

**Comparison study between
CHROMagar™ Listeria medium versus ALOA™
medium**

CONFIDENTIAL

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Table of contents

1. Introduction.....	3
2. Materials and methods.....	3
2.1. Materials	3
2.1.1. Devices	3
2.1.2. Culture media.....	3
2.1.3. Strains	3
2.1.3.1. Inclusivity study	3
2.1.3.1. Exclusivity study.....	5
2.1.4. Matrices	6
2.2. Methods	6
2.2.1. Inclusivity study protocol	6
2.2.2. Exclusivity study protocol.....	6
2.2.3. Sensitivity study protocol.....	7
3. Results	8
3.1. Inclusivity and exclusivity study.....	8
3.1.1. Raw results	8
3.2. Sensitivity study.....	12
3.2.1. Raw results	12
4. Interpretation	15
4.1. Inclusivity study	15
4.2. Exclusivity study	15
4.3. Sensitivity study.....	15
5. Conclusion	16

1. Introduction

The aim of this study is to verify the analytical performance of the CHROMagar™ *Listeria* medium versus the ALOA™ medium for the detection and differentiation of *Listeria* spp. According to EN ISO 11290-1.

In order to compare the performances of the two-culture media, 50 target strains of *Listeria* spp. excluding *Listeria monocytogenes* (inclusivity study) and 40 non-target strains (exclusivity study) were tested and fifty representatives food matrices were analyzed (sensitivity study).

2. Materials and methods

2.1. Materials

2.1.1. Devices

The devices used in this study are the ones usually encountered in a laboratory. All tests were carried out in accordance with good laboratory practices.

2.1.2. Culture media

The culture media used were as follows:

- Brain Heart Infusion Broth - BioMérieux – reference: AEB110110
- Peptone Salt Broth – BioMérieux – reference: AEB111499
- Half-Fraser – BioMérieux – reference: AEB910915/2
- Tryptone Soy Agar – BioMérieux – reference : 417475
- ALOA™ medium – BioMérieux – reference : 424133
- CHROMagar™ *Listeria* - CHROMagar™ - reference: LM852
- Skimmed milk powder – OXOID – reference: LP0033B

2.1.3. Strains

2.1.3.1. Inclusivity study

The 50 strains chosen for the inclusivity study represent the 9 genus of *Listeria* species other than *Listeria monocytogenes*. The majority of them are of wild origin and representative of the different categories of food encountered or of the environment.

The list is shown in table 1.

Table 1 : *Listeria* species strains

#	Strains	Origin	Reference
1	<i>Listeria grayi</i>	Sausage patty	SWZ606
2	<i>Listeria grayi</i>	Delicatessen	SWE117
3	<i>Listeria grayi</i>	Salmon marinated in dill	RZM251
4	<i>Listeria grayi</i>	Green bean	RZK366
5	<i>Listeria grayi</i>	Coconut	BFSC26
6	<i>Listeria grayi</i>	Lemon scallop prawns	TXG292
7	<i>Listeria innocua</i>	Indian pizza	CCPU75
8	<i>Listeria innocua</i>	Shrimp shell	RXY158
9	<i>Listeria innocua</i>	Egg product environment swab	HQM372
10	<i>Listeria innocua</i>	Environment	BZQK45
11	<i>Listeria innocua</i>	Environment	RVL329
12	<i>Listeria innocua</i>	Meat environment	BGPC63
13	<i>Listeria innocua</i>	Dairy environment	BVG975
14	<i>Listeria innocua</i>	Chopped steak	CADA98
15	<i>Listeria innocua</i>	Smoked mackerel paprika	BJSC68
16	<i>Listeria innocua</i>	Smoked herring	AMUS89
17	<i>Listeria innocua</i>	Dairy environment	CQTB35
18	<i>Listeria innocua</i>	Diced onions	TTZ273
19	<i>Listeria innocua</i>	Salmon shell	XEN574
20	<i>Listeria innocua</i>	Potatoes, rillaud, vinaigrette	BWWF66
21	<i>Listeria innocua</i>	Poultry environment	CDBJ23
22	<i>Listeria innocua</i>	Duck rillettes	ANAC98
23	<i>Listeria innocua</i>	Delicatessen	CLF414
24	<i>Listeria innocua</i>	Chicken wing	XJG961
25	<i>Listeria ivanovii</i>	Composite food environment	GJP629
26	<i>Listeria ivanovii</i>	Turkey cutlet scraps	AAZ671
27	<i>Listeria ivanovii</i>	Merguez - Delicatessen	APE161
28	<i>Listeria ivanovii</i>	Composite food environment	GQD028
29	<i>Listeria marthii</i>	Collection strain – DSM 23813	TKK147
30	<i>Listeria seeligeri</i>	Collection strain DSM 20751	TJM186
31	<i>Listeria seeligeri</i>	Zucchini	ADTW22
32	<i>Listeria seeligeri</i>	Herring salad	BTRF33
33	<i>Listeria welshimeri</i>	Wipe poultry environment	PNW846
34	<i>Listeria welshimeri</i>	Composite food environment	BFSL26
35	<i>Listeria welshimeri</i>	Fat	THL274
36	<i>Listeria welshimeri</i>	Raw turkey meat	ZAY286
37	<i>Listeria welshimeri</i>	Monkfish	AMAB65
38	<i>Listeria welshimeri</i>	Salmon steak	AJP106
39	<i>Listeria welshimeri</i>	Fresh beef	CBTF93
40	<i>Listeria welshimeri</i>	Delicatessen	CQAJ98
41	<i>Listeria welshimeri</i>	Vegetables	TLJ742
42	<i>Listeria welshimeri</i>	Toulouse sausage	TPR354
43	<i>Listeria welshimeri</i>	Organic salmon ready to smoke	BHZK94
44	<i>Listeria welshimeri</i>	Chinese noddles	TXR109
45	<i>Listeria welshimeri</i>	Delicatessen	RYH463
46	<i>Listeria welshimeri</i>	Salmon shell	XCW614
47	<i>Listeria welshimeri</i>	Plastic pallet	RVG428
48	<i>Listeria fleishmannii subsp. fleishmannii</i>	Collection strain – DSM 24998	HKKZ77
49	<i>Listeria rocourtiae</i>	Collection strain – DSM 22097	HLSJ38
50	<i>Listeria weihenstephanensis</i>	Collection strain – DSM 24698	HLUW69

2.1.3.1. Exclusivity study

40 non-*Listeria* strains were chosen from species close to *Listeria* or known to be capable of growing on *Listeria* detection media.

The list is shown in table 2.

