



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

051703

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

BACSpec Listeria

manufactured by

Eurofins Technologies Hungary Kft.

1047 Budapest

Fóti út 56. A. ép.

Hungary

This method has been evaluated in the AOAC[®] *Performance Tested MethodsSM* 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 TestedSM* 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 15, 2020 – December 31, 2020). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

A handwritten signature in black ink that reads "Scott Coates".

Scott Coates, Senior Director
Signature for AOAC Research Institute

January 15, 2020

Date

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KIT NAME(S)BACSpec *Listeria***CATALOG NUMBERS**

4323410201, 4323410205

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APPLICABILITY OF METHOD

Target organism – *Listeria* spp. (including *L. monocytogenes*, *L. seeligeri*, *L. welshimeri*, *L. marthii*, *L. invanovii*, *L. grayi*, *L. innocua*, and *L. rocourtia*)

Matrices – (25 g) - vegetable salad, frozen cantaloupe, soft white cheese, raw whole milk, Frankfurter sausages, process water, smoked salmon, frozen cooked shrimp
 stainless steel (1 x 1 in), sealed ceramic tile (4 x 4 in)

Performance claims – Performance equivalent to reference method for the food matrixes and environmental surfaces tested.

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 (3)

ORIGINAL CERTIFICATION DATE

May 12, 2017

CERTIFICATION RENEWAL RECORD

Renewed annually through December 2020

METHOD MODIFICATION RECORD

1. January 2019 Level 2
2. January 2020 Level 2

SUMMARY OF MODIFICATION

1. Long-term stability change from 12 to 24 months
2. Manufacturing locations: addition of Budapest, Hungary to compliment Freiburg, Germany location

Under this AOAC® *Performance Tested*SM License Number, 051703 this method is distributed by:
 NONE

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PRINCIPLE OF THE METHOD (1)

Introduction to the BACSpec Listeria method

The BACSpec Listeria method is a qualitative sandwich ELISA (Enzyme-linked Immunosorbent Assay) for the detection of the flagella antigen of *Listeria* spp. in selected foodstuffs, process water and environmental samples. Utilizing antigen-antibody-binding and enzyme catalyzed color reactions. The assay ensures the potential for high specificity and sensitivity in detection of *Listeria* with flagella. Samples are first enriched in Half Fraser broth followed secondary enrichment in Eurofins Listeria Enrichment Broth (ELEB) to resuscitate any injured *Listeria* cells and support their subsequent growth. After completion of the enrichment steps, an aliquot is removed and heat-inactivated. These sample preparations are then analyzed by ELISA.

BACSpec Listeria ELISA detects specifically the flagella protein of *Listeria* (*Listeria* antigen), which is produced by the organism. There are two types of ELISA: Sandwich and Competitive. For detection of large molecules like the protein-antigens from *Listeria* flagella, the principle of sandwich ELISA is applied. The method BACSpec Listeria is carried out on a 96-well microtiter plate (MTP). In each well of the MTP antibodies against *Listeria* antigens are immobilized. If samples containing *Listeria* antigens are added into the wells, the antibodies recognize and capture the antigens. After a washing step an antibody-peroxidase conjugate is added which binds to the antigen and is subsequently captured by the immobilized antibodies on the wells of the MTP. After a second washing step, a substrate (TMB) solution for peroxidase is added. The peroxidase then catalyzes a reaction, which generates a blue color in the substrate solution. After incubation the reaction is stopped and the color turns yellow. The optical density (OD) of the yellow-colored solution can be measured with a microplate reader (Fig 1).

The OD is proportional to the concentration of *Listeria* antigen in the sample. A threshold is defined to evaluate the results. Samples with OD signals above the threshold will be designated as positive.

Brief description of the BACSpec Listeria method

The test portion is first enriched in Half Fraser medium for 24±2 h at 30±1°C. Thereafter, 0.2 mL of the Half Fraser medium is transferred into 10 mL of Eurofins Listeria Enrichment Broth (ELEB) and incubated for 24±2 h at 30±1°C. After the second enrichment step, aliquots of 0.5-1.0 mL are heat inactivated at 85-100°C, cooled and subjected to ELISA.

