AFNOR CERTIFICATION VALIDATION STUDY
ADIAFOOD LISTERIA MONOCYTOGENES TEST SYSTEM

SYNTHESIS REPORT

ADIAFOOD LISTERIA MONOCYTOGENES TEST SYSTEM - S.R.(V0)
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Only certain assays reported in this document are covered by the accreditation of the Section Laboratory of COFRAC. They are identified by the symbol (*).

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For the AFNOR Certification validation of the ADIAFOOD *Listeria monocytogenes* test kit with confirmation according to the NF EN ISO 16140 standard

SUMMARY

1. Introduction

1.1. Validation referential

The aim of this validation study is to evaluate the performance of the alternative method against the reference method ISO 11290-1 (1997) and ISO 11290-1/A1 (2005). It consists in a preliminary study and a collaborative study.

1.2. Alternative method

The ADIAFOOD system is based on real-time Polymerase Chain Reaction (PCR) technology. The system provides rapid detection by specifically identifying the DNA sequence of pathogens in a series of sequential steps that include Sample Preparation and Enrichment, DNA Extraction, and Pathogen Detection.
The thermocycler used for the validation was a MX 3005P from Stratagene, used with the Sentinel software number 2.0.0 – R 9 3.
The protocol of the method is showed in figure 1.

STEP 1: PRE-ENRICHMENT
X g (mL) sample + 9X mL Listerboost broth
Incubation at (37±1)°C for 18 to 22 hours
Make a 1 mL aliquot of the suspension in a 1,2 mL tube

STEP 2: DNA EXTRACTION
Place the tubes in a 96 well microplate and centrifuge for 2 min at 500 g
Transfer 500 µL of the supernatant in new tubes, place them in a microplate and centrifuge the plate for 5 min at (4000±500)g
Discard the supernatant and add 100 µL of the solution EX-2 followed by a succion-discharge step
Transfer the 100 µL in an extraction microplate
Place the closed microplate in a thermocycler begin the extraction
Centrifuge the extraction microplate for 5 min at 1800 g

STEP 3: DNA AMPLIFICATION
Place 15 µL of the solution DT-2 in the wells of the detection microplate* and add 10 µL of the DNA extract from the extraction microplate
Close hermetically the microplate with optical quality corks
Tape and shake manually the microplate
Centrifuge the detection microplate for 1 min at 1800 g
Place the detection microplate in the thermocycler and begin the detection

STEP 4: RESULTS
Results appear automatically at the end of the detection (+ or -)
With inhibited samples, make another assay with DNA extracts diluted at $1/10^{th}$ or make a washing of the concerned wells

STEP 5: CONFIRMATION OF PRESUMED POSITIVE RESULTS
The confirmation consists in an isolation of 100 µL of enriched Listerboost broth on ALOA™ agar medium

*: Ability to use barrettes unit

Figure 1: alternative method protocol

During the detection step, the target pathogen DNA is amplified and detected using specific primers and molecular beacons, the key components of the AES CHEMUNEX technology. Molecular beacons probes consist in a unique sequence probe that allows the identification of the pathogen with a high level of specificity. Once bound to their target,

the molecular beacons emit a fluorescent signal that is proportional to the amount of amplified pathogenic DNA. In the absence of target bacteria in food samples, no fluorescent signal is detected. Results are recorded and analyzed automatically with AES CHEMUNEX' proprietary Sentinel software.

1.3. Scope of application

The alternative method was tested for all food and feed products and environmental samples.

1.4. Reference method(*)

The standards NF EN ISO 11290-1 (1997) and NF EN ISO 11290-1/A1 (2005), horizontal method for the detection and the enumeration of *Listeria monocytogenes* were applied. The protocol of this method is shown in figure 2.