Table 2: non-*Listeria* strains

#	Strains	Origin	Reference
1	<i>Bacillus circulans</i>	Infant milk powder	CQND74
2	<i>Bacillus cereus cereus</i> grp III	Cheddar ham sandwich	EAA229-BC
3	<i>Bacillus cereus thuringiensis</i>	Curry	GGN787
4	<i>Bacillus kochii</i>	Cosmetic environment	DPP171
5	<i>Bacillus licheniformis</i>	Dehydrated soup made with asparagus	WHA424
6	<i>Carnobacterium divergens</i>	Salmon steak	YFJ492
7	<i>Staphylococcus caprae</i>	Dairy product	UJC759
8	<i>Staphylococcus epidermidis</i>	Composite foods	VCW959
9	<i>Staphylococcus lentus</i>	Meat product	FAS312
10	<i>Staphylococcus aureus</i>	Seafood product	BATB46
11	<i>Lactobacillus gasseri</i>	Probiotics	EFP530
12	<i>Lactobacillus paracasei</i>	Dairy product	ANW492
13	<i>Lactobacillus plantarum</i>	Vegetables products	DLTB68
14	<i>Lactobacillus sakei</i>	Probiotics	EEX511
15	<i>Brochothrix thermosphacta</i>	Sushi	CXZ948
16	<i>Brochotrix thermosphacta</i>	Minced beef	LPF985
17	<i>Corynebacterium phoceense</i>	Chicken fillet	CCQT66
18	<i>Corynebacterium sp</i>	Halal plain sausage	MPFH54
19	<i>Corynebacterium sp</i>	Piece of turkey cutlet	VLS691
20	<i>Corynebacterium callunae</i>	Calzone	EFG554
21	<i>Corynebacterium flavescens</i>	Raw milk cheese	FBM018
22	<i>Corynebacterium frankenforstense</i>	Chicken sleeve	UMP052
23	<i>Enterococcus hirae</i>	Raw milk goat cheese	WLT854
24	<i>Enterococcus faecalis</i>	Raw milk cheese	GQC471
25	<i>Enterococcus faecalis</i>	Raw milk cheese	UDL722
26	<i>Enterococcus faecium</i>	Bechamel ham puff pastry	UYS555
27	<i>Enterococcus faecium</i>	Raw milk cheese	WDF839
28	<i>Enterococcus faecium</i>	Dry sausage	WQU904
29	<i>Enterococcus faecium</i>	Plantain	GZA327
30	<i>Kocuria kristinae</i>	Pasteurized milk cheese	VGJ971
31	<i>Niallia sp</i>	Walnut kernels	XHE898
32	<i>Niallia sp</i>	Jambalaya	XJG782
33	<i>Niallia taxi</i>	Celery remoulade	BGZL52
34	<i>Niallia taxi</i>	Pastry	CANM24
35	<i>Niallia taxi</i>	Chocolate	CYTW88
36	<i>Mixta calida</i>	Plant	WYZ121
37	<i>Mixta sp</i>	Grilled yellow powder onion	BYYG32
38	<i>Lelliottia amnigena</i>	Pasteurized milk cheese	HKU627
39	<i>Lelliottia amnigena</i>	Dairy environment	HVB243
40	<i>Lelliottia amnigena</i>	Lamb feed	BXFU93

2.1.4. Matrices

The food matrices represent a broad range of foods grouped into different categories and types. The fifty samples of food products were divided into 5 categories and 3 types (a, b, c) per category. The categories and types are presented in table 3:

Table 3: categories and types

Category	Type
Meat products	a Raw
	b Ready-to-reheat (RTRH)
	c Delicatessen
Dairy products	a Raw milk cheese
	b Other raw milk products
	c Pasteurized dairy products
Seafood products	a Raw
	b Smoked and marinated
	c Processed products
Vegetables	a Raw
	b Seasoned and ready-to-eat (RTE)
	c Transformed vegetables
Composite foods	a Ready-to-eat (RTE)
	b Ready-to-reheat (RTRH)
	c Pastries and egg products

Ten samples per category were analyzed.

2.2. Methods

2.2.1. Inclusivity study protocol

Fifty pure strains were inoculated in Brain Heart Infusion Broth and incubated for 24 hours at $37\pm 1^{\circ}\text{C}$. The purity and typicality of each strain were checked by streaking 10 μL of Brain Heart Infusion Broth on ALOA medium.

Dilutions were carried out in 9 mL of peptone salt and enumerated on tryptone soy agar medium to inoculate 10 to 100 cells in 100 mL of Half-Fraser broth.

Half-Fraser broth was incubated 24 hours at $30\pm 1^{\circ}\text{C}$ then 10 μL were seeded on CHROMagar™ Listeria and ALOA™ media.

CHROMagar™ Listeria and ALOA™ media were incubated at $37\pm 1^{\circ}\text{C}$ and results were read after 24 and 48 hours.

If no-growth was observed after incubation of the Half-Fraser broth, the strain was retested by the addition of 10g/100 mL of enrichment broth supplemented with skimmed milk powder.

2.2.2. Exclusivity study protocol

Forty pure strains were inoculated in Brain Heart Infusion Broth and incubated for 24 hours at $37\pm 1^{\circ}\text{C}$. Dilutions were carried out in 9 mL of peptone salt and enumerated on tryptone soy agar medium to obtain a level of 10^6 cells/mL. A sample of 10 μL of the concentrated suspension at 10^6 cells/mL was seeded on CHROMagar™ Listeria and ALOA™ media. The two media were incubated at $37\pm 1^{\circ}\text{C}$ and

results were read after 24 and 48 hours. A confirmation by CAMP test was carried out on characteristic colonies (blue colonies with or without halo) observed on CHROMagar™ Listeria medium.

2.2.3. Sensitivity study protocol

A test sample of 25 g of each matrix was analyzed with 225 mL of half-Fraser broth incubated for $24\text{h}\pm 2\text{h}$ at 30°C . After incubation, $10\ \mu\text{l}$ of enrichment broth was streaking on CHROMagar™ Listeria and ALOA media. The 2 agar media were incubated at 37°C for $24\text{h}\pm 2\text{h}$. A confirmation was carried out on characteristic colonies observed on CHROMagar™ Listeria and ALOA media after purification with biochemical gallery. Naturally contaminated samples were preferentially analyzed, but to complete the categories, artificial contaminations were done using the seeding method during 72 hours at $5\pm 3^{\circ}\text{C}$ and at contamination levels allowing positive results to be obtained. The strains chosen to contaminate the samples have an origin similar to that of the sample.

The list of the samples analyzed, and the strains used is shown in table 4.

Table 4: matrix and strains used.