The ELISA plate has 96 wells. Since three wells are needed for the Blank, NC and PC, each plate can be used for a maximum of 93 samples. The procedure is described as follows:

- Pipette 100 µl of NC, PC and all samples into the wells. The well for the Blank is left empty.
- Incubate the plate for 30-35 min at 37±1°C
- Wash the wells with washing buffer 5-7 times.
- Add 100 µL of conjugate to the wells. The well for Blank is left empty.
- Incubate the plate for 30-35 min at 37±1°C
- Wash the wells with washing buffer 5-7 times.
- Add 100 µL of substrate to all wells.
- Incubate the plate for 30±1 min at room temperature (20-25°C).
- Add 100 µL of stop solution to all wells
- Take the OD measurement at 450 nm
- Subtract the Blank from the OD values for NC, PC and all samples.
- Evaluate the results.

Confirmation of presumptive positive ELISA results is conducted by streaking 10 µL of ELEB onto two selective media - Ottiviani and Agosti (O&A) and PALCAM, followed by 24-48 h of incubation at 37±1°C. Presumptive *Listeria* colonies are further confirmed by either the tests described in the reference method (ISO 11290-1) or a biochemical gallery directly without a further purification step.

Certification by AFNOR according to ISO 16140

The method was also certified according to ISO 16140-2 (2016) by AFNOR (Reference No. EGS38/04-01/17) for the following food categories: meat and meat products; milk and dairy products; vegetables; fish and seafood; composite foods and environmental samples.

DISCUSSION OF THE VALIDATION STUDY (1)

BACSpec Listeria is a Sandwich-ELISA-based method used for the detection of *Listeria* in various food and environmental samples. This report presents the AOAC Research Institute Performance Tested MethodsSM validation study according to the current AOAC validation guidelines. After a two-step enrichment in Half Fraser Broth and Eurofins Listeria Enrichment Broth (ELEB), an aliquot of the secondary enrichment in ELEB is heat-inactivated and then tested by Sandwich-ELISA for the presence of *Listeria* including *L. monocytogenes*, *L. seeligeri*, *L. welshimeri*, *L. innocua*, *L. marthii*, *L. invanovii*, *L. grayi*, and *L. rocourtia*. The study consists of an inclusivity/exclusivity study and a method comparison study for seven food matrixes (mayonnaise-based vegetable salad, frankfurters, raw whole milk, soft white cheese, frozen cantaloupe balls, smoked salmon, and frozen shrimp), one process water (from a vegetable sausage production site), and two environmental surfaces (stainless steel 304L and sealed ceramic tile). The method comparison was conducted using a paired study design for all matrixes. The screening results were confirmed using EN ISO 11290-1 and using Ottiviani and Agosti (O&A) and PALCAM plates by streaking 10 µL of the secondary enrichment (ELEB) onto the plates with subsequent incubation at 37±1°C for 24-48 hours. Presumptive colonies were then identified by either the tests described in the reference method or by using a biochemical gallery directly on a colony without a purification step. The performance of the candidate method was compared to EN 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. For the inclusivity, all 87 of the *Listeria* spp. organisms tested were correctly identified. For the exclusivity, all 30 of the non-*Listeria* organisms were correctly called negative. For the matrix study, all samples enriched and tested with the BACSpec Listeria methods were culturally confirmed by the BACSpec Listeria confirmation procedures according to ISO 11290-1/A1 (2004). Statistical analysis was conducted according to the Probability of Detection (POD) statistical model and there were no statistically significant differences in the number of positive samples detected by the BACSpec Listeria and the reference method for all 10 matrixes. Additionally, this report includes a ruggedness study and a lot-to-lot comparison study. The ruggedness study showed no significant impact of the variation of critical parameters, indicating that the BACSpec Listeria method is robust under the study conditions. The lot-to-lot comparison study demonstrated that the production at Eurofins GeneScan GmbH can be realized in a standardized fashion, and the BACSpec Listeria kit gave a constant performance over three independently produced lots.

