

NF VALIDATION
Validation of alternative analytical methods
Application in food microbiology

Summary report

Validation study according to the EN ISO 16140-2:2016

Solus *Listeria monocytogenes* ELISA
(Certificate number: SOL 37/05-10/22)
for detection of *Listeria monocytogenes*
in food and environmental samples

Qualitative method

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This report consists of 89 pages, including 7 appendices.

Only copies including the totality of this report are authorised.

Competencies of the laboratory are certified by COFRAC accreditation for the analyses marked with the symbol♦.

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Quality Assurance documents related to this study can be consulted upon request from **SOLUS SCIENTIFIC SOLUTIONS Ltd.**

The technical protocol and the result interpretation were carried out according to the EN ISO 16140-2:2016 and the AFNOR technical rules (PR Revision 7).

Validation protocols	<ul style="list-style-type: none"> ▪ EN ISO 16140-1 (June 2016): Microbiology of the food chain - Method validation - <i>Part 1: Vocabulary</i> ▪ EN ISO 16140-2 (June 2016): Microbiology of the food chain - Method validation - <i>Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method</i> ▪ AFNOR Technical Rules (PR Rev 7)
Reference method*	ISO 11290-1 (May 2017): Microbiology of the food chain - Horizontal method for the detection and enumeration of <i>Listeria monocytogenes</i> and of <i>Listeria</i> spp.- Part 1: detection method
Alternative method	Solus <i>Listeria monocytogenes</i> ELISA
Scope	<input checked="" type="checkbox"/> Food products <input checked="" type="checkbox"/> Environmental samples
Certification organism	AFNOR Certification (http://nf-validation.afnor.org/)

* Analyses performed according to the COFRAC accreditation

1 AIM OF THE STUDY

The Solus *Listeria monocytogenes* ELISA for detection of *Listeria monocytogenes* in food and environmental samples was validated on 7th of October 2022 (certificate number: SOL 37/05-10/22).

2 METHOD PROTOCOLS

2.1 Alternative method

The flow diagram of the alternative method is provided in **Appendix 1**.

2.1.1 Principle

The Solus *Listeria monocytogenes* ELISA is based on an ELISA principle. The test provides a negative or a presumptive positive result from a two enrichment steps.

2.1.2 Protocol

The protocol is the following:

- Pre-enrichment step (25 g + 225 ml) in Half Fraser broth for 24 - 28 h at 30°C ± 1°C;
- Subculture in PAC supplemented Solus Palcam broth (0.2 ml + 10 ml) incubated for 24 - 28 h at 37°C ± 1°C;
- Heat treatment of an aliquot of the enrichment broth (15 - 20 min at 85 - 100°C);
- ELISA test using either the manual protocol or the DS2 automate;
- Confirmation of the positive results by streaking 100 µl of the non-heated enriched sample onto O&A plates. The only presence of typical colonies allows to confirm the positive ELISA tests.

It is possible to store the PAC supplemented Solus Palcam broth for 72 h at 5°C ± 3°C before proceeding to ELISA test and confirmation.

2.1.3 Restrictions

There is no restriction for use.

2.2 Reference method♦

The reference method is the ISO 11290-1 (May 2017): Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 1: detection method (See **Appendix 2**).

2.3 Study design

The study is a paired study design as the reference and alternative method have a common enrichment step.

3 METHOD COMPARISON STUDY

The method comparison study is a study performed by the expert laboratory to compare the alternative method with the reference method.

The study was carried out on a diversity of samples and strains representative of agri-food products. This does not constitute an exhaustive list of the different matrices included in the scope.

For any comment on the alternative method, please contact AFNOR Certification at <http://nf-validation.afnor.org/contact-2/>.

3.1 Sensitivity study

The sensitivity (SE) is the ability of the method to detect the analyte by either the reference or alternative method.

3.1.1 Number and nature of samples

413 samples were analysed providing 187 positive and 226 negative results. The distribution per tested category and type is given in Table 1.

♦ Analysis performed according to the COFRAC accreditation

Table 1 – Distribution per tested category and type

Category	Type	Positive	Negative	Total
1 Composite foods / Ready-to-eat and ready-to-reheat	a Ready-to-eat	12	9	21
	b Ready-to-reheat	9	11	20
	c Confectionaries, pastries and egg products	10	10	20
	Total	31	30	61
2 Meat products	a Raw products (frozen or fresh)	10	22	32
	b Meat based products ready to reheat	8	19	27
	c Raw and cooked delicatessen	12	13	25
	Total	30	54	84
3 Milk & dairy products	a Raw milk cheeses	8	12	20
	b Other products based on raw milk	15	10	25
	c Heat treated products	12	10	22
	Total	35	32	67
4 Vegetables	a Raw products (fresh and frozen)	9	12	21
	b Pre-cooked vegetables, vegetables under modified atmosphere	12	20	32
	c Ready to eat	9	11	20
	Total	30	43	73
5 Seafood and fishery products	a Raw products (fresh and frozen)	9	11	20
	b Cured & smoked	10	11	21
	c Ready to eat, ready to reheat	12	14	26
	Total	31	36	67
6 Environmental Samples	a Dusts and Residues	8	13	21
	b Cleaning and Process Waters	10	10	20
	c Surface samples	12	8	20
	Total	30	31	61
All categories		187	226	413

3.1.2 Artificial contamination of samples

Artificial contaminations were done by seeding (storage for 48 h or 72 h at $3^{\circ}\text{C} \pm 2^{\circ}\text{C}$) or spiking protocol (heat treatment for 8 min at 56°C). The artificial contaminations are presented in **Appendix 3**.

106 samples were artificially contaminated, using 34 different strains. 76 gave a positive result. 74 samples were inoculated using the seeding protocol. 33.2 % of samples were inoculated at level ≤ 3 CFU and 6.4 % were inoculated between 3 and 10 CFU.

Two positive samples were inoculated using the spiking protocol; they were inoculated between 5 and 10 CFU.

The repartition of the positive samples per inoculation protocol and inoculation level is given in Table 2.

Table 2 - Repartition of the positive samples per inoculation protocol and inoculation level

Naturally contaminated	Artificially contaminated						Total	
	Seeding protocol			Spiking protocol				
	≤ 3 CFU	3 < x ≤ 10 CFU	10 < x < 30 CFU	≤ 5 CFU	5 < x ≤ 10 CFU	10 < x < 30 CFU		
Number of samples	111	62	12	0	0	2	0	187
%	59.4	33.2	6.4	0,0	0,0	1,1	0,0	100,0

59.4 % of the samples were naturally contaminated.

3.1.3 Protocols applied during the validation study

Enrichment broth incubation time

The Half Fraser broths were incubated for 24 h at 30°C and the PAC supplemented Solus Palcam broth for 24h at 37°C.

ELISA test protocol

Automatic equipment ELISA was used: the Dynex DS2 with the software DS-Matrix 1.34.1.

Confirmation protocols

For all the samples, positive and negative, the PAC supplemented Solus Palcam broth (100 µl) was streaked onto O&A plates. The only presence of typical colonies on the plates allows to confirm the positive ELISA tests but during the validation, the typical colonies were confirmed by haemolysis and biochemical tests (MICROGEN Listeria-ID) on isolated colonies without purification step.

Note that for the negative samples, as the incubation time for the alternative method is equivalent to the incubation time for the reference method, no additional subculture was required to be in agreement with the ISO 16140-2 requirements.

Enrichment broth storage 72 h at 5°C ± 3°C

The enrichment broths (PAC supplemented Solus Palcam broth) from positive and discordant samples were stored for 72 h at 5°C ± 3°C and tested again (ELISA and confirmatory tests).

3.1.4 Test results

Raw data per category are given in **Appendix 4**. The results are given in Table 3.

Table 3 – Interpretation of sample results between the reference and alternative method (based on the confirmed alternative method results)

Category		PA	NA*	PD	ND**	PPND	PPNA	Total
1	Composite foods / Ready-to-eat and ready-to-reheat	26	29	1	4	0	1	61
2	Meat products	25	53	1	4	0	1	84
3	Milk and dairy products	34	31	0	1	0	1	67
4	Vegetables	28	43	2	0	0	0	73
5	Seafood and fishery products	30	36	1	0	0	0	67
6	Environmental samples	28	31	1	1	0	0	61
All categories		171	223	6	10	0	3	413

* PPNA not included

** PPND not included

3.1.5 Calculation of relative trueness (RT), sensitivity (SE) and false positive ratio (FPR)

The calculations are presented in Table 4.

Table 4 – Calculation of the relative trueness (RT), the sensitivity (SE) and the false positive ratio (FPR)

Category		Type	PA	NA	PD	ND	PPND	PPNA	SE alt %	SE ref %	RT %	FPR %
1	Composite foods / Ready-to-eat and ready-to-reheat	a Ready-to-eat	12	9	0	0	0	0	100,0	100,0	100,0	0,0
		b Ready-to-reheat	5	11	0	4	0	0	55,6	100,0	80,0	0,0
		c Confectionaries, pastries and egg products	9	9	1	0	0	1	100,0	90,0	95,0	11,1
		Total	26	29	1	4	0	1	87,1	96,8	91,8	3,3
2	Meat products	a Raw products (frozen or fresh)	10	22	0	0	0	0	100,0	100,0	100,0	0,0
		b Meat based products ready to reheat	6	18	0	2	0	1	75,0	100,0	92,6	5,6
		c Raw and cooked delicatessen	9	13	1	2	0	0	83,3	91,7	88,0	0,0
		Total	25	53	1	4	0	1	86,7	96,7	94,0	1,9
3	Milk & dairy products	a Raw milk cheeses	7	11	0	1	0	1	87,5	100,0	95,0	9,1
		b Other products based on raw milk	15	10	0	0	0	0	100,0	100,0	100,0	0,0
		c Heat treated products	12	10	0	0	0	0	100,0	100,0	100,0	0,0
		Total	34	31	0	1	0	1	97,1	100,0	98,5	3,1
4	Vegetables	a Raw products (fresh and frozen)	9	12	0	0	0	0	100,0	100,0	100,0	0,0
		b Pre-cooked vegetables, vegetables under modified atmosphere	11	20	1	0	0	0	100,0	91,7	96,9	0,0
		c Ready to eat	8	11	1	0	0	0	100,0	88,9	95,0	0,0
		Total	28	43	2	0	0	0	100,0	93,3	97,3	0,0
5	Seafood and fishery products	a Raw products (fresh and frozen)	9	11	0	0	0	0	100,0	100,0	100,0	0,0
		b Cured & smoked	9	11	1	0	0	0	100,0	90,0	95,2	0,0
		c Ready to heat, ready to reheat	12	14	0	0	0	0	100,0	100,0	100,0	0,0
		Total	30	36	1	0	0	0	100,0	96,8	98,5	0,0
6	Environmental Samples	a Dusts and Residues	7	13	0	1	0	0	87,5	100,0	95,2	0,0
		b Cleaning and Process Waters	9	10	1	0	0	0	100,0	90,0	95,0	0,0
		c Surface samples	12	8	0	0	0	0	100,0	100,0	100,0	0,0
		Total	28	31	1	1	0	0	96,7	96,7	96,7	0,0
All categories			171	223	6	10	0	3	94,7	96,8	96,1	1,3

* PPNA not included

** PPND not included

A summary of the results is given in Table 5.

Table 5 - Summary of results

Sensitivity for the alternative method	$SE_{alt} = \frac{(PA + PD)}{(PA + ND + PD)} \times 100\%$	94.7 %
Sensitivity for the reference method	$SE_{ref} = \frac{(PA + ND)}{(PA + ND + PD)} \times 100\%$	96.8 %
Relative trueness	$RT = \frac{(PA + NA)}{N} \times 100\%$	96.1 %
False positive ratio for the alternative method* FP = PPNA + PPND	$FPR = \frac{(FP)}{NA} \times 100\%$	1.3 %

With $ND = ND + PPND$
 $NA = NA + PPNA$

3.1.6 Analysis of discordant results

The negative deviations are given in Table 6 and the positive deviations in Table 7.

Negative deviations

Ten negative deviations were observed for the overall categories. All the samples were naturally contaminated.

For Sample No 7080, the confirmatory tests concluded to the presence of *Listeria monocytogenes* in the PAC supplemented Solus PALCAM broth. The ELISA test was repeated twice with the same heat treated aliquot and a new heat treated aliquot. All the ELISA tests were negative.

The strain recovered in the enriched broth was grown in BHI medium and tested with the Solus ELISA *L. monocytogenes* kit. Positive ELISA result was obtained. The detection level of the alternative method was probably not reached for this sample.

For all other samples, the confirmatory tests did not allow to recover *Listeria monocytogenes* strains in the enriched broths.

Positive deviations

Six positive deviations were observed; all the samples were naturally contaminated.

Table 6 - Negative deviations

Year of analysis	N° Sample	Product (French name)	Product	Global result	Reference method: ISO 11290-1				Alternative method: SOLUS <i>Listeria monocytogenes</i> ELISA					Agreement Ref/Alt	Category	Type		
					Half Fraser		Fraser 1		Identification	Final result	O.D.	Result	O&A (100µl)	All confirmatory tests		Final result		
					O&A	Palcam	O&A	Palcam										
2021	6229	Nuggets avec Emmental	Nuggets with cheese	+	H+	-	H-	+(2)	<i>L. monocytogenes</i>	+	0,052	-	H-	-	-	ND	1	b
2021	6466	Feuilleté chèvre et pesto	RTRH food with goat milk cheese and pesto	+	H+d/H-	+	H-	+	<i>L. monocytogenes</i>	+	0,027	-	H-	-	-	ND	1	b
2021	6474	Feuilleté saumon-épinard	RTRH food with salmon and spinach	+	H+/H-	+d	H+/H-	+	<i>L. monocytogenes</i>	+	0,110	-	H-	-	-	ND	1	b
2021	7080	Kiev provençal	RTRH food	+	H+(2)	-	H+	-	<i>L. monocytogenes</i>	+	0,091/0,093/0,100/0,035*/0,098*/0,051*/3,000 (BHI)	-/-/-*/-*/-*/+(BHI)	H+ (x5:H+)	+ <i>L. monocytogenes</i>	-	ND	1	b
2021	5346	Emincés kebab rôti dinde/poulet	Roasted poultry and chicken	+	H+d/H-	+	H+d/H-	+	<i>L. monocytogenes</i>	+	0,096	-	H-	-	-	ND	2	b
2021	5702	Sauté de dinde	Poultry meat	+	H+/H-	+	H+/H-	+	<i>L. monocytogenes</i>	+	0,115	-	H-	-	-	ND	2	b
2021	5528	Rillettes du Mans pur porc	Rillettes (pork meat)	+	H+/H-	+	H-	+	<i>L. monocytogenes</i>	+	0,198	-	H-	-	-	ND	2	c
2021	5851	Saucisson sec	Sausage	+	H+(1)	-	H+	-	<i>L. monocytogenes</i>	+	0,026	-	-	-	-	ND	2	c
2021	6609	Fromage non affiné, lait cru de vache	Raw cow milk cheese	+	-	-	H+	+d	<i>L. monocytogenes</i>	+	0,106	-	-	-	-	ND	3	a
2021	5960	Déchet viande de bœuf ramassée au sol (industrie de produits carnés)	Residues (meat products industry)	+	H+	+	H+	+	<i>L. monocytogenes</i>	+	0,041	-	H-	-	-	ND	6	a

RTRH: Ready to reheat

Table 7 - Positive deviations

Year of analysis	N° Sample	Product (french name)	Product	Global result	Reference method: ISO11290-1*		Alternative method: SOLUS <i>Listeria monocytogenes</i> ELISA					Agreement Ref/Alt PAC 37°C	Category	Type
					Final result	O.D.	Result	O&A (100 µl)	All confirmatory tests	Final result 37°C				
2021	5345	Truite de mer fumée Norvege	Smoked trout	+	-	0,220	+	H+d/H-	<i>L. monocytogenes</i>	+	PD	5	b	
2021	5359	Houmous	Hummus	+	-	3,000	+	H+d	<i>L. monocytogenes</i>	+	PD	4	c	
2021	5516	Saucisse Muscadet vrac	Sausage	+	-	3,000	+	H+	<i>L. monocytogenes</i>	+	PD	2	c	
2021	5540	Croissant cru	Croissant	+	-	1,378	+	H+	<i>L. monocytogenes</i>	+	PD	1	c	
2021	5856	Eau de process	Process water	+	-	3,000	+	H+	<i>L. monocytogenes</i>	+	PD	6	b	
2021	6467	Jeunes carottes	Baby carrots	+	-	3,000	+	H+	<i>L. monocytogenes</i>	+	PD	4	b	

* Analyses performed according to the COFRAC accreditation

The analyses of discordant results according to the EN ISO 16140-2:2016 is the following (See Table 8):

Table 8 - Analyses of discordant results

Category		Type	N+	ND	PPND	PD	Paired			
							(ND+PPND)-PD	AL	(ND+PPND)+PD	AL
1	Composite foods / Ready-to-eat and ready-to-reheat	a Ready-to-eat	12	0	0	0				
		b Ready-to-reheat	9	4	0	0				
		c Confectionaries, pastries and egg products	10	0	0	1				
		Total	31	4	0	1	3	3	5	6
2	Meat products	a Raw products (frozen or fresh)	10	0	0	0				
		b Meat based products ready to reheat	8	2	0	0				
		c Raw and cooked delicatessen	12	2	0	1				
		Total	30	4	0	1	3	3	5	6
3	Milk & dairy products	a Raw milk cheeses	8	1	0	0				
		b Other products based on raw milk	15	0	0	0				
		c Heat treated products	12	0	0	0				
		Total	35	1	0	0	1	3	1	6
4	Vegetables	a Raw products (fresh and frozen)	9	0	0	0				
		b Pre-cooked vegetables, vegetables under modified atmosphere	12	0	0	1				
		c Ready to eat	9	0	0	1				
		Total	30	0	0	2	-2	3	2	6
5	Seafood and fishery products	a Raw products (fresh and frozen)	9	0	0	0				
		b Cured & smoked	10	0	0	1				
		c Ready to eat, ready to reheat	12	0	0	0				
		Total	31	0	0	1	-1	3	1	6
6	Environmental Samples	a Dusts and Residues	8	1	0	0				
		b Cleaning and Process Waters	10	0	0	1				
		c Surface samples	12	0	0	0				
		Total	30	1	0	1	0	3	2	6
All categories			187	10	0	6	4	6	16	16

* PPNA not included

** PPND not included

The observed values for ND + PPPND – PD and ND + PPND + PD meet the acceptability limit for each individual category and for all the combined categories (calculated values \leq AL).