Category	Type	#	Matrix	Strains				
				Strain	Reference	Origin	Stress applied	Inoculation level CFU/25g
Meat products	a	2588571	Chicken mechanically separated meat	<i>Naturally contaminated</i>	/	/	/	/
	a	2588572	Ground beef meat	<i>Naturally contaminated</i>	/	/	/	/
	a	2588573	Quail	<i>Naturally contaminated</i>	/	/	/	/
	a	2588574	Pork fillet	<i>L.welshimeri</i>	BEY899	Chicken	Seeding 72h at 5±3°C	10,6
	b	2588575	Turkey nem	<i>Naturally contaminated</i>	/	/	/	/
	b	2588576	Beef burger	<i>L.welshimeri</i>	RYH463	Delicatessen	Seeding 72h at 5±3°C	28,6
	b	2588577	Chicken puff pastry	<i>L.welshimeri</i>	CQAJ98	Ham rillettes	Seeding 72h at 5±3°C	32,6
	c	2588578	Delicatessen (cervelas)	<i>Naturally contaminated</i>	/	/	/	/
	c	2588579	Delicatessen (boudin noir)	<i>Naturally contaminated</i>	/	/	/	/
c	2588580	Herb sausage	<i>Naturally contaminated</i>	/	/	/	/	
Dairy products	a	2588581	Raw milk cheese (Reblochon)	<i>L. innocua</i>	QBB281	Raw milk cheese	Seeding 72h at 5±3°C	24,4
	a	2588582	Raw milk cheese (Morbier)	<i>L. ivanovii</i>	GJP629	Dairy environment	Seeding 72h at 5±3°C	6,0
	a	2588583	Raw milk cheese (Le petit gris)	<i>Naturally contaminated</i>	/	/	/	/
	a	2588584	Raw milk cheese (Abondance)	<i>Naturally contaminated</i>	/	/	/	/
	b	2588585	Raw cow's milk	<i>L. innocua</i>	TGW734	Pasteurized cheese	Seeding 72h at 5±3°C	6,8
	b	2588586	Cottage with raw milk	<i>L.welshimeri</i>	GLX736	Dairy environment	Seeding 72h at 5±3°C	25,0
	b	2588587	Raw goat's milk	<i>L. ivanovii</i>	GJP629	Composite food environment	Seeding 72h at 5±3°C	6,0
	c	2588588	Pasteurized milk	<i>L. innocua</i>	ADQC53	Butter	Seeding 72h at 5±3°C	22,6
	c	2588589	Butter with pasteurized milk	<i>L.welshimeri</i>	GLX736	Dairy environment	Seeding 72h at 5±3°C	25,0
c	2588590	Ice cream cookie	<i>L. grayi</i>	GRY999	Composite food environment	Seeding 72h at 5±3°C	13,6	
Seafood products	a	2588591	Mackerel	<i>L. innocua</i>	TYA050	Tataki with tuna	Seeding 72h at 5±3°C	22,2
	a	2588592	Saithe fillet	<i>L. ivanovii</i>	GQD028	Composite food environment	Seeding 72h at 5±3°C	7,0
	a	2588593	Ray	<i>L.welshimeri</i>	AMAB65	Raw fish	Seeding 72h at 5±3°C	30,8
	a	2588594	Cod fillet	<i>L. innocua</i>	BJSC68	Smoked mackerel	Seeding 72h at 5±3°C	26,2
	b	2588595	Smoked herring	<i>Naturally contaminated</i>	/	/	/	/
	b	2588596	Smoked salmon	<i>L.seeligeri</i>	BTRF33	Herring salad	Seeding 72h at 5±3°C	30,6
	b	2588597	Marinated salmon	<i>L.welshimeri</i>	BZVD88	Shrimps	Seeding 72h at 5±3°C	22,6
	c	2588598	Cod brandade	<i>L.grayi</i>	TXG292	Prawns and scallops	Seeding 72h at 5±3°C	19,0
	c	2588599	Piémontaise with tuna	<i>Naturally contaminated</i>	/	/	/	/
c	2588600	Surimi shell	<i>Naturally contaminated</i>	/	/	/	/	
Vegetables	a	2588601	Butternut	<i>L. innocua</i>	TTZ273	Cubed onions	Seeding 72h at 5±3°C	33,2
	a	2588602	Pineapple	<i>L. grayi</i>	BFSC26	Coconut	Seeding 72h at 5±3°C	12,4
	a	2588603	Cucumber	<i>L. ivanovii</i>	GJP629	Composite food environment	Seeding 72h at 5±3°C	6,0
	a	2588604	Grated carrots	<i>L.seeligeri</i>	TJM186	Vegetables	Seeding 72h at 5±3°C	24,4
	b	2588605	Remoulade celery	<i>L. grayi</i>	RZK366	Green beans	Seeding 72h at 5±3°C	13,8
	b	2588606	Greek mushrooms	<i>L. innocua</i>	BNEQ97	Remoulade celery	Seeding 72h at 5±3°C	21,6
	b	2588607	Endive salad, walnuts, tomatoes, chives	<i>L.welshimeri</i>	TXR109	Chinese noodles	Seeding 72h at 5±3°C	14,4
	c	2588608	Cooked mung beans	<i>L. grayi</i>	GSCM68	Mushrooms	Seeding 72h at 5±3°C	12,6
	c	2588609	Zucchini soup	<i>L. innocua</i>	BJCK82	Zucchini gratin	Seeding 72h at 5±3°C	17,0
c	2588610	Couscous vegetables	<i>L.welshimeri</i>	TLJ742	Cooked vegetables	Seeding 72h at 5±3°C	36,4	
Composite foods	a	2588611	Tabbouleh	<i>L. innocua</i>	TTW844	Sandwich	Seeding 72h at 5±3°C	22,2
	a	2588612	Shrimps and cucumber	<i>L. ivanovii</i>	GJP629	Composite food environment	Seeding 72h at 5±3°C	6,0
	a	2588613	Vegetables with rice	<i>L.seeligeri</i>	ADTW22	Zucchini	Seeding 72h at 5±3°C	3,8
	a	2588614	Cooked delicatessen in sauce	<i>L.welshimeri</i>	DEG545	Smoked bacon	Seeding 72h at 5±3°C	12,2
	b	2588615	4 cheese pizza	<i>L. innocua</i>	CCPU75	Indiana pizza	Seeding 72h at 5±3°C	27,0
	b	2588616	Lasagna	<i>L.welshimeri</i>	TDV458	Delicatessen	Seeding 72h at 5±3°C	15,2
	b	2588617	Parisienne salad	<i>Naturally contaminated</i>	/	/	/	/
	c	2588618	Pastry (flan)	<i>L. grayi</i>	DHP264	Caramel apple pancake	Seeding 72h at 5±3°C	15,6
	c	2588619	Liquid egg yolk	<i>L. innocua</i>	BWHS39	Cream brioche	Seeding 72h at 5±3°C	14,6
c	2588620	Pastry (chocolate mousse)	<i>Naturally contaminated</i>	/	/	/	/	

3. Results

3.1. Inclusivity and exclusivity study

3.1.1. Raw results

The inclusivity results are given in table 5 and exclusivity results in table 6.

Table 5: raw results of inclusivity study (*Listeria* spp. strains)