Table 1: Inclusivity study results for BACSpec Listeria (1)

Strains							BACSpec Listeria			
No.	Genus	Species	Source ^a	Molecular serotypes	Origin	CFU/mL enriched ELEB	ELISA		Confirmation	
							O.D.	Result	O&A	PALCAM
1	Listeria	monocytogenes	Adria 153	VI b	Soft cheese (Munster)	8.3 x 10 ⁶	2.181	+ ^b	H+ ^c	+
2	Listeria	monocytogenes	1011/1410	II a	Frozen broccoli	4.2 x 10 ⁶	2.155	+	H+	+
3	Listeria	monocytogenes	1972/2399	VI b	Puff pastry with mushrooms	6.3 x 10 ⁶	1.502	+	H+	+
4	Listeria	monocytogenes	1973/2400	VI b	Puff pastry egg and ham (Quiche-lorraine)	3.6 x 10 ⁶	1.703	+	H+	+
5	Listeria	monocytogenes	2407/3139	IV b	Tripes with tomatoes	6.7 x 10 ⁶	1.420	+	H+	+
6	Listeria	monocytogenes	2760/3145	II a	Raw bacon	5.1 x 10 ⁶	1.520	+	H+	+
7	Listeria	monocytogenes	32.183	II b	Croque-Monsieur	5.1 x 10 ⁶	2.275	+	H+	+
8	Listeria	monocytogenes	38/181	II a	Toulouse sausages	5.6 x 10 ⁶	1.205	+	H+	+
9	Listeria	monocytogenes	5721/6179	IV b	Smoked bacon	7.1 x 10 ⁶	1.135	+	H+	+
10	Listeria	monocytogenes	7111/7516	IV b	Pâté (Rillettes)	5.6 x 10 ⁶	1.667	+	H+	+
11	Listeria	monocytogenes	850/109	II a	Ready-to-eat food (deli salad with seafood)	4.4 x 10 ⁶	1.454	+	H+	+
12	Listeria	monocytogenes	877/113	II a	Environmental sample (pastry)	4.9 x 10 ⁶	1.667	+	H+	+
13	Listeria	monocytogenes	913/1048	IV b	Black pudding	7.5 x 10 ⁶	1.778	+	H+	+
14	Listeria	monocytogenes	A00C014	II a	Sausage	4.7 x 10 ⁶	1.002	+	H+	+
15	Listeria	monocytogenes	A00C022	II a	Merguez	5.8 x 10 ⁶	1.392	+	H+	+
16	Listeria	monocytogenes	A00C024	II a	Sausage	4.9 x 10 ⁶	2.060	+	H+	+
17	Listeria	monocytogenes	A00C036	II a	Poultry (guinea)	8.3 x 10 ⁶	1.446	+	H+	+
18	Listeria	monocytogenes	A00C039	II a	Sausages	6.7 x 10 ⁶	1.339	+	H+	+
19	Listeria	monocytogenes	A00C040	IV b	Cooked delicatessen (Museau)	7.9 x 10 ⁶	1.650	+	H+	+
20	Listeria	monocytogenes	A00C041	La	Sausage	1.0 x 10 ⁷	1.432	+	H+	+
21	Listeria	monocytogenes	A00C042	IV b	Raw sausage	1.2 x 10 ⁷	1.664	+	H+	+
22	Listeria	monocytogenes	A00C043	II a	Smoked bacon	8.3 x 10 ⁶	1.374	+	H+	+
23	Listeria	monocytogenes	A00C044	II b	Poultry (duck)	6.7 x 10 ⁶	1.950	+	H+	+
24	Listeria	monocytogenes	A00C052	II b	Ready-to-eat food (Osso bucco with turkey)	6.7 x 10 ⁶	2.239	+	H+	+
25	Listeria	monocytogenes	A00C053	II a	Gizzards	1.2 x 10 ⁷	0.639	+	H+	+
26	Listeria	monocytogenes	A00C054	IV b	Beef heart	4.7 x 10 ⁶	1.491	+	H+	+
27	Listeria	monocytogenes	A00C055	II a	Raw sausages	7.9 x 10 ⁶	1.763	+	H+	+
28	Listeria	monocytogenes	A00E008	II a	Environmental sample	8.3 x 10 ⁶	1.290	+	H+	+
29	Listeria	monocytogenes	A00E049	II a	Environmental sample (samoked salmon)	7.1 x 10 ⁶	0.867	+	H+	+
30	Listeria	monocytogenes	A00E082	II a	Environmental sample (smoked salmon)	7.1 x 10 ⁶	1.685	+	H+	+
31	Listeria	monocytogenes	A00L097	II a	Milk	8.7 x 10 ⁶	1.666	+	H+	+
32	Listeria	monocytogenes	A00M009	II a	Smoked salmon	6.7 x 10 ⁶	1.489	+	H+	+
33	Listeria	monocytogenes	A00M032	IV b	Smoked salmon	5.1 x 10 ⁶	1.453	+	H+	+
34	Listeria	monocytogenes	A00M045	II a	Smoked salmon	7.9 x 10 ⁶	1.173	+	H+	+
35	Listeria	monocytogenes	A00M088	II a	Smoked salmon	4.2 x 10 ⁶	1.532	+	H+	+
36	Listeria	monocytogenes	Ad235	II b	Poultry	8.7 x 10 ⁶	2.029	+	H+	+
37	Listeria	monocytogenes	Ad253	II b	Hard cheese	8.7 x 10 ⁶	0.718	+	H+	+

