CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

061703

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

BAC*Gene Listeria monocytogenes*

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 05, 2019 – December 31, 2019). 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

January 05, 2019
Date

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Eurofins GeneScan GmbH, now Eurofins GeneScan Technologies GmbH
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KIT NAME(S)
BAC*Gene Listeria monocytogenes*

CATALOG NUMBERS
5123222001 (96 rxn) and 5123222010 (10 x 96 rxn)

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APPLICABILITY OF METHOD
Target organisms – *Listeria monocytogenes*

Matrices – mayonnaise-based vegetable salad (25 g), frankfurters (25 g), raw whole milk (25 g), frozen cantaloupe balls (25 g), smoked salmon (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
Renewed annually through December 2019

METHOD MODIFICATION RECORD
1. **January 2018 Level 1**
2. **January 2019 Level 2**

SUMMARY OF MODIFICATION
1. **Name change to Eurofins GeneScan Technologies GmbH and editorial changes**
2. **Long-term storage changed from 12 to 24 months.**

Under this AOAC® *Performance Tested*[SM] License Number, 061703 this method is distributed by:
NONE

Under this AOAC® *Performance Tested*[SM] License Number, 061703 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)

Introduction to the BACGene Listeria monocytogenes method

The BAC*Gene Listeria monocytogenes* method is a qualitative real-time PCR assay for the detection of *Listeria monocytogenes* in selected food, environmental surfaces and process water. The BAC*Gene Listeria monocytogenes* kit specifically detects *L. monocytogenes* only.

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 *L. monocytogenes*, nucleotide sequences for *L. monocytogenes* are amplified during PCR. The primers do not react with DNA derived from closely related species from the *Bacillales* order or with *Listeria* species other than *L. monocytogenes*. 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 monocytogenes 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 monocytogenes* 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 *L. monocytogenes* 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 monocytogenes* 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 monocytogenes* assay successfully recovered *Listeria monocytogenes* from 5 food matrices (mayonnaise based vegetable salad, smoked salmon, raw milk, cantaloupe (frozen balls) and Frankfurter sausages), 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 monocytogenes* method and the reference method for all tested matrixes, except for detection of *L. monocytogenes* on ceramic tile, where the BAC*Gene L. monocytogenes* method recovered significantly more positives than the reference method at the low level of inoculation.

The AriaMx and CFX96 PCR instruments showed equivalent overall results with a minor difference in raw milk. One false negative result was observed for *L. monocytogenes* detection on the CFX. C_q values for this sample were very high (negative on the CFX and 36.55 on the AriaMx) and a cultural confirmation result was obtained only after enrichment in Fraser broth for 48h. This leads to the assumption that a very low amount of DNA was present in the sample, which might be due to a very low level of contamination.

For the matrix study, all samples enriched and assayed with the BAC*Gene Listeria* methods 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 of the BAC*Gene Listeria monocytogenes* method 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* 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 monocytogenes* 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 3: Inclusivity study results for BAC*Gene Listeria monocytogenes* (1)

