CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

061702

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

BAC*Gene Listeria* spp.

manufactured by

Eurofins GeneScan Technologies GmbH
Engesserstrasse 4
79108 Freiburg
Germany

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 (January 16, 2018 – December 31, 2018). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Deborah McKenzie

Deborah McKenzie, Senior Director
Signature for AOAC Research Institute

January 16, 2018
Date

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KIT NAME(S)
BAC*Gene Listeria* spp.

CATALOG NUMBERS
5123222101 (96 rxn) and 5123222110 (10 x 96 rxn)

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APPLICABILITY OF METHOD
Target organisms – *Listeria* spp. (Including *L. monocytogenes, L. seeligeri, L. welshimeri, L. marthii, L. ivanovii, L. grayi, L. innocua, and L. rocourtiae*)

Matrices – mayonnaise-based vegetable salad (25 g), frankfurters (25 g), raw whole milk (25 g), soft white cheese (25 g), frozen cantaloupe balls (25 g), smoked salmon (25 g), frozen cooked shrimp (25 g), stainless steel 304L (1 x 1 in swab), ceramic tile (4 x 4 in sponge), and process water (25 g) (vegetable sausage production)

Performance claims - Performance equivalent to ISO 11290-1/A1 (2004) for a selection of food matrixes, process water and environmental surfaces.

REFERENCE METHOD
ISO 11290-1/A1 (2004). Microbiology of food and animal feeding stuffs Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: detection method (1)

ORIGINAL CERTIFICATION DATE
June 02, 2017

CERTIFICATION RENEWAL RECORD
New Approval 2018

METHOD MODIFICATION RECORD
1. **January 2018 Level 1**

SUMMARY OF MODIFICATION
1. **Name change to Eurofins GeneScan Technologies GmbH and editorial changes**

Under this AOAC® *Performance Tested*℠ License Number, 061702 this method is distributed by:
NONE

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NONE

PRINCIPLE OF THE METHOD (1)

Introduction to the BACGene Listeria spp. method

The BAC*Gene Listeria* spp. method is a qualitative real-time PCR assays for the detection of select *Listeria* spp. in selected food, environmental surfaces and process water. The BAC*Gene Listeria* spp. detects *Listeria* species, including *L. monocytogenes*, *L. seeligeri, L. welshimeri, L. marthii, L. invanovii, L. grayi, L. innocua,* and *L. rocourtiae.*

DNA amplification and detection methods take advantage of the nucleotide sequence conservation found in bacterial genomes that ensures the potential for high specificity and sensitivity in detection of food-borne pathogenic bacteria. After enrichment, the microbial DNA is released by a simple thermal lysis step and rapidly analyzed by real-time PCR. In this way, *Listeria* can be detected in enrichment cultures of food products, process water and environmental samples with extraordinary high sensitivity. Using specific primers for *Listeria* spp., nucleotide sequences for *Listeria* species are amplified during PCR. The primers do not react with DNA derived from closely related species from the *Bacillales* order. The amplified fragments are detected with a R6G™ fluorescence-labeled hybridization probe quenched by non-fluorescent Tide Quencher™ 2 (TQ2). An internal positive control (IPC) is included in the MasterMix. IPC DNA is amplified in parallel and detected using a Cy5™ fluorescence-labeled hybridization probe, quenched by non-fluorescent Tide Quencher™ 3 (TQ3). IPC detection indicates the proper functioning of the PCR.

Brief description of the BACGene Listeria spp. method

The test portion is enriched in pre-warmed (37 ±1°C) Actero™ *Listeria* Enrichment media for 21±3 h. A 30 µL aliquot of enrichment is sampled and thermally and enzymatically lysed to release the DNA and 5 µL of the lysate is then analyzed by real-time PCR using either the CFX96 Touch™ Deep Well (CFX Deep Well) or the AriaMx instruments. Eurofins GeneScan GmbH has developed a specific PCR run file template for each of the PCR instruments and associated software platform. Once the PCR run is completed, the PCR data sets are exported to the BAC*Gene* evaluation spreadsheet with final interpretation of the results automatically performed. The laboratory analyst also has access to the amplification curves for the *Listeria* spp. specific target and the IPC.