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38	<i>Listeria monocytogenes</i>	Ad260	II a	Semi hard cheese	7.5 x 10 ⁶	2.550	+	H+	+
39	<i>Listeria monocytogenes</i>	Ad265	II b	Tongue	5.6 x 10 ⁶	2.147	+	H+	+
40	<i>Listeria monocytogenes</i>	Ad266	II a	Poultry	4.2 x 10 ⁶	2.212	+	H+	+
41	<i>Listeria monocytogenes</i>	Ad267	II b	Dry sausage	6.0 x 10 ⁶	1.641	+	H+	+
42	<i>Listeria monocytogenes</i>	Ad268	IV b	Cured ham	1.1 x 10 ⁶	0.428	+	H+	+
43	<i>Listeria monocytogenes</i>	Ad270	IV b	Fermented sausage	2.0 x 10 ⁶	1.632	+	H+	+
44	<i>Listeria monocytogenes</i>	Ad272	IV b	Fermented sausage	9.6 x 10 ⁵	1.791	+	H+	+
45	<i>Listeria monocytogenes</i>	Ad273	II b	Cured delicatessen	3.6 x 10 ⁵	1.449	+	H+	+
46	<i>Listeria monocytogenes</i>	Ad274	II a	Ready-to-eat food (Asiatic meal)	3.2 x 10 ⁵	1.483	+	H+	+
47	<i>Listeria monocytogenes</i>	Ad534	II b	Fruits	6.8 x 10 ⁵	1.752	+	H+	+
48	<i>Listeria monocytogenes</i>	Ad544	II a	Onion	1.0 x 10 ⁶	1.754	+	H+	+
49	<i>Listeria monocytogenes</i>	Ad548	II a	Environment (seafood)	2.3 x 10 ⁶	1.577	+	H+	+
50	<i>Listeria monocytogenes</i>	Ad546	II a	Flour	1.8 x 10 ⁵	1.646	+	H+	+
51	<i>Listeria monocytogenes</i>	Ad623	II b	Bread crumbs	9.8 x 10 ⁵	1.858	+	H+	+
52	<i>Listeria monocytogenes</i>	Ad665	II a	Raw milk	1.8 x 10 ⁵	1.841	+	H+	+
53	<i>Listeria grayi</i>	Ad1198	N/A	Smoked salmon	9.6 x 10 ⁵	0.855	+	H-	st ^d
54	<i>Listeria grayi</i>	Ad1443	N/A	Pork meat sausages	8.7 x 10 ⁵	1.727	+	H-	st
55	<i>Listeria grayi</i>	Ad1295	N/A	Spinach	3.6 x 10 ⁶	0.323	+	H-	st
56	<i>Listeria grayi</i>	Ad1490	N/A	Salmon terrine	1.4 x 10 ⁷	0.342	+	H-	st
57	<i>Listeria grayi</i>	Ad2148	N/A	Pork rillettes	1.6 x 10 ⁷	0.305	+	H-	st
58	<i>Listeria innocua</i>	Adria 1	N/A	Smoked salmon	1.8 x 10 ⁶	1.998	+	H-	+
59	<i>Listeria innocua</i>	Ad658	N/A	Gorgonzola	5.7 x 10 ⁶	1.629	+	H-	+
60	<i>Listeria innocua</i>	Ad655	N/A	Brine	2.4 x 10 ⁶	1.976	+	H-	+
61	<i>Listeria innocua</i>	Ad660	N/A	Bread crumbs	2.2 x 10 ⁶	1.448	+	H-	+
62	<i>Listeria innocua</i>	Ad663	N/A	Environment (dairy industry)	2.8 x 10 ⁶	2.773	+	H-	+
63	<i>Listeria innocua</i>	Ad671	N/A	Smoked bacon	5.0 x 10 ⁶	1.885	+	H-	+
64	<i>Listeria innocua</i>	Ad661	N/A	Soft cheese (Pont L'Evêque)	2.8 x 10 ⁶	1.769	+	H-	+
65	<i>Listeria innocua</i>	Ad659	N/A	Environment (dairy industry)	3.0 x 10 ⁶	2.580	+	H-	+
66	<i>Listeria ivanovii</i>	Ad466	N/A	Raw veal meat	5.4 x 10 ⁶	2.770	+	H+	+
67	<i>Listeria ivanovii</i>	Ad662	N/A	Environment (dairy industry)	2.0 x 10 ⁴	0.487	+	H+	+
68	<i>Listeria ivanovii</i>	BR11	N/A	Environment (fish)	6.0 x 10 ⁴	0.445	+	H+	+
69	<i>Listeria ivanovii</i>	Ad1289	N/A	Raw milk cheese	1.4 x 10 ⁵	0.609	+	H+	+
70	<i>Listeria ivanovii</i>	Ad1290	N/A	Milk powder	6.0 x 10 ⁴	0.487	+	H+	+
71	<i>Listeria ivanovii</i>	Ad1291	N/A	Poultry	1.3 x 10 ⁶	0.749	+	H+	+
72	<i>Listeria ivanovii</i>	Ad1288	N/A	Sheep milk	5.7 x 10 ⁴	0.413	+	H+	+
73	<i>Listeria ivanovii londoniensis</i>	CIP103466	N/A	N/A	1.0 x 10 ⁵	1.321	+	H+	+
74	<i>Listeria seeligeri</i>	Ad649	N/A	Cheese	4.0 x 10 ⁴	0.908	+	H-	+
75	<i>Listeria seeligeri</i>	Ad651	N/A	Environment	7.2 x 10 ⁵	1.096	+	H-	+
76	<i>Listeria seeligeri</i>	Ad652	N/A	Environment (dairy industry)	6.0 x 10 ⁴	0.586	+	H-	+
77	<i>Listeria seeligeri</i>	Ad674	N/A	Soft cheese (Munster)	1.8 x 10 ⁵	0.871	+	H-	+
78	<i>Listeria seeligeri</i>	BR1	N/A	Trout	9.0 x 10 ⁵	1.200	+	H-	+