#	Strains	Origin	Reference	Aspect on ALOA before analysis	Level of contamination CFU/100mL	Broth	ALOA 24h at 37°C	ALOA 48h at 37°C	CHROMagar™ <i>Listeria</i> 24h at 37°C	CHROMagar™ <i>Listeria</i> 48h at 37°C
1	<i>Listeria grayi</i>	Pancake with sausage	SWZ606	Blue-green star-shaped colonie wo halo	63	Half-fraser	Blue-white star-shaped colonies wo halo 2 mm	Blue-white star-shaped colonies wo halo 3 mm	Absence of colonie	Absence of colonie
	<i>Listeria grayi</i> (retest)	Pancake with sausage	SWZ606	Blue-green star-shaped colonie wo halo	20	Half-fraser + skimmed milk	Blue-white star-shaped colonies wo halo 2 mm	Blue-white star-shaped colonies wo halo 3 mm	Blue star-shaped colonies wo halo 1 mm	Blue star-shaped colonies wo halo 1 mm
2	<i>Listeria grayi</i>	Delicatessen	SWE117	Blue-green star-shaped colonie wo halo	25	Half-fraser	2 blue-white star-shaped colonies wo halo 2 mm	Blue-white star-shaped colonies wo halo 3 mm	Absence of colonie	Absence of colonie
	<i>Listeria grayi</i> (retest)	Delicatessen	SWE117	Blue-green star-shaped colonie wo halo	90	Half-fraser + skimmed milk	Blue-white star-shaped colonies wo halo 2 mm	Blue-white star-shaped colonies wo halo 3 mm	Blue star-shaped colonies wo halo 2 mm	Blue star-shaped colonies wo halo 2 mm
3	<i>Listeria grayi</i>	Salmon marinated in dill	RZM251	Blue-green star-shaped colonie wo halo	86	Half-fraser	Blue-white star-shaped colonies wo halo 2 mm	Blue-white star-shaped colonies wo halo 3 mm	Absence of colonie	Absence of colonie
	<i>Listeria grayi</i> (retest)	Salmon marinated in dill	RZM251	Blue-green star-shaped colonie wo halo	21	Half-fraser + skimmed milk	Blue-white star-shaped colonies wo halo 2 mm	Blue-white star-shaped colonies wo halo 3 mm	Blue star-shaped colonies wo halo 2 mm	Blue star-shaped colonies wo halo 2 mm
4	<i>Listeria grayi</i>	Green bean	RZK366	Blue-green colonie wo halo	107	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 1 mm
5	<i>Listeria grayi</i>	Coconut	BFSC26	Blue-green star-shaped colonie wo halo	78	Half-fraser	Blue-white star-shaped colonies wo halo 2 mm	Blue-white star-shaped colonies wo halo 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 1 mm
6	<i>Listeria grayi</i>	Lemon scallop prawns	TXG292	Blue-green star-shaped colonie wo halo	70	Half-fraser	Blue-white star-shaped colonies wo halo 1 mm	Blue-white star-shaped colonies wo halo 2 mm	Absence of colonie	Absence of colonie
	<i>Listeria grayi</i>	Lemon scallop prawns	TXG292	Blue-green star-shaped colonie wo halo	27	Half-fraser + skimmed milk	Blue-white star-shaped colonies wo halo 2 mm	Blue-white star-shaped colonies wo halo 3 mm	Absence of colonie	Absence of colonie
7	<i>Listeria innocua</i>	Indian pizza	CCPU75	Blue-green colonie wo halo	90	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
8	<i>Listeria innocua</i>	Shrimp shell	RXY158	Blue-green colonie wo halo	100	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
9	<i>Listeria innocua</i>	Egg product environment swab	HQM372	Blue-green colonie wo halo	59	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
10	<i>Listeria innocua</i>	Egg product environment	BZQK45	Blue-green colonie wo halo	80	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
11	<i>Listeria innocua</i>	Composite food environment	RVL329	Blue-green colonie wo halo	100	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
12	<i>Listeria innocua</i>	Meat environment	BGPC63	Blue-green colonie wo halo	100	Half-fraser	Blue-green colonies wo halo 1 mm	Blue-green colonies wo halo, 2 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
13	<i>Listeria innocua</i>	Dairy environment	BVG975	Blue-green colonie wo halo	10	Half-fraser	Blue-green colonies wo halo 1 mm	Blue-green colonies wo halo, 2 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
14	<i>Listeria innocua</i>	Chopped steak	CADA98	Blue-green colonie wo halo	95	Half-fraser	Blue-green colonies wo halo 1 mm	Blue-green colonies wo halo, 2 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
15	<i>Listeria innocua</i>	Smoked mackerel paprika	BJSC68	Blue-green colonie wo halo	90	Half-fraser	Blue-green colonies wo halo 1 mm	Blue-green colonies wo halo, 2 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
16	<i>Listeria innocua</i>	Smoked herring	AMUS89	Blue-green colonie wo halo	18	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
17	<i>Listeria innocua</i>	Dairy environment	CQTB35	Blue-green colonie wo halo	46	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
18	<i>Listeria innocua</i>	Diced onions	TTZ273	Blue-green colonie wo halo	36	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
19	<i>Listeria innocua</i>	Salmon shell	XEN574	Blue-green colonie wo halo	43	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
20	<i>Listeria innocua</i>	Potatoes, rillaud, vinaigrette	BWWF66	Blue-green colonie wo halo	57	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
21	<i>Listeria innocua</i>	Poultry environment	CDBJ23	Blue-green colonie wo halo	47	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
22	<i>Listeria innocua</i>	Duck rillettes	ANAC98	Blue-green colonie wo halo	41	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm

#	Strains	Origin	Reference	Aspect on ALOA before analysis	Level of contamination CFU/100mL	Broth	ALOA 24h at 37°C	ALOA 48h at 37°C	CHROMagar™ Listeria 24h at 37°C	CHROMagar™ Listeria 48h at 37°C
23	<i>Listeria innocua</i>	Delicatessen	CLF414	Blue-green colonie wo halo	34	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
24	<i>Listeria innocua</i>	Chicken wing	XJG961	Blue-green colonie wo halo	51	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
25	<i>Listeria ivanovii</i>	Composite food environment	GJP629	Blue-green colonie with halo	84	Half-fraser	Blue-green colonies with halo <1mm	Blue-green colonies with halo 1mm	Blue colonies with halo <1 mm	Blue colonies with halo 1 mm
26	<i>Listeria ivanovii</i>	Turkey cutlet scraps	AAZ671	Blue-green colonie with weak and small halo	35	Half-fraser	Blue-green colonies with weak and small halo, 2 mm	Blue-green colonies with weak and small halo, 3 mm	Blue colonies with very weak and small halo, 1 mm	Blue colonies with very weak and small halo, 2 mm
27	<i>Listeria ivanovii</i>	Merguez - Delicatessen	APE161	Blue-green colonie with halo	14	Half-fraser	Blue-green colonies with halo 1 mm	Blue-green colonies with halo, 2 mm	Blue colonies with halo <1 mm	Blue colonies with halo 1 mm
28	<i>Listeria ivanovii</i>	Composite food environment	GQD028	Blue-green colonie with weak and small halo	46	Half-fraser	Blue-green colonies with weak and small halo, 2 mm	Blue-green colonies with weak and small halo, 3 mm	Blue colonies with very weak and small halo, 1 mm	Blue colonies with very weak and small halo, 2 mm
29	<i>Listeria marthii</i>	Collection strain - DSM 23813	TKK147	Blue-green colonie wo halo	45	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
30	<i>Listeria seeligeri</i>	Vegetables - Collection strain DSM 20751	TJM186	Blue-green colonie wo halo	57	Half-fraser	Blue-green colonies wo halo <1mm	Blue-green colonies wo halo 1mm	Blue colonies wo halo, <1 mm	Blue colonies wo halo, 1 mm
31	<i>Listeria seeligeri</i>	Zucchini	ADTW22	Blue-green colonie wo halo	19	Half-fraser	Absence of colonie	Blue-green colonies wo halo <1mm	1 blue colonie wo halo <1mm	Blue colonies wo halo, 1 mm
32	<i>Listeria seeligeri</i>	Herring salad	BTRF33	Blue-green colonie wo halo	103	Half-fraser	Blue-green colonie wo halo <1 mm	Blue-green colonies wo halo 1mm	Blue colonies wo halo, <1 mm	Blue colonies wo halo, 1 mm
33	<i>Listeria welshimeri</i>	Wipe poultry environment	PNW846	Blue-green colonie wo halo and rare dark blue colonies	47	Half-fraser	Blue-green colonies wo halo <1mm	Blue-green colonies wo halo 1mm	Blue colonies wo halo, <1 mm	Blue colonies wo halo, 1 mm
34	<i>Listeria welshimeri</i>	Composite food environment	BFSL26	Blue-green colonie wo halo	38	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
35	<i>Listeria welshimeri</i>	Fat	THL274	Blue-green colonie wo halo	20	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
36	<i>Listeria welshimeri</i>	Raw turkey meat	ZAY286	Blue-green colonie wo halo and rare dark blue colonies	31	Half-fraser	Blue-green colonies wo halo 1 mm	Blue-green colonies wo halo, 2 mm	Blue colonies wo halo, <1 mm	Blue colonies wo halo, 1 mm
37	<i>Listeria welshimeri</i>	Monkfish	AMAB65	Blue-green colonie wo halo	23	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
38	<i>Listeria welshimeri</i>	Salmon steak	AJP106	Blue-green colonie wo halo	17	Half-fraser	Blue-green colonies wo halo 1 mm	Blue-green colonies wo halo, 2 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
39	<i>Listeria welshimeri</i>	Fresh beef	CBTF93	Blue-green colonie wo halo and rare dark blue colonies	25	Half-fraser	Blue-green colonies wo halo 1 mm	Blue-green colonies wo halo, 2 mm	Blue colonies wo halo, <1 mm	Blue colonies wo halo, 1 mm
40	<i>Listeria welshimeri</i>	Delicatessen	CQAJ98	Blue-green colonie wo halo	46	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
41	<i>Listeria welshimeri</i>	Vegetables	TLJ742	Blue-green colonie wo halo	17	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
42	<i>Listeria welshimeri</i>	Toulouse sausage	TPR354	Blue-green colonie wo halo	70	Half-fraser	Blue-green colonies wo halo 1 mm	Blue-green colonies wo halo, 2 mm	Blue colonies wo halo, <1 mm	Blue colonies wo halo, 1 mm
43	<i>Listeria welshimeri</i>	Organic salmon ready to smoke	BHZK94	Blue-green colonie wo halo and rare dark blue colonies	57	Half-fraser	Blue-green colonies wo halo, <1 mm	Blue-green colonies wo halo 1 mm	Blue colonies wo halo, <1 mm	Blue colonies wo halo, 1 mm
44	<i>Listeria welshimeri</i>	Chinese noddles	TXR109	Blue-green colonie wo halo	117	Half-fraser	Blue-green colonies wo halo, 2 mm	Blue-green colonies wo halo, 3 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
45	<i>Listeria welshimeri</i>	Delicatessen	RYH463	Blue-green colonie wo halo	10	Half-fraser	Blue-green colonies wo halo 1 mm	Blue-green colonies wo halo, 2 mm	Blue colonies wo halo, 1 mm	Blue colonies wo halo, 2 mm
46	<i>Listeria welshimeri</i>	Salmon shell	XCW614	Blue-green colonie wo halo and with halo	16	Half-fraser	Blue-green colonies wo halo 1 mm	Blue-green colonies wo halo, 2 mm	Blue colonies wo halo, <1 mm	Blue colonies wo halo, 1 mm
47	<i>Listeria welshimeri</i>	Plastic pallet	RVG428	Blue-green colonie wo halo and rare dark blue colonies	41	Half-fraser	Blue-green colonies wo halo, 1 mm	Blue-green colonies wo halo, 2 mm	Blue colonies wo halo, <1 mm	Blue colonies wo halo, 1 mm
48	<i>Listeria fleishmannii</i> subsp. <i>fleischmannii</i>	Collection strain - DSM 24998	HKKZ77	Blue-green colonie wo halo	21	Half-fraser	Blue-green colonies wo halo, 1 mm	Blue-green colonies wo halo, 2 mm	Blue colonie wo halo 1mm	Blue colonie wo halo 2mm
49	<i>Listeria rocourtaiae</i>	Collection strain - DSM 22097	HLSJ38	Blue-green star-shaped colonie wo halo	37	Half-fraser	Blue-green star-shaped colonie wo halo 1 mm	Blue-green star-shaped colonie wo halo 2 mm	Blue colonie wo halo 1mm	Blue colonie wo halo 1mm
50	<i>Listeria weihenstephanensis</i>	Collection strain - DSM 24698	HLUW69	Blue-green star-shaped colonie wo halo	44	Half-fraser	Blue-green star-shaped colonie wo halo 1-2 mm	Blue-green star-shaped colonie wo halo 2 mm	Blue colonie wo halo 1-2mm	Blue colonie wo halo 2mm

Microsept

Table 6: raw results of exclusivity study (non-Listeria strains)