The confirmation of presumptive positive PCR screening results is conducted by streaking 10 µL of enrichment onto O&A and PALCAM plates with 24-48 h of incubation at 37±1°C. Characteristic colonies presumed to be *Listeria* spp. are confirmed by either the tests described in the ISO 11290-1/A1 (2004) reference method or by API Listeria.

DISCUSSION OF THE VALIDATION STUDY (1)

In the Inclusivity and Exclusivity study, the BAC*Gene Listeria* spp. assay demonstrated 100% agreement with the expected results for both the inclusivity and exclusivity panels. Results were equal for the AriaMx and CFX Deep Well instruments.

The BAC*Gene Listeria* spp. method successfully recovered *Listeria* spp. from 7 food matrixes (mayonnaise based vegetable salad, soft white cheese, smoked salmon, raw milk, cantaloupe (frozen balls), Frankfurter sausages and frozen cooked shrimps), one process water and two environmental samples (stainless steel and ceramic tiles). Statistical analysis was conducted according to the Probability of Detection (POD) statistical model, and there was no statistically significant difference in the number of positive samples detected by the BAC*Gene Listeria* spp. methods and the reference method for all tested matrixes, except from frozen cantaloupe, where a significantly better detection for the BAC*Gene Listeria* spp. method over the ISO reference method was achieved. The AriaMx and CFX96 PCR instruments showed equivalent results. All samples enriched and assayed with the BAC*Gene Listeria* spp method were culturally confirmed by the BAC*Gene Listeria* confirmation procedures. Additionally, all presumptive negative samples were culturally confirmed negative by the reference method according to ISO 11290-1.

Robustness was evaluated by varying critical steps of the lysis and PCR procedures. These variations could happen by user error or due to equipment being out of calibration. The lysis incubation temperature was varied to 35°C and 39°C instead of 37°C and lysis incubation time was changed to 18 and 22 min instead of 20 min, which may be suboptimal for enzymatic digestion of *Listeria* cell walls. Furthermore, the amount of lysate was changed to 4.5 µL, which may lead to a lower amount of DNA at the fractional recovery level or to 5.5 µL, which might have an effect on PCR performance due to variations in the final concentrations of the reagents in the mix. Nevertheless, it is recommended to follow the BAC*Gene Listeria* spp method exactly by using calibrated equipment such as incubators, heating blocks or pipettes and to strictly follow the incubation times.

The product consistency study showed consistent and reproducible performance of all components for lysis and PCR for the BAC*Gene Listeria* spp. kit. Evaluation using the BAC*Gene* Evaluation sheet, which is based on defined criteria for C_q and dR last values, showed positive (and valid) results for all positive samples and negative valid results for all negative samples. Therefore, this study showed that the production process is standardized and reproducible.

Table 1: Inclusivity study results for BAC*Gene Listeria* spp. (1)