79	<i>Listeria seeligeri</i>	BR18	N/A	Environment (fish)	4.6 x 10 ⁵	0.652	+	H-	+
80	<i>Listeria seeligeri</i>	CIP100100	N/A	N/A	4.8 x 10 ⁵	1.258	+	H-	+
81	<i>Listeria welshimeri</i>	Ad1276	N/A	Environment (Slaughterhouse)	5.0 x 10 ⁵	1.473	+	H-	+
82	<i>Listeria welshimeri</i>	Ad1235	N/A	Beef meat	8.7 x 10 ⁵	1.332	+	H-	+
83	<i>Listeria welshimeri</i>	191424	N/A	Poultry	5.7 x 10 ⁵	1.613	+	H-	+
84	<i>Listeria welshimeri</i>	Ad1175	N/A	Ready-to-eat food	6.0 x 10 ⁴	1.700	+	H-	+
85	<i>Listeria welshimeri</i>	Ad 650	N/A	Poultry	1.2 x 10 ⁵	1.931	+	H-	+
86	<i>Listeria marthii</i>	DSM 23813T	N/A	Environment (flour)	4 x 10 ⁵	0.554	+	H-	+
87	<i>Listeria rocourtiae</i>	DSM 22097T	N/A	Vegetables (salad)	9.9 x 10 ⁶	0.983	+	H-	+