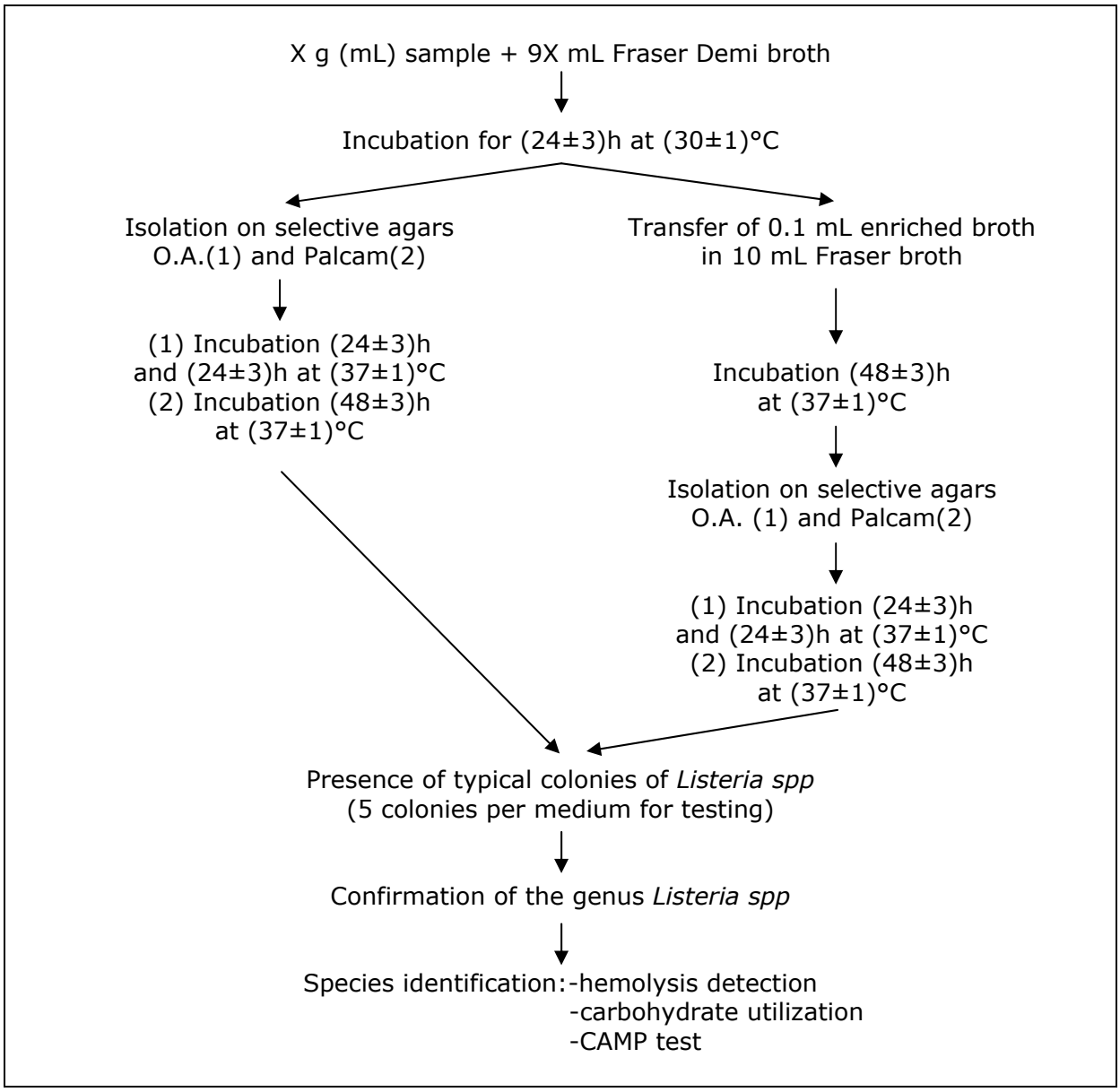


Figure 2: reference method protocol

2. Comparative study

The following characteristics are studied during the preliminary study:

- Relative accuracy (AC), relative specificity (SP) and relative sensitivity (SE)
- Relative detection level of the alternative method and the reference method
- Selectivity of the alternative method
- Practicability of the alternative method

2.1. Relative accuracy, relative specificity, relative sensitivity

The relative accuracy is the degree of correspondence between the response obtained by the reference method and the response obtained by the alternative method on identical samples.

The relative specificity is the ability of the alternative method to not detect the target microorganism when it is not detected by the reference method.

The relative sensitivity is the ability of the alternative method to detect the analyte when it is detected by the reference method.

The objective of this study is to evaluate the performance of both methods on contaminated and non-contaminated samples.

2.1.1. Number and nature of samples

The following categories are studied: meat products, dairy products, seafood products, vegetable products and environmental samples.

A number of 320 samples was analysed. Types of products are indicated in table 1.

Category	Type	Number of positive*	Number of negative	Total
Meat products	Raw meat	9	7	16
	Cooked meat	6	9	15
	Delicatessen	9	9	18
	Meat dishes	7	5	12
	Total	**31**	**30**	**61**
Dairy products	Raw milk cheese	10	10	20
	Pasteurized milk cheese	1	5	6
	Yoghurts and puddings	15	12	27
	Milks and creams	9	4	13
	Total	**35**	**31**	**66**
Seafood products	Smoked fish	11	9	20
	Raw fish and shellfish	8	5	13
	Cooked fish and shellfish	6	8	14
	Fish dishes	5	9	14
	Total	**30**	**31**	**61**
Vegetable products	Frozen vegetables	2	3	5
	Dishes	7	8	15
	Raw fruit and vegetables	13	9	22
	Cooked fruit and vegetables	8	15	23
	Total	**30**	**35**	**65**
Environ-mental samples	Swabs	17	36	53
	Process waters	14	0	14
	Total	**31**	**36**	**67**
Total		**157**	**163**	**320**

Table 1: nature and number of analysed samples (*=positive results by either method)

2.1.2. Artificial contamination of samples

Naturally contaminated samples are seldom available. Therefore, artificial contaminations of food samples were mostly performed. For spiking, several strains were stressed using different treatments and the stress intensity was evaluated (logarithmic difference between enumeration on non selective agar –TSA- and selective agar –ALOA-).

77 naturally contaminated samples were analysed.49,3% of positive samples are the results of artificial spiking.

2.1.3. Confirmation protocol

The confirmation of presumed positive results obtained by the alternative method was realized from 100 µL of the enrichment broth in Listerboost which were isolated on ALOA media which were then incubated at (37±1)°C for 24 to 48 hours.

2.1.4. Results

Each sample was analysed once by the alternative method and once by the reference method. Table 2 presents paired results of both methods.

Biochemical tests were realized from colonies isolated on ALOA in the case of positive results confirmed by the alternative method to explain prospective discordant results.

Category	Response	Reference method(*) positive (R+)	Reference method (*) négative (R-)
Meat products	**Alternative method positive (A+)**	PA=22	PD=3
	Alternative method négative (A-)	ND=6 including 1 PPND	NA=30 including 3 PPNA
Dairy products	**Alternative method positive (A+)**	PA=31	PD=2
	Alternative method négative (A-)	ND=2 including 0 PPND	NA=31 including 1 PPNA
Seafood products	**Alternative method positive (A+)**	PA=21	PD=4
	Alternative method négative (A-)	ND=5 including 2 PPND	NA=31 including 1 PPNA
Vegetable products	**Alternative method positive (A+)**	PA=26	PD=3
	Alternative method négative (A-)	ND=1 including 0 PPND	NA=35 including 1 PPNA
Environmental samples	**Alternative method positive (A+)**	PA=28	PD=2
	Alternative method négative (A-)	ND=1 including 0 PPND	NA=36 including 1 PPNA
All products	**Alternative method positive (A+)**	PA=128	PD=14
	Alternative method négative (A-)	ND=15 including 4 PPND	NA=163 including 7 PPNA

Table 2: results of relative accuracy for both methods (PA: positive agreement, NA: negative agreement, ND: negative deviation, PD: positive deviation, PP: presumed positive before confirmation, A+: confirmed positive, A-: negative immediately and negative after confirmation when presumed positive)

2.1.5. Calculation of relative accuracy (AC), relative specificity (SP) and relative sensitivity (SE)

For all products categories, these results permit to calculate the relative accuracy, relative specificity and relative sensitivity according to NF EN ISO standard. Results are indicated in table 3.