Strains						C_q values and final results (+/-)		
						CFU/mL enriched Actero	BAC*Gene Listeria spp*	
No.	Genus	Species	Source[1]	Molecular serotypes	Origin		CFX DeepWell	AriaMx
1	*Listeria*	*monocytogenes*	1011/1410	II a	Frozen broccoli	5.5×10^3	+(31.34)	+(29.82)
2	*Listeria*	*monocytogenes*	Adria 153	VI b	Soft cheese (Munster)	4.7×10^3	+(32.50)	+(30.37)
3	*Listeria*	*monocytogenes*	1973/2400	VI b	Egg and ham pastry (Quiche Lorraine)	3.4×10^3	+(32.39)	+(30.68)
4	*Listeria*	*monocytogenes*	38/181	II a	Toulouse sausages	3.0×10^3	+(31.01)	+(30.15)
5	*Listeria*	*monocytogenes*	7111/7516	IV b	Pâté (Rillettes)	7.1×10^3	+(31.54)	+(29.82)
6	*Listeria*	*monocytogenes*	913/1048	IV b	Black pudding	5.5×10^3	+(32.74)	+(29.77)
7	*Listeria*	*monocytogenes*	A00C036	II a	Poultry (guinea)	2.9×10^3	+(31.04)	+(30.15)
8	*Listeria*	*monocytogenes*	A00C041	L a	Sausage	9.6×10^3	+(35.21)	+(31.88)
9	*Listeria*	*monocytogenes*	A00C044	II b	Poultry (Duck)	4.0×10^3	+(33.32)	+(30.67)
10	*Listeria*	*monocytogenes*	A00L097	II a	Milk	2.9×10^3	+(34.08)	+(31.06)
11	*Listeria*	*monocytogenes*	A00M009	II a	Smoked salmon	8.6×10^2	+(33.71)	+(32.67)
12	*Listeria*	*monocytogenes*	Ad253	II b	Semi-hard cheese	1.2×10^2	+(34.80)	+(33.61)
13	*Listeria*	*monocytogenes*	Ad266	II a	Poultry	9.6×10^3	+(34.36)	+(31.96)
14	*Listeria*	*monocytogenes*	Ad270	IV b	Fermented sausage	2.9×10^3	+(32.61)	+(29.84)
15	*Listeria*	*monocytogenes*	Ad273	II b	Cured delicatessen	4.9×10^3	+(34.76)	+(31.87)
16	*Listeria*	*monocytogenes*	Ad274	II a	Ready-to-eat food (Asiatic meal)	8.3×10^3	+(34.57)	+(32.73)
17	*Listeria*	*monocytogenes*	Ad534	II b	Fruits	6.2×10^3	+(34.02)	+(31.84)
18	*Listeria*	*monocytogenes*	Ad548	II a	Environment (Seafood)	2.2×10^3	+(33.25)	+(30.77)
19	*Listeria*	*monocytogenes*	Ad623	II b	Bread crumbs	5.8×10^3	+(34.02)	+(31.23)
20	*Listeria*	*monocytogenes*	Ad665	II a	Raw milk	4.3×10^3	+(33.72)	+(31.82)
21	*Listeria*	*grayi*	Ad1198	/	Smoked salmon	6.8×10^3	+(37.28)	+(35.80)
22	*Listeria*	*grayi*	Ad1443	/	Pork meat	2.5×10^4	+(37.85)	+(33.20)

