10 (1)

Matrix	Strain	MPN/Test Portion; CFU/Test Area[a]	N[b]	Listeria CANARY Zephyr Assay			BAM Ch. 10			dPOD$_{CP}$[f]	95% CI[g]
				x[c]	POD$_C$[d]	95% CI	x	POD$_R$[e]	95% CI		
Sprout irrigation water (25 mL)	*L. monocytogenes* ATCC 19114 & E. faecalis ATCC 29212	-	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0	(-0.43, 0.43)
		0.66 (0.42, 1.04)	20	9	0.45	(0.26, 0.66)	8	0.40	(0.22, 0.61)	0.05	(-0.24, 0.33)
		2.52 (1.26, 5.02)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0	(-0.43, 0.43)
Sprout irrigation water (25 mL)[j]	*L. monocytogenes* ATCC 19115	-	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.47, 0.47)
		0.62 (0.37, 0.94)	20	10	0.50	0.30, 0.70	9	0.45	(0.26, 0.66)	0.05	(-0.24, 0.33)
		2.10 (1.13, 4.99)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0	(-0.47, 0.47)
Stainless steel swab (1" x 1")	*L. monocytogenes* FSL-J2-064 & *E. faecalis* ATCC 2912	0/100	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0	(-0.43, 0.43)
		2.5/100	20	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0	(-0.28, 0.28)
		10/100	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0	(-0.43, 0.43)
Stainless steel swab (1" x 1")[j]	*L. monocytogenes* ATCC BAA-751 & E. faecalis *ATCC 29212*	0/100	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		47/500	20	10	0.50	(0.30, 0.70)	8	0.40	(0.22, 0.61)	0.10	(-0.19, 0.37)
		180/2200	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Silicone Rubber Swab (1" x 1")	*L. fleischmannii* FSL F6-1016	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0	(-0.43, 0.43)
		2.8	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0	(-0.27, 0.27)
		10	5	5	1.00	(0.57, 1.00)	5	1.00	(0.00, 0.43)	0	(-0.43, 0.43)
HDPE Swab (1" x 1")	*L. innocua* ATCC 33090	0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0	(-0.43, 0.43)
		110	20	13	0.65	(0.43, 0.82)	12	0.60	(0.39, 0.78)	0.05	(-0.23, 0.32)
		200	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0	(-0.43, 0.43)

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.
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.
[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.

REFERENCES CITED

1. Wright, S., Davey, N., Lehett, J.J., and Flannery, A.R., Validation Report for the Listeria CANARY® Zephyr Assay for the Detection of *Listeria* in Irrigation Water and Environmental Surface Samples, AOAC® *Performance Tested*SM certification number 122005.
2. Food and Drug Administration Bacteriological Analytical Manual Chapter 10: *Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods*. October 2017. (Accessed August 2020) https://www.fda.gov/food/laboratory-methods-food/bam-chapter-10-detection-listeria-monocytogenes-foods-and-environmental-samples-and-enumeration