Category	**PA**	**NA**	**ND**	**PD**	**N**	Relative accuracy **AC** [(PA+NA)/N]	**N+** PA+ND	Relative sensitivity **SE** [PA/N+]	**N-** NA+PD	Relative specificity **SP** [NA/N-]
Meat products	22	30	6	3	61	85.2%	28	78.6%	33	90.9%
Dairy products	31	31	2	2	66	93.9%	33	93.9%	33	93.9%
Seafood products	21	31	5	4	61	85.2%	26	80.8%	35	88.6%
Vegetable products	26	35	1	3	65	93.8%	27	96.3%	38	92.1%
Environmental samples	28	36	1	2	67	95.5%	29	96.6%	38	94.7%
All products	128	163	15	14	320	90.9%	143	89.5%	177	92.1%

Table 3: relative accuracy, relative specificity and relative sensitivity of alternative method (PA: positive agreement, NA: negative agreement, ND: negative deviation, PD: positive deviation, AC = (PA+NA)/N x 100%, SE = PA/N+ x 100%, SP = NA/N- x 100%, N+ = PA+ND and N- = NA+PD)

Criteria values in percent are shown in table 4.

	Alternative method
Relative accuracy	90.9%
Relative sensitivity	89.5%
Relative specificity	92.1%

Table 4: AC, SE and SP in percent for alternative method

Sensitivity of both methods was recalculated considering all confirmed positive (including alternative method positive deviations). Results are shown in table 5.

	Alternative method (PA+PD)/(PA+PD+ND)	**Reference method** (PA+ND)/(PA+PD+ND)
Sensitivity	90.4%	91.1%

Table 5: sensitivity of both methods including all confirmed positive

2.1.6. Analysis of discordant results

Discordant results are examined according to annex F of NF EN ISO 16140 standard, with Y as the number of discordant results and m as the smallest of the two values of PD and ND.

In the present case, Y = 14 + 15 = 29, the McNemar's test is used with the chi square distribution for 1 degree of freedom to compare the two methods.

The following formula $X^2 = d2/Y$, with d = l PD – ND l permits to calculate $X^2 = 0.034$. The X^2 value is inferior to 3.841, both methods are comparable for $\alpha=0.05$.

- Negative deviations

-Sample numbers: RD 2005 / RD 2038 / RD 2068 / RD 2578 / RD 2589 / RD 2000 / RD 2800 / RD 2803 / RD 2559 / RD 2651:
A positive result is obtained by the reference method whereas a negative result is obtained by the alternative method. However the isolation of the Listerboost broth on ALOA agar medium didn't allow finding typical colonies or showed typical colonies not confirmed. Due to the difference of sampling between both methods, no cell of *Listeria* may have been taken in the sampling for the alternative method.

-Sample numbers: RD 2377 / RD 2383
A positive result is obtained by the reference method whereas a negative result is obtained by the alternative method from naturally contaminated samples. However the isolation of the Listerboost broth on ALOA agar medium allowed finding typical colonies which were confirmed as *L. monocytogenes.* The *s*trains isolated by the reference method were analyzed by the alternative method in pure culture in Listerboost between 10 and 30 CFU/225 mL. They gave a positive result.
This lack of detection may be due to a lack of sensitivity of the method in a low bacterial burden sample.

-Sample numbers: RD 2056 / RD 2058 / RD 2632
A positive result is obtained by both methods. However the isolation of the Listerboost broth on ALOA agar medium didn't allow finding typical colonies. The positive reaction of the PCR test may be due to a low bacterial burden sample which didn't permit to isolate L. monocytogenes cells on a selective medium.

- Positive deviations

-Sample numbers: RD 2059 / RD 2060 / RD 2376 / RD 2590 / RD 2591 / RD 2045 / RD 2618 / RD 2797 / RD 2798 / RD 2562 / RD 2563 / RD 2566 / RD 2652 / RD 2811
A positive result is obtained by the alternative medium whereas a negative result is obtained by the reference method. Due to the difference of sampling between both methods, no cell of *Listeria* may have been taken in the sampling for the reference method.

2.2. Relative detection level

The objective of this study is to determine the level of contamination for which less than 50% of the responses obtained are positive and that for which more than 50% of the responses obtained are positive.

2.2.1. Matrices

A couple "matrix-strain" was studied in parallel with the reference method and the alternative method for each category. The total viable count of each matrix was enumerated. Characteristics of the strain and the matrix are shown in table 6.

Matrix	Strain	ISHA code	Origin
Rillettes	*Listeria monocytogenes* 1/2a	LIS.4.26	Ham
Raw milk	*Listeria monocytogenes* 1/2b	LIS.4.32	Raw milk
Smoked salmon	*Listeria monocytogenes* 4b	LIS.4.47	Salmon pieces
Celery	*Listeria monocytogenes* 1/2c	LIS.4.35	Sandwich
Process water	*Listeria monocytogenes* 3a	LIS.4.44	Surface swab

Table 6: "matrix-strain" couples of the relative detection level

2.2.2. Spiking protocol

Six levels of contamination were tested including the negative control.
Six replicates for each level of contamination were inoculated and analysed by the reference method and the alternative method.
As the two methods have no common step, 12 test portions of 25 g were prepared for each level of contamination and individually inoculated with a calibrated bacterial

suspension. Bacterial suspension of about 10 cells per mL was prepared. From this initial suspension, volumes of 0.9 mL, 0.3 mL and 0.1 mL were used to spike 25 g of sample respectively for the 3 first levels. In parallel, the initial suspension was diluted ratio ½ and ¼ in order to inoculate the lower levels of contamination with 0.1 mL. For all the levels of contamination, homogeneity of the inoculums was checked by enumeration on 30 TSA Petri dishes. Then, the confidence interval was determined according to Poisson law.

2.2.3. Results

Tables 7 and 8 present the relative detection level for each method.

		Relative detection level according to Spearman-Kärber method (cells in 25 g)	
Strain	**Matrix**	**Reference method (*)**	**Alternative method**
L. mono 1/2a	Rillettes	0.797 [0.448 ; 1.419]	0.772 [0.455 ; 1.310]
L. mono 1/2b	Raw milk	1.134 [0.772 ; 1.666]	1.634 [1.112 ; 2.399]
L. mono 4b	Smoked salmon	0.466 [0.350 ; 0.622]	0.466 [0.350 ; 0.622]
L. mono 1/2c	Celery	0.919 [0.625 ; 1.349]	1.092 [0.708 ; 1.682]
L. mono 3a	Process water	0.672 [0.378 ; 1.196]	0.546 [0.292 ; 1.020]

Table 7: relative detection level (3 significant numbers)

		Relative detection level according to Spearman-Kärber method (cells in 25 g)	
Strain	**Matrix**	**Reference method (*)**	**Alternative method**
L. mono 1/2a	Rillettes	0.8 [0.4 ; 1.4]	0.8 [0.5 ; 1.3]
L. mono 1/2b	Raw milk	1.1 [0.8 ; 1.7]	1.6 [1.1 ; 2.4]
L. mono 4b	Smoked salmon	0.5 [0.4 ; 0.6]	0.5 [0.4 ; 0.6]
L. mono 1/2c	Celery	0.9 [0.6 ; 1.3]	1.1 [0.7 ; 1.9]
L. mono 3a	Process water	0.7 [0.4 ; 1.2]	0.5 [0.3 ; 1.0]

Table 8: relative detection level (1 significant number)

The alternative and the reference method show similar detection levels. The detection limit obtained with the alternative method is comprised between 0.3 and 2.4 CFU in 25 g. The detection limit obtained with the reference method is comprised between 0.4 and 1.7 CFU in 25 g.