#	Strains	Origin	Reference	ALOA 24h at 37°C	ALOA 48h at 37°C	CHROMagar™ Listeria 24h at 37°C	CHROMagar™ Listeria 48h at 37°C	Confirmation of CHROMagar™ (on blue colony)
1	<i>Bacillus circulans</i>	Infant milk powder	CQND74	no-characteristic blue colonie 1mm	no-characteristic blue colonie 1mm	no-characteristic blue colonie 1mm	no-characteristic blue colonie 1mm	/
2	<i>Bacillus cereus cereus grp III</i>	Cheddar ham sandwich	EAA229-BC	white colonie 1mm	white colonie 1mm	white star-shaped colonie 2mm	white star-shaped colonie 2mm	/
3	<i>Bacillus cereus thuringiensis</i>	Curry	GGN787	brownish veil	brownish veil	whitish veil	whitish veil	/
4	<i>Bacillus kachii</i>	Cosmetic environment	DPP171	∅	∅	white colonies <1mm	white colonies <1mm	/
5	<i>Bacillus licheniformis</i>	Dehydrated soup made with asparagus	WHA424	∅	∅	white colonies <1mm	white colonies <1mm	/
6	<i>Carnobacterium divergens</i>	Salmon steak	YFJ492	∅	∅	∅	∅	/
7	<i>Staphylococcus caprae</i>	Dairy product	UJC759	∅	∅	white colonies 1mm	white colonies 1mm	/
8	<i>Staphylococcus epidermidis</i>	Composite foods	VCW959	∅	∅	white colonies 1mm	white colonies 1mm	/
9	<i>Staphylococcus lentus</i>	Meat product	FAS312	∅	∅	∅	∅	/
10	<i>Staphylococcus aureus</i>	Seafood product	BATB46	∅	∅	white colonies <1mm	white colonies <1mm	/
11	<i>Lactobacillus gasseri</i>	Probiotics	EPF530	∅	∅	white colonies <1mm	white colonies <1mm	/
12	<i>Lactobacillus paracasei</i>	Dairy product	ANW492	∅	∅	∅	∅	/
13	<i>Lactobacillus plantarum</i>	Vegetables products	DLTB68	∅	∅	white colonie 1mm	white colonie 1mm	/
14	<i>Lactobacillus sakei</i>	Probiotics	EEX511	∅	∅	mixture of very small, not characteristic blue and white colonies	mixture of very small, not characteristic blue and white colonies	Camp test: -
15	<i>Brochothrix thermosphacta</i>	Sushi	CXZ948	∅	∅	white colonies <1mm	white colonies 1mm	/
16	<i>Brochothrix thermosphacta</i>	Minced beef	LPF985	∅	∅	white colonies <1mm	white colonies 1mm	/
17	<i>Corynebacterium phoceense</i>	Chicken fillet	CCQT66	∅	∅	very small, blue colonies	very small, blue colonies	Camp test: -
18	<i>Corynebacterium sp</i>	Halal plain sausage	MPFH54	∅	∅	white colonies <1mm	white colonies <1mm	/
19	<i>Corynebacterium sp</i>	Piece of turkey cutlet	VLS691	∅	∅	white colonies <1mm	white colonies <1mm	/
20	<i>Corynebacterium callunae</i>	Calzone	EFG554	slight brownish veil	slight brownish veil	slight whitish veil	slight whitish veil	/
21	<i>Corynebacterium flavescens</i>	Raw milk cheese	FBM018	∅	∅	∅	∅	/
22	<i>Corynebacterium frankenforstense</i>	Chicken sleeve	UMP052	∅	∅	white colonies <1mm	white colonies <1mm	/
23	<i>Enterococcus hirae</i>	Raw milk goat cheese	WLT854	∅	∅	∅	∅	/
24	<i>Enterococcus faecalis</i>	Raw milk cheese	GCQ471	∅	∅	very small no- characteristic light blue colonies	very small no- characteristic light blue colonies	/
25	<i>Enterococcus faecalis</i>	Raw milk cheese	UDL722	∅	∅	very small no- characteristic light blue colonies	very small no- characteristic light blue colonies	/
26	<i>Enterococcus faecium</i>	Bechamel ham puff pastry	UY555	∅	∅	no-characteristic light blue colonies <1mm	no-characteristic light blue colonies <1mm	/
27	<i>Enterococcus faecium</i>	Raw milk cheese	WDF839	∅	∅	very small no- characteristic light blue colonies	very small no- characteristic light blue colonies	/
28	<i>Enterococcus faecium</i>	Dry sausage	WQU904	∅	∅	very small no- characteristic light blue colonies	very small no- characteristic light blue colonies	/
29	<i>Enterococcus faecium</i>	Plantain	GZA327	slight brownish veil	slight brownish veil	slight whitish haze	slight whitish haze	/
30	<i>Kocuria kristinae</i>	Pasteurized milk cheese	VGJ971	∅	∅	mixture of very small, no- characteristic light blue and white colonies	mixture of very small, no- characteristic light blue and white colonies	/
31	<i>Niallia sp</i>	Walnut kernels	XHE898	1 blue colonie 1mm	blue colonie 1mm	no-characteristic blue star- shaped colonies 2 mm	no-characteristic blue star- shaped colonies 2 mm	/
32	<i>Niallia sp</i>	Jambalaya	XJG782	∅	∅	no-characteristic light blue colonies	no-characteristic light blue colonies	/
33	<i>Niallia taxi</i>	Celery remoulade	BGZL52	∅	∅	blue colonie 1mm	blue colonie 1mm	Camp test: -
34	<i>Niallia taxi</i>	Pastry	CANM24	∅	∅	star-shaped light blue colonies 2mm	star-shaped light blue colonies 2mm	Camp test: -
35	<i>Niallia taxi</i>	Chocolate	CYTW88	no-characteristic light blue colonie 1mm	no-characteristic light blue colonie 1mm	no-characteristic light blue colonie 1mm	no-characteristic light blue colonie 1mm	/
36	<i>Mixta calida</i>	Plant	WYZ121	∅	∅	∅	∅	/
37	<i>Mixta sp</i>	Grilled yellow powder onion	BYYG32	∅	∅	∅	∅	/
38	<i>Lelliottia amnigena</i>	Pasteurized milk cheese	HKU627	∅	∅	∅	∅	/
39	<i>Lelliottia amnigena</i>	Dairy environment	HVB243	∅	∅	white colonies <1mm	white colonies 1mm	/
40	<i>Lelliottia amnigena</i>	Lamb feed	BXFU93	∅	∅	white colonies <1mm	white colonies 1mm	/

∅: no growth

veil: no colonies formed

3.2. Sensitivity study

3.2.1. Raw results

The sensitivity study results are given in table 7.

Caption for the table 6:

Bacterial burden

∅: no culture

L = low

M = moderate

H = high

/: not done

PA: positive agreement

NA: negative agreement

ND: negative deviation

PD: positive deviation

/: not done

Distribution of flora

A = pure culture of suspect colonies

B = mixture with a majority of suspect colonies

C = mixture with a minority of suspect colonies

D = mixture with rare suspect colonies

E = absence of suspect colonies

(x): x colonies characteristic of *Listeria* if $x \leq 5$

Example: +(AL)