3.1.7 Enrichment broth storage at $5 \pm 3^{\circ}\text{C}$ for 72 h

221 samples were tested again after storage of the PAC supplemented Solus PALCAM broth for 72 h at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$. No change was observed.

The analyses of discordant results are (See Table 9).

Table 9 - Analysis of discordant results after storage 72 h at $5 \pm 3^{\circ}\text{C}$

		Paired								
Category		Type	N+	ND	PPND	PD	(ND+PPND)-PD	AL	(ND+PPND)+PD	AL
1	Composite foods / Ready-to-eat and ready-to-reheat	a	12	0	0	0				
		b	9	0	4	0				
		c	10	1	0	0				
		Total	31	1	4	0	3	3	5	6
2	Meat products	a	10	0	0	0				
		b	8	0	2	0				
		c	12	1	2	0				
		Total	30	1	4	0	3	3	5	6
3	Milk & dairy products	a	8	0	1	0				
		b	15	0	0	0				
		c	12	0	0	0				
		Total	35	0	1	0	1	3	1	6
4	Vegetables	a	9	0	0	0				
		b	12	1	0	0				
		c	9	1	0	0				
		Total	30	2	0	0	-2	3	2	6
5	Seafood and fishery products	a	9	0	0	0				
		b	10	1	0	0				
		c	12	0	0	0				
		Total	31	1	0	0	-1	3	1	6
6	Environmental Samples	a	8	0	1	0				
		b	10	1	0	0				
		c	12	0	0	0				
		Total	30	1	1	0	0	3	2	6
All categories			187	6	10	0	4	6	16	16

* PPNA not included

** PPND not included

The observed values for ND + PPND - PD and ND + PPND + PD meet the acceptability limit for each individual category and for all the combined categories (calculated values \leq AL).

3.1.8 Confirmation

Confirmations were carried out by streaking 0.1 mL of the PAC supplemented PALCAM broth onto O&A plates. All ELISA positive tests were confirmed by streaking on the plates except for three samples (No 5164, 5360 and 6617). It was not possible to confirm these three samples. In each case, the ELISA test was tested three times; all the tests gave positive result with low O.D. values (See Table 10).

Two of these samples gave also positive results after storage for 72 h at 5°C ± 3°C (No 5164 and 6617).

For all the confirmed positive samples, the haemolysis test and biochemical tests gave positive results.

Table 10 – PPNA samples

Year of analysis	N° Sample	Product (french name)	Product	Global result	ISO 11290-1*	SOLUS <i>Listeria monocytogenes</i> ELISA					Agreement Ref/Alt PAC 37°C	SOLUS <i>Listeria monocytogenes</i> ELISA after storage 72h at 5±3°C					Category	Type		
						Final result	O.D	Result	O&A (100 µl)	All confirmatory tests		O.D	Result	O&A (100 µl)	All confirmatory tests	Final result 37°C				
2021	5164	Fromage non affiné au lait cru de vache	Raw cow milk cheese	-	-	1,704/ 0,648/ 0,779	+/-/+	-	- (Palcam plate x5: -, Palcam broth x5: -)	-	-	PPNA	0,227/ 0,377/ 0,350	+/-/+	- (Palcam plate x5: -, Palcam broth x5: -)	-	-	PPNA	3	a
2021	5360	Croissant	Croissant	-	-	0,221/ 0,276/ 0,397	+/-/+	-	- (Palcam plate x5: -, Palcam broth x5: -)	-	-	PPNA	0,090	-	-	-	-	NA	1	c
2021	6617	Emincés poulet mariné au citron, cuits	Seasoned and cooked chicken meat	-	-	0,367/ 0,374/ 0,362	+/-/+	st	(Palcam plate x5: st, Palcam broth x5: -)	-	-	PPNA	0,345/ 0,306/ 0,281	+/-/+	st (x5: st, Palcam broth x5: -)	-	-	PPNA	2	b

* Analyses performed according to the COFRAC accreditation

3.2 Relative level of detection

The relative level of detection is the level of detection at P = 0.50 (LOD₅₀) of the alternative (proprietary) method divided by the level of detection at P = 0.50 (LOD₅₀) of the reference method.

The RLOD is defined as the ratio of the alternative and reference methods:

$$RLOD = \frac{LOD_{Alt.}}{LOD_{Ref.}}$$

The relative detection level is the smallest number of culturable micro-organisms that can be detected in the sample in 50% of occasions by the alternative and reference methods.

3.2.1 Experimental design

Six (matrix/strain) pairs were analysed by the reference method and by the alternative method (See Table 11):

The following protocol was applied:

- A negative control: 5 samples,
- A low contamination level providing fractional recovery data, with 20 replicates,
- A high contamination level, with 5 replicates.

A total plate count determination on each matrix was performed to estimate the total microbial load on the day of analysis.

Table 11 - Defined (matrix/strain) pairs for the RLOD determination

Category		Matrix	Inoculated strain	Origin	Storage conditions before analysis
1	Composite foods	Deli salad: piémontaise	<i>Listeria monocytogenes</i> Ad494	Piémontaise	Seeding 3°C ± 2°C for 48 h
2	Meat products	Rillettes	<i>Listeria monocytogenes</i> Ad669	Rillettes	
3	Milk and dairy products	Raw milk	<i>Listeria monocytogenes</i> 153	Cheese	
4	Seafood and fishery products	Smoked salmon	<i>Listeria monocytogenes</i> Ad670	Smoked salmon	
5	Vegetables	Ready-to-cook vegetables	<i>Listeria monocytogenes</i> Ad279	Ready-to-cook vegetables	
6	Production environmental samples	Process water	<i>Listeria monocytogenes</i> Ad551	Environment	

3.2.2 Calculation and interpretation of the RLOD

The raw data are given in **Appendix 5**.

The RLOD calculations were performed using the Excel spreadsheet available at <http://standards.iso.org/iso/16140> - RLOD (clause 5-1-4-2 Calculation and interpretation of RLOD) version 06.07.2015. The RLOD are given Table 12.

Table 12 – Presentation of RLOD before and after confirmation of the alternative method results

Category	Name	AL	RLOD	RLODL	RLODU	b=ln(RLOD)	sd(b)	z-Test statistic	p-value
1	Piemontaise / <i>L. monocytogenes</i> Ad494	1,5	1,000	0,477	2,097	0,000	0,37	0,000	1,000
2	Rillettes / <i>L. monocytogenes</i> Ad669		1,000	0,387	2,582	0,000	0,474	0,000	1,000
3	Raw milk / <i>L. monocytogenes</i> 153		1,000	0,457	2,187	0,000	0,391	0,000	1,000
4	Smoked salmon / <i>L. monocytogenes</i> Ad670		1,000	0,396	2,525	0,000	0,463	0,000	1,000
5	Ready to cook vegetables / <i>L. monocytogenes</i> Ad279		0,826	0,322	2,120	-0,191	0,472	0,406	1,315
6	Process water / <i>L. monocytogenes</i> Ad551		1,000	0,485	2,063	0,000	0,362	0,000	1,000
Combined			0,974	0,699	1,356	-0,027	0,166	0,162	1,129

The LOD₅₀ % calculations according to Wilrich & Wilrich POD-LOD calculation program - version 10, 2021-03-02 test are given in Table 13.

Table 13 - LOD₅₀ results

Category	(Strain / matrix) pair	Level of detection at 50% (CFU / test portion) according to Wilrich & Wilrich ¹	
		Reference method	Alternative method
1	Piemontaise / <i>L. monocytogenes</i> Ad494	0,7 [0,4-1,2]	0,7 [0,4-1,2]
2	Rillettes / <i>L. monocytogenes</i> Ad669	1,4 [0,7-2,7]	1,4 [0,7-2,7]
3	Raw milk / <i>L. monocytogenes</i> 153	0,3 [0,2-0,5]	0,3 [0,2-0,5]
4	Smoked salmon / <i>L. monocytogenes</i> Ad670	1,0 [0,5-1,9]	1,0 [0,5-1,9]
5	Ready to cook vegetables / <i>L. monocytogenes</i> Ad279	1,4 [0,7-2,9]	1,2 [0,6-2,2]
6	Process water / <i>L. monocytogenes</i> Ad551	0,6 [0,3-1,0]	0,6 [0,3-1,0]
Combined results		0,8 [0,6-1,0]	0,7 [0,6-1,0]

3.2.3 Conclusion

The RLOD values (using the confirmed alternative method results) meet the acceptability limit of 1.5 for paired studies, for all matrix/strain pairs tested.

The LOD₅₀ varies from 0.3 to 1.4 CFU/test portion for both the reference and the alternative methods.

¹ Wilrich, C., and P.-Th. Wilrich: Estimation of the POD function and the LOD of a qualitative microbiological measurement method. AOAC International **92** (2009) 1763 - 1772.

3.3 Inclusivity / exclusivity

The inclusivity is the ability of the alternative method to detect the target analyte from a wide range of strains. The exclusivity is the lack of interference from a relevant range of non-target strains of the alternative method.

3.3.1 Test protocols

> Inclusivity

Listeria monocytogenes strain cultures were performed in BHI medium at 37°C. Dilutions were done in order to inoculate 10 - 100 cells/225 ml of Half Fraser broth. The broths were incubated for 24 h at 30°C and sub-cultured in PAC supplemented Solus Palcam broth (0.2 ml + 10 ml) incubated for 24 h at 37°C ± 1°C before performing the alternative method protocol (test ELISA and confirmation).

> Exclusivity

Negative strains cultures were performed in BHI at 37°C. Dilutions were done in order to inoculate 10^5 cells/ml BPW. The BPW was incubated for 24h at 37°C ± 1°C. The alternative method was then performed (ELISA test).

3.3.2 Results

Raw data are given in **Appendix 6**.

> Inclusivity

The 50 *Listeria monocytogenes* strains tested gave positive results.

> Exclusivity

No cross reaction was observed among the 30 negative strains tested.

3.4 Practicability

The alternative method practicability was evaluated according to the AFNOR criteria relative to method comparison study.

Storage conditions, shelf-life and modalities of utilisation after first use	The storage temperature is 2-8°C. The shelf-life is given on the package. All the reagents shall be stored at the temperature mentioned on the package.		
Time to result	Steps	Reference method	Alternative method
Negative samples			
Sample enrichment	Day 0	Day 0	
Subculture in Fraser 1 or PAC supplemented Solus PALCAM broth	Day 1	Day 1	
ELISA test	/	Day 2	
Streaking onto plates (O1/P1)	Day 1	/	
Second streaking (O2/P2)	Day 2	/	
Reading plates (O1/P1)	Day 2 - Day 3	/	
Reading plates (O2/P2)	Day 3 - Day 4	/	
Result	Day 4	Day 2	
Presumptive positive or positive results			
Subculture of typical colonies	Day 3 - Day 4	/	
Streaking onto plates	/	Day 2	
Reading plates	/	Day 3 - Day 4	
Confirmatory test	Day 3 - Day 5	Day 3 - Day 4	
Result	Day 4 - Day 6	Day 3 - Day 4	
Common step with the reference method	Primary enrichment step		

The negative results are available in 2 days and the positive results in 3 or 4 days.

4 INTER-LABORATORY STUDY

The inter-laboratory study is a study performed by multiple laboratories testing identical samples at the same time, the results of which are used to estimate alternative-method performance parameters.

4.1 Study organisation

> Collaborators number

Samples were sent to 15 laboratories (See **Appendix 3**).

> Matrix and strain used

A fresh goat cheese was inoculated by *Listeria monocytogenes* 153 isolated from a raw milk cheese.

> Samples

Samples were prepared and inoculated on Monday 20th of June 2022 as described below:

- 24 blind coded samples (25 g) for detection of *Listeria monocytogenes* by the **Solus Listeria monocytogenes ELISA method** and by the **ISO 11290-1 reference method**.
- 1 sample (labelled “Sample for Total Count enumeration”) for Aerobic mesophilic flora enumeration by the ISO 4833-1 method
- 1 water flask labelled “Temperature Control” with a temperature probe.

> Inoculation

The targeted inoculation levels were the following:

- Level 0: CFU/25 g,
- Level 1: 1.5 CFU/25 g, inoculation level providing fractional positive results;
- Level 2: 6 CFU/25 g.

> *Labelling and shipping*

Blind coded samples were placed in isothermal boxes, which contained cooling blocks, and express-shipped to the different laboratories.

A temperature control flask containing a sensor was added to the package to register the temperature profiles of the samples during the transport, the package delivery and storage until analyses.

Samples were supposed to be shipped in 24 h to 48 h to the involved laboratories. The temperature conditions had to stay lower or equal to 8°C during transport, and between 0°C – 8°C in the labs.

> *Analyses*

Collaborative study laboratories and the expert laboratory carried out the analyses on Tuesday 21st June or Wednesday 22nd June with the alternative and reference methods.

4.2 Experimental parameters controls

4.2.1 Strain stability and background microflora stability

Strain stability was checked by inoculating the matrix at 1.5 CFU/g (detection) and 1.10³ CFU/g (enumeration). Enumerations were performed for the high contamination level and detection analyses were performed for the low contamination level after 24 h and 48 h storage at 3 ± 2°C. *Triplicate* samples were analysed. The aerobic mesophilic flora was also enumerated; the results are given in Table 14.

Table 14 - Sample stability

Day	Reference method (detection)			Enumeration (CFU/g)			Aerobic mesophilic flora (CFU/g)
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	
Day 0	+	+	+	8,0.10 ²	8,0.10 ²	8,2.10 ²	6,0.10 ⁵
Day 1	-	+	+	8,0.10 ²	7,0.10 ²	1,5.10 ³	1,2.10 ⁵
Day 2	+	+	+	6,7.10 ²	8,3.10 ²	7,9.10 ²	1,4.10 ⁵
Day 3	-	+	+	1,1.10 ³	9,8.10 ²	9,5.10 ²	1,8.10 ⁵

No evolution was observed during storage at 3°C ± 2°C for both *Listeria monocytogenes* enumeration and detection and as well as for the mesophilic flora enumeration.

4.2.2 Contamination levels

The contamination levels and the sample codification were the following (see Table 15).

Table 15 - Contamination levels

Level	Samples	Theoretical target level (CFU/test portion)	True level (CFU/test portion)	Low limit (CFU/test portion)	High limit (CFU/test portion)
0	3-7-8-11-14-18-19-24	0	0	/	/
1	1-6-10-13-16-17-21-23	1,5	1,3	1,0	1,7
2	2-4-5-9-12-15-20-22	6	4,5	3,3	6,0

4.2.3 Logistic conditions

Temperature conditions are given in Table 16.

Table 16 - Sample temperatures at receipt

Collaborators	Temperature measured		Receipt		Analysis	
	By the probe (°C)	At receipt (°C)	Date	Time	Date	Time
A	3,5	5,4	22/06/2022	1:00 PM	22/06/2022	2:00 PM
B	16,5	14°C	23/06/2022	11:00 AM	23/06/2022	1:00 PM
C	Samples not received					
D	3,5	6,2	21/06/2022	11:30 AM	22/06/2022	2:00 PM
F	3,0	10,0	21/06/2022	11:00 AM	22/06/2022	10:00 AM
G	4,0	5,6	21/06/2022	09:30 AM	21/06/2022	10:00 AM
H	5,0	5,3	22/06/2022	12:30 AM	22/06/2022	1:30 PM
I	2,0	5,7	22/06/2022	11:30 AM	22/06/2022	4:00 PM
J	Analyses not performed					
L	2,5	6,2	22/06/2022	12:30 AM	22/06/2022	1:30 PM
M	2,0	3,1	21/06/2022	09:30 AM	21/06/2022	01:00 PM
N	3,0	5,8	22/06/2022	09:00 AM	22/06/2022	10:30 AM
O	Samples not received					
P	1,5	3,9	21/06/2022	10:30 AM	21/06/2022	11:30 AM
Q	5,0	15	22/06/2022	10:45 AM	22/06/2022	10:30 AM
S (ADRIA)	2,5	3,6	21/06/2022	08:30 AM	22/06/2022	08:30 AM

For 9 collaborators (A, D, G, H, I, L, M, N and P) no problem was encountered during the transport or at samples receipt. All the samples were delivered on time and in appropriate conditions. The temperatures during transport and at receipt were correct. For two collaborators (F and Q), no problem was encountered during the transport or receipt of samples. However, the collaborators have measured high temperature at receipt (10.0°C for collaborator F and 15.0°C for Q) but the temperature probe indicated a correct temperature (3.0°C for F and 5.0°C for Q).