2.3. Inclusivity / exclusivity (selectivity)

The objective of this study is to test:

-the inclusivity: the detection of the target microorganism from a wide range of strains,

-the exclusivity: the lack of interference from a relevant range of non-target microorganisms.

According to the requirements of NF EN ISO 16140, 50 strains of *Listeria monocytogenes* and 32 non-target strains were tested. A list of the strains figures in annex 1.

2.3.1. Test protocols

- **Inclusivity**

Each *L. monocytogenes* strain was cultivated twice before inoculation in Listerboost broth (about 1 to 100 CFU/225 mL). The complete protocol of alternative method was applied with the minimum time of incubation.

- **Exclusivity**

Each non-target strain was cultivated twice before inoculation in growth medium (Tryticase Soy Broth) with a level of contamination expected to occur in the food

matrices (about 10^5 CFU/mL). After 24 hours of incubation, the ADIAFOOD test was performed.
In cases where the target strains or non-target strains results were unexpected to interpret by the alternative method, the analysis was conducted once again in parallel with the alternative method and the reference method (complete protocol).

2.3.2. Results

The 50 *Listeria monocytogenes* strains tested were detected by the alternative method.
No non target strain was detected by the alternative method.

2.3.3. Conclusion

The selectivity of the method is satisfactory.

3. Collaborative study

The main object of the collaborative study is to determine the variability of the results obtained by different laboratories analysing identical samples and to compare these results within the framework of the comparative study of the methods.

3.1. Collaborative study implementation

3.1.1. Participating laboratories

The collaborative study was realized by the expert laboratory and fourteen participating laboratories.

3.1.2. *L. monocytogenes* absence in the matrix

Before spiking, the absence of *Listeria monocytogenes* was verified in the batch of pasteurized milk used according to the reference method.

3.1.3. Strain stability in the matrix

The total viable count (TVC) of several pasteurized milks was enumerated to choose a matrix which contains an annex microflora. The results showed a TVC inferior to 1 CFU/mL for all the matrices analysed. The pasteurized milk used for the collaborative study was consequently supplemented with raw milk (0.25mL for 25 mL).

The strain stability in the supplemented pasteurized milk matrix was evaluated for 4 days at (4±2)°C. The strain used was *Listeria monocytogenes* 1/2b (ISHA code: LIS.4.32) isolated from a raw milk.

Two kind of analysis were realized:

(1) Inoculation of 10 cells in 25 mL pasteurized milk. The samples were analysed at D0, D+1, D+2 and D+3 by the reference method (detection part) and by the alternative method.

(2) Inoculation of 300 cells in 25 mL pasteurized milk. The samples were analysed at D0, D+1, D+2 and D+3 by the reference method (enumeration part).

The results are summarized in table 9.

Day	Alternative method (1)	Reference method (1)	Reference method (2)
D0	Presence in 25 mL	Presence in 25 mL	$2{,}8.10^2$ CFU/mL
D+1	Presence in 25 mL	Presence in 25 mL	$3{,}0.10^2$ CFU /mL
D+2	Presence in 25 mL	Presence in 25 mL	$2{,}8.10^2$ CFU /mL
D+3	Presence in 25 mL	Presence in 25 mL	$3{,}5.10^2$ CFU /mL

Table 9: results of the stability study of the strain LIS.4.32 in supplemented pasteurized milk

The results show that the *L. monocytogenes* strain used is stable for 3 days at (4±2)°C in the supplemented pasteurized milk matrix.

3.1.4. Samples preparation and spiking

The matrix was inoculated with the target strain suspension to obtain 3 contamination levels:

-L0: 0 cell in 25 mL
-L1: 3 cells in 25 mL
-L2: 30 cells in 25 mL

The matrix was distributed at 25 mL in sterile vials. Every vial was individually spiked and homogenized. Eight samples per level, per laboratory and per method were prepared. Each laboratory received 48 samples to analyse, 1 sample to quantify the endogenous microflora and 1 water sample containing a temperature probe.
The results of the enumerations of the TVC, the target levels and the real levels of contamination are presented in table 10.

Matrix	Total viable count (CFU/mL)	Target level (cells / 25 mL	Real level (cells / 25 mL)	Confidence interval
Pasteurized milk	$1{,}2.10^2$	0	0	0
		3	3	[0 ; 7]
		30	26	[17 ; 36]

Table 10: target level, real level and TVC of the matrix

3.1.5. Samples labeling

The labelling of the vials was realized as follows: a code to identify the laboratory: from A to N (cf. table 11) and a code to identify each sample, only known by the expert laboratory. The samples and the temperature control vials (water sample with a temperature probe) were stored at 4°C before shipping.

Contamination level	Sample code
L0	4/7/8/9/11/18/19/24
L1	1/2/3/10/12/20/22/23
L2	5/6/13/14/15/16/17/21

Table 11: sample code by contamination level

3.1.6. Samples shipping

The samples were shipped in a coolbox the 12th of October 2009.

3.1.7. Samples reception and analysis

The coolboxes were received the 13th of October 2009 by all the participating laboratories. The control temperature was recorded upon receipt of the package and the temperature probe sent to the expert laboratory. The samples were analysed the same day. The expert laboratory concurrently analysed a set of samples under the same conditions with both methods.

3.2. Results

3.2.1. Temperature and state of the samples

The temperature readings upon reception and the state of the samples are shown in table 12.