					sausages			
23	*Listeria*	*grayi*	Ad1295	/	Spinach	2.0×10^3	+(39.25)	+(36.37)
24	*Listeria*	*grayi*	Ad1490	/	Salmon terrine	6.0×10^3	+(34.62)	+(33.05)
25	*Listeria*	*grayi*	Ad2148	/	Pork rillettes	2.0×10^3	+(37.93)	+(38.72)
26	*Listeria*	*innocua*	Adria 1	/	Smoked salmon	4.0×10^3	+(34.30)	+(31.75)
27	*Listeria*	*innocua*	Ad658	/	Gorgonzola	7.1×10^3	+(33.39)	+(31.39)
28	*Listeria*	*innocua*	Ad655	/	Brine	7.6×10^3	+(33.44)	+(30.66)
29	*Listeria*	*innocua*	Ad660	/	Bread crumbs	4.1×10^3	+(33.24)	+(30.72)
30	*Listeria*	*innocua*	Ad663	/	Environment (dairy industry)	1.3×10^3	+(33.94)	+(31.61)
31	*Listeria*	*innocua*	Ad671	/	Smoked bacon	4.9×10^3	+(34.00)	+(31.98)
32	*Listeria*	*innocua*	Ad661	/	Soft cheese (Pont L'Evêque)	3.9×10^3	+(33.65)	+(31.13)
33	*Listeria*	*innocua*	Ad659	/	Environment (dairy industry)	2.6×10^3	+(33.22)	+(31.08)
34	*Listeria*	*ivanovii*	Ad466	/	Raw veal meat	4.1×10^3	+(36.88)	+(33.66)
35	*Listeria*	*ivanovii*	Ad662	/	Environment (dairy industry)	4.7×10^3	+(40.42)	+(37.91)
36	*Listeria*	*ivanovii*	BR11	/	Environment (fish)	5.3×10^4	+(28.81 (b)	+(27.00)
37	*Listeria*	*ivanovii*	Ad1289	/	Raw milk cheese	5.1×10^3	+(37.53)	+(35.21)
38	*Listeria*	*ivanovii*	Ad1290	/	Milk powder	7.1×10^3	+(33.17)	+(30.61)
39	*Listeria*	*ivanovii*	Ad1291	/	Poultry	4.8×10^4	+(33.11)	+(30.63)
40	*Listeria*	*ivanovii*	Ad1288	/	Sheep milk	5.3×10^4	+(34.91)	+(34.41)
41	*Listeria*	*ivanovii londoniensis*	CIP103466	/	unknown	6.7×10^4	+(37.21)	+(35.72)
42	*Listeria*	*seeligeri*	Ad649	/	Cheese	8.3×10^3	+(34.07)	+(31.07)
43	*Listeria*	*seeligeri*	Ad651	/	Environment	6.0×10^3	+(37.00)	+(33.92)
44	*Listeria*	*seeligeri*	Ad652	/	Environment (dairy industry)	4.9×10^3	+(36.55)	+(34.12)
45	*Listeria*	*seeligeri*	Ad674	/	Soft cheese (Munster)	5.1×10^3	+(35.52)	+(32.93)
46	*Listeria*	*seeligeri*	BR1	/	Trout	4.8×10^3	+(35.12)	+(33.10)
47	*Listeria*	*seeligeri*	BR18	/	Environment (fish)	8.3×10^3	+(35.29)	+(33.20)
48	*Listeria*	*seeligeri*	CIP100100	/	unknown	6.0×10^5	+(35.06)	+(33.73)
49	*Listeria*	*welshimeri*	Ad1276	/	Environment (Slaughterhouse)	3.8×10^2	+(35.68)	+(33.43)
50	*Listeria*	*welshimeri*	Ad1235	/	Beef meat	5.6×10^3	+(38.06)	+(33.55)
51	*Listeria*	*welshimeri*	191424	/	Poultry	4.1×10^3	+(37.74)	+(34.75)
52	*Listeria*	*welshimeri*	Ad1175	/	Ready-to-eat-food	3.4×10^3	+(30.70)	+(29.22)
53	*Listeria*	*welshimeri*	A 650	/	Poultry	4.7×10^3	+(36.31)	+(35.09)
54	*Listeria*	*marthii*	DSM 23813		Environment (flour)	4.2×10^3	+(32.13)	+(28.67)
55	*Listeria*	*rocourtiae*	DSM 22097		Vegetables (salad)	5.6×10^3	+(37.44)	+(34.57)

Note: [1]For strain source see under section "Sources for Reference and Test Cultures" on page 9. Strains from Strain Collection of Adria Developpement; / = Origin or serotype unknown; +(00.00)= positive result (C_q value); - = negative result

Table 2: Exclusivity study results for BAC*Gene Listeria* spp. (1)