Strains						CFU/mL enriched Actero	C_q values and final results (+/-)	
							BAC*Gene Listeria monocytogenes*	
No.	Genus	Species	Source[1]	Molecular serotypes	Origin		CFX DeepWell	AriaMx
1	*Listeria*	*monocytogenes*	Adria 153	VI b	Soft cheese (Munster)	4.7×10^3	+(37.04)	+(32.81)
2	*Listeria*	*monocytogenes*	1011/1410	II a	Frozen broccoli	5.5×10^3	+(36.10)	+(33.55)
3	*Listeria*	*monocytogenes*	1972/2399	VI b	Puff pastry with mushrooms	4.4×10^3	+(36.03)	+(32.37)
4	*Listeria*	*monocytogenes*	1973/2400	VI b	Puff pastry egg and ham (Quiche-lorraine)	3.4×10^3	+(38.20)	+(33.78)
5	*Listeria*	*monocytogenes*	2407/3139	IV b	Tripes with tomatoes	2.5×10^3	+(39.32)	+(34.10)
6	*Listeria*	*monocytogenes*	2760/3145	II a	Raw bacon	4.4×10^3	+(36.29)	+(34.93)
7	*Listeria*	*monocytogenes*	32.183	II b	Croque-Monsieur	4.7×10^3	+(38.77)	+(35.07)
8	*Listeria*	*monocytogenes*	38/181	II a	Toulouse sausages	3.0×10^3	+(37.86)	+(33.56)
9	*Listeria*	*monocytogenes*	5721/6179	IV b	Smoked bacon	3.1×10^3	+(37.40)	+(34.26)
10	*Listeria*	*monocytogenes*	7111/7516	IV b	Pâté (Rillettes)	7.1×10^3	+(37.02)	+(33.21)
11	*Listeria*	*monocytogenes*	850/109	II a	RTE food (deli salad with seafood)	1.6×10^3	+(35.96)	+(33.34)
12	*Listeria*	*monocytogenes*	877/113	II a	Environmental sample (pastry)	4.7×10^3	+(38.25)	+(34.28)
13	*Listeria*	*monocytogenes*	913/1048	IV b	Black pudding	5.5×10^3	+(35.94)	+(34.13)
14	*Listeria*	*monocytogenes*	A00C014	II a	Sausage	6.7×10^3	+(37.37)	+(32.80)
15	*Listeria*	*monocytogenes*	A00C022	II a	Merguez	7.3×10^3	+(36.87)	+(32.53)
16	*Listeria*	*monocytogenes*	A00C024	II a	Sausage	4.0×10^3	+(37.62)	+(33.69)