Laboratory	Temperature (°C)	State of the samples
A	9.7	Correct
B	5.0	Correct
C	4.1	Correct
D	7.1	Correct
E	4.4	Correct
F	6.0	Correct
G	5.7	Correct
H	4.1	Correct
I	4.0	Correct
J	3.2	Correct
K	5.2	Correct
L	8.2	Correct
M	6.1	Correct
N	3.9	Correct

Table 12: temperature and state of the samples upon reception

The temperature measurements are inferior to 8.4°C for all the laboratories, except for laboratory A for which it was at 9.7°C upon reception. However the temperature probe

indicated a correct mean temperature between the shipping and the reception of the coolbox for this laboratory. The analysis of thermal profiles is shown in table 13.

Laboratory		A	B	C	D	E	F	G	H	I	J	K	L	M	N
Tempera-ture (°C)	Mean	3.4	2.5	1.2	4.0	2.5	2.4	2.4	2.0	2.6	2.2	3.2	2.2	4.5	3.6
	SD	1.4	0.7	0.9	0.9	0.5	1.9	0.6	1.3	0.7	0.5	0.7	0.4	0.3	0.5

Table 13: data of the temperature probes for the transportation time of samples

The thermal profiles analysis indicates for all laboratories mean temperatures comprises between 1.2 and 4.5°C.

3.2.2. Total viable counts

For the whole laboratories, the total viable counts at 30°C vary between $8.6x10^1$ and $9.7x10^2$ CFU/mL.

3.2.3. Expert laboratory results

The results obtained by the expert laboratory are summarized in table 14.

Contamination level	Alternative method	Reference method (*)
L0	0/8	0/8
L1	8/8	7/8
L2	8/8	8/8

Table 14: positive results obtained by expert laboratory by both methods

The results are consistent with those expected, except for 1 sample at low level contamination which appears negative by reference method and positive by alternative method. Due to the low level of contamination of this sample (3 CFU/25 mL), no Listeria cell may have been inoculated in the matrix.

3.2.4. Participating laboratories results

The results are summarized in tables 15 and 16.

- Alternative method results

	Contamination level		
Laboratory	**L0**	**L1**	**L2**
A	0/8	8/8	**7/8**
B	**2/8**	8/8	8/8
C	0/8	7/8	8/8
D	0/8	8/8	8/8
E	0/8	8/8	8/8
F	0/8	8/8	8/8
G	0/8	8/8	8/8
H	0/8	7/8	8/8
I	0/8	7/8	8/8
J	0/8	8/8	8/8
K	0/8	8/8	8/8
L	0/8	8/8	8/8
M	0/8	7/8	8/8
N	0/8	8/8	8/8

Table 15: alternative method positive results for all laboratories

Only the laboratory I reported inhibitions of the PCR reaction for 4 samples (I2, I6, I10 and I16). The protocol which consists in diluting the extract was applied and permitted to "lift" this inhibition.

- Reference method results

	Contamination level		
Laboratory	**L0**	**L1**	**L2**
A	0/8	8/8	8/8
B	0/8	8/8	8/8
C	0/8	8/8	8/8
D	0/8	7/8	8/8
E	0/8	8/8	8/8
F	0/8	7/8	8/8
G	0/8	8/8	8/8
H	0/8	7/8	8/8
I	0/8	8/8	8/8
J	0/8	8/8	8/8
K	0/8	7/8	8/8
L	0/8	8/8	8/8
M	0/8	7/8	8/8
N	0/8	8/8	8/8

Table 16: reference method positive results for all laboratories

- Results analysis

The laboratory A presented a negative result by the alternative method and positive by the reference method for a high level contaminated sample (A6). A second PCR analysis of the extract gave a negative result and the isolation of 100 µL of Listerboost on ALOA showed the absence of typical colonies. Two hypotheses are possible: a non-contamination of the sample or an inversion of two samples during the analysis.
The laboratory B showed a positive result by the alternative method and negative by the reference method for two non contaminated samples (B4 and B24). The isolation of 100 µL of listerboost broth on ALOA showed the presence of typical colonies for only one of the samples (B4).
PCR analyses from these stored extracts and from new extracts confirmed the presence of Listeria monocytogenes in the samples. A new isolation of the Listerboost broth confirmed the presence of typical colonies for the two samples. The participating laboratory confirmed the hypothesis of a cross-contamination of these two samples.
According to these findings, the expert laboratory proposed to exclude the results of laboratory B of the statistical analysis of the results. This proposition was accepted by the Technical Committee.

Final analysis was consequently conducted using data supplied by thirteen laboratories.

3.2.5. Specificity (SP) and sensitivity (SE) calculations

The specificity and sensitivity calculations of both methods are presented in table 17, with the low critical value (LCL). Formulas used are:

For level L0, SP = [1-(FP/N-)] x 100%, N-: total number of L0 tests
FP: number of false positive

For levels L1 and L2, SE = (TP/N+) x 100%, N+: total numbers of L1 or L2 tests
TP: number of true positive

Specificity / sensitivity	**Alternative method**	**LCL**	**Reference method**	**LCL**
SP (level L0)	100%	98%	100%	98%
SE (level L1)	96%	93%	95%	89%
SE (level L2)	99%	98%	100%	98%
SE (level L1+L2)	98%	96%	98%	96%

Table 17: specificity (SP), sensitivity (SE) and LCL of alternative and reference method

3.2.6. Relative accuracy calculations

Pairs of results of the different levels of contamination are presented in table 18.

Level	Alternative method	Reference method		
		RM+	RM-	Total
L0	AM+	PA=0	PD=0	0
	AM-	ND=0	NA=104	104
	Total	0	104	104
L1	AM+	PA=95	PD=5	100
	AM-	ND=4	NA=0	4
	Total	99	5	104
L2	AM+	PA=103	PD=0	103
	AM-	ND=1	NA=0	1
	Total	104	0	104
L0+L1+L2	AM+	PA=198	PD=5	203
	AM-	ND=5	NA=104	109
	Total	203	109	312

Table 18: tests results for both methods (PA: positive agreement, NA: negative agreement, ND: negative deviation, PD: positive deviation)

Relative accuracy values of the different contamination levels are presented in table 19 with their LCL. Formula used is the following:

AC = (PA+NA)/N x 100%, PA: number of positive agreements
NA: number of negative agreements

Level	Relative accuracy (AC)	LCL (Low Critical Value)
L0	100%	98%
L1	91%	84%
L2	99%	98%
L1+L2	95%	93%
Total	97%	93%

Table 19: relative accuracy values (AC) and LCL of alternative method

3.2.7. Discordant results analysis

Discordant results are analysed according to the annex F of ISO 16140 standard. The total number of discordant results is given by the following formula: Y = PD + ND.
In the present case, Y = 5 + 5 = 10, the binomial law test was used to compare the two methods:

	Alternative method
Y = PD + ND	Y = 5 + 5 = 10
m	5
M (for 9≤Y≤11)	1
Conclusion	m > M the two methods are not different for α = 0,05

The ADIAFOOD ADIAFOOD *Listeria monocytogenes* test and the reference method can be considered as equivalent.