For one collaborator (B), the samples arrived at day 3 (Thursday 23rd June) with high temperature at receipt (14.0°C) which was confirmed by the temperature probe (16.5°C). According to the ISO16140-2:2016, if transit conditions and times fall outside the specified acceptable tolerances, the data are disregarded for interpretation. Data set from collaborator B will not be considered.

Two collaborators (C and O) did not receive the samples on time (2 weeks after) caused by delivery destination error by the carrier.

One collaborator (J) had received the samples in appropriate conditions, but they could not proceed to the analyses (lack of personnel related to COVID).

4.3 Results analysis

The raw data are given in **Appendix 7**.

4.3.1 Expert laboratory results

The results obtained by the expert laboratory are given in Table 17.

Table 17 – Results obtained by the expert Lab

Level	Reference method	Alternative method
L0	0/8	0/8
L1	7/8	7/8
L2	8/8	8/8

Only one sample inoculated at the low level gave a negative result on the 8 samples inoculated.

4.3.2 Results observed by the collaborative laboratories

> **Aerobic mesophilic flora enumeration**

Depending on the Lab results, the enumeration levels varied from $8.0 \cdot 10^3$ CFU/g to $6.6 \cdot 10^8$ CFU/g.

> **Protocol used for the ELISA test**

2 collaborators used the manual protocol and 10 collaborators the DS2 automate. See Table 18.

Table 18 - ELISA protocol used by the collaborators

Collaborators	ELISA protocol
A	DS2 automate
B	Manual protocol
C	/
D	DS2 automate
F	DS2 automate
G	Manual protocol
H	DS2 automate
I	DS2 automate
J	/
L	DS2 automate
M	DS2 automate
N	DS2 automate
O	/
P	DS2 automate
Q	DS2 automate
S (ADRIA)	DS2 automate

> **Listeria monocytogenes detection**

12 collaborators participated to the study. The results obtained are provided in Table 19 (reference method) and Table 20 (alternative method).

Table 19 - Positive results by the reference method (ALL the collaborators)

Collaborator	Contamination level		
	L0	L1	L2
A	0	5	8
B	0	5	8
C	/	/	/
D	0	3	8
F	0	7	8
G	0	5	8
H	0	5	8
I	0	5	8
J	/	/	/
L	0	6	7
M	0	6	8
N	0	7	8
O	/	/	/
P	0	6	8
Q	0	7	8
TOTAL	P₀ = 0	P₁ = 67	P₂ = 95

Table 20 - Positive results (before and after confirmation) by the alternative method (ALL the collaborators)

Collaborators	Contamination level								
	L0			L1			L2		
	ELISA result	Confirmation result	Final result	ELISA result	Confirmation result	Final result	ELISA result	Confirmation result	Final result
A	0	0	0	5	5	5	8	8	8
B	1	0	0	6	5	5	8	8	8
C	/	/	/	/	/	/	/	/	/
D	0	0	0	3	3	3	8	8	8
F	5	0	0	8	7	7	8	8	8
G	0	0	0	5	5	5	8	8	8
H	0	0	0	5	5	5	8	8	8
I	0	0	0	5	5	5	8	8	8
J	/	/	/	/	/	/	/	/	/
L	0	0	0	6	6	6	7	7	7
M	0	0	0	6	6	6	8	8	8
N	0	0	0	7	7	7	8	8	8
O	/	/	/	/	/	/	/	/	/
P	0	0	0	6	6	6	8	8	8
Q	2	0	0	7	7	7	8	8	8
Total	8	0	0	69	67	67	95	95	95

Three collaborators obtained non-confirmed positive ELISA results on unspiked samples for the alternative method:

- Collaborator B: 1 sample (n°7)
- Collaborator F: 5 samples (n°3, 8, 11, 14 and 18)
- Collaborator Q: 2 samples (n°7 and 24).

For the collaborator F, in addition of the 5 PPNA samples obtained at the level 0, 1 non confirmed ELISA positive result was observed at level 1. The ELISA test was carried out again with the same heat treated aliquot and the same results were obtained. A new aliquot of the stored, enriched PAC supplemented Solus Palcam broth from each of the 6 PPNA samples observed at level 0 and 1, were heat treated and tested, with 5 of the repeat samples giving ELISA negative results. Note that for 3 analytical series run by collaborator F, the negative control O.D was above 0.100 for two analytical series, acceptance criteria fixed to validate the protocol. See Table 21.

These results could be explained by an ineffective washing step carried out by the DS2 automate.

The following explanation was given by collaborator F: “*The rack of the Solus Listeria monocytogenes Kit has not the same height compared to the Solus Salmonella Kit, we normally used. Actually, I do not know if there was a note of Solus but the technician (from DS2) informed me we should have change the different height of the Listeria kit rack in the DS2 assay protocol. This would explain the “ineffective “washing step.”*

Due to this washing step issue during the ELISA test resulting from an incorrect plate wash height setting, it was decided to not take into account the data set from collaborator F.

Table 21 – PPNA samples obtained by the collaborator F

		Heat treated aliquots n°1 : 23/06/2022				Heat treated aliquots n°2 : 14/07/2022		Confirmations	
		Test 1		Test 2		ELISA test (O.D.)	Result		
		ELISA test (O.D.)	Result	ELISA test (O.D.)	Result				
Control	Negative control (<0,100)	0,212	FAILED	0,188	PASS	0,213	FAILED	O&A (100µl)	
	Positive control (>0,500)	5,000	PASS	5,000	PASS	5,000	PASS		
Level 0	3	0,271	+	0,213	+	0,177	-	-	
	8	0,201	+	0,233	+	0,184	-	-	
	11	0,220	+	0,238	+	0,161	-	-	
	14	0,203	+	0,210	+	0,160	-	-	
	18	0,219	+	0,213	+	0,211	+	-	
Level 1	23	0,212	+	0,253	+	0,175	-	-	

For collaborators B and Q, they were asked to test the samples again by ELISA test, but unfortunately, due to all the samples being disposed of, this was not possible.

As a reminder, the data from collaborator B could not be considered because of the high temperature at receipt anyway.

According to the AFNOR technical rules, it is possible to include the results from a collaborator with maximum one cross contamination at Level 0.

If this rule is applied, the results from the collaborator Q should be excluded: this means that only 9 data sets are available.

It was proposed to the AFNOR Technical committee to keep the data from collaborator Q as according to the O.D. values obtained (2,118 and 1,514) with a negative cultural confirmation for the two unspiked samples could not be linked to a cross contamination during the cultural process but most likely due to sample mixing during testing. The AFNOR Technical committee agreed with this proposition and the interpretation was performed with 10 data sets including collaborator Q.

4.3.3 Results of the collaborators retained for interpretation

The results obtained with the 10 collaborators kept for interpretation are presented in Table 22 (reference method) and Table 23 (alternative method).

**Table 22 - Positive results by the reference method
(Without collaborators B, C, F, J and O)**

Collaborators	Contamination level		
	L0	L1	L2
A	0	5	8
D	0	3	8
G	0	5	8
H	0	5	8
I	0	5	8
L	0	6	7
M	0	6	8
N	0	7	8
P	0	6	8
Q	0	7	8
TOTAL	P₀ = 0	P₁ = 55	P₂ = 79

**Table 23 - Positive results (before and after confirmation)
by the alternative method (Without collaborators B, C, F, J and O)**

Collaborators	Contamination level								
	L0			L1			L2		
	ELISA result	Confirmation	Final result	ELISA result	Confirmation	Final result	ELISA result	Confirmation	Final result
A	0	0	0	5	5	5	8	8	8
D	0	0	0	3	3	3	8	8	8
G	0	0	0	5	5	5	8	8	8
H	0	0	0	5	5	5	8	8	8
I	0	0	0	5	5	5	8	8	8
L	0	0	0	6	6	6	7	7	7
M	0	0	0	6	6	6	8	8	8
N	0	0	0	7	7	7	8	8	8
P	0	0	0	6	6	6	8	8	8
Q	2	0	0	7	7	7	8	8	8
TOTAL	P₀ = 2	C₀ = 0	CP₀ = 0	P₁ = 55	C₁ = 55	CP₁ = 55	P₂ = 79	C₂ = 79	CP₂ = 79

Fractional positive results were observed for the reference and the alternative method (68.8%) for the low inoculation level (L1)

4.4 Calculation and interpretation

4.4.1 Calculation of the specificity percentage (SP)

The Specificity percentage (SP) of the reference method and of the alternative method, using the data after confirmation, based on the results of level L0 are presented in **Table 11**.

Table 24 - Specificity percentage

Specificity for the reference method	$SP_{ref} = \left(1 - \left(\frac{P_0}{N_-}\right)\right) \times 100 \% =$	100 %
Specificity for the alternative method	$SP_{alt} = \left(1 - \left(\frac{CP_0}{N_-}\right)\right) \times 100 \% =$	100 %

N: number of all L0 tests

P_0 = total number of false-positive results obtained with the blank samples before confirmation

CP_0 = total number of false-positive results obtained with the blank samples

4.4.2 Calculation of the sensitivity of the alternative method (SE_{alt}), the sensitivity for the reference method (SE_{ref}), the relative trueness (RT) and the false positive ratio for the alternative method (FPR)

Fractional positive results were obtained for the low and the high inoculation levels (L1 + L2). The two inoculation levels were retained for calculation.

A summary of the results of the collaborators retained for interpretation, obtained with the reference and the alternative methods for Level 1 and Level 2 are provided in Table 25.

Table 25 - Summary of the obtained results with the reference method and the alternative method for Level 1 and Level 2

Level	Response	Reference method positive (R+)	Reference method negative (R-)
1	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 55	Positive deviation (R-/A+) PD = 0
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 0 (0 PPND)	Negative agreement (A-/R-) NA = 25 (0 PPNA)
2	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 79	Positive deviation (R-/A+) PD = 0
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 0 (0 PPND)	Negative agreement (A-/R-) NA = 1 (0 PPNA)

Based on the data summarized in Table 25, the calculated values of the sensitivity of the alternative and reference methods, as well as the relative trueness and false positive ratio for the alternative method (taking account the confirmations) are presented in Table 27 for Level 1.

Table 26 - Sensitivity, relative trueness, and false positive ratio percentages

		Level 1
Sensitivity for the alternative method	$SE_{alt} = \frac{(PA+PD)}{(PA+PD+ND)} \times 100\% =$	100.0%
Sensitivity for the reference method	$SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)} \times 100\% =$	100.0%
Relative trueness	$RT = \frac{(PA+NA)}{N} \times 100\% =$	100.0%
False positive ratio for the alternative method	$FPR = \frac{FP}{NA} \times 100\% =$	0.0%

4.4.3 Interpretation of data

No negative and no positive deviation was obtained for this interlaboratory study for the 10 collaborators kept for interpretation.

For a **paired study design**, the difference between (ND – PD) and the addition (ND + PD) are calculated for the level(s) where fractional recovery is obtained (so L_1 and possibly L_2). The observed value found for (ND – PD) and (ND + PD) shall not be higher than the AL.

For 10 Labs, the limits are the following for Level 1:

		Calculated values	AL	Conclusion
Level 1	ND - PD	0	3	ND - PD < AL
	ND + PD	0	4	ND + PD < AL

The EN ISO 16140-2:2016 requirements are fulfilled as (ND - PD) and (ND + PD) meet the AL.

4.4.4 Evaluation of the LOD_{50%}, LOD_{95%} and RLOD between laboratories

The RLOD was calculated using the EN ISO 16140-2:2016 Excel spreadsheet available at https://standards.iso.org/iso/16140/-5/ed-1/en/RLOD_inter-lab-study_16140-2_AnnexF_ver1_28-06-2017.xls. The results are used only for information (see Table 27).

Table 27 - LOD_{50%}, LOD_{95%} and RLOD

Method	LOD 50%	LOD 95%	RLOD
Reference	0.8 [0.6-1.0]	3.3 [2.6-4.1]	1.0 [0.8-1.3]
Alternative	0.8 [0.6-1.0]	3.3 [2.6-4.1]	

5 CONCLUSION

The **method comparison study conclusions** are:

- ☒ The method comparison study scheme corresponds to a PAIRED STUDY design as the alternative and reference methods have a common enrichment procedure.
- ☒ In the sensitivity study, 6 categories were tested: 5 food categories and the environmental samples. The protocol of the alternative method shows 6 positive deviations (PD) and 10 negative deviations (ND) for all the combined categories. The ND + PPND - PD and ND + PPND + PD meet the acceptability limits (AL) whatever the categories, and as well for the 6 tested categories.
- ☒ The Relative Levels of Detection (RLOD) meet the AL fixed 1.5 for the paired data study whatever the matrix/strain pairs tested.
- ☒ The inclusivity and exclusivity testing gave the expected results for the 50 target strains and the 30 non-target strains.
- ☒ It is possible to store the primary enrichment broth for 72 h at $5 \pm 3^\circ\text{C}$.
- ☒ The alternative method allows to obtain results of the negative samples in 2 days.
- ☒ The alternative method fulfils all the EN ISO 16140-2:2016 and AFNOR technical rules (PR revision 7).

The **inter-laboratory study conclusions** are:

- ☒ These data and interpretations comply with the EN ISO 16140-2:2016 requirements.

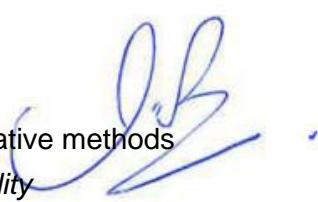
The Solus *Listeria monocytogenes* ELISA method is considered equivalent to the ISO standard method.

Quimper, 08 November 2022

Lizaïg GOUGUET
Technical Study Manager
Validation of Alternative methods
Food Safety & Quality



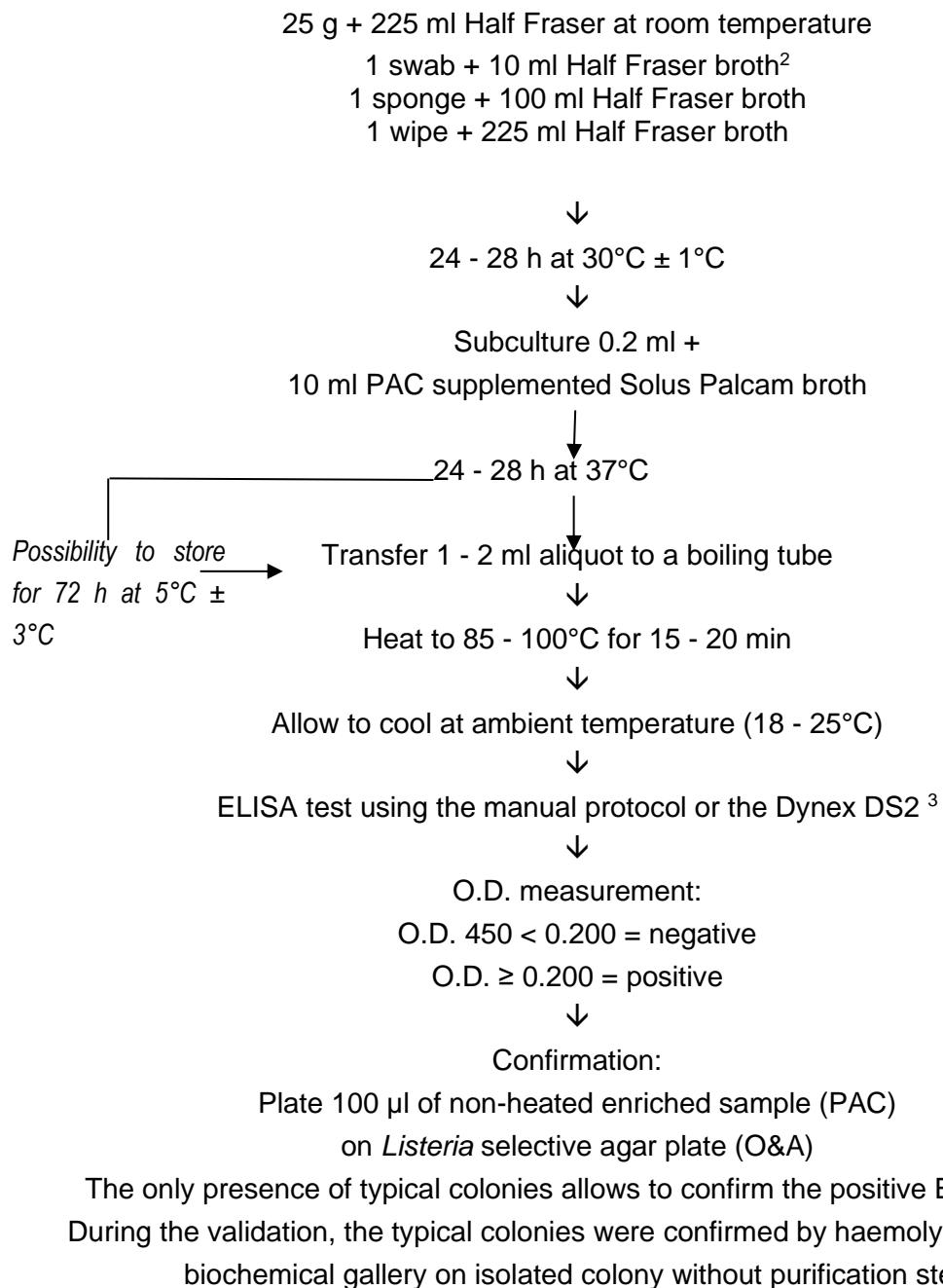
Maryse RANNOU
Project Manager
Validation of Alternative methods
Food Safety & Quality



I hereby attest to the validation of the results of the analyses carried out under the COFRAC accreditation.