17	*Listeria*	*monocytogenes*	A00C036	II a	Poultry (guinea)	2.9×10^3	+(37.19)	+(34.64)
18	*Listeria*	*monocytogenes*	A00C039	II a	Sausages	4.2×10^3	+(37.36)	+(33.48)
19	*Listeria*	*monocytogenes*	A00C040	IV b	Cooked delicatessen (Museau)	9.3×10^3	+(36.77)	+(33.66)
20	*Listeria*	*monocytogenes*	A00C041	La	Sausage	9.6×10^3	+(37.73)	+(35.75)
21	*Listeria*	*monocytogenes*	A00C042	IV b	Raw sausage	3.6×10^3	+(36.57)	+(33.27)
22	*Listeria*	*monocytogenes*	A00C043	II a	Smoked Bacon	3.6×10^3	+(36.88)	+(32.80)
23	*Listeria*	*monocytogenes*	A00C044	II b	Poultry (duck)	4.0×10^3	+(37.89)	+(34.56)
24	*Listeria*	*monocytogenes*	A00C052	II b	RTE food (Osso bucco with turkey)	8.3×10^3	+(36.04)	+(32.88)
25	*Listeria*	*monocytogenes*	A00C053	II a	Gizzards	3.2×10^3	+(36.94)	+(34.21)
26	*Listeria*	*monocytogenes*	A00C054	IV b	Beef hart	7.9×10^3	+(35.78)	+(33.27)
27	*Listeria*	*monocytogenes*	A00C055	II a	Raw sausages	1.8×10^3	+(38.27)	+(33.96)
28	*Listeria*	*monocytogenes*	A00E008	II a	Environmental sample	1.4×10^3	+(38.14)	+(35.46)
29	*Listeria*	*monocytogenes*	A00E049	II a	Environmental sample (samoked salmon)	6.0×10^3	+(36.85)	+(33.90)
30	*Listeria*	*monocytogenes*	A00E082	II a	Environmental sample (smoked salmon)	3.8×10^3	+(37.10)	+(33.13)
31	*Listeria*	*monocytogenes*	A00L097	II a	Milk	2.9×10^3	+(39.23)	+(34.38)
32	*Listeria*	*monocytogenes*	A00M009	II a	Smoked salmon	8.6×10^2	+(39.05)	+(36.98)
33	*Listeria*	*monocytogenes*	A00M032	IV b	Smoked salmon	4.6×10^3	+(35.59)	+(32.78)
34	*Listeria*	*monocytogenes*	A00M045	II a	Smoked salmon	6.3×10^3	+(36.26)	+(32.72)
35	*Listeria*	*monocytogenes*	A00M088	II a	Smoked salmon	4.4×10^2	+(39.03)	+(36.69)
36	*Listeria*	*monocytogenes*	Ad235	II b	Poultry	1.4×10^3	+(37.70)	+(33.22)
37	*Listeria*	*monocytogenes*	Ad253	II b	Hard cheese	1.2×10^2	+(39.25)	-(noCq)
38	*Listeria*	*monocytogenes*	Ad260	II a	Semi hard cheese	2.2×10^3	+(37.48)	+(35.25)
39	*Listeria*	*monocytogenes*	Ad265	II b	Tong	3.7×10^3	+(37.72)	+(33.41)
40	*Listeria*	*monocytogenes*	Ad266	II a	Poultry	9.6×10^3	+(38.81)	+(36.60)
41	*Listeria*	*monocytogenes*	Ad267	II b	Dry sausage	1.7×10^3	+(40.88)	+(36.72)
42	*Listeria*	*monocytogenes*	Ad268	IV b	Cured ham	5.4×10^4	+(36.89)	+(34.30)
43	*Listeria*	*monocytogenes*	Ad270	IV b	Fermented sausage	2.9×10^3	+(36.14)	+(32.84)
44	*Listeria*	*monocytogenes*	Ad272	IV b	Fermented sausage	5.3×10^3	+(38.36)	+(34.45)
45	*Listeria*	*monocytogenes*	Ad273	II b	Cured delicatessen	4.9×10^3	+(37.74)	+(34.43)
46	*Listeria*	*monocytogenes*	Ad274	II a	Ready-to-eat food (Asiatic meal)	8.3×10^3	+(38.26)	+(36.10)
47	*Listeria*	*monocytogenes*	Ad534	II b	Fruits	6.2×10^3	+(37.87)	+(33.41)
48	*Listeria*	*monocytogenes*	Ad544	II a	Onion	6.3×10^3	+(37.98)	+(32.53)
49	*Listeria*	*monocytogenes*	Ad546	II a	Flour	4.4×10^3	+(40.10)	+(35.43)
50	*Listeria*	*monocytogenes*	Ad623	II b	Bread crumbs	5.8×10^3	+(39.06)	+(28.44)

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 4: Exclusivity study results for BAC*Gene Listeria monocytogenes* (1)							
No.	Strains				Inoculation level CFU/mL BPW	C_q values and final results (+/-)	
	Genus	Species	Source[1]	Origin		BAC*Gene Listeria monocytogenes*	
						CFX DeepWell	AriaMx
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	$1.0 \times .10^5$	-	-
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	9.9×10^6	-	-
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	8.3×10^5	-	-
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	-	-
31	*Listeria*	*grayi*	Ad1198	Smoked salmon	1.2×10^4	-	-
32	*Listeria*	*grayi*	Ad1443	Pork meat sausages	1.0×10^5	-	-

33	*Listeria*	*innocua*	1	Smoked salmon	8.6×10^4	-	-
34	*Listeria*	*innocua*	Ad658	Gorgonzola	3.2×10^5	-	-
35	*Listeria*	*ivanovii*	Ad466	Raw veal meat	7.4×10^4	-	-
36	*Listeria*	*ivanovii*	Ad662	Environment (dairy industry)	1.8×10^5	-	-
37	*Listeria*	*seeligeri*	Ad649	Cheese	1.2×10^5	-	-
38	*Listeria*	*seeligeri*	BR1	Trout	8.0×10^4	-	-
39	*Listeria*	*welshimeri*	Ad1276	Environ-ment (Slaughterhouse)	1.2×10^5	-	-
40	*Listeria*	*welshimeri*	Ad1175	Ready-to-eat-food	2.2×10^5	-	-
41	*Listeria*	*marthii*	DSM 23813T	Environment (flour)	3.78×10^5	-	-
42	*Listeria*	*rocourtiae*	DSM 22097T	Vegetables (salad)	5.11×10^5	-	-