All the discordant results are found at the contamination level L1 (3 CFU in 25 mL). A positive result is obtained only by one or the other methods. Due to the inoculation way of the samples, no Listeria cell may have been inoculated in some samples at this contamination level.

3.3. Interpretation

3.3.1. Accordance

The accordance is the percentage chance of finding the same result (i.e. both negative or both positive) from two identical test portions analysed in the same laboratory, under repeatability conditions (i.e. one operator using the same apparatus and same reagents within the shortest feasible time interval).

To derive the accordance from the results of an interlaboratory study, the probability that two samples give the same result is calculated for each participating laboratory in turn, and this probability is then averaged over all laboratories. Values of accordance are shown in table 20. Calculations of accordance by level and method are presented in annex 2.

Level	Alternative method	Reference method
L0	100%	100%
L1	93%	92%
L2	98%	100%

Table 20: accordance by level and method

3.3.2. Concordance

The concordance is the percentage chance of finding the same result for two identical samples analysed in two different laboratories.

To calculate the concordance from the results of an interlaboratory study, take in turn each replicate in each participating laboratory, pair it with identical results of all the other laboratories. The concordance is the percentage of all pairings giving the same results on all the possible pairings of data. Values of concordance are shown in table 21. Calculations of concordance by level and method are presented in annex 3.

Level	Alternative method	Reference method
L0	100%	100%
L1	92%	92%
L2	98%	100%

Table 21: concordance by level and method

3.3.3. Concordance odds ratio

If the concordance is smaller than the accordance, it indicates that two identical samples are more likely to give the same result if they are analysed by the same laboratory than if they are analysed by different ones, suggesting that there can be variability in performance between laboratories. Unfortunately, the magnitude of the concordance and accordance is strongly dependent on the level of accuracy, making it difficult to assess easily the degree of between-laboratory variation.

It is therefore helpful to calculate the concordance odds ratio (COR) defined as follows:
COR = accordance x (100-concordance)/concordance x (100 – accordance).

Values of COR for both methods are shown in table 22.

A value for the odds ratio of 1.00 would be expected if accordance and concordance were equal, and the larger the odds ratio is, the more inter-laboratory variation is predominant. Nevertheless, values above 1.00 can occur by chance variation, and so a statistical significance test should be used to confirm whether the evidence for extra variation between laboratories is convincing. The "exact test" is the best recommended test for this). The philosophy behind such tests is that the probabilities of occurrence are calculated for all sets of replicate results that could have produced the overall numbers of positives and negatives.

Level	Alternative method			Reference method		
	Accordance	Concordance		Accordance	Concordance	
L0	100	100	1,0	100	100	1,0
L1	93	92	1,2	92	92	1,0
L2	98	98	1,0	100	100	1,0

Table 22: COR values for each method by contamination level

3.3.4. AC, SP, SE comparison

Table 23 summarizes the values obtained for AC, SP and SE parameters for the preliminary study and the interlaboratory study.

Parameter	Preliminary study	Interlaboratory study
AC	91%	97%
SP	92%	100%
SE	90%	98%

Table 23: AC, SP and SE comparison between preliminary and interlaboratory study

The values obtained during the collaborative study are better than those obtained during the preliminary study, probably because of the greater variety of samples and strains tested during the preliminary study.

The sensitivity of both methods is recalculated in table 24 by including all confirmed positive results.

Alternative method (PA+PD)/(PA+PD+ND)	Reference method (PA+ND)/(PA+PD+ND)
98%	98%

Table 24: sensitivity recalculated by both methods

4. Practicability

The practicability was evaluated according to the 13 criteria defined by AFNOR Technical Committee.

1- Mode of packaging of test components
2- Volume of reagents

COMPOSANT	DESCRIPTION	Format ou volume
Detection microplates or barrettes	Microplates / barrettes for the real-time PCR detection	ADIF1303 : 5 microplates - 88 tests ADIF6103 : 12 barrettes - 8 tests
Control barrettes	Control barretttes specific of the pathogen	ADIF8103 : 8 control barrettes
DT-1	solution used for the reconstitution of lyophilised DT-2	2.2 mL
DT-2	Lyophilised compound for the detection solution	/
EX-1	solution used for the reconstitution of lyophilised EX-2	10 mL
EX-2	Lyophilised compound for the extraction solution	/
Optical quality PCR corks	Flat corks	/
User guide	Protocol	/

3- Storage conditions of components and shelf-life of unopened products (expiration of not opened products)
4- Modalities after first use

The ADIAFOOD detection kits must be stored at 4°C (from 2 to 8°C) in their original packaging.
After extraction, microplates can be stored at 4°C (from 2 to 8°C) for a maximum of 24 hours or at -20°C for several months. Do not remove the domed caps before storing.
The shelf life of the reconstituted solutions EX-2 and DT-2 is 14 days at 4°C (from 2 to 8°C) in their original vials, sealed by a Parafilm.
Never exchange the components of different lots or from other sources.

5- Equipment and specific local requirements

Equipment

- Real-time PCR thermocycler validated by AES : STRATAGENE MX3005P ou MX3000P
- Centrifuge
- Capping tool
- Barrettes supports
- Two multi-channel pipettes
- Two single channel pipettes
- PCR encloser
- *Stomacher* (homogenizer)
- Incubators
- Dilutor
- Bunsen burner
- Serological pipette pump
- Stomacher bag holder
- Refrigerator 4°C (2 to 8°C)
- Colour printer
- Pipettes supports
- Tubes racks

Consumables

- ADIAFOOD extraction kit
- Aerosol barrier tips for pipetors
- Sterile microcentrifuge cap tubes
- Free powder gloves
- Denatured alcool and bleach
- Disposable basins for multi-channel pipettes
- Filter stomacher bags
- Disposable serological pipettes
- Parafilm
- Listerboost enrichment media

6- Reagents ready to use or for reconstitution

DT-2 and EX-2 components must be reconstituted in DT-1 and EX-1.