I hereby attest to the validation of the verification of the conformity of the report (opinion and interpretation).

**Appendix 1 – Flow diagram of the alternative method:
Solus *Listeria monocytogenes* ELISA**



² For sampling after cleaning process pre-moisten
 - 1 swab + 1 ml broth universal neutralizing (+ 9 ml Half-Fraser)
 - 1 sponge + 10 ml broth universal neutralizing (+ 90 ml Half-Fraser)
 - 1 wipe + BPW + 10 % neutralizing agent (+ 225 ml Half-Fraser)

³ During the validation study, only the DS2 was tested

Appendix 2 – Flow diagram of the reference method: ISO 11290-1 (2017)
Microbiology of the food chain - Horizontal method for the detection and
enumeration of *Listeria monocytogenes* and of *Listeria* spp.-

Part 1: detection method

25 g + 225 ml Half Fraser broth at room temperature

1 swab + 10 ml Half Fraser broth⁴

1 sponge + 100 ml Half Fraser broth

1 wipe + 225 ml Half Fraser broth

↓

Incubation at 30°C ± for 25 h ± 1 h

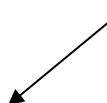
0.1 ml of culture

in 10 ml of secondary
enrichment medium (Fraser broth)

↓

Incubation at 37°C ± 1°C
for 24 h ± 2 h

↓
Streaking 10 µl onto
Ottaviani and Agosti (O&A) and Palcam plates



↓
Incubation for 24 h ± 2 h and
an additional 24 h ± 3 h at 37°C ± 1°C

↓

Confirmation:
Gram
β-haemolysis
Rhamnose
Xylose⁴

⁴ For sampling after cleaning process pre-moisten

- 1 swab + 1 ml broth universal neutralizing (+ 9 ml Half-Fraser)
- 1 sponge + 10 ml broth universal neutralizing (+ 90 ml Half-Fraser)
- 1 wipe + BPW + 10 % neutralizing agent (+ 225 ml Half-Fraser)

⁴ During the validation study, biochemical galleries were used

Year of analysis	N° Sample	Product (french name)	Product	Artificial contaminations						Global result	Category	Type			
				Strain	Origin	Injury protocol	Injury measurement	Inoculation level/sample							
								Enumeration	Mean						
2021	5968	Lingette vaisselle, avant nettoyage (production de glace)	Wipe before cleaning (ice cream production)	<i>L. monocytogenes</i> Ad1784	Dairy products	Seeding 72h 3±2°C	/	3-1-2-0-2	1,6	+	6	c			
2021	5969	Lingette homogénéisateur crème glacée, avant nettoyage (production de glace)	Wipe before cleaning (ice cream production)	<i>L. monocytogenes</i> Ad1784	Dairy products	Seeding 72h 3±2°C	/	3-1-2-0-2	1,6	+	6	c			
2021	5970	Eponge nacelle abattoir, avant nettoyage (industrie de produits carnés)	Sponge, before cleaning (meat products industry)	<i>L. monocytogenes</i> Ad2453	Meat product	Seeding 72h 3±2°C	/	2-0-0-1-1	0,8	-	6	c			
2021	5971	Eponge abattoir, avant nettoyage (industrie de produits carnés)	Sponge, before cleaning (meat products industry)	<i>L. monocytogenes</i> Ad2453	Meat product	Seeding 72h 3±2°C	/	2-0-0-1-1	0,8	-	6	c			
2021	6577	Ecouvillon mélangeur rhule crème glacée avant nettoyage (production de glace)	Swab, blender before cleaning (ice cream production)	<i>L. monocytogenes</i> Ad249	Dairy products	Seeding 72h 3±2°C	/	2-2-2-2-2	2,0	+	6	c			
2021	6578	Ecouvillon homogénéisateur après nettoyage (production de glace)	Swab, homogenizer after cleaning (ice cream production)	<i>L. monocytogenes</i> Ad249	Dairy products	Seeding 72h 3±2°C	/	2-2-2-2-2	2,0	+	6	c			
2021	6579	Ecouvillon freezer crème glacée avant nettoyage (production de glace)	Swab, freezer before cleaning (ice cream production)	<i>L. monocytogenes</i> Ad249	Dairy products	Seeding 72h 3±2°C	/	2-2-2-2-2	2,0	+	6	c			
2021	6580	Chiffonnette pompe crème glacée (production de glace)	Wipe, pump after cleaning (ice cream production)	<i>L. monocytogenes</i> Ad249	Dairy products	Seeding 72h 3±2°C	/	2-2-2-2-2	2,0	+	6	c			
2021	6581	Chiffonnette CDH crème glacée après nettoyage (production de glace)	Wipe, pump after cleaning (ice cream production)	<i>L. monocytogenes</i> Ad249	Dairy products	Seeding 72h 3±2°C	/	2-2-2-2-2	2,0	+	6	c			
2021	6582	Chiffonnette n°26 (industrie de produits laitiers)	Wipe before cleaning (dairy products industry)	<i>L. monocytogenes</i> Ad1781	Dairy products	Seeding 72h 3±2°C	/	2-0-1-3-4	2,0	+	6	c			
2021	6583	Chiffonnette n°47 sol zone feed 1 avant nettoyage (industrie de produits laitiers)	Wipe, ground, before cleaning (dairy products industry)	<i>L. monocytogenes</i> Ad1781	Dairy products	Seeding 72h 3±2°C	/	2-0-1-3-4	2,0	+	6	c			
2021	6584	Chiffonnette n°38 tapis intralock têtes échaudées (intérieur) après nettoyage (industrie de produits carnés)	Wipe, carpet after cleaning (meat products industry)	<i>L. monocytogenes</i> Ad271	Meat product	Seeding 72h 3±2°C	/	3-5-4-3-2	3,4	-	6	c			
2021	6585	Chiffonnette n°33 couteau désosseur 2tr (industrie de produits carnés)	Wipe, shaping knife, before cleaning (meat products industry)	<i>L. monocytogenes</i> Ad271	Meat product	Seeding 72h 3±2°C	/	3-5-4-3-2	3,4	-	6	c			
2021	6586	Chiffonnette n°28 support pour fromage steak avant nettoyage (industrie de produits carnés)	Wipe, working plan, before cleaning (meat products industry)	<i>L. monocytogenes</i> Ad271	Meat product	Seeding 72h 3±2°C	/	3-5-4-3-2	3,4	-	6	c			
2021	7732	Chiffonnette plant67 après nettoyage (industrie biscuit chocolat)	Wipe after cleaning (biscuit and chocolate)	<i>L. monocytogenes</i> Ad26000	Environment	Seeding 72h 3±2°C	/	6-4-2-4-6	4,4	+	6	c			
2021	7733	Chiffonnette plant69 avant nettoyage (industrie biscuit chocolat)	Wipe before cleaning (biscuit and chocolate)	<i>L. monocytogenes</i> Ad26000	Environment	Seeding 72h 3±2°C	/	6-4-2-4-6	4,4	+	6	c			
2021	7734	Eponge avant nettoyage cross screen cover plant 68 (industrie biscuit chocolat)	Sponge before cleaning (biscuit and chocolate)	<i>L. monocytogenes</i> Ad26000	Environment	Seeding 72h 3±2°C	/	6-4-2-4-6	4,4	+	6	c			

RTE: ready to eat

Appendix 4 – Sensitivity study: raw data

Bold typing: artificially inoculated samples

Listeria detection results:

H+: characteristic *Listeria* colonies with halo
-: no typical colonies but presence of background microflora
st: plate without any colony
PA: positive agreement
NA: negative agreement
ND: negative deviation
PD: positive deviation
PPNA: positive presumptive negative agreement
PPND : positive presumptive negative deviation
NC: non-characteristic colony on TSYEA
d: doubtful colony
*: result after enrichment broth dilution at 1/10
NI: no identification
ni : non isolated colony
RTE: ready-to-eat
RTRH: ready-to-reheat

COMPOSITE FOODS / READY-TO-EAT AND READY-TO-REHEAT																								
Year of analysis	N°Sample	Product (French name)	Product	Reference method: ISO 11290-1 *				Alternative method: SOLUS <i>Listeria monocytogenes</i> ELISA												Category	Type			
				Half Fraser		Fraser 1		Identification	Final result	25g (or swab/sponge/wipe) + 225ml (or 9mL/90mL/225mL) Half Fraser broth - 24h at 30°C						Subculture: 0.2 ml + 10 ml PAC supp SOLUS Palcam broth - 24 h at 37°C								
										Subculture: 0.2 ml + 10 ml PAC supp SOLUS Palcam broth 24 h at 37°C + 72 h at 5°C ± 3°C														
				O&A	Palcam	O&A	Palcam			Solus <i>Listeria monocytogenes</i> ELISA		O&A (100 µl)	Confirmations		All confirmatory tests	Final result 37°C	Agreement Ref/Alt PAC 37°C	Solus <i>Listeria monocytogenes</i> ELISA	Confirmations	O&A (100 µl)	All confirmatory tests	Final result 37°C 72H	Agreement Ref/Alt 37°C 72h	
2021	7276	Snickers (chocolat, caramel et noisettes)	Confectionary with chocolate, caramel and nuts	st	-	-	-	/	-	O.D.	Result	Typical colonies	Biochemical gallery	Haemolysis			NA					1 c		
2021	7277	Bounty (chocolat, noix de coco)	Confectionary with chocolate and coconut	-	-	-	-	/	-	O.D.	Result	Typical colonies	Biochemical gallery	Haemolysis			NA					1 c		

MEAT PRODUCTS																											
Year of analysis	N°Sample	Product (French name)	Product	Reference method: ISO 11290-1 *						Alternative method: SOLUS <i>Listeria monocytogenes</i> ELISA																	
				Half Fraser		Fraser 1		Identification	Final result	25g (or swab/sponge/wipe) + 225ml (or 9mL/90mL/225mL) Half Fraser broth - 24h at 30°C						Subculture: 0.2 ml + 10 ml PAC supp SOLUS Palcam broth - 24 h at 37°C						Subculture: 0.2 ml + 10 ml PAC supp SOLUS Palcam broth 24 h at 37°C + 72 h at 5°C ± 3°C					
										Solus <i>Listeria monocytogenes</i> ELISA		O&A (100 µl)		Without purification step		All confirmatory tests	Final result 37°C	Agreement Ref/Alt PAC 37°C	Solus <i>Listeria monocytogenes</i> ELISA		Confirmations		Final result 37°C 72H	Agreement Ref/Alt 37°C 72h			
				O&A	Palcam	O&A	Palcam			O.D.	Result	Typical colonies	Biochemical gallery	Haemolysis	O.D.		Result	Typical colonies	O&A (100 µl)	All confirmatory tests							
2021	7078	Mineraï de bœuf	Raw beef meat	H+/H-	+	H+/H-	+	L. monocytogenes	+	3,000	+	H+/H-	L. monocytogenes	+	+	+	PA	3,000	+	H+/H-	+	+	PA	2	a		
2021	7079	Haut de cuisse de dinde	Raw poultry meat	st	-	st	st	/	-	0,023	-	st	/	/	-	-	NA							2	a		
2021	5346	Emincés kebab rôti dinde/poulet	Roasted poultry and chicken	H+d/H-	+	H+d/H-	+	L. monocytogenes	+	0,096	-	H-	/	/	-	-	ND	0,099	-	H-	-	-	ND	2	b		
2021	5348	Aile de poulet pimentée cuite	RTRH spiced chicken wings	-	-	-	-	/	-	0,080	-	-	/	/	-	-	NA							2	b		
2021	5532	Donuts de filet de poulet frais	RTRH donut chicken meat	-	-	st	-	/	-	0,095	-	H-	/	/	-	-	NA							2	b		
2021	5533	Emincés de kebab rôti dinde/poulet	Sliced and seasoned poultry meat	st	-	st	st	/	-	0,068	-	-	/	/	-	-	NA							2	b		
2021	5534	Paupiettes de dinde surgelées	RTRH poultry meat	H+	-	H+	+	L. monocytogenes	+	3,000	+	H+	L. monocytogenes	+	+	+	PA	3,000	+	H+	+	+	PA	2	b		
2021	5537	Cubes de filet et viande de cuisse de poulet rôti	Roasted chicken meat	st	st	st	st	/	-	0,060	-	st	/	/	-	-	NA							2	b		
2021	5538	Poulet façon fajitas	Seasoned chicken meat	-	-	-	-	/	-	0,056	-	-	/	/	-	-	NA							2	b		
2021	5702	Sauté de dinde	Poultry meat	H+/H-	+	H+/H-	+	L. monocytogenes	+	0,115	-	H-	/	/	-	-	ND	0,104	-	H-	-	-	ND	2	b		
2021	5706	Blanquette de dinde	RTRH turkey meat	-	-	-	st	/	-	0,150	-	-	/	/	-	-	NA							2	b		
2021	5707	Wings hoties	Wings hoties	-	-	st	-	/	-	0,088	-	-	/	/	-	-	NA							2	b		
2021	5709	Nuggets cuit à DLC	Chicken nuggets	H-	+	H-	+	/	-	0,098	-	H-	/	/	-	-	NA							2	b		
2021	5712	Ballotine de volaille et légumes	RTRH poultry meat and vegetables	st	-	st	-	/	-	0,073	-	st	/	/	-	-	NA							2	b		
2021	5713	Emincés de filet poulet rôti	Sliced roasted chicken meat	st	-	-	-	/	-	0,095	-	st	/	/	-	-	NA							2	b		
2021	6225	Emincés de poulet rôti	Roasted chicken meat	st	st	st	st	/	-	0,022	-	-	/	/	-	-	NA							2	b		
2021	6226	Filet de poulet pané	Paned breaded chicken	st	st	st	st	/	-	0,023	-	-	/	/	-	-	NA							2	b		
2021	6456	Wings de poulet BBQ	Chicken meat (seasoned wing)	st	st	st	st	/	-	0,041	-	st	/	/	-	-	NA							2	b		
2021	6457	Ribs kebab	Kebab meat	H+	+	H+	+	L. monocytogenes	+	3,000	+	H+	L. monocytogenes	+	+	+	PA	3,000	+	H+	+	+	PA	2	b		
2021	6461	Lamelle de volaille kebab rôti	Seasoned and cooked poultry meat	-	-	st	-	/	-	0,030	-	-	/	/	-	-	NA							2	b		
2021	6614	Emincés de filet de poulet rôti frais	Roasted chicken meat	-	-	-	-	/	-	0,042	-	-	/	/	-	-	NA							2	b		
2021	6615	Emincés de filet rôti de poulet	Roasted chicken meat	H+	+	H+	+	L. monocytogenes	+	3,000	+	H+	L. monocytogenes	+	+	+	PA	3,000	+	H+	+	+	PA	2	b		
2021	6616	Lamelles de volaille kebab rôti	Seasoned and cooked poultry meat	st	-	st	st	/	-	0,035	-	-	/	/	-	-	NA							2	b		

MEAT PRODUCTS																										
Year of analysis	N°Sample	Product (French name)	Product	Reference method: ISO 11290-1 *					Alternative method: SOLUS <i>Listeria monocytogenes</i> ELISA															Category	Type	
				Half Fraser		Fraser 1		Identification	Final result	25g (or swab/sponge/wipe) + 225ml (or 9mL/90mL/225mL) Half Fraser broth - 24h at 30°C							Subculture: 0.2 ml + 10 ml PAC supp SOLUS Palcam broth - 24 h at 37°C									
										Subculture: 0.2 ml + 10 ml PAC supp SOLUS Palcam broth 24 h at 37°C + 72 h at 5°C ± 3°C																
				O&A	Palcam	O&A	Palcam			Solus <i>Listeria monocytogenes</i> ELISA		O&A (100 µl)	Without purification step		All confirmatory tests	Final result 37°C	Agreement Ref/Alt PAC 37°C	Solus <i>Listeria monocytogenes</i> ELISA		Confirmations		Final result 37°C 72H	Agreement Ref/Alt 37°C 72h			
				O.D.	Result	Typical colonies	Biochemical gallery	Haemolysis		O.D.	Result	Typical colonies	Agreement Ref/Alt PAC 37°C	O&A (100 µl)	All confirmatory tests		Final result 37°C 72H	Agreement Ref/Alt 37°C 72h								
2021	5851	Saucisson sec	Sausage	H+(1)	-	H+	-	<i>L. monocytogenes</i>	+	0,026	-	-	/	/	-	-	ND	0,030	-	-	-	-	ND	2	c	
2021	5854	Saucisson (Indication Géographique Protégée) auvergne	Sausage	H+	-	H+	-	<i>L. monocytogenes</i>	+	3,000	+	H+	<i>L. monocytogenes</i>	+	+	+	PA	3,000	+	H+	+	+	PA	2	c	
2021	5855	Saucisson au noisettes	Sausage	H+	+	H+	-	<i>L. monocytogenes</i>	+	3,000	+	H+	<i>L. monocytogenes</i>	+	+	+	PA	3,000	+	H+	+	+	PA	2	c	
2021	5873	Miettes de bacon (porc)	Bacon	-	-	st	-	/	-	0,024	-	-	/	/	-	-	NA							2	c	
2021	6222	Dés de jambon	Diced ham	H+	+	H+	+	<i>L. monocytogenes</i>	+	3,000	+	H+	<i>L. monocytogenes</i>	+	+	+	PA	3,000	+	H+	+	+	PA	2	c	
2021	6224	Dés de jambon	Diced ham	st	st	st	st	/	-	0,024	-	st	/	/	-	-	NA							2	c	
2021	6459	Jambon de Vendée à l'ancienne	Ham	-	-	-	-	/	-	0,042	-	-	/	/	-	-	NA							2	c	