No.	Strains				Inoculation level CFU/mL	C_q values and final results (+/-)	
						BAC*Gene Listeria* spp.	
	Genus	Species	Source[1]	Origin		CFX DeepWell	Aria Mx
1	*Bacillus*	*cereus*	Ad465	Salmon Terrine	2.6×10^5	-	-
2	*Bacillus*	*circulans*	Ad760	Vegetables	2.6×10^4	-	-
3	*Bacillus*	*coagulans*	Ad731	Dairy product	2.0×10^4	-	-
4	*Bacillus*	*licheniformis*	Ad978	Dairy product	1.0×10^5	-	-
5	*Bacillus*	*mycoïdes*	Ad762	Milk	4.4×10^5	-	-
6	*Bacillus*	*pseudomycoides*	Ad765	Vegetables	6.0×10^4	-	-
7	*Bacillus*	*pumilus*	Ad284	Ready-to-eat	5.0×10^5	-	-
8	*Bacillus*	*weihenstephanensis*	Ad726	Egg product	6.6×10^4	-	-
9	*Brochothrix*	*thermosphacta*	EN 15129	Trout	2.6×10^4	-	-
10	*Brochrotrix*	*campestris*	CIP 102920T	Environment	6.0×10^4	-	-
11	*Carnobacterium*	*divergens*	CIP 101029^T	unknown	3.0×10^4	-	-
12	*Carnobacterium*	*piscicola*	Ad369	Raw milk	9.2×10^4	-	-
13	*Enterococcus*	*durans*	Ad149	Ham	4.6×10^4	-	-
14	*Enterococcus*	*faecalis*	Adria 89L326	Soft cheese (Vacherin)	3.4×10^6	-	-
15	*Lactobacillus*	*brevis*	Adria 86L126	Ham	8.0×10^5	-	-
16	*Lactobacillus*	*curvatus*	Ad380	Delicatessen	5.0×10^5	-	-
17	*Lactobacillus*	*fermentum*	Ad482	Tomatoes juice	9.86×10^6	-	-
18	*Lactobacillus*	*sakei*	Ad473	Ham	4.2×10^4	-	-
19	*Lactococcus*	*lactis subsp cremoris*	Ad137	Dairy product	6.0×10^5	-	-
20	*Leuconostoc*	*carnosum*	Ad411	Ham	2.2×10^5	-	-
21	*Leuconostoc*	*citreum*	Ad396	Ham	4.8×10^4	-	-
22	*Micrococcus*	*luteus*	Ad432	Cocktail	5.0×10^5	-	-
23	*Pediococcus*	*pentosaceus*	ATCC 33316	unknown	8.28×10^5	-	-
24	*Propionibacterium*	*freundenreichii*	CNRZ 725	Dairy product	1.0×10^4	-	-
25	*Staphylococcus*	*aureus*	Ad165	Smoked delicatessen	1.20×10^5	-	-
26	*Staphylococcus*	*epidermidis*	Ad931	Fruits	2.0×10^4	-	-
27	*Staphylococcus*	*haemoliticus*	Ad989	Dairy product	8.0×10^4	-	-
28	*Streptococcus*	*bovis*	Adria 92L622	Dairy product	8.0×10^5	-	-
29	*Streptococcus*	*salivarius sps thermophilus*	Ad441	Dairy product	4.8×10^4	-	-
30	*Macrococcus*	*caseolyticus*	CIP100755	Milk	8.3×10^5	-	-

Note: [1]For strain source see under section “Sources for Reference and Test Cultures” on page 9. Strains from Strain Collection of Adria Developpement; / = Origin or serotype unknown; +(00.00)= positive result (C_q value);