Table 7: raw results of sensitivity study

Category	Type	#	Matrix	Strains				Inoculation level CFU/25g	ALOA 24h at 37°C	ALOA 48h at 37°C	Confirmation	Final result	CHROMagar™ Listeria 24h at 37°C	CHROMagar™ Listeria 48h at 37°C	Confirmation	Final result	Agreement
				Strain	Reference	Origin	Stress applied										
Meat products	a	2588571	Chicken mechanically separated meat	Naturally contaminated	/	/	/	/	-(EL)	-(EL)	/	A	-(EL)	-(EL)	/	A	NA
	a	2588572	Ground beef meat	Naturally contaminated	/	/	/	/	∅	∅	/	A	-(EL)	-(EL)	/	A	NA
	a	2588573	Quail	Naturally contaminated	/	/	/	/	∅	∅	/	A	∅	-(EL)	/	A	NA
	a	2588574	Pork fillet	<i>L.welshimeri</i>	BEY899	Chicken	Seeding 72h at 5±3°C	10,6	+(AL)	+(AL)	<i>L. welshimeri</i>	P	+(AL)	+(AL)	<i>L. welshimeri</i>	P	PA
	b	2588575	Turkey nem	Naturally contaminated	/	/	/	/	-(EL)	+(BL)	<i>L. welshimeri</i>	P	+(BL)	+(BL)	<i>L. welshimeri</i>	P	PA
	b	2588576	Beef burger	<i>L.welshimeri</i>	RYH463	Delicatessen	Seeding 72h at 5±3°C	28,6	+(AM)	+(AH)	<i>L. welshimeri</i>	P	+(BM)	+(BM)	<i>L. welshimeri</i>	P	PA
	b	2588577	Chicken puff pastry	<i>L.welshimeri</i>	CQAJ98	Ham rillettes	Seeding 72h at 5±3°C	32,6	+(AM)	+(AH)	<i>L. welshimeri</i>	P	+(BM)	+(BM)	<i>L. welshimeri</i>	P	PA
	c	2588578	Delicatessen (cervelas)	Naturally contaminated	/	/	/	/	∅	∅	/	A	-(EL)	-(EL)	/	A	NA
	c	2588579	Delicatessen (boudin noir)	Naturally contaminated	/	/	/	/	-(EL)	-(EL)	/	A	-(EL)	-(EL)	/	A	NA
	c	2588580	Herb sausage	Naturally contaminated	/	/	/	/	∅	∅	/	A	+(AL) halo (1)	+(BL) halo	<i>L. ivanovii</i>	P	PD
Dairy products	a	2588581	Raw milk cheese (Reblochon)	<i>L. innocua</i>	QBB281	Raw milk cheese	Seeding 72h at 5±3°C	24,4	+(AM)	+(AM)	<i>L. innocua</i>	P	+(BM)	+(BM)	<i>L. innocua</i>	P	PA
	a	2588582	Raw milk cheese (Morbier)	<i>L. ivanovii</i>	GJP629	Composite food environment	Seeding 72h at 5±3°C	6,0	-(EL)	+(BM) halo	<i>L. ivanovii</i>	P	+(BL) halo	+(BL) halo	<i>L. ivanovii</i>	P	PA
	a	2588583	Raw milk cheese (Le petit gris)	Naturally contaminated	/	/	/	/	+(AM)	+(AM)	<i>L. innocua</i>	P	+(AM)	+(AM)	<i>L. innocua</i>	P	PA
	a	2588584	Raw milk cheese (Abondance)	Naturally contaminated	/	/	/	/	+(AM)	+(AM)	<i>L. welshimeri</i>	P	+(BM)	+(BM)	<i>L. welshimeri</i>	P	PA
	b	2588585	Raw cow's milk	<i>L. innocua</i>	TGW734	Pasteurized cheese	Seeding 72h at 5±3°C	6,8	+(AM)	+(AM)	<i>L. innocua</i>	P	+(AM)	+(AM)	<i>L. innocua</i>	P	PA
	b	2588586	Cottage with raw milk	<i>L.welshimeri</i>	GLX736	Dairy environment	Seeding 72h at 5±3°C	25,0	∅	∅	/	A	-(EL)	-(EL)	/	A	NA
	b	2588587	Raw goat's milk	<i>L. ivanovii</i>	GJP629	Composite food environment	Seeding 72h at 5±3°C	6,0	+(AL) halo	+(BL) halo	<i>L. ivanovii</i>	P	+(AL) halo	+(BL) halo	<i>L. ivanovii</i>	P	PA
	c	2588588	Pasteurized milk	<i>L. innocua</i>	ADQC53	Butter	Seeding 72h at 5±3°C	22,6	+(AM)	+(BL)	<i>L. innocua</i>	P	+(BM)	+(BM)	<i>L. innocua</i>	P	PA
	c	2588589	Butter with pasteurized milk	<i>L.welshimeri</i>	GLX736	Dairy environment	Seeding 72h at 5±3°C	25,0	+(AM)	+(AM)	<i>L. welshimeri</i>	P	+(BM)	+(BM)	<i>L. welshimeri</i>	P	PA
	c	2588590	Ice cream cookie	<i>L. grayi</i>	GRY99	Composite food environment	Seeding 72h at 5±3°C	13,6	+(AH)	+(AH)	<i>L. grayi</i>	P	+(BH)	+(BH)	<i>L. grayi</i>	P	PA
Seafood products	a	2588591	Mackerel	<i>L. innocua</i>	TYA050	Tataki with tuna	Seeding 72h at 5±3°C	22,2	+(AL)	+(AL)	<i>L. innocua</i>	P	+(AL)	+(BL)	<i>L. innocua</i>	P	PA
	a	2588592	Saithe fillet	<i>L. ivanovii</i>	GQD028	Composite food environment	Seeding 72h at 5±3°C	7,0	∅	∅	/	A	-(EL)	-(EL)	/	A	NA
	a	2588593	Ray	<i>L.welshimeri</i>	AMAB65	Raw fish	Seeding 72h at 5±3°C	30,8	+(AL)	+(AL)	<i>L. welshimeri</i>	P	+(AM)	+(AM)	<i>L. welshimeri</i>	P	PA
	a	2588594	Cod fillet	<i>L. innocua</i>	BJS68	Smoked mackerel	Seeding 72h at 5±3°C	26,2	+(AL)	+(AL)	<i>L. innocua</i>	P	+(AL)	+(AL)	<i>L. innocua</i>	P	PA
	b	2588595	Smoked herring	Naturally contaminated	/	/	/	/	+(AM)	+(AM)	<i>L. innocua</i>	P	+(AM)	+(AM)	<i>L. innocua</i>	P	PA
	b	2588596	Smoked salmon	<i>L.seeligeri</i>	BTRF33	Herring salad	Seeding 72h at 5±3°C	30,6	+(AL)	+(AM)	<i>L.seeligeri</i>	P	+(AL)	+(AM)	<i>L.seeligeri</i>	P	PA
	b	2588597	Marinated salmon	<i>L.welshimeri</i>	BZVD88	Shrimps	Seeding 72h at 5±3°C	22,6	+(AM) with & w/o halo	+(AM) with & w/o halo	<i>L. monocytogenes + L. welshimeri</i>	P	+(AM) with & w/o halo	+(AM) with & w/o halo	<i>L. monocytogenes + L. welshimeri</i>	P	PA
	c	2588598	Cod brandade	<i>L.grayi</i>	TXG292	Prawns and scallops	Seeding 72h at 5±3°C	19,0	+(AL)	+(AL)	<i>L. grayi</i>	P	+(AL)	+(AL)	<i>L. grayi</i>	P	PA
	c	2588599	Piémontaise with tuna	Naturally contaminated	/	/	/	/	∅	-(EL)	/	A	-(EL)	-(EL)	/	A	NA
	c	2588600	Surimi shell	Naturally contaminated	/	/	/	/	∅	∅	/	A	∅	∅	/	A	NA