MILK AND DAIRY PRODUCTS																											
Year of analysis	N°Sample	Product (French name)	Product	Reference method: ISO 11290-1 *						Alternative method: SOLUS <i>Listeria monocytogenes</i> ELISA																	
				Half Fraser		Fraser 1		Identification	Final result	25g (or swab/sponge/wipe) + 225ml (or 9mL/90mL/225mL) Half Fraser broth - 24h at 30°C						Subculture: 0.2 ml + 10 ml PAC supp SOLUS Palcam broth - 24 h at 37°C						Subculture: 0.2 ml + 10 ml PAC supp SOLUS Palcam broth 24 h at 37°C + 72 h at 5°C ± 3°C					
										Solus <i>Listeria monocytogenes</i> ELISA		O&A (100 µl)		Without purification step		All confirmatory tests	Final result 37°C	Agreement Ref/Alt PAC 37°C	Solus <i>Listeria monocytogenes</i> ELISA		Confirmations		Final result 37°C 72H	Agreement Ref/Alt 37°C 72h			
				O&A	Palcam	O&A	Palcam			O.D.	Result	Typical colonies	Biochemical gallery	Haemolysis	O.D.		Result	Typical colonies	O&A (100 µl)	All confirmatory tests	Final result 37°C 72H	Agreement Ref/Alt 37°C 72h					
2021	5158	Fromage non affiné au lait cru de vache	Raw cow milk cheese	H+	+	H+	+	L. monocytogenes	+	1,417	+	H+	L. monocytogenes	+	+	+	PA	0,813	+	H+	+	+	PA	3	a		
2021	5164	Fromage non affiné au lait cru de vache	Raw cow milk cheese	-	-	-	-	/	-	1,704/0,648/0,779	+/-/+	- (Palcam broth x5: -, Palcam plate x5: -)	/	/	-	-	PPNA	0,227/0,377/0,350	+/-/+	- (Palcam broth x5: -, Palcam plate x5: -)	-	-	PPNA	3	a		
2021	5165	Fromage pâte persillée au lait cru de brebis	Raw ewe milk cheese	st	-	-	-	/	-	0,170	-	-	/	/	-	-	NA							3	a		
2021	5186	Fromage pâte persillée au lait cru de brebis	Raw ewe milk cheese	H+	+	H+	+	L. monocytogenes	+	3,000	+	H+	L. monocytogenes	+	+	+	PA	3,000	+	H+	+	+	PA	3	a		
2021	5187	Fromage pâte persillée au lait cru de brebis	Raw ewe milk cheese	-	-	-	-	/	-	0,085	-	-	/	/	-	-	NA							3	a		
2021	5188	Fromage pâte persillée au lait cru de brebis	Raw ewe milk cheese	H+	+	H+	+	L. monocytogenes	+	3,000	+	H+	L. monocytogenes	+	+	+	PA	3,000	+	H+	+	+	PA	3	a		
2021	5720	Fromage au lait cru de vache	Raw cow milk cheese	st	st	st	st	/	-	0,083	-	-	/	/	-	-	NA							3	a		
2021	5721	Gorgonzola AOP	Raw milk cheese	H+(1)	-	H+	-	L. monocytogenes	+	2,806	+	H+ (5x: H+)	L. monocytogenes	+	+	+	PA	2,296	+	H+ (5x: H+)	+	+	PA	3	a		
2021	5722	Fromage pâte persillée au lait cru de brebis	Raw ewe milk cheese	-	st	st	st	/	-	0,111	-	st	/	/	-	-	NA	0,077	-	st	-	-	NA	3	a		
2021	5723	Fromage pâte persillée au lait cru de brebis	Raw ewe milk cheese	st	st	st	st	/	-	0,085	-	st	/	/	-	-	NA							3	a		
2021	6608	Fromage pâte persillée au lait cru de brebis n°226	Raw ewe milk cheese	-	-	st	st	/	-	0,030	-	-	/	/	-	-	NA							3	a		
2021	6609	Fromage non affiné, lait cru de vache n°225	Raw cow milk cheese	-	-	H+	+d	L. monocytogenes	+	0,106	-	-	/	/	-	-	ND	0,071	-	-	-	-	ND	3	a		
2021	6610	Fromage pâte persillée au lait cru de brebis	Raw ewe milk cheese	H+(5)	st	H+	+	L. monocytogenes	+	3,000	+	H+	L. monocytogenes	+	+	+	PA	3,000	+	H+	+	+	PA	3	a		
2021	7087	Roquefort AOP lait cru de brebis	Raw ewe milk cheese	st	-	st	st	/	-	0,026	-	st	/	/	-	-	NA							3	a		
2021	7088	Osseau iraty AOP lait cru de brebis	Raw ewe milk cheese	-	-	st	-	/	-	0,020	-	-	/	/	-	-	NA							3	a		
2021	7089	Morbier AOP lait cru de vache	Raw cow milk cheese	-	-	st	st	/	-	0,067	-	-	/	/	-	-	NA							3	a		
2021	7625	Tome des bauges au lait cru de vache	Raw cow milk cheese (Tome)	H+	+	H+	+	L. monocytogenes	+	2,932	+	H+	L. monocytogenes	+	+	+	PA	3,000	+	H+	+	+	PA	3	a		

* Analyses performed according to the COFRAC accreditation

MILK AND DAIRY PRODUCTS																						
Year of analysis	N°Sample	Product (French name)	Product	Reference method: ISO 11290-1 *				Alternative method: SOLUS <i>Listeria monocytogenes</i> ELISA												Category	Type	
				Half Fraser		Fraser 1		Identification	Final result	25g (or swab/sponge/wipe) + 225ml (or 9mL/90mL/225mL) Half Fraser broth - 24h at 30°C						Subculture: 0.2 ml + 10 ml PAC supp SOLUS Palcam broth - 24 h at 37°C						
				Solus <i>Listeria monocytogenes</i> ELISA		Confirmations				O&A (100 µl)	Without purification step		All confirmatory tests	Final result 37°C	Agreement Ref/Alt PAC 37°C	Solus <i>Listeria monocytogenes</i> ELISA		Confirmations		Category	Type	
				O.D.	Result	Typical colonies	Biochemical gallery	Haemolysis		O.D.						Result	Typical colonies					
2021	7267	Riz au lait saveur vanille	Rice pudding with pasteurised milk, vanilla	st	st	st	st	/	-	0,022	-	st	/	/	-	-	NA			3 c		
2021	7268	Riz au lait nature	Rice pudding with pasteurised milk	st	st	st	st	/	-	0,021	-	st	/	/	-	-	NA			3 c		
2021	7269	Semoule au lait à la vanille	Semolina pudding, flavour vanilla	st	st	st	st	/	-	0,031	-	st	/	/	-	-	NA			3 c		
2021	7270	Semoule au lait à la vanille	Semolina pudding, flavour vanilla	st	st	st	-	/	-	0,019	-	st	/	/	-	-	NA			3 c		

ENVIRONMENTAL SAMPLES																											
Year of analysis	N°Sample	Product (French name)	Product	Reference method: ISO 11290-1 *						Alternative method: SOLUS <i>Listeria monocytogenes</i> ELISA																	
				Half Fraser		Fraser 1		Identification	Final result	25g (or swab/sponge/wipe) + 225ml (or 9mL/90mL/225mL) Half Fraser broth - 24h at 30°C						Subculture: 0.2 ml + 10 ml PAC supp SOLUS Palcam broth - 24 h at 37°C						Subculture: 0.2 ml + 10 ml PAC supp SOLUS Palcam broth 24 h at 37°C + 72 h at 5°C ± 3°C					
										Solus <i>Listeria monocytogenes</i> ELISA		O&A (100 µl)		Without purification step		All confirmatory tests	Final result 37°C	Agreement Ref/Alt PAC 37°C	Solus <i>Listeria monocytogenes</i> ELISA		Confirmations		Final result 37°C 72H	Agreement Ref/Alt 37°C 72h			
				O&A	Palcam	O&A	Palcam			O.D.	Result	Typical colonies	Biochemical gallery	Haemolysis	O.D.		Result	Typical colonies	O&A (100 µl)	All confirmatory tests	Final result 37°C 72H	Agreement Ref/Alt 37°C 72h					
2021	6577	Ecouvillon mélangeur rhule crème glacée avant nettoyage (production de glace)	Swab, blender before cleaning (ice cream production)	H+	+	H+	+	L. monocytogenes	+	3,000	+	H+	L. monocytogenes	+	+	+	PA	3,000	+	H+	+	+	PA	6	c		
2021	6578	Ecouvillon homogénéisateur après nettoyage (production de glace)	Swab, homogenizer after cleaning (ice cream production)	H+	+	H+	+	L. monocytogenes	+	3,000	+	H+	L. monocytogenes	+	+	+	PA	3,000	+	H+	+	+	PA	6	c		
2021	6579	Ecouvillon freezer crème glacée avant nettoyage (production de glace)	Swab, freezer before cleaning (ice cream production)	H+	+	H+	+	L. monocytogenes	+	3,000	+	H+	L. monocytogenes	+	+	+	PA	3,000	+	H+	+	+	PA	6	c		
2021	6580	Chiffonnette pompe crème glacée (production de glace)	Wipe, pump after cleaning (ice cream production)	H+	+	H+	+	L. monocytogenes	+	3,000	+	H+	L. monocytogenes	+	+	+	PA	3,000	+	H+	+	+	PA	6	c		
2021	6581	Chiffonnette CDH crème glacée après nettoyage (production de glace)	Wipe, pump after cleaning (ice cream production)	H+	+	H+	+	L. monocytogenes	+	3,000	+	H+	L. monocytogenes	+	+	+	PA	3,000	+	H+	+	+	PA	6	c		
2021	6582	Chiffonnette n°26 (industrie de produits laitiers)	Wipe before cleaning (dairy products industry)	H+	+	H+	+	L. monocytogenes	+	3,000	+	H+	L. monocytogenes	+	+	+	PA	3,000	+	H+	+	+	PA	6	c		
2021	6583	Chiffonnette n°47 sol zone feed 1 avant nettoyage (industrie de produits laitiers)	Wipe, ground, before cleaning (dairy products industry)	H+	+	H+	+	L. monocytogenes	+	3,000	+	H+	L. monocytogenes	+	+	+	PA	3,000	+	H+	+	+	PA	6	c		
2021	6584	Chiffonnette tapis intralock têtes échaudées (intérieur) après nettoyage (industrie de produits carnés)	Wipe, carpet after cleaning (meat products industry)	st	st	st	st	/	-	0,021	-	st	/	/	-	-	NA							6	c		
2021	6585	Chiffonnette couteau désosseur 2tr (industrie de produits carnés)	Wipe, shaping knife, before cleaning (meat products industry)	st	st	st	st	/	-	0,017	-	st	/	/	-	-	NA							6	c		

ENVIRONMENTAL SAMPLES																									
Year of analysis	N°Sample	Product (French name)	Product	Reference method: ISO 11290-1 *					Alternative method: SOLUS <i>Listeria monocytogenes</i> ELISA															Category	Type
				Half Fraser		Fraser 1		Identification	Final result	25g (or swab/sponge/wipe) + 225ml (or 9mL/90mL/225mL) Half Fraser broth - 24h at 30°C							Subculture: 0.2 ml + 10 ml PAC supp SOLUS Palcam broth - 24 h at 37°C								
				Solus <i>Listeria monocytogenes</i> ELISA		Confirmations				O&A (100 µl)		Without purification step		All confirmatory tests	Final result 37°C	Agreement Ref/Alt PAC 37°C	Solus <i>Listeria monocytogenes</i> ELISA		Confirmations		Final result 37°C 72H	Agreement Ref/Alt 37°C 72h			
				O.D.	Result	Typical colonies	Biochemical gallery	Haemolysis																	
2021	6586	Chiffonnette support pour fromage steak avant nettoyage (industrie de produits carnés)	Wipe, working plan, before cleaning (meat products industry)	st	st	st	st	/	-	0,017	-	st	/	/	-	-	NA							6	c
2021	7732	Chiffonnette après nettoyage (industrie biscuit chocolat)	Wipe after cleaning (biscuit and chocolate)	H+	+	H+	+	<i>L. monocytogenes</i>	+	1,538	+	H+	<i>L. monocytogenes</i>	+	+	+	PA	1,058	+	H+	+	+	PA	6	c
2021	7733	Chiffonnette avant nettoyage (industrie biscuit chocolat)	Wipe before cleaning (biscuit and chocolate)	H+	+	H+	+	<i>L. monocytogenes</i>	+	1,454	+	H+	<i>L. monocytogenes</i>	+	+	+	PA	1,038	+	H+	+	+	PA	6	c
2021	7734	Eponge avant nettoyage cross screen cover plant 68 (industrie biscuit chocolat)	Sponge before cleaning (biscuit and chocolate)	H+	+	H+	+	<i>L. monocytogenes</i>	+	0,662	+	H+	<i>L. monocytogenes</i>	+	+	+	PA	0,600	+	H+	+	+	PA	6	c

Appendix 5 – Relative level of detection study: raw data

Matrix : Deli-salad: piemontaise

Strain : *Listeria monocytogenes* Ad494

Seeding 72h at 3±2°C

Aerobic mesophilic flora: 3,0.10⁴ CFU/g

N° sample	Level	Inoculation level (CFU/sample)	Reference method: ISO 11290-1 *					Alternative method: SOLUS <i>Listeria monocytogenes</i> ELISA					
			Half Fraser		Fraser		Final result	Number positive samples/Total	Subculture: 0.2 ml+10 ml PAC supp SOLUS Palcam broth - 24h at 37°C		Confirmation		Final result 37°C
			O&A	Palcam	O&A	Palcam			Solus <i>Listeria monocytogenes</i> ELISA	O&A (100 µl)	All confirmatory tests		
7433	0	0	-	-	-	-	-	0/5	0,096	-	-	-	-
7434			-	-	-	-	-		0,096	-	-	-	-
7435			-	-	-	-	-		0,088	-	-	-	-
7436			-	-	-	-	-		0,092	-	-	-	-
7437			-	-	-	-	-		0,117	-	-	-	-
7438	Low	0,7	H+	+	H+	+	+	12/20	3,000	+	H+	+	+
7439			-	-	-	-	-		0,088	-	-	-	-
7440			H+	st	H+	+	+		3,000	+	H+	+	+
7441			-	-	-	-	-		0,078	-	st	-	-
7442			st	-	-	-	-		0,129	-	-	-	-
7443			H+	+	H+	+	+		3,000	+	H+	+	+
7444			-	-	-	-	-		0,082	-	st	-	-
7445			-	-	-	-	-		0,101	-	st	-	-
7446			H+	+	H+	+	+		3,000	+	H+	+	+
7447			H+	+	H+	+	+		3,000	+	H+	+	+
7448			H+	+	H+	+	+		3,000	+	H+	+	+
7449			H+	+	H+	+	+		3,000	+	H+	+	+
7450			-	-	-	-	-		0,094	-	-	-	-
7451			H+	+	H+	+	+		3,000	+	H+	+	+
7452			H+	+	H+	+	+		3,000	+	H+	+	+
7453			-	-	-	-	-		0,107	-	-	-	-
7454			-	-	-	-	-		0,101	-	-	-	-
7455			H+	+	H+	+	+		3,000	+	H+	+	+
7456			H+	+	H+	+	+		3,000	+	H+	+	+
7457			H+	+	H+	+	+		3,000	+	H+	+	+
7458	High	2,9	H+	+	H+	+	+	4/5	3,000	+	H+	+	+
7459			H+	+	H+	+	+		3,000	+	H+	+	+
7460			-	-	-	-	-		0,099	-	-	-	-
7461			H+	+	H+	+	+		3,000	+	H+	+	+
7462			H+	+	H+	+	+		3,000	+	H+	+	+

* Analyses performed according to the COFRAC accreditation

ADRIA Développement

Summary report (Version 0)