Table 7a: POD statistics of candidate presumptive vs. confirmed results for BAC*Gene Listeria* spp. in AriaMx and CFX96™ Touch Deep Well (1)										
Matrix (APC[i])	Strain	MPN[b]/ test portion	N[a]	Instru-ment	Presumptive		Confirmed		$dPOD_{CP}$[f]	95% CI[g]
					X[c]	POD_{CP}[d] (95% CI[g])	X[c]	POD_{CC}[e] (95% CI[g])		
Vegetable salad (8.0×10^2 CFU/g)	*L. mono-cytogenes* Ad494	N/A	5	Aria	0	0.00 (0.00. 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00. 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
		1.04 (0.64, 1.76)	20	Aria	16	0.80 (0.58. 0.92)	16	0.80 (0.58, 0.92)	0.00	N/A
				CFX	16	0.80 (0.58. 0.92)	16	0.80 (0.58, 0.92)	0.00	N/A
		5.02 (2.18, 12.17)	5	Aria	5	1.00 (0.57. 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
				CFX	5	1.00 (0.57. 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
Frankfurter sausages (8.1×10^6 CFU/g)	*L. mono-cytogenes* Ad669	N/A	5	Aria	0	0.00 (0.00. 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00. 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
		1.14 (0.7, 1.93)	20	Aria	16	0.80 (0.58. 0.92)	16	0.80 (0.58, 0.92)	0.00	N/A
				CFX	16	0.80 (0.58. 0.92)	16	0.80 (0.58, 0.92)	0.00	N/A
		1.75 (0.66, 5.65)	5	Aria	4	0.80 (0.38. 1.00)	4	0.80 (0.38, 1.00)	0.00	N/A
				CFX	4	0.80 (0.38. 1.00)	4	0.80 (0.38, 1.00)	0.00	N/A
Frozen cantaloupe (2.0×10^6 CFU/g)	*Listeria seeligeri Ad1754*	N/A	5	Aria	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00. 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
		0.65 (0.37, 1.93)	20	Aria	18	0.90 (0.70, 0.97)	18	0.90 (0.70, 0.97)	0.00	N/A
				CFX	18	0.90 (0.70, 0.97)	18	0.90 (0.70, 0.97)	0.00	N/A
		2.5 (1.02, 9.4)	5	Aria	4	0.80 (0.38. 1.00)	4	0.80 (0.38, 1.00)	0.00	N/A
				CFX	4	0.80 (0.38. 1.00)	4	0.80 (0.38, 1.00)	0.00	N/A
Soft White Cheese (9.2×10^5 CFU/g)	*Listeria ivanovii* Ad1337	N/A	5	Aria	0	0.00 (0.00. 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00. 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
		1.2 (0.74,2.08)	20	Aria	12	0.60 (0.39, 0.78)	12	0.60 (0.39, 0.78)	0.00	N/A
				CFX	12	0.60 (0.39, 0.78)	12	0.60 (0.39, 0.78)	0.00	N/A
		3.42 (2.09, 6.92)	5	Aria	5	1.00 (0.57. 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
				CFX	5	1.00 (0.57. 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
Frozen Cooked Shrimp (1.6×10^3 CFU/g)	*Listeria innocua* Ad1200	N/A	5	Aria	0	0.00 (0.00. 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00. 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
		0.99 (0.61, 1.69)	20	Aria	12	0.60 (0.39, 0.78)	12	0.60 (0.39, 0.78)	0.00	N/A
				CFX	12	0.60 (0.39, 0.78)	12	0.60 (0.39, 0.78)	0.00	N/A
		1.64 (1.64, 1.64)	5	Aria	5	1.00 (0.57. 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
				CFX	5	1.00 (0.57. 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
Smoked Salmon (7.0×10^5 CFU/g)	*L. mono-cytogenes* Ad670	N/A	5	Aria	0	0.00 (0.00. 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00. 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A

		0.91 (0.64. 1.76)	20	Aria	16	0.80 (0.58, 0.92)	16	0.80 (0.58, 0.92)	0.00	N/A
				CFX	16	0.80 (0.58, 0.92)	16	0.80 (0.58, 0.92)	0.00	N/A
		7.34 (3.65, 16.76)	5	Aria	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
				CFX	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
Raw Milk (9.4×10^7 CFU/mL)	*L. mono-cytogenes* Ad618	N/A	5	Aria	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
		1.57 (1.01. 2.59)	20	Aria	19	0.95 (0.76, 1.00)	19	0.95 (0.76, 1.00)	0.00	N/A
				CFX	19	0.95 (0.76, 1.00)	19	0.95 (0.76, 1.00)	0.00	N/A
		5.02 (2.18.12.17)	5	Aria	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
				CFX	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
Process water (1.2×10^3 CFU/mL)	*L. mono-cytogenes* Ad551	N/A	5	Aria	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
		0.45 (0.24. 0.71)	20	Aria	13	0.65 (0.43. 0.82)	13	0.65 (0.43, 0.82)	0.00	N/A
				CFX	13	0.65 (0.43. 0.82)	13	0.65 (0.43, 0.82)	0.00	N/A
		1.64 (1.64. 1.64)	5	Aria	5	1.00 (0.57. 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
				CFX	5	1.00 (0.57. 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
Stainless Steel	*L. mono-cytogenes* Ad551	N/A	5	Aria	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
		N/A	20	Aria	15	0.75 (0.53, 0.89)	15	0.75 (0.53, 0.89)	0.00	N/A
				CFX	15	0.75 (0.53, 0.89)	15	0.75 (0.53, 0.89)	0.00	N/A
		N/A	5	Aria	5	1.00 (0.57. 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
				CFX	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
Ceramic tiles	*L. mono-cytogenes* Ad551	N/A	5	Aria	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
	Listeria innocua Ad653	N/A	20	Aria	16	0.8 (0.58, 0.92)	16	0.8 (0.58, 0.92)	0.00	N/A
				CFX	16	0.8 (0.58, 0.92)	16	0.8 (0.58, 0.92)	0.00	N/A
	E. faecalis Ad1350	N/A	5	Aria	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
				CFX	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A