Category	Type	#	Matrix	Strains				Inoculation level CFU/25g	ALOA 24h at 37°C	ALOA 48h at 37°C	Confirmation	Final result	CHROMagar™ Listeria 24h at 37°C	CHROMagar™ Listeria 48h at 37°C	Confirmation	Final result	Agreement
				Strain	Reference	Origin	Stress applied										
Vegetables	a	2588601	Butternut	<i>L. innocua</i>	TTZ273	Cubed onions	Seeding 72h at 5±3°C	33,2	+(AL)	+(AL)	<i>L. innocua</i>	P	+(AL)	+(AL)	<i>L. innocua</i>	P	PA
	a	2588602	Pineapple	<i>L. grayi</i>	BFSC26	Coconut	Seeding 72h at 5±3°C	12,4	∅	∅	/	A	∅	∅	/	A	NA
	a	2588603	Cucumber	<i>L. ivanovii</i>	GJP629	Composite food environment	Seeding 72h at 5±3°C	6,0	+(AL) halo	+(AL) halo	<i>L. ivanovii</i>	P	+(AL) halo	+(BL) halo	<i>L. ivanovii</i>	P	PA
	a	2588604	Gratted carrots	<i>L. seeligeri</i>	TJM186	Vegetables	Seeding 72h at 5±3°C	24,4	∅	∅	/	A	-(EM)	+(DM) doubt	Colonies not characteristic on TSA YE	A	NA
	b	2588605	Remoulade celery	<i>L. grayi</i>	RZK366	Green beans	Seeding 72h at 5±3°C	13,8	∅	∅	/	A	-(EL)	-(EL)	/	A	NA
	b	2588606	Greek mushrooms	<i>L. innocua</i>	BNEQ97	Remoulade celery	Seeding 72h at 5±3°C	21,6	∅	∅	/	A	∅	-(EL)	/	A	NA
	b	2588607	Endive salad, walnuts, tomatoes, chives	<i>L. welshimeri</i>	TXR109	Chinese noodles	Seeding 72h at 5±3°C	14,4	+(AM)	+(AM)	<i>L. welshimeri</i>	P	+(AM)	+(AM)	<i>L. welshimeri</i>	P	PA
	c	2588608	Cooked mung beans	<i>L. grayi</i>	GSCM68	Mushrooms	Seeding 72h at 5±3°C	12,6	-(EL)	-(EL)	/	A	-(EL)	-(EL)	/	A	NA
	c	2588609	Zucchini soup	<i>L. innocua</i>	BJCK82	Zucchini gratin	Seeding 72h at 5±3°C	17,0	+(AL)	+(AL)	<i>L. innocua</i>	P	+(AL)	+(AL)	<i>L. innocua</i>	P	PA
	c	2588610	Couscous vegetables	<i>L. welshimeri</i>	TLJ742	Cooked vegetables	Seeding 72h at 5±3°C	36,4	+(AM)	+(AM)	<i>L. welshimeri</i>	P	+(BM)	+(BM)	<i>L. welshimeri</i>	P	PA
Composite foods	a	2588611	Tabbouleh	<i>L. innocua</i>	TTW844	Sandwich	Seeding 72h at 5±3°C	22,2	+(BM)	+(BM)	<i>L. innocua</i>	P	+(CM)	+(CM)	<i>L. innocua</i>	P	PA
	a	2588612	Shrimps and cucumber	<i>L. ivanovii</i>	GJP629	Composite food environment	Seeding 72h at 5±3°C	6,0	∅	+(AL) halo	<i>L. ivanovii</i>	P	+(AL) halo (1)	+(AL) halo	<i>L. ivanovii</i>	P	PA
	a	2588613	Vegetables with rice	<i>L. seeligeri</i>	ADTW22	Zucchini	Seeding 72h at 5±3°C	3,8	-(EL)	-(EL)	/	A	-(EL)	-(EL)	/	A	NA
	a	2588614	Cooked delicatessen in sauce	<i>L. welshimeri</i>	DEG545	Smoked bacon	Seeding 72h at 5±3°C	12,2	+(AM)	+(BM)	<i>L. welshimeri</i>	P	+(BL)	+(BM)	<i>L. welshimeri</i>	P	PA
	b	2588615	4 cheese pizza	<i>L. innocua</i>	CCPU75	Indiana pizza	Seeding 72h at 5±3°C	27,0	+(AH)	+(AH)	<i>L. innocua</i>	P	+(BM)	+(BM)	<i>L. innocua</i>	P	PA
	b	2588616	Lasagna	<i>L. welshimeri</i>	TDV458	Delicatessen	Seeding 72h at 5±3°C	15,2	+(AL)	+(AM)	<i>L. welshimeri</i>	P	+(AL)	+(AL)	<i>L. welshimeri</i>	P	PA
	b	2588617	Parisienne salad	Naturally contaminated	/	/	/	/	-(EL)	+(DL) (2)	<i>L. innocua</i>	P	-(EL)	-(EL)	/	A	ND
	c	2588618	Pastry (flan)	<i>L. grayi</i>	DHPZ64	Apple pancake	Seeding 72h at 5±3°C	15,6	-(EL)	-(EL)	/	A	∅	-(EL)	/	A	NA
	c	2588619	Liquid egg yolk	<i>L. innocua</i>	BWHS39	Cream brioche	Seeding 72h at 5±3°C	14,6	+(AL)	+(AL)	<i>L. innocua</i>	P	+(AL)	+(AL)	<i>L. innocua</i>	P	PA
	c	2588620	Pastry (chocolate mousse)	Naturally contaminated	/	/	/	/	+(CL)	+(DL)	<i>L. innocua</i>	P	+(CL)	+(CL)	<i>L. innocua</i>	P	PA

4. Interpretation

4.1. Inclusivity study

The 50 strains tested were pure and characteristic on the ALOA™ medium before inoculation in half Fraser broth.

The 50 strains isolated from half-Fraser broth gave characteristic colonies on ALOA™ medium after 24 and 48 hours of incubation of the agar.

On the CHROMagar™ Listeria medium, 46 strains gave characteristic colonies: blue colonies with or without a halo depending on the species. For 3 strains of *Listeria grayi* (n° SWZ606, n° SWE117, n° RZM251) growth on CHROMagar™ Listeria medium was possible only by adding skimmed milk to half Fraser broth. The colonies observed were characteristic after 24 and 48 hours of incubation on the agar. For a strain of *Listeria grayi* (n°TXG292), the addition of skimmed milk did not allow its growth in the broth and its detection on the CHROMagar™ Listeria medium.

4.2. Exclusivity study

Among the 40 strains tested, 36 did not grow or gave uncharacteristic colonies on the CHROMagar™ Listeria medium. For 4 strains (*Lactobacillus sakei* EEX511, *Corynebacterium phoceense* CCQT66, *Niallia taxi* BGZ152 et *Niallia taxi* CANM24) blue colonies without a halo were observed on the CHROMagar™ Listeria medium but the CAMP test confirmed that they were not *Listeria* strains.

4.3. Sensitivity study

Among the fifty samples tested, sixteen gave a negative result with the two-culture media tested. Thirty-two samples tested positive by both methods. Among these samples, five were naturally contaminated and twenty-seven were the result of artificial contamination.

For two samples, a discrepancy was observed between the result of the ALOA plates and that of the CHROMagar™ Listeria plates:

- Sample n°2588580 (Herb sausage), naturally contaminated, gave a positive result only with the CHROMagar™ Listeria plate and with a single colony observed after 24 hours of incubation. Biochemical identification revealed the presence of *Listeria ivanovii*.
- Sample no. 2588617 (Parisienne salad), naturally contaminated, gave a positive result only with the ALOA plate. The detection of *Listeria innocua* was possible after 48 hours of incubation of the ALOA plate and only 2 colonies were observed.

The two-discordance observed are probably due to the low level of contamination of the target analyte in the samples. This phenomenon is classically observed in these types of study.

5. Conclusion

Fifty strains of *Listeria* spp. other than *Listeria monocytogenes* were tested and forty-nine of them gave characteristic colonies on the CHROMagar™ *Listeria* medium after 24 and 48 hours of incubation of the plates. A single strain of *Listeria grayi* could not be detected on the CHROMagar™ *Listeria* medium but four other strains were tested and gave satisfactory results.

Fourty non-*Listeria* spp. strains were tested and for four of them blue colonies without halo were observed on the CHROMagar™ *Listeria* medium but the confirmation was negative.

Fifty representative matrices were tested on CHROMagar™ *Listeria* and ALOA agar media and only two discordances were observed: one in favor of ALOA medium and one in favor of CHROMagar™ *Listeria* medium.

The selectivity and the sensitivity of the CHROMagar™ *Listeria* medium are satisfactory.

The microbiological performance of CHROMagar™ *Listeria* medium is equivalent to that of a medium meeting the requirements of the EN ISO 11290 standard.

Le Lion d'Angers, January 18, 2024
Guillaume MESNARD,
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