Solus *Listeria monocytogenes* ELISA

Matrix: Raw milk

Strain: *Listeria monocytogenes* 153

Seeding 48h at 3±2°C

Aerobic mesophilic flora: 2.10⁶ CFU/mL

N° sample	Level	Inoculation level (CFU/sample)	Reference method: ISO 11290-1/A1*					Alternative method: SOLUS <i>Listeria monocytogenes</i> ELISA					
			Half Fraser		Fraser		Final result	Number positive samples/Total	Solus <i>Listeria monocytogenes</i> ELISA		Confirmation		Final result 37°C
			O&A	Palcam	O&A	Palcam			O.D.	Result	O&A (100 µl)	Typical colonies	
6933	0	0	st	st	-	st	-	0/5	0,069	-	-	-	-
6934			st	-	-	-	-		0,039	-	-	-	-
6935			-	-	-	-	-		0,048	-	-	-	-
6936			-	-	-	-	-		0,041	-	-	-	-
6937			-	-	-	-	-		0,044	-	-	-	-
6938	Low	0,4	H+	+	H+	+	+	12/20	3,000	+	H+		+
6939			st	-	-	-	-		0,040	-	-	-	-
6940			-	-	-	-	-		0,036	-	-	-	-
6941			-	-	-	-	-		0,044	-	-	-	-
6942			H+	+	H+	+	+		3,000	+	H+	+	+
6943			-	-	-	-	-		0,031	-	-	-	-
6944			H+	+	H+	+	+		3,000	+	H+	+	+
6945			H+	+	H+	+	+		3,000	+	H+	+	+
6946			H+	+	H+	+	+		3,000	+	H+	+	+
6947			H+	+	H+	+	+		3,000	+	H+	+	+
6948			H+	+	H+	+	+		3,000	+	H+	+	+
6949			-	-	-	-	-		0,043	-	-	-	-
6950			-	-	-	-	-		0,036	-	-	-	-
6951			H+	+	H+	+	+		3,000	+	H+	+	+
6952			-	-	-	-	-		0,036	-	-	-	-
6953			H+(3)	+	H+	+	+		3,000	+	H+	+	+
6954			H+	+	H+	+	+		3,000	+	H+	+	+
6955			H+	+	H+	+	+		3,000	+	H+	+	+
6956			H+	+	H+	+	+		3,000	+	H+	+	+
6957			-	-	-	-	-		0,042	-	-	-	-
6958	High	1,6	H+	+	H+	+	+	5/5	3,000	+	H+	+	+
6959			H+	+	H+	+	+		3,000	+	H+	+	+
6960			H+	+	H+	+	+		3,000	+	H+	+	+
6961			H+	+	H+	+	+		3,000	+	H+	+	+
6962			H+	+	H+	+	+		3,000	+	H+	+	+

* Analyses performed according to the COFRAC accreditation

ADRIA Développement

Summary report (Version 0)

Solus *Listeria monocytogenes* ELISA

Matrix: Smoked salmon

Strain: *Listeria monocytogenes* Ad670

Seeding 48h at 3±2°C

Aerobic mesophilic flora: 1,0.10² CFU/g

N° sample	Level	Inoculation level (CFU/sample)	Reference method: ISO 11290-1/A1*					Alternative method : SOLUS <i>Listeria monocytogenes</i> ELISA						
			Half Fraser		Fraser		Final result	Number positive samples/Total	Solus <i>Listeria monocytogenes</i> ELISA		Confirmation		Final result 37°C	Number positive samples/Total
			O&A	Palcam	O&A	Palcam			O.D.	Result	Typical colonies	All confirmatory tests		
7278	0	0	st	-	st	st	-	0/5	0,020	-	-	-	-	0/5
7279			-	-	-	-	-		0,022	-	st	-	-	
7280			-	-	-	-	-		0,025	-	st	-	-	
7281			-	-	-	-	-		0,020	-	st	-	-	
7282			-	-	-	-	-		0,020	-	-	-	-	
7394	Low	0,5	-	-	-	-	-	6/20	0,100	-	-	-	-	6/20
7395			st	-	st	-	-		0,097	-	-	-	-	
7396			st	-	st	-	-		0,102	-	st	-	-	
7397			st	-	st	-	-		0,118	-	-	-	-	
7398			st	-	-	-	-		0,115	-	-	-	-	
7399			-	-	-	-	-		0,113	-	-	-	-	
7400			H+	-	H+	-	+		3,000	+	H+	+	+	
7401			st	-	-	-	-		0,104	-	st	-	-	
7402			H+/H-	-	H+/H-	-	+		3,000	+	H+	+	+	
7403			-	-	-	-	-		0,113	-	-	-	-	
7404			H+	+	H+/H-	+	+		3,000	+	H+	+	+	
7405			H+/H-	+	H+/H-	+	+		3,000	+	H+	+	+	
7406			st	-	st	-	-		0,198	-	-	-	-	
7407			H+	+	H+/H-	+	+		3,000	+	H+	+	+	
7408			-	-	-	-	-		0,127	-	-	-	-	
7409			st	st	st	st	-		0,119	-	st	-	-	
7410			st	-	st	-	-		0,133	-	-	-	-	
7411			st	st	st	st	-		0,130	-	st	-	-	
7412			st	st	st	st	-		0,126	-	st	-	-	
7413			H+	+	H+/H-	+	+		3,000	+	H+	+	+	
7414	High	2,3	H+	+	H+	+	+	4/5	3,000	+	H+	+	+	4/5
7415			H+	+	H+	+	+		3,000	+	H+	+	+	
7416			-	-	-	-	-		0,134	-	-	-	-	
7417			H+	+	H+	+	+		3,000	+	H+	+	+	
7418			H+	+	H+/H-	+	+		3,000	+	H+	+	+	

* Analyses performed according to the COFRAC accreditation

ADRIA Développement

Summary report (Version 0)

Solus *Listeria monocytogenes* ELISA

Matrix : Ready to cook vegetables (cut white cabbage, cut carrots and cut celery under modified atmosphere)

Strain : *Listeria monocytogenes* Ad279

Seeding 48h at 3±2°C

Aerobic mesophilic flora: 1,3.10⁸ CFU/g

N° sample	Level	Inoculation level (CFU/sample)	Reference method: ISO 11290-1/A1*				Number positive samples/Total	Alternative method: SOLUS <i>Listeria monocytogenes</i> ELISA					
			Subculture: 0,2ml+10 ml PAC supp SOLUS Palcam broth - 24h at 37°C					Solus Listeria monocytogenes ELISA		Confirmation			
			O&A	Palcam	O&A	Palcam		O.D.	Result	Typical colonies	All confirmatory tests	Final result 37°C	Number positive samples/Total
6742	0	0	st	st	st	st	-	0/5	0,045	-	st	-	-
6743			st	st	st	st	-		0,031	-	st	-	-
6744			st	st	st	st	-		0,028	-	st	-	-
6745			st	st	st	st	-		0,028	-	st	-	-
6746			st	st	st	st	-		0,034	-	st	-	-
6747	Low	0,6	st	st	st	st	-	6/20	0,031	-	st	-	-
6448			st	st	st	st	-		0,026	-	st	-	-
6749			H+	+	H+	+	+		3,000	+	H+	+	+
6750			st	st	st	st	-		0,027	-	st	-	-
6751			H+	+	H+	+	+		3,000	+	H+	+	+
6752			st	st	H-	+	-		0,027	-	H-	-	-
6753			H+	+	H+	+	+		3,000	+	H+	+	+
6754			H+	+	H+	+	+		3,000	+	H+	+	+
6755			H-	+	H-	+	-		0,027	-	H-	-	-
6756			H+	+	H+	+	+		3,000	+	H+	+	+
6757			st	st	st	st	-		0,026	-	st	-	-
6758			H-	+	H-	+	-		0,025	-	H-	-	-
6759			st	st	st	st	-		0,024	-	st	-	-
6760			st	st	st	st	-		0,023	-	st	-	-
6761			st	st	st	st	-		0,021	-	st	-	-
6762			st	st	st	st	-		0,024	-	st	-	-
6763			st	st	st	st	-		0,029	-	st	-	-
6764			st	st	st	st	-		0,027	-	st	-	-
6765			st	st	st	st	-		0,037	-	st	-	-
6766			H+	+	H+	+	+		3,000	+	H+	+	+
6767	High	2,6	st	st	st	st	-	3/5	0,057	-	st	-	-
6768			H+	+	H+	+	+		3,000	+	H+	+	+
6769			H+	+	H+	+	+		3,000	+	H+	+	+
6770			H+(2)	+(2)	H+	+	+		3,000	+	H+	+	+
6771			st	st	st	st	-		3,000	+	H+	+	+

* Analyses performed according to the COFRAC accreditation

ADRIA Développement

Summary report (Version 0)

Solus Listeria monocytogenes ELISA

Matrix : Process water (dairy products industry)

Strain : *Listeria monocytogenes* Ad551

Seeding 48h at 3±2°C

Aerobic mesophilic flora: 1,1.10² CFU/g

N° sample	Level	Inoculation level (CFU/sample)	Reference method: ISO 11290-1/A1*					Alternative method : SOLUS <i>Listeria monocytogenes</i> ELISA						
			Half Fraser		Fraser		Final result	Number positive samples/Total	Solus <i>Listeria monocytogenes</i> ELISA		Confirmation		Final result 37°C	Number positive samples/Total
			O&A	Palcam	O&A	Palcam			O.D.	Result	Typical colonies	All confirmatory tests		
7588	0	0	st	st	st	st	-	0/5	0,074	-	st	-	-	0/5
7589			st	st	-	st	-		0,083	-	st	-	-	
7590			st	st	-	st	-		0,075	-	st	-	-	
7591			st	st	-	st	-		0,088	-	st	-	-	
7592			st	st	st	st	-		0,089	-	st	-	-	
7593	Low	0,7	st	st	st	st	-	13/20	0,076	-	st	-	-	13/20
7594			H+	st	H+	+	+		3,000	+	H+	+	+	
7595			H+	+	H+	+	+		2,743	+	H+	+	+	
7596			H+	st	H+	+	+		2,807	+	H+	+	+	
7597			st	st	st	st	-		0,073	-	st	-	-	
7598			H+	st	H+	+	+		3,000	+	H+	+	+	
7599			st	st	st	st	-		0,084	-	st	-	-	
7600			H+	st	H+	+	+		3,000	+	H+	+	+	
7601			st	st	st	st	-		0,092	-	st	-	-	
7602			H+	st	H+	+	+		3,000	+	H+	+	+	
7603			H+	+ (2)	H+	+	+		3,000	+	H+	+	+	
7604			H+	st	H+	+	+		3,000	+	H+	+	+	
7605			st	st	st	st	-		0,086	-	st	-	-	
7606			st	st	st	st	-		0,083	-	st	-	-	
7607			H+	st	H+	+	+		3,000	+	H+	+	+	
7608			H+	st	H+	+	+		3,000	+	H+	+	+	
7609			H+	+ (2)	H+	+	+		0,081	-	H+	+	+	
7610			st	st	st	st	-		3,000	+	st	-	-	
7611			H+(5)	st	H+	+	+		3,000	+	H+	+	+	
7612			H+	st	H+	+	+		3,000	+	H+	+	+	
7613	High	2,2	H+	+	H+	+	+	4/5	3,000	+	H+	+	+	4/5
7614			H+	st	H+	+	+		3,000	+	H+	+	+	
7615			H+	+	H+	+	+		3,000	+	H+	+	+	
7616			st	st	st	st	-		0,085	-	st	-	-	
7617			H+	st	H+	+	+		3,000	+	H+	+	+	

* Analyses performed according to the COFRAC accreditation

ADRIA Développement

Summary report (Version 0)

Solus *Listeria monocytogenes* ELISA

Appendix 6 – Inclusivity and exclusivity study: raw data

INCLUSIVITY									
No	Strain	Species	Reference	Molecular serotypes	Origin	Inoculation level (CFU/225ml)	SOLUS <i>Listeria monocytogenes</i> ELISA		
							Subculture: 0.2 ml+10 ml PAC supp SOLUS Palcam broth - 24h à 37°C		
							Solus <i>Listeria monocytogenes</i> ELISA	Confirmations	
							O&A (100µl)	Typical colonies	
O.D.	Result								
1	<i>Listeria</i>	<i>monocytogenes</i>	153	VI b	Soft cheese (Munster)	56	3,000	+	H+
2	<i>Listeria</i>	<i>monocytogenes</i>	1011/1410	II a	Frozen broccoli	40	3,000	+	H+
3	<i>Listeria</i>	<i>monocytogenes</i>	1972/2399	VI b	Puff pastry with mushrooms	71	3,000	+	H+
4	<i>Listeria</i>	<i>monocytogenes</i>	1973/2400	VI b	Puff pastry egg and ham (Quiche lorraine)	46	3,000	+	H+
5	<i>Listeria</i>	<i>monocytogenes</i>	2407/3139	IV b	Tripes with tomatoes	61	3,000	+	H+
6	<i>Listeria</i>	<i>monocytogenes</i>	2760/3145	II a	Raw bacon	47	3,000	+	H+
7	<i>Listeria</i>	<i>monocytogenes</i>	32.183	II b	Croque-Monsieur	48	3,000	+	H+
8	<i>Listeria</i>	<i>monocytogenes</i>	38/181	II a	Toulouse sausages	40	3,000	+	H+
9	<i>Listeria</i>	<i>monocytogenes</i>	5721/6179	IV b	Smoked bacon	55	3,000	+	H+
10	<i>Listeria</i>	<i>monocytogenes</i>	7111/7516	IV b	Pâté (Rillettes)	37	3,000	+	H+
11	<i>Listeria</i>	<i>monocytogenes</i>	850/109	II a	RTE food (deli salad with seafood)	43	3,000	+	H+
12	<i>Listeria</i>	<i>monocytogenes</i>	877/113	II a	Environmental sample (pastry)	63	3,000	+	H+
13	<i>Listeria</i>	<i>monocytogenes</i>	913/1048	IV b	Black pudding	49	3,000	+	H+
14	<i>Listeria</i>	<i>monocytogenes</i>	A00C014	II a	Sausage	65	3,000	+	H+
15	<i>Listeria</i>	<i>monocytogenes</i>	A00C022	II a	Merguez	47	3,000	+	H+
16	<i>Listeria</i>	<i>monocytogenes</i>	A00C024	II a	Sausage	62	3,000	+	H+
17	<i>Listeria</i>	<i>monocytogenes</i>	A00C036	II a	Poultry (guinea)	59	3,000	+	H+
18	<i>Listeria</i>	<i>monocytogenes</i>	A00C039	II a	Sausages	33	3,000	+	H+
19	<i>Listeria</i>	<i>monocytogenes</i>	A00C040	IV b	Cooked delicatessen (Museau)	55	3,000	+	H+
20	<i>Listeria</i>	<i>monocytogenes</i>	A00C041	La	Sausage	80	3,000	+	H+
21	<i>Listeria</i>	<i>monocytogenes</i>	A00C042	IV b	Raw sausage	45	3,000	+	H+
22	<i>Listeria</i>	<i>monocytogenes</i>	A00C043	II a	Smoked Bacon	61	3,000	+	H+
23	<i>Listeria</i>	<i>monocytogenes</i>	A00C044	II b	Poultry (duck)	42	3,000	+	H+

INCLUSIVITY									
No	Strain	Species	Reference	Molecular serotypes	Origin	Inoculation level (CFU/225ml)	SOLUS <i>Listeria monocytogenes</i> ELISA		
							Subculture: 0.2 ml+10 ml PAC supp SOLUS Palcam broth - 24h à 37°C		
							Solus <i>Listeria monocytogenes</i> ELISA		Confirmations
							O&A (100µl)		
								Typical colonies	
24	<i>Listeria</i>	<i>monocytogenes</i>	A00C052	II b	RTE food (Osso bucco with turkey)	58	3,000	+	H+
25	<i>Listeria</i>	<i>monocytogenes</i>	A00C053	II a	Gizzards	37	3,000	+	H+
26	<i>Listeria</i>	<i>monocytogenes</i>	A00C054	IV b	Beef hart	63	3,000	+	H+
27	<i>Listeria</i>	<i>monocytogenes</i>	A00C055	II a	Raw sausages	55	3,000	+	H+
28	<i>Listeria</i>	<i>monocytogenes</i>	A00E008	II a	Environmental sample	41	3,000	+	H+
29	<i>Listeria</i>	<i>monocytogenes</i>	A00E049	II a	Environmental sample (smoked salmon)	46	3,000	+	H+
30	<i>Listeria</i>	<i>monocytogenes</i>	A00E082	II a	Environmental sample (smoked salmon)	83	3,000	+	H+
31	<i>Listeria</i>	<i>monocytogenes</i>	A00L097	II a	Milk	74	3,000	+	H+
32	<i>Listeria</i>	<i>monocytogenes</i>	A00M009	II a	Smoked salmon	66	3,000	+	H+
33	<i>Listeria</i>	<i>monocytogenes</i>	A00M032	IV b	Smoked salmon	59	3,000	+	H+
34	<i>Listeria</i>	<i>monocytogenes</i>	A00M045	II a	Smoked salmon	57	3,000	+	H+
35	<i>Listeria</i>	<i>monocytogenes</i>	A00M088	II a	Smoked salmon	37	3,000	+	H+
36	<i>Listeria</i>	<i>monocytogenes</i>	Ad235	II b	Poultry	81	3,000	+	H+
37	<i>Listeria</i>	<i>monocytogenes</i>	Ad253	II b	Hard cheese	86	3,000	+	H+
38	<i>Listeria</i>	<i>monocytogenes</i>	Ad260	II a	Semi hard cheese	83	3,000	+	H+
39	<i>Listeria</i>	<i>monocytogenes</i>	Ad265	II b	Tong	68	3,000	+	H+
40	<i>Listeria</i>	<i>monocytogenes</i>	Ad266	II a	Poultry	37	3,000	+	H+
41	<i>Listeria</i>	<i>monocytogenes</i>	Ad267	II b	Dry sausage	50	3,000	+	H+
42	<i>Listeria</i>	<i>monocytogenes</i>	Ad268	IV b	Cured ham	54	3,000	+	H+
43	<i>Listeria</i>	<i>monocytogenes</i>	Ad270	IV b	Fermented sausage	72	3,000	+	H+
44	<i>Listeria</i>	<i>monocytogenes</i>	Ad272	IV b	Fermented sausage	49	3,000	+	H+
45	<i>Listeria</i>	<i>monocytogenes</i>	Ad273	II b	Cured delicatessen	36	3,000	+	H+
46	<i>Listeria</i>	<i>monocytogenes</i>	Ad274	II a	Ready-to-eat food (Asiatic meal)	9	3,000	+	H+
47	<i>Listeria</i>	<i>monocytogenes</i>	Ad534	II b	Fruits	72	3,000	+	H+
48	<i>Listeria</i>	<i>monocytogenes</i>	Ad544	II a	Onion	49	3,000	+	H+