[a]N = Number of test potions. [b]MPN = Most Probable Number with 95% confidence interval. [c]x = Number of positive test portions. [d]POD_{CP} = Probability of Detection of candidate presumptive results. [e]POD_{CC} = probability of detection for the candidate confirmed results. [f]$dPOD_{CP}$ = POD_{CP} minus POD_{CC}. [g]95% Confidence Interval. [h]N/A not applicable. [i]APC = Aerobic Plate Counts

Table 7b: POD statistics of confirmed candidate vs. reference method results for BAC*Gene Listeria* spp. in AriaMx and CFX96™ Touch Deep Well (1)

Matrix	Strain	MPN[b]/ test portion	N[a]	Instrument	Confirmed candidate		Reference		dPOD$_c$[j]	95% CI[g]
					X[c]	POD$_c$[h] (95% CI[g])	X[c]	POD$_R$[k] (95% CI[g])		
Mayonnaise based vegetable salad	*L. mono-cytogenes* Ad494	N/A	5	Aria	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
		1.04 (0.64. 1.76)	20	Aria	16	0.80 (0.58, 0.92)	14	0.70 (0.48, 0.85)	0.10	(-0.17. 0.35)
				CFX	16	0.80 (0.58, 0.92)	14	0.70 (0.48, 0.85)	0.10	(-0.17. 0.35)
		5.02 (2.18. 12.17)	5	Aria	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
				CFX	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
Frankfurter sausages	*L. mono-cytogenes* Ad669	N/A	5	Aria	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
		1.1 (0.71. 1.93)	20	Aria	16	0.80 (0.58, 0.92)	15	0.75 (0.53, 0.89)	0.05	(-0.21. 0.30)
				CFX	16	0.80 (0.58, 0.92)	15	0.75 (0.53, 0.89)	0.05	(-0.21. 0.30)
		1.75 (0.66. 5.65)	5	Aria	4	0.80 (0.38, 1.00)	4	0.80 (0.38. 1.00)	0.00	N/A
				CFX	4	0.80 (0.38, 1.00)	4	0.8 (0.38. 1.00)	0.00	N/A
Frozen cantaloupe	*Listeria seeligeri Ad1754*	N/A	5	Aria	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
		1.09 (0.67. 1.76)	20	Aria	18	0.90 (0.70, 0.97)	11	0.55 (0.34, 0.74)	0.35	(0.07. 0.57)
				CFX	18	0.90 (0.70, 0.97)	11	0.55 (0.34, 0.74)	0.35	(0.07. 0.57)
		11.86 (5.02. 40.25)	5	Aria	4	0.8 (0.38, 1.00)	4	0.8 (0.38, 1.00)	0.00	N/A
				CFX	4	0.8 (0.38, 1.00)	4	0.8 (0.38, 1.00)	0.00	N/A
Soft White Cheese	*Listeria ivanovii* Ad1337	N/A	5	Aria	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
		1.2 (0.74. 2.08)	20	Aria	12	0.60 (0.39, 0.78)	14	0.70 (0.48, 0.85)	-0.10	(-0.36. 0.18)
				CFX	12	0.60 (0.39, 0.78)	14	0.70 (0.48, 0.85)	-0.10	(-0.36. 0.18)
		3.42 (2.09. 6.92)	5	Aria	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
				CFX	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
Frozen Cooked Shrimp	*Listeria innocua* Ad1200	N/A	5	Aria	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
		0.99 (0.61. 1.69)	20	Aria	12	0.60 (0.39, 0.78)	15	0.75 (0.53, 0.89)	-0.15	(-0.40. 0.13)
				CFX	12	0.60 (0.39, 0.78)	15	0.75 (0.53. 0.89)	-0.15	(-0.40. 0.13)
		1.64 (1.64. 1.64)	5	Aria	5	1.00 (0.57. 1.00)	5	1.00 (0.57. 1.00)	0.00	N/A
				CFX	5	1.00 (0.57. 1.00)	5	1.00 (0.57. 1.00)	0.00	N/A
Smoked Salmon	*L. mono-cytogenes* Ad670	N/A	5	Aria	0	0.00 (0.00. 0.43)	0	0.00 (0.00. 0.43)	0.00	N/A
				CFX	0	0.00 (0.00. 0.43)	0	0.00 (0.00. 0.43)	0.00	N/A