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); - = negative result

Table 9a: POD statistics of candidate presumptive vs. confirmed results and of candidate confirmed versus reference method results of BAC*Gene Listeria monocytogenes* in AriaMx and CFX96™ Touch Deep Well (1)

Matrix (APC[i])	Strain	MPN/test portion	N[a]	Instru-ment	Presumptive		Confirmed		dPOD$_{CP}$[d]	95% CI[d]
					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 (0.71, 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
Cantaloupe (2.0×10^5 cfu/g)	*L. mono-cytogenes* Ad532	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	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
		11.86 (5.02, 40.25)	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	18	0.90 (0.70, 0.97)	19	0.95 (0.76, 1.00)	-0.05	(-0.15, 0.05)
		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
Smoked Salmon (7.0 x 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
Process water (1.2 x 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. monocytogenes* 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	6	0.30 (0,15, 0,52)	7	0.35 (0,18, 0,57)	-0.05	(-0.15, 0.05)
				CFX	6	0.30 (0,15, 0,52)	7	0.35 (0,18, 0,57)	-0.05	(-0.15, 0.05)
	E. faecalis Ad1350 (background flora)	N/A	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

[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 9b: POD statistics of confirmed candidate vs. reference method results for BAC*Gene Listeria monocytogenes* in AriaMx and CFX96™ Touch Deep Well (1)

Matrix	Strain	MPN[b]/ test portion	N[a]	Instru-ment	Confirmed candidate method		Reference method		dPOD$_{(c)}$[j]	95% CI[g]
					X[c]	POD$_c$[h] (95% CI[g])	X[c]	POD$_R$[k] (95% CI[g])		
Vegetable salad (8.0 x 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)	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 (8.1 x 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 (0.71, 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	0.8 (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 (2.0 x 10^5 cfu/g)	*L. mono-cytogenes* Ad532	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	15	0.75 (0.53. 0.89)	13	0.65 (0.43. 0.82)	0.10	(-0.18, 0.36)
				CFX	15	0.75 (0.53. 0.89)	13	0.65 (0.43. 0.82)	0.10	(-0.18, 0.36)
		11.86 (5.02, 40.25)	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 x 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)	15	0.75 (0.53, 0.89)	0.20	(-0.03, 0.42)
				CFX	18	0.90 (0.70. 0.97)	15	0.75 (0.53, 0.89)	0.15	(-0.09, 0.38)
		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
Smoked Salmon (7.0 x 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)	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
Process water	*L. mono-cytogenes*	N/A	5	Aria	0	0.00 (0.00. 0.43)	0	0.00 (0.00. 0.43)	0.00	N/A

	Ad551			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	(-0.1, 0.46)
				CFX	13	0.65 (0.43. 0.82)	9	0.45 (0.26, 0.66)	0.20	(-0.1, 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	6	0.30 (0.15, 0.52)	1	0.05 (0.00, 0.24)	0.25	(0.01, 0.47)
				CFX	6	0.30 (0.15, 0.52)	1	0.05 (0.00, 0.24)	0.25	(0.01, 0.47)
	E. faecalis Ad1350	N/A	5	Aria	4	0.80 (0.38, 1.00)	3	0.6 (0.23, 0.88)	0.20	(-0.31, 0.62)
				CFX	4	0.80 (0.38, 1.00)	3	0.6 (0.23, 0.88)	0.20	(-0.31, 0.62)

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