INCLUSIVITY									
No	Strain	Species	Reference	Molecular serotypes	Origin	Inoculation level (CFU/225ml)	SOLUS <i>Listeria monocytogenes</i> ELISA		
							Subculture: 0.2 ml+10 ml PAC supp SOLUS Palcam broth - 24h à 37°C		
							Solus <i>Listeria monocytogenes</i> ELISA	Confirmations	O&A (100µl)
49	<i>Listeria</i>	<i>monocytogenes</i>	Ad546	II a	Flour	67	3,000	+	H+
50	<i>Listeria</i>	<i>monocytogenes</i>	Ad623	II b	Breadcrumbs	69	3,000	+	H+

H+: typical blue green colonies with opaque halo

EXCLUSIVITY						
No	Strain	Species	Reference	Origin	Inoculation level (CFU/ml)	SOLUS <i>Listeria monocytogenes</i> ELISA
						BPW 24h-37°C
						Solus <i>Listeria monocytogenes</i> ELISA
						O.D. Result
1	<i>Bacillus</i>	<i>cereus</i>	Ad 465	Salmon Terrine	2,6.10 ⁴	0,021 -
2	<i>Bacillus</i>	<i>circulans</i>	Ad 760	Vegetables	3,2.10 ⁴	0,028 -
3	<i>Bacillus</i>	<i>coagulans</i>	Ad731	Dairy product	1,8.10 ⁴	0,013 -
4	<i>Bacillus</i>	<i>licheniformis</i>	Ad 978	Dairy product	1,1.10 ⁵	0,015 -
5	<i>Bacillus</i>	<i>pumilus</i>	Ad 284	Ready-to-eat	1,2.10 ⁴	0,014 -
6	<i>Brochrotrix</i>	<i>campestris</i>	CIP 102920T	Environment	4,0.10 ⁵	0,011 -
7	<i>Carnobacterium</i>	<i>piscicola</i>	Ad369	Raw milk	8,2.10 ⁴	0,012 -
8	<i>Enterococcus</i>	<i>durans</i>	Ad 149	Ham	8,0.10 ⁵	0,012 -
9	<i>Enterococcus</i>	<i>faecalis</i>	89L326	Soft cheese (Vacherin)	1,4.10 ⁵	0,011 -
10	<i>Lactobacillus</i>	<i>brevis</i>	86L126	Ham	1,6.10 ⁵	0,018 -
11	<i>Lactobacillus</i>	<i>curvatus</i>	Ad 380	Delicatessen	6,0.10 ⁵	0,011 -
12	<i>Lactobacillus</i>	<i>sakei</i>	Ad 473	Ham	1,4.10 ⁴	0,010 -
13	<i>Leuconostoc</i>	<i>carnosum</i>	Ad 411	Dairy product	1,2.10 ⁴	0,012 -
14	<i>Leuconostoc</i>	<i>citreum</i>	Ad396	Ham	1,1.10 ⁴	0,009 -
15	<i>Micococcus</i>	<i>luteus</i>	Ad432	Cocktail	1,0.10 ⁵	0,010 -
16	<i>Staphylococcus</i>	<i>aureus</i>	Ad165	Smoked delicatessen	1,2.10 ⁵	0,022 -
17	<i>Staphylococcus</i>	<i>epidermidis</i>	Ad931	Fruits	1,1.10 ⁵	0,016 -
18	<i>Staphylococcus</i>	<i>haemoliticus</i>	Ad989	Dairy product	2,2.10 ⁴	0,015 -
19	<i>Steptococcus</i>	<i>bovis</i>	92L622	Cheese	3,4.10 ⁴	0,013 -
20	<i>Steptococcus</i>	<i>salivarius</i>	Ad441	Dairy product	4,0.10 ⁵	0,011 -
21	<i>Listeria</i>	<i>grayi</i>	Ad1198	Smoked salmon	3,7.10 ⁵	0,012 -
22	<i>Listeria</i>	<i>grayi</i>	Ad1443	Pork meat sausages	6,2.10 ⁵	0,010 -
23	<i>Listeria</i>	<i>innocua</i>	1	Smoked salmon	2,4.10 ⁵	0,011 -
24	<i>Listeria</i>	<i>innocua</i>	Ad658	Gorgonzola	2,9.10 ⁵	0,011 -
25	<i>Listeria</i>	<i>ivanovii</i>	Ad466	Raw veal meat	2,0.10 ⁵	0,010 -

EXCLUSIVITY							
No	Strain	Species	Reference	Origin	Inoculation level (CFU/ml)	SOLUS <i>Listeria monocytogenes</i> ELISA	
						BPW 24h-37°C	Solus <i>Listeria monocytogenes</i> ELISA
						O.D.	Result
26	<i>Listeria</i>	<i>ivanovii</i>	Ad462	Environment (dairy industry)	4,4.10 ⁵	0,010	-
27	<i>Listeria</i>	<i>seeligeri</i>	Ad649	Cheese	3,8.10 ⁵	0,010	-
28	<i>Listeria</i>	<i>seeligeri</i>	BR1	Environment (fish)	3,0.10 ⁵	0,066	-
29	<i>Listeria</i>	<i>welshimeri</i>	Ad1276	Environment (slaughterhouse))	3,8.10 ⁵	0,060	-
30	<i>Listeria</i>	<i>welshimeri</i>	Ad1175	Ready-to-eat food	5,1.10 ⁵	0,059	-

Appendix 7 - Results obtained by the collaborative laboratories and the expert laboratory

Laboratory A

Aerobic mesophilic flora: $2,5 \cdot 10^6$ CFU/g

N° Sample	Reference method: ISO 11290-1				Final result	SOLUS Listeria monocytogenes ELISA				Agreement		
	Half Fraser		Fraser			Elisa test (Optical Density)	Result	Confirmation	Final result			
	O&A	Palcam	O&A	Palcam				O&A (100µl)				
3	-	-	-	-	-	0,020	-	-	-	NA		
7	-	-	-	-	-	0,057	-	-	-	NA		
8	-	-	-	-	-	0,026	-	-	-	NA		
11	-	-	-	-	-	0,019	-	-	-	NA		
14	-	-	-	-	-	0,058	-	-	-	NA		
18	-	-	-	-	-	0,032	-	-	-	NA		
19	-	-	-	-	-	0,019	-	-	-	NA		
24	-	-	-	-	-	0,021	-	-	-	NA		
1	H+	+	H+	+	+	9,999	+	H+	+	PA		
6	H+	+	H+	+	+	9,999	+	H+	+	PA		
10	-	-	H+	+	+	9,999	+	H+	+	PA		
13	H+	+	H+	+	+	9,999	+	H+	+	PA		
16	-	-	-	-	-	0,017	-	-	-	NA		
17	H+	+	H+	+	+	9,999	+	H+	+	PA		
21	-	-	-	-	-	0,026	-	-	-	NA		
23	-	-	-	-	-	0,023	-	-	-	NA		
2	H+	+	H+	+	+	9,999	+	H+	+	PA		
4	H+	+	H+	+	+	9,999	+	H+	+	PA		
5	H+	+	H+	+	+	9,999	+	H+	+	PA		
9	H+	+	H+	+	+	9,999	+	H+	+	PA		
12	H+	+	H+	+	+	9,999	+	H+	+	PA		
15	H+	+	H+	+	+	9,999	+	H+	+	PA		
20	H+	+	H+	+	+	9,999	+	H+	+	PA		
22	H+	+	H+	+	+	9,999	+	H+	+	PA		

Laboratory**B**Aerobic mesophilic flora: 9,0.10⁶ CFU/g

N° Sample	Reference method: ISO 11290-1					SOLUS <i>Listeria monocytogenes</i> ELISA				Agreement	
	Half Fraser		Fraser		Final result	Elisa test (Optical Density)	Result	Confirmation	Final result		
	O&A	Palcam	O&A	Palcam				O&A (100µl)			
3	-	-	-	-	-	0,015	-	-	-	NA	
7	-	-	-	-	-	0,425	+	-	-	PPNA	
8	-	-	-	-	-	0,015	-	-	-	NA	
11	-	-	-	-	-	0,010	-	-	-	NA	
14	-	-	-	-	-	0,023	-	-	-	NA	
18	-	-	-	-	-	0,084	-	-	-	NA	
19	-	-	-	-	-	0,001	-	-	-	NA	
24	-	-	-	-	-	0,027	-	-	-	NA	
1	-	-	-	-	-	0,014	-	-	-	NA	
6	+	+	+	+	+	3,000	+	+	+	PA	
10	+	+	+	+	+	2,783	+	+	+	PA	
13	-	-	-	-	-	0,017	-	-	-	NA	
16	+	+	+	+	+	2,880	+	+	+	PA	
17	-	-	-	-	-	0,478	+	-	-	PPNA	
21	-	-	+	+	+	3,000	+	+	+	PA	
23	+	+	+	+	+	2,866	+	+	+	PA	
2	+	+	+	+	+	2,908	+	+	+	PA	
4	+	+	+	+	+	2,353	+	+	+	PA	
5	+	+	+	+	+	3,000	+	+	+	PA	
9	+	+	+	+	+	2,816	+	+	+	PA	
12	+	+	+	+	+	2,996	+	+	+	PA	
15	+	+	+	+	+	2,901	+	+	+	PA	
20	+	+	+	+	+	2,982	+	+	+	PA	
22	+	+	+	+	+	2,996	+	+	+	PA	

Laboratory D
Aerobic mesophilic flora: $5,3 \cdot 10^5$ CFU/g

N° Sample	Reference method: ISO 11290-1					SOLUS <i>Listeria monocytogenes</i> ELISA				Agreement	
	Half Fraser		Fraser		Final result	Elisa test (Optical Density)	Result	Confirmation	Final result		
	O&A	Palcam	O&A	Palcam				O&A (100µl)			
3	-	-	-	-	-	0,017	-	-	-	NA	
7	-	-	-	-	-	0,013	-	-	-	NA	
8	-	-	-	-	-	0,013	-	-	-	NA	
11	-	-	-	-	-	0,014	-	-	-	NA	
14	-	-	-	-	-	0,032	-	-	-	NA	
18	-	-	-	-	-	0,042	-	-	-	NA	
19	-	-	-	-	-	0,034	-	-	-	NA	
24	-	-	-	-	-	0,014	-	-	-	NA	
1	-	-	-	-	-	0,012	-	-	-	NA	
6	-	-	-	-	-	0,022	-	-	-	NA	
10	-	-	-	-	-	0,013	-	-	-	NA	
13	-	-	-	-	-	0,021	-	-	-	NA	
16	H+	H+	H+	H+	+	3,000	+	H+	+	PA	
17	H+	H+	H+	H+	+	3,000	+	H+	+	PA	
21	H+	H+	H+	H+	+	3,000	+	H+	+	PA	
23	-	-	-	-	-	0,017	-	-	-	NA	
2	H+	H+	H+	H+	+	3,000	+	H+	+	PA	
4	H+	H+	H+	H+	+	3,000	+	H+	+	PA	
5	H+	H+	H+	H+	+	3,000	+	H+	+	PA	
9	H+	H+	H+	H+	+	3,000	+	H+	+	PA	
12	H+	H+	H+	H+	+	3,000	+	H+	+	PA	
15	H+	H+	H+	H+	+	3,000	+	H+	+	PA	
20	H+	H+	H+	H+	+	3,000	+	H+	+	PA	
22	H+	H+	H+	H+	+	3,000	+	H+	+	PA	

Laboratory FAerobic mesophilic flora: $2,9 \cdot 10^5$ CFU/g

N° Sample	Reference method: ISO 11290-1				SOLUS <i>Listeria monocytogenes</i> ELISA				Confirmation	Final result		Agreement		
					First test (23/06/2022)		Second test (14/07/2022)							
	Half Fraser		Fraser		Final result	Elisa test (Optical Density)	Result	Elisa test (Optical Density)	Result	O&A (100µl)	First test (23/06/2022)	Second test (14/07/2022)	First test (23/06/2022)	Second test (14/07/2022)
O&A	Palcam	O&A	Palcam											
3	H-	-	-	-	-	0,271/0,213	+	0,177	-	-	-	-	PPNA	NA
7	-	-	-	-	-	0,188	-	0,142	-	-	-	-	NA	NA
8	-	-	-	-	-	0,201/0,233	+	0,184	-	-	-	-	PPNA	NA
11	-	-	-	-	-	0,220/0,238	+	0,161	-	-	-	-	PPNA	NA
14	-	-	-	-	-	0,203/0,210	+	0,160	-	-	-	-	PPNA	NA
18	-	-	-	-	-	0,219/0,213	+	0,211	+	-	-	-	PPNA	PPNA
19	-	-	-	-	-	0,198	-	0,153	-	-	-	-	NA	NA
24	-	-	-	-	-	0,199	-	0,143	-	-	-	-	NA	NA
1	H+	+	H+	+	+	5,000	+	5,000	+	+	+	+	PA	PA
6	H+	+	H+	+	+	5,000	+	5,000	+	+	+	+	PA	PA
10	H+	+	H+	+	+	5,000	+	5,000	+	+	+	+	PA	PA
13	H+	+	H+	+	+	5,000	+	5,000	+	+	+	+	PA	PA
16	H+	+	H+	+	+	5,000	+	5,000	+	+	+	+	PA	PA
17	H+	+	H+	+	+	5,000	+	5,000	+	+	+	+	PA	PA
21	H+	+	H+	+	+	5,000	+	5,000	+	+	+	+	PA	PA
23	-	-	-	-	-	0,212/0,253	+	0,175	-	-	-	-	PPNA	NA
2	H+	+	H+	+	+	5,000	+	5,000	+	+	+	+	PA	PA
4	H+	+	H+	+	+	5,000	+	5,000	+	+	+	+	PA	PA
5	H+	+	H+	+	+	5,000	+	5,000	+	+	+	+	PA	PA
9	H+	+	H+	+	+	5,000	+	5,000	+	+	+	+	PA	PA
12	H+	+	H+	+	+	5,000	+	5,000	+	+	+	+	PA	PA
15	H+	+	H+	+	+	5,000	+	5,000	+	+	+	+	PA	PA
20	H+	+	H+	+	+	5,000	+	5,000	+	+	+	+	PA	PA
22	H+	+	H+	+	+	5,000	+	5,000	+	+	+	+	PA	PA

Negative
controls 0,212
(> 0,100)Negative controls
0,213 (> 0,100)

Laboratory**G**Aerobic mesophilic flora: 2,6;10⁵ CFU/g

N° Sample	Reference method: ISO 11290-1				Final result	SOLUS <i>Listeria monocytogenes</i> ELISA				Agreement		
	Half Fraser		Fraser			Elisa test (Optical Density)	Result	Confirmation	Final result			
	O&A	Palcam	O&A	Palcam				O&A (100µl)				
3	-	-	-	-	-	-0,002	-	H-	-	NA		
7	-	-	-	-	-	0,012	-	-	-	NA		
8	-	-	-	-	-	0,011	-	-	-	NA		
11	-	-	-	-	-	0,066	-	-	-	NA		
14	-	-	-	-	-	0,018	-	H-	-	NA		
18	-	-	-	-	-	0,020	-	-	-	NA		
19	-	-	-	-	-	0,029	-	-	-	NA		
24	-	-	-	-	-	0,046	-	H-	-	NA		
1	H+	+	H+	+	+	2,880	+	H+	+	PA		
6	H+	+	H+	+	+	3,034	+	H+	+	PA		
10	H+	+	H+	+	+	2,919	+	H+	+	PA		
13	-	-	-	-	-	0,009	-	H-	-	NA		
16	H+	+	H+	+	+	2,779	+	H+	+	PA		
17	H+	+	H+	+	+	2,920	+	H+	+	PA		
21	-	-	-	-	-	-0,016	-	H-	-	NA		
23	-	-	-	-	-	0,007	-	-	-	NA		
2	H+	+	H+	+	+	2,997	+	H+	+	PA		
4	H+	+	H+	+	+	2,959	+	H+	+	PA		
5	H+	+	H+	+	+	2,729	+	H+	+	PA		
9	H+	+	H+	+	+	2,970	+	H+	+	PA		
12	H+	+	H+	+	+	2,901	+	H+	+	PA		
15	H+	+	H+	+	+	2,842	+	H+	+	PA		
20	H+	+	H+	+	+	2,873	+	H+	+	PA		
22	H+	+	H+	+	+	2,763	+	H+	+	PA		