^a Source: Adria, Ad, A000, 000/000 and EN: collection of Adria Développement, France. CIP: Collection of Institute Pasteur, France. DSM: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany. BR: Collection of L'agence Française de Sécurité Sanitaire, France. ^b + = positive, Listeria colonies on plate; ^c H+/H- = positive, Listeria colonies with/without halo; ^d st = plate without any colony

Table 2: Exclusivity study results for BACSpec Listeria (1)

No.	Strains					BACSpec Listeria	
	Genus	Species	Source ^a	Origin	Inoculation level CFU/mL	ELISA	
						O.D.	Result
1	<i>Bacillus</i>	<i>cereus</i>	Ad465	Salmon Terrine	2.6 x 10 ⁵	0.051	-
2	<i>Bacillus</i>	<i>circulans</i>	Ad760	Vegetables	2.6 x 10 ⁴	0.135	-
3	<i>Bacillus</i>	<i>coagulans</i>	Ad731	Dairy product	2.0 x 10 ⁴	0.068	-
4	<i>Bacillus</i>	<i>licheniformis</i>	Ad978	Dairy product	1.0 x 10 ⁵	0.082	-
5	<i>Bacillus</i>	<i>mycoïdes</i>	Ad762	Milk	4.4 x 10 ⁵	0.065	-
6	<i>Bacillus</i>	<i>pseudomycoïdes</i>	Ad765	Vegetables	6.0 x 10 ⁴	0.064	-
7	<i>Bacillus</i>	<i>pumilus</i>	Ad284	Ready-to-eat	5.0 x 10 ⁵	0.076	-
8	<i>Bacillus</i>	<i>weihenstephanensis</i>	Ad726	Egg product	6.6 x 10 ⁴	0.104	-
9	<i>Brochothrix</i>	<i>thermosphacta</i>	EN 15129	Trout	2.6 x 10 ⁴	0.036	-
10	<i>Brochothrix</i>	<i>campestris</i>	CIP 102920 T	Environment	6.0 x 10 ⁴	0.033	-
11	<i>Carnobacterium</i>	<i>divergens</i>	CIP 101029T	unknown	3.0 x 10 ⁴	0.044	-
12	<i>Carnobacterium</i>	<i>piscicola</i>	Ad369	Raw milk	9.2 x 10 ⁴	0.043	-
13	<i>Enterococcus</i>	<i>durans</i>	Ad149	Ham	4.6 x 10 ⁴	0.035	-
14	<i>Enterococcus</i>	<i>faecalis</i>	Adria 89L326	Soft cheese (Vacherin)	3.4 x 10 ⁶	0.032	-
15	<i>Lactobacillus</i>	<i>brevis</i>	Adria 86L126	Ham	8.0 x 10 ⁵	0.037	-
16	<i>Lactobacillus</i>	<i>curvatus</i>	Ad380	Delicatessen	5.0 x 10 ⁵	0.044	-
17	<i>Lactobacillus</i>	<i>fermentum</i>	Ad482	Tomatoes juice	9.9 x 10 ⁶	0.030	-
18	<i>Lactobacillus</i>	<i>sakei</i>	Ad473	Ham	4.2 x 10 ⁴	0.040	-
19	<i>Lactococcus</i>	<i>lactis subsp. cremoris</i>	Ad137	Dairy product	6.0 x 10 ⁵	0.048	-
20	<i>Leuconostoc</i>	<i>carnosum</i>	Ad411	Ham	2.2 x 10 ⁵	0.047	-
21	<i>Leuconostoc</i>	<i>citreum</i>	Ad396	Ham	4.8 x 10 ⁴	0.039	-
22	<i>Micrococcus</i>	<i>luteus</i>	Ad432	Cocktail	5.0 x 10 ⁵	0.037	-
23	<i>Pediococcus</i>	<i>pentosaceus</i>	ATCC 33316	N/A	8.3 x 10 ⁵	0.059	-
24	<i>Propionibacterium</i>	<i>freundenreichii</i>	CNRZ 725	Dairy product	1.0 x 10 ⁴	0.031	-
25	<i>Staphylococcus</i>	<i>aureus</i>	Ad165	Smoked delicatessen	1.2 x 10 ⁵	0.084	-
26	<i>Staphylococcus</i>	<i>epidermidis</i>	Ad931	Fruits	2.0 x 10 ⁴	0.035	-
27	<i>Staphylococcus</i>	<i>haemoliticus</i>	Ad989	Dairy product	8.0 x 10 ⁴	0.050	-
28	<i>Streptococcus</i>	<i>bovis</i>	Adria 92L622	Dairy product	8.0 x 10 ⁵	0.036	-
29	<i>Streptococcus</i>	<i>salivarius subsp. thermophilus</i>	Ad441	Dairy product	4.8 x 10 ⁴	0.036	-
30	<i>Macrooccus</i>	<i>caseolyticus</i>	CIP100755	Milk	8.3 x 10 ⁵	0.033	-