7- Training period for operator with no experience with the method

1 day is required for technicians with microbiology knowledge.

8- Handling time and flexibility of the method in relation to the number of samples

Steps– Manipulation time – Negative samples	**Time (minutes)**			
	Alternative method		**Reference method**	
	1 analysis	**20 analyses**	**1 analysis**	**20 analyses**
Suspension	3	23	3	23
Sampling	0.5	15	/	/
Isolation + 2ème enrichment	/	/	2	30
Extraction	8.5	15	/	/
Amplification	7.5	10.5	/	/
PCR reading	0.1	3	/	/
Isolation from Fraser	/	/	1	13
1rst reading	/	/	1	12
2nd reading	/	/	1	12
Total	**19.6**	**66.5**	**8**	**90**

Steps– Manipulation time – Positive samples	**Time (minutes)**			
	Alternative method		**Reference method**	
	1 analyse	**20 analyses**	**1 analyse**	**20 analyses**
Suspension	3	23	3	23
Sampling	0.5	15	/	/
Isolation + 2ème enrichment	/	/	2	30
Extraction	8.5	15	/	/
Amplification	7.5	10.5	/	/
PCR reading	0.1	3	/	/
Isolation from Fraser	/	/	1	13
1rst reading	/	/	1	12
2nd reading	/	/	1	12
Confirmation PCR – isolation on ALOA	0.5	7	/	/
ALOA reading	0.2	3	/	/
Biochemical confirmation	/	/	8	80
Total	**20.3**	**76.5**	**16**	**170**

9- Time required for results

Steps –Time for negative results	Alternative method	Reference method
Suspension	D0	D0
Extraction	D1	/
Amplification	D1	/
PCR reading	D1	/
Isolation + inoculation Fraser	/	D1
1rst reading	/	D3
2nd reading	/	D5

Steps –Time for positive results	Alternative method	Reference method
Suspension	D0	D0
Extraction	D1	/
Amplification	D1	/
PCR reading and isolation on ALOA	D1	/
ALOA PCR Reading	D3	/
Isolation + inoculation Fraser	/	D1
Isolation TSYE	/	/
Biochemical confirmation	/	/
1rst reading	/	D3
2nd reading	/	D5
Isolation TSYE	/	D7
Biochemical confirmation	/	D8

10- Operator qualification
Identical as necessary for the reference method

11- Steps common with the reference method
None.

12- Traceability of analysis results
Traceability realized by the Sentinel software

13- Maintenance by laboratory
None.

5. Conclusion

Concerning the preliminary study, the performances of the ADIAFOOD *Listeria monocytogenes* test for the detection of *L. monocytogenes* are comparable to those of the standard NF EN ISO 11290-1 (1997) et NF EN ISO 11290-1/A1 (2005).
This study concerned 320 samples of five categories of products (meat, dairy, seafood, vegetable and environmental products).

Values obtained for the 3 criteria are the following:

- -relative accuracy: 90.9%
- -relative sensitivity: 89.5%
- -relative specificity: 92.1 %

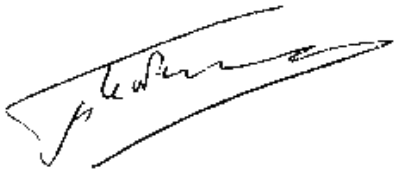
Several discordant results were observed. Mostly of them may be explained because the first culture step of each method differs. Consequently, 2 replicates were prepared for each sample. Because of the low level of artificial contamination, is it possible that no cell of *L. monocytogenes* was present in the test portion replicate analysed with either method.

The relative level of detection of the alternative method and the reference method was evaluated for all categories. The results are comparable because the detection limit of the alternative method varies from 0.3 to 2.4 CFU /25 g and the detection limit of the reference method varies from 0.4 to 1.7 CFU / 25 g for all categories.

The specificity of the method is satisfactory.

Concerning the interlaboratory study, the results obtained for the 13 selected laboratories showed that the values of relative accuracy, relative sensitivity and relative specificity are comparable to those obtained during the preliminary study. The variability of the alternative method, demonstrated by the calculations of accordance, concordance and concordance odds ratio, is similar to that of the reference method.

The study of the practicability of the alternative method shows a simple and easy-to-use method and a significant time savings compared to the reference method.