Laboratory H
Aerobic mesophilic flora: $4,4 \cdot 10^4$ CFU/g

N° Sample	Reference method: ISO 11290-1				Final result	SOLUS <i>Listeria monocytogenes</i> ELISA				Agreement		
	Half Fraser		Fraser			Elisa test (Optical Density)	Result	Confirmation	Final result			
	O&A	Palcam	O&A	Palcam				O&A (100µl)				
3	-	-	-	-	-	0,003	-	-	-	NA		
7	-	-	-	H-	-	0,003	-	-	-	NA		
8	-	-	-	-	-	0,003	-	-	-	NA		
11	-	-	-	H-	-	0,016	-	-	-	NA		
14	-	-	-	-	-	0,001	-	-	-	NA		
18	-	-	-	-	-	0,005	-	-	-	NA		
19	-	-	-	H-	-	0,000	-	-	-	NA		
24	-	-	-	H-	-	0,001	-	-	-	NA		
1	-	-	-	-	-	0,002	-	-	-	NA		
6	-	-	-	-	-	0,002	-	-	-	NA		
10	H+	-	H+	+	+	3,000	+	H+	+	PA		
13	-	-	-	-	-	0,001	-	-	-	NA		
16	H+	+	H+	+	+	3,000	+	H+	+	PA		
17	H+	+	H+	+	+	3,000	+	H+	+	PA		
21	H+	-	H+	+	+	3,000	+	H+	+	PA		
23	H+	+	H+	+	+	3,000	+	H+	+	PA		
2	H+	-	H+	+	+	3,000	+	H+	+	PA		
4	H+	+	H+	+	+	3,000	+	H+	+	PA		
5	H+	+	H+	+	+	3,000	+	H+	+	PA		
9	H+	+	H+	+	+	3,000	+	H+	+	PA		
12	H+	+	H+	+	+	3,000	+	H+	+	PA		
15	H+	+	H+	+	+	3,000	+	H+	+	PA		
20	H+	+	H+	+	+	3,000	+	H+	+	PA		
22	H+	+	H+	+	+	2,627	+	H+	+	PA		

Laboratory

Aerobic mesophilic flora: 8,0.10³ CFU/g

N°Sample	Reference method: ISO 11290-1				Final result	SOLUS <i>Listeria monocytogenes</i> ELISA			Agreement		
	Half Fraser		Fraser			Elisa test (Optical Density)	Result	Confirmation	Final result		
	O&A	Palcam	O&A	Palcam				O&A (100µl)			
3	-	-	-	-	-	0,008	-	-	-	NA	
7	-	-	-	-	-	0,009	-	-	-	NA	
8	-	-	-	-	-	0,004	-	-	-	NA	
11	-	-	-	-	-	0,006	-	-	-	NA	
14	-	-	-	-	-	0,067	-	-	-	NA	
18	-	-	-	-	-	0,006	-	-	-	NA	
19	-	-	-	-	-	0,004	-	-	-	NA	
24	-	-	-	-	-	0,014	-	-	-	NA	
1	-	-	-	-	-	0,030	-	-	-	NA	
6	H+	+	H+	+	+	3,000	+	H+	+	PA	
10	H+	+	H+	+	+	3,000	+	H+	+	PA	
13	-	-	-	-	-	0,016	-	-	-	NA	
16	H+	+	H+	+	+	3,000	+	H+	+	PA	
17	H+	+	H+	+	+	3,000	+	H+	+	PA	
21	-	-	-	-	-	0,049	-	-	-	NA	
23	H+	+	H+	+	+	3,000	+	H+	+	PA	
2	H+	+	H+	+	+	3,000	+	H+	+	PA	
4	H+	+	H+	+	+	3,000	+	H+	+	PA	
5	H+	+	H+	+	+	3,000	+	H+	+	PA	
9	H+	+	H+	+	+	3,000	+	H+	+	PA	
12	H+	+	H+	+	+	3,000	+	H+	+	PA	
15	H+	+	H+	+	+	3,000	+	H+	+	PA	
20	H+	+	H+	+	+	3,000	+	H+	+	PA	
22	H+	+	H+	+	+	3,000	+	H+	+	PA	

Laboratory

L

Aerobic mesophilic flora: $2,7 \cdot 10^5$ CFU/g

N° Sample	Reference method: ISO 11290-1				Final result	SOLUS <i>Listeria monocytogenes</i> ELISA				Agreement		
	Half Fraser		Fraser			Elisa test (Optical Density)	Result	Confirmation	Final result			
	O&A	Palcam	O&A	Palcam				O&A (100µl)				
3	-	-	-	-	-	0,021	-	-	-	NA		
7	-	-	-	-	-	0,106	-	-	-	NA		
8	-	-	-	-	-	0,013	-	-	-	NA		
11	-	-	-	-	-	0,014	-	-	-	NA		
14	-	-	-	-	-	0,015	-	-	-	NA		
18	-	-	-	-	-	0,011	-	-	-	NA		
19	-	-	-	-	-	0,011	-	-	-	NA		
24	-	-	-	-	-	0,135	-	-	-	NA		
1	H+	-	H+	+	+	9,999	+	H+	+	PA		
6	H+	-	H+	+	+	9,999	+	H+	+	PA		
10	H+	-	H+	+	+	9,999	+	H+	+	PA		
13	H+	-	H+	+	+	9,999	+	H+	+	PA		
16	H+	-	H+	+	+	9,999	+	H+	+	PA		
17	-	-	-	-	-	0,008	-	-	-	NA		
21	H+	-	H+	+	+	9,999	+	H+	+	PA		
23	-	-	-	-	-	0,015	-	-	-	NA		
2	H+	-	H+	+	+	9,999	+	H+	+	PA		
4	H+	-	H+	+	+	9,999	+	H+	+	PA		
5	-	-	-	-	-	0,013	-	-	-	NA		
9	H+	-	H+	+	+	9,999	+	H+	+	PA		
12	H+	-	H+	+	+	9,999	+	H+	+	PA		
15	H+	-	H+	+	+	9,999	+	H+	+	PA		
20	H+	-	H+	+	+	9,999	+	H+	+	PA		
22	H+	-	H+	+	+	9,999	+	H+	+	PA		

Laboratory

M

Aerobic mesophilic flora: $6,6 \cdot 10^8$ CFU/g

N° Sample	Reference method: ISO 11290-1				Final result	SOLUS <i>Listeria monocytogenes</i> ELISA				Agreement		
	Half Fraser		Fraser			Elisa test (Optical Density)	Result	Confirmation	Final result			
	O&A	Palcam	O&A	Palcam				O&A (100µl)				
3	-	-	-	-	-	0,016	-	-	-	NA		
7	-	-	-	-	-	0,017	-	-	-	NA		
8	-	-	-	-	-	0,018	-	-	-	NA		
11	-	-	-	-	-	0,014	-	-	-	NA		
14	-	-	-	-	-	0,018	-	-	-	NA		
18	-	-	-	-	-	0,015	-	-	-	NA		
19	-	-	-	-	-	0,011	-	-	-	NA		
24	-	-	-	-	-	0,019	-	-	-	NA		
1	-	-	-	-	-	0,014	-	-	-	NA		
6	H+	-	H+	+	+	3,000	+	H+	+	PA		
10	H+	+	H+	+	+	3,000	+	H+	+	PA		
13	H+	+	H+	+	+	3,000	+	H+	+	PA		
16	-	-	-	-	-	0,017	-	-	-	NA		
17	H+	+	H+	+	+	3,000	+	H+	+	PA		
21	H+	+	H+	+	+	3,000	+	H+	+	PA		
23	H+	+	H+	+	+	3,000	+	H+	+	PA		
2	H+	+	H+	+	+	3,000	+	H+	+	PA		
4	H+	+	H+	+	+	3,000	+	H+	+	PA		
5	H+	+	H+	+	+	3,000	+	H+	+	PA		
9	H+	+	H+	+	+	3,000	+	H+	+	PA		
12	H+	+	H+	+	+	3,000	+	H+	+	PA		
15	H+	+	H+	+	+	3,000	+	H+	+	PA		
20	H+	+	H+	+	+	3,000	+	H+	+	PA		
22	H+	+	H+	+	+	3,000	+	H+	+	PA		

Laboratory N
Aerobic mesophilic flora: $9,7 \cdot 10^4$ CFU/g

N°Sample	Reference method: ISO 11290-1				Final result	SOLUS <i>Listeria monocytogenes</i> ELISA				Agreement		
	Half Fraser		Fraser			Elisa test (Optical Density)	Result	Confirmation	Final result			
	O&A	Palcam	O&A	Palcam				O&A (100µl)				
3	-	-	-	-	-	0,001	-	-	-	NA		
7	-	-	-	-	-	0,000	-	-	-	NA		
8	-	-	-	-	-	0,000	-	-	-	NA		
11	-	-	-	-	-	0,000	-	-	-	NA		
14	-	-	-	-	-	0,001	-	-	-	NA		
18	-	-	-	-	-	0,019	-	-	-	NA		
19	-	-	-	-	-	0,000	-	-	-	NA		
24	-	-	-	-	-	0,000	-	-	-	NA		
1	-	-	-	-	-	0,001	-	-	-	NA		
6	H+	+	H+	+	+	3,000	+	H+	+	PA		
10	H+	+	H+	+	+	3,000	+	H+	+	PA		
13	H+	+	H+	+	+	3,000	+	H+	+	PA		
16	H+	+	H+	+	+	3,000	+	H+	+	PA		
17	H+	+	H+	+	+	3,000	+	H+	+	PA		
21	H+	+	H+	+	+	3,000	+	H+	+	PA		
23	H+	+	H+	+	+	3,000	+	H+	+	PA		
2	H+	+	H+	+	+	3,000	+	H+	+	PA		
4	H+	+	H+	+	+	3,000	+	H+	+	PA		
5	H+	+	H+	+	+	3,000	+	H+	+	PA		
9	H+	+	H+	+	+	3,000	+	H+	+	PA		
12	H+	+	H+	+	+	3,000	+	H+	+	PA		
15	H+	+	H+	+	+	3,000	+	H+	+	PA		
20	H+	+	H+	+	+	3,000	+	H+	+	PA		
22	H+	+	H+	+	+	3,000	+	H+	+	PA		

Laboratory P
Aerobic mesophilic flora: $8,5 \cdot 10^4$ CFU/g

N° Sample	Reference method: ISO 11290-1				Final result	SOLUS <i>Listeria monocytogenes</i> ELISA				Agreement		
	Half Fraser		Fraser			Elisa test (Optical Density)	Result	Confirmation	Final result			
	O&A	Palcam	O&A	Palcam				O&A (100µl)				
3	-	-	-	-	-	0,043	-	-	-	NA		
7	-	-	-	-	-	0,020	-	-	-	NA		
8	-	-	-	-	-	0,026	-	-	-	NA		
11	-	-	-	-	-	0,020	-	-	-	NA		
14	-	-	-	-	-	0,030	-	-	-	NA		
18	-	-	-	-	-	0,016	-	-	-	NA		
19	-	-	-	-	-	0,017	-	-	-	NA		
24	-	-	-	-	-	0,018	-	-	-	NA		
1	H+	+	H+	+	+	3,000	+	H+	+	PA		
6	-	-	-	-	-	0,026	-	-	-	NA		
10	H+	+	H+	+	+	3,000	+	H+	+	PA		
13	H+	+	H+	+	+	3,000	+	H+	+	PA		
16	-	-	-	-	-	0,018	-	-	-	NA		
17	H+	+	H+	+	+	3,000	+	H+	+	PA		
21	H+	+	H+	+	+	3,000	+	H+	+	PA		
23	H+	+	H+	+	+	3,000	+	H+	+	PA		
2	H+	+	H+	+	+	3,000	+	H+	+	PA		
4	H+	+	H+	+	+	3,000	+	H+	+	PA		
5	H+	+	H+	+	+	3,000	+	H+	+	PA		
9	H+	+	H+	+	+	3,000	+	H+	+	PA		
12	H+	+	H+	+	+	3,000	+	H+	+	PA		
15	H+	+	H+	+	+	3,000	+	H+	+	PA		
20	H+	+	H+	+	+	3,000	+	H+	+	PA		
22	H+	+	H+	+	+	3,000	+	H+	+	PA		

Laboratory Q
Aerobic mesophilic flora: $2,8 \cdot 10^5$ CFU/g

N° Sample	Reference method: ISO 11290-1				Final result	SOLUS <i>Listeria monocytogenes</i> ELISA				Agreement		
	Half Fraser		Fraser			Elisa test (Optical Density)	Result	Confirmation	Final result			
	O&A	Palcam	O&A	Palcam				O&A (100µl)				
3	-	-	-	-	-	0,011	-	-	-	NA		
7	-	-	-	-	-	2,118	+	-	-	PPNA		
8	-	-	-	-	-	0,081	-	-	-	NA		
11	-	-	-	-	-	0,010	-	-	-	NA		
14	-	-	-	-	-	0,016	-	-	-	NA		
18	-	-	-	-	-	0,024	-	-	-	NA		
19	-	-	-	-	-	0,017	-	-	-	NA		
24	-	-	-	-	-	1,574	+	-	-	PPNA		
1	H+	H+	H+	H+	+	3,000	+	H+	+	PA		
6	H+	H+	H+	H+	+	3,000	+	H+	+	PA		
10	H+	H+	H+	H+	+	3,000	+	H+	+	PA		
13	-	-	-	-	-	0,017	-	-	-	NA		
16	H+	H+	H+	H+	+	2,929	+	H+	+	PA		
17	H+	H+	H+	H+	+	2,908	+	H+	+	PA		
21	H+	H+	H+	H+	+	3,000	+	H+	+	PA		
23	H+	H+	H+	H+	+	2,662	+	H+	+	PA		
2	H+	H+	H+	H+	+	3,000	+	H+	+	PA		
4	H+	H+	H+	H+	+	3,000	+	H+	+	PA		
5	H+	H+	H+	H+	+	3,000	+	H+	+	PA		
9	H+	H+	H+	H+	+	3,000	+	H+	+	PA		
12	H+	H+	H+	H+	+	3,000	+	H+	+	PA		
15	H+	H+	H+	H+	+	3,000	+	H+	+	PA		
20	H+	H+	H+	H+	+	2,964	+	H+	+	PA		
22	H+	H+	H+	H+	+	3,000	+	H+	+	PA		

Laboratory S ADRIA
Aerobic mesophilic flora: 1,4.10⁵ CFU/g

N°Sample	Reference method: ISO 11290-1*				Final result	SOLUS <i>Listeria monocytogenes</i> ELISA				Agreement		
	Half Fraser		Fraser			Elisa test (Optical Density)	Result	Confirmation	Final result			
	O&A	Palcam	O&A	Palcam				O&A (100µl)				
3	-	-	-	-	-	0,019	-	-	-	NA		
7	-	-	-	-	-	0,011	-	-	-	NA		
8	-	-	-	-	-	0,015	-	-	-	NA		
11	-	-	-	-	-	0,018	-	-	-	NA		
14	-	-	-	-	-	0,009	-	-	-	NA		
18	-	-	-	-	-	0,013	-	-	-	NA		
19	-	-	-	-	-	0,013	-	-	-	NA		
24	-	-	-	-	-	0,013	-	-	-	NA		
1	H+	+	H+	+	+	3,000	+	H+	+	PA		
6	H+	+	H+	+	+	3,000	+	H+	+	PA		
10	H+	+	H+	+	+	3,000	+	H+	+	PA		
13	H+	+	H+	+	+	3,000	+	H+	+	PA		
16	H+	+	H+	+	+	3,000	+	H+	+	PA		
17	H+	+	H+	+	+	3,000	+	H+	+	PA		
21	-	-	-	-	-	0,025	-	-	-	NA		
23	H+	+	H+	+	+	3,000	+	H+	+	PA		
2	H+	+	H+	+	+	3,000	+	H+	+	PA		
4	H+	+	H+	+	+	3,000	+	H+	+	PA		
5	H+	+	H+	+	+	3,000	+	H+	+	PA		
9	H+	+	H+	+	+	3,000	+	H+	+	PA		
12	H+	+	H+	+	+	3,000	+	H+	+	PA		
15	H+	+	H+	+	+	3,000	+	H+	+	PA		
20	H+	+	H+	+	+	3,000	+	H+	+	PA		
22	H+	+	H+	+	+	3,000	+	H+	+	PA		

* Analysis performed according to the COFRAC accreditation