^a Source: Adria, Ad, A000, 000/000 and EN: collection of Adria Développement, France. CIP: Collection of Institute Pasteur, France. ATCC: American Type Culture Collection, USA. CNRZ: Centre National de Recherches Zootechniques (Animal Research Centre), France

Table 5: POD statistics of candidate presumptive vs. candidate confirmed method results of BACSpec Listeria (1)

Matrix	Strain	MPN ^a /test portion	N ^b	Presumptive			Confirmed			dPOD _{CP,CC} ^f	95% CI ^g
				X ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI		
Deli Salad	<i>L. monocytogenes</i> Ad494	N/A ^h	5	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	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	N/A
		5.02 (2.18, 12.17)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	N/A
Frankfurter	<i>L. monocytogenes</i> Ad669	N/A	5	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	15	0.75	(0.53, 0.89)	15	0.75	(0.53, 0.89)	0.00	N/A
		1.75 (0.66, 5.65)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	N/A
Soft White Cheese	<i>L. ivanovii</i> Ad1337	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	N/A
		1.20 (0.74, 2.08)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	N/A
		3.42 (2.09, 6.92)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	N/A
Cantaloupe	<i>L. seeligeri</i> Ad1754	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	N/A
		0.65 (0.37, 1.09)	20	16	0.80	(0.58, 0.92)	16	0.80	(0.58, 0.92)	0.00	N/A
		2.5 (1.02, 9.4)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	N/A
Frozen Shrimp	<i>L. innocua</i> Ad1200	N/A	5	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	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	N/A
		1.64 (1.64, 1.64)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	N/A
Smoked Salmon	<i>L. monocytogenes</i> Ad670	N/A	5	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	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	N/A
		7.34 (3.65, 16.76)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	N/A
Raw Milk	<i>L. monocytogenes</i> Ad618	N/A	5	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	15	0.75	(0.53, 0.89)	15	0.75	(0.53, 0.89)	0.00	N/A
		5.02 (2.18, 12.17)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	N/A
Process Water	<i>L. monocytogenes</i> Ad551	N/A	5	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	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	N/A
		1.64 (1.64, 1.64)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	N/A
Stainless Steel 304L	<i>L. monocytogenes</i> Ad551	N/A ⁱ	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	N/A
		N/A ⁱ	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	N/A
		N/A ⁱ	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	N/A
Sealed ceramic Surface	<i>L. monocytogenes</i> Ad551 & <i>L. innocua</i> Ad653	N/A ⁱ	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	N/A
		N/A ⁱ	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	N/A
		N/A ⁱ	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	N/A

^aMPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulation MPN calculator with 95% confidence interval. ^bN = Number of test portions; ^cx = Number of positive test portions; ^dPOD_{CP} = Probability of Detection of presumptive candidate method - positive outcomes divided by the total number of trials; ^ePOD_{CC} = probability of detection for the confirmation method according to ISO 11290-1/A1 - positive outcomes divided by the total number of trials; ^fdPOD_{CP,CC} = Difference between POD values of presumptive candidate method (POD_{CP}) and confirmation (POD_{CC}) results; ^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level; ^hN/A = not applicable; ⁱ Instead MPN, CFU/test area were determined for environmental surfaces by plating the inoculum in triplicate onto non-selective agar

Table 6: POD statistics of candidate confirmed results versus reference method results of BACSpec Listeria (1)

Matrix	Strain	MPN ^a /test portion	N ^b	BACSpec Listeria confirmed			Reference Method			dPOD _{c,R} ^f	95% CI ^g
				X ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI		
Deli Salad	<i>L. monocytogenes</i> Ad494	N/A ^h	5	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	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	N/A
		5.02 (2.18, 12.17)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	N/A
Frankfurter	<i>L. monocytogenes</i> Ad669	N/A	5	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	15	0.75	(0.53, 0.89)	15	0.75	(0.53, 0.89)	0.00	N/A
		1.75 (0.66, 5.65)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	N/A
Soft White Cheese	<i>L. ivanovii</i> Ad1337	N/A	5	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	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	N/A
		3.42 (2.09, 6.92)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	N/A
Cantaloupe	<i>L. seeligeri</i> Ad1754	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	N/A
		0.65 (0.37, 1.09)	20	16	0.80	(0.58, 0.92)	11	0.55	(0.34, 0.74)	0.25	(-0.04, 0.49)
		2.5 (1.02, 9.4)	5	5	1.00	(0.57, 1.00)	4	0.80	(0.38, 1.00)	0.20	(-0.28, 0.62)
Frozen Shrimp	<i>L. innocua</i> Ad1200	N/A	5	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	14	0.70	(0.48, 0.85)	15	0.75	(0.53, 0.89)	-0.05	(-0.31, 0.22)
		1.64 (1.64, 1.64)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	N/A
Smoked Salmon	<i>L. monocytogenes</i> Ad670	N/A	5	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	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	N/A
		7.34 (3.65, 16.76)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	N/A
Raw Milk	<i>L. monocytogenes</i> Ad618	N/A	5	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	15	0.75	(0.53, 0.89)	15	0.75	(0.53, 0.89)	0.00	N/A
		5.02 (2.18, 12.17)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	N/A
Process Water	<i>L. monocytogenes</i> Ad551	N/A	5	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	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	N/A
		1.64 (1.64, 1.64)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	N/A
Stainless Steel 304L	<i>L. monocytogenes</i> Ad551	N/A ⁱ	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	N/A
		N/A ⁱ	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	N/A
		N/A ⁱ	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	N/A
Sealed ceramic Surface	<i>L. monocytogenes</i> Ad551 & <i>L. innocua</i> Ad653	N/A ⁱ	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	N/A
		N/A ⁱ	20	14	0.70	(0.48, 0.85)	13	0.65	(0.43, 0.82)	0.05	(-0.23, 0.32)
		N/A ⁱ	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	N/A

^a MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulation MPN calculator with 95% confidence interval. ^b N = Number of test portions; ^c x = Number of positive test portions; ^d POD_c = Probability of Detection of confirmed candidate method - positive outcomes divided by the total number of trials; ^e POD_R = probability of detection of reference method - positive outcomes divided by the total number of trials; ^f dPOD_{c,R} = Difference between POD values of confirmed candidate method (POD_c) and reference method (POD_R) results; ^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 N/A not applicable; ⁱ Instead MPN, CFU/test area were determined for environmental surfaces by plating the inoculum in triplicate onto non-selective agar

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