		0.91 (0.64. 1.76)	20	Aria	16	0.80 (0.58, 0.92)	14	0.70 (0.48, 0.85)	0.10	(-0.17, 0.35)
				CFX	16	0.80 (0.58, 0.92)	14	0.70 (0.48, 0.85)	0.10	(-0.17, 0.35)
		7.34 (3.65. 16.76)	5	Aria	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
				CFX	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
Raw Milk	*L. mono-cytogenes* Ad618	N/A	5	Aria	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
		1.57 (1.01. 2.59)	20	Aria	19	0.95 (0.76, 1.00)	15	0.75 (0.53, 0.89)	0.20	(-0.03, 0.42)
				CFX	19	0.95 (0.76, 1.00)	15	0.75 (0.53, 0.89)	0.20	(-0.03, 0.42)
		5.02 (2.18.12.2)	5	Aria	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
				CFX	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
Process water	*L. mono-cytogenes* Ad551	N/A	5	Aria	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
		0.45 (0.24. 0.71)	20	Aria	13	0.65 (0.43, 0.82)	9	0.45 (0.26, 0.66)	0.20	(-0.10, 0.46)
				CFX	13	0.65 (0.43, 0.82)	9	0.45 (0.26, 0.66)	0.20	(-0.10, 0.46)
		1.64 (1.64. 1.64)	5	Aria	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
				CFX	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
Stainless Steel	*L. mono-cytogenes* Ad551	N/A	5	Aria	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
		N/A	20	Aria	15	0.75 (0.53, 0.89)	18	0.90 (0.70, 0.97)	-0.15	(-0.38. 0.09)
				CFX	15	0.75 (0.53, 0.89)	18	0.90 (0.70, 0.97)	-0.15	(-0.38. 0.09)
		N/A	5	Aria	5	1.00 (0.57. 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
				CFX	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
Ceramic tiles	*L. mono-cytogenes* Ad551	N/A	5	Aria	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
				CFX	0	0.00 (0.00, 0.43)	0	0.00 (0.00, 0.43)	0.00	N/A
	Listeria innocua Ad653	N/A	20	Aria	16	0.80 (0.58, 0.92)	13	0.65 (0.43, 0.82)	0.15	(-0.12. 0.40)
				CFX	16	0.80 (0.58, 0.92)	13	0.65 (0.43, 0.82)	0.15	(-0.12. 0.40)
	E. faecalis Ad1350	N/A	5	Aria	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A
				CFX	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00	N/A

Note: [a]N = Number of test potions, [b]MPN = Most Probable Number with 95% confidence interval, [c]x = Number of positive test portions, [g]95% Confidence Interval, [h]POD_C = probability of detection for the confirmed candidate results, [k]POD_R = Probability of Detection of reference method results, [j]$dPOD_C$ = POD_C minus POD, N/A not applicable

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