Massy, the 26th of August 2010
François Le Nestour
Research engineer

Annex 1 : selectivity

Inclusivity list

Code ISHA	Microorganism	Origin (French designation)
LIS.4.1	*Listeria monocytogenes*	CIP 78.31
LIS.4.2	*Listeria monocytogenes*	environnement clinique
LIS.4.4	*Listeria monocytogenes 1/2a*	brochette courgette chèvre
LIS.4.5	*Listeria monocytogenes 1/2a*	jambon crudité
LIS.4.6	*Listeria monocytogenes 1/2a*	sandwich jambon emmental
LIS.4.7	*Listeria monocytogenes 1/2a*	sandwich jambon emmental
LIS.4.8	*Listeria monocytogenes 1/2a*	sandwich thon œuf surimi
LIS.4.9	*Listeria monocytogenes 1/2a*	granulé de bœuf rôti
LIS.4.10	*Listeria monocytogenes 1/2a*	salade
LIS.4.11	*Listeria monocytogenes 1/2a*	poulet curry
LIS.4.12	*Listeria monocytogenes 1/2a*	saumon fumé
LIS.4.13	*Listeria monocytogenes 1/2a*	foie gras
LIS.4.14	*Listeria monocytogenes 1/2a*	sauce ktipiti
LIS.4.15	*Listeria monocytogenes 1/2a*	tartare de saumon
LIS.4.16	*Listeria monocytogenes 1/2a*	contrôle surface égoût
LIS.4.17	*Listeria monocytogenes 1/2a*	crudités
LIS.4.18	*Listeria monocytogenes 1/2a*	salade de légumes
LIS.4.19	*Listeria monocytogenes 1/2a*	pintade fermière
LIS.4.20	*Listeria monocytogenes 1/2a*	sandwich bacon crudité
LIS.4.21	*Listeria monocytogenes 1/2a*	CIP 103574
LIS.4.22	*Listeria monocytogenes 1/2a*	CIP 104794
LIS.4.23	*Listeria monocytogenes 1/2a*	fromage frais
LIS.4.24	*Listeria monocytogenes 1/2a*	repas fromager
LIS.4.25	*Listeria monocytogenes 1/2a*	poisson et légumes à la provençale
LIS.4.26	*Listeria monocytogenes 1/2a*	Jambon
LIS.4.27	*Listeria monocytogenes 1/2a*	viande hachée
LIS.4.28	*Listeria monocytogenes 1/2b*	cuisse de canard
LIS.4.29	*Listeria monocytogenes 1/2b*	pâte praliné
LIS.4.30	*Listeria monocytogenes 1/2b*	roulé de dinde cru
LIS.4.31	*Listeria monocytogenes 1/2b*	hareng aux épices
LIS.4.32	*Listeria monocytogenes 1/2b*	lait cru
LIS.4.33	*Listeria monocytogenes 1/2c*	viande hachée
LIS.4.34	*Listeria monocytogenes 1/2c*	gouda
LIS.4.35	*Listeria monocytogenes 1/2c*	sandwich salade du chef
LIS.4.36	*Listeria monocytogenes 1/2c*	CIP 103573
LIS.4.37	*Listeria monocytogenes 1/2c*	foie gras de canard
LIS.4.38	*Listeria monocytogenes 1/2c*	foie gras de canard
LIS.4.39	*Listeria monocytogenes 1/2c*	tartare saumon
LIS.4.40	*Listeria monocytogenes 1/2c*	sauce ktipiti
LIS.4.41	*Listeria monocytogenes 1/2c*	foie gras
LIS.4.42	*Listeria monocytogenes 3a*	saumon fumé
LIS.4.43	*Listeria monocytogenes 3a*	bacon tranché
LIS.4.44	*Listeria monocytogenes 3a*	contrôle de surface
LIS.4.45	*Listeria monocytogenes 3a*	bacon grillé
LIS.4.46	*Listeria monocytogenes 3a*	sandwich chèvre
LIS.4.47	*Listeria monocytogenes 4b*	lanières de saumon
LIS.4.48	*Listeria monocytogenes 4b*	CIP 103575
LIS.4.49	*Listeria monocytogenes 4b*	CIP 78.38
LIS.4.50	*Listeria monocytogenes 4b*	contrôle de surface sur saumon
LIS.4.51	*Listeria monocytogenes 4c*	CIP 78.39

Exclusivity list

Code	Microorganism	Origin (French designation)
LIS.1.1	*Listeria grayi*	CIP 105447T
LIS.2.1	*Listeria innocua*	sandwich légumes
LIS.2.2	*Listeria innocua*	sandwich bacon crudité
LIS.2.3	*Listeria innocua*	contrôle surface porte
LIS.2.4	*Listeria innocua*	CIP 80.12
LIS.2.5	*Listeria innocua*	CTSCCV
LIS.2.6	*Listeria innocua*	17765 (viande de porc)
LIS.2.7	*Listeria innocua*	sandwich poulet bacon
LIS.2.8	*Listeria innocua*	langue de bœuf
LIS.2.9	*Listeria innocua*	viande hachée
LIS.2.10	*Listeria innocua*	CIP 80.11
LIS.3.1	*Listeria ivanovii*	lait cru
LIS.3.2	*Listeria ivanovii*	CTSCCV
LIS.3.3	*Listeria ivanovii*	CIP 78.42
LIS.3.4	*Listeria ivanovii subsp. Londoniensis*	CIP 103505
LIS.5.1	*Listeria seeligeri*	CIP 79.46
LIS.5.2	*Listeria seeligeri*	CTSCCV
LIS.6.1	*Listeria welshimeri*	CIP 81.48
LIS.6.2	*Listeria welshimeri*	CIP 81.94 T
LIS.6.3	*Listeria welshimeri*	CTSCCV
BAC.1.1	*Bacillus cereus*	industrie laitière
BAC.2.1	*Bacillus circulans*	industrie laitière
BAC.4.1	*Bacillus subtilis*	crème dessert
BRE.1.1	*Brevibacterium casei*	produit laitier
ENTC.1.2	*Enterococcus faecalis*	ATCC 33186
ENTC.2.1	*Enterococcus faecium*	industrie laitière
LACB.1.1	*Lactobacillus casei*	produit laitier
LACB.3.1	*Lactobacillus leishmanii*	CIP 53.61
MIC.1.1	*Micrococcus luteus*	industrie laitière
RHO.1.1	*Rhodococcus equi*	CIP 58.69
STA.2.1	*Staphylococcus epidermidis*	produit laitier
BAC.5.1	*Bacillus licheniformis*	fromage

Annex 2 - Accordance calculation

Alternative method Number of replicates: 8

Level L0

Laboratory	Number of positive	Probability of positive	Probability of pair of positives	Probability of negative	Probability of pair of negatives	Probability of pair of same results
C	0	0,000	0,000	1,000	1,000	1,000
D	0	0,000	0,000	1,000	1,000	1,000
E	0	0,000	0,000	1,000	1,000	1,000
F	0	0,000	0,000	1,000	1,000	1,000
G	0	0,000	0,000	1,000	1,000	1,000
H	0	0,000	0,000	1,000	1,000	1,000
I	0	0,000	0,000	1,000	1,000	1,000
J	0	0,000	0,000	1,000	1,000	1,000
K	0	0,000	0,000	1,000	1,000	1,000
L	0	0,000	0,000	1,000	1,000	1,000
M	0	0,000	0,000	1,000	1,000	1,000
N	0	0,000	0,000	1,000	1,000	1,000
Mean						100,0%

Level L1

Laboratory	Number of positive	Probability of positive	Probability of pair of positives	Probability of negative	Probability of pair of negatives	Probability of pair of same results
C	7	0,875	0,766	0,125	0,016	0,781
D	8	1,000	1,000	0,000	0,000	1,000
E	8	1,000	1,000	0,000	0,000	1,000
F	8	1,000	1,000	0,000	0,000	1,000
G	8	1,000	1,000	0,000	0,000	1,000
H	7	0,875	0,766	0,125	0,016	0,781
I	7	0,875	0,766	0,125	0,016	0,781
J	8	1,000	1,000	0,000	0,000	1,000
K	8	1,000	1,000	0,000	0,000	1,000
L	8	1,000	1,000	0,000	0,000	1,000
M	7	0,875	0,766	0,125	0,016	0,781
N	8	1,000	1,000	0,000	0,000	1,000
Mean						92,7%

Level L2

Laboratory	Number of positive	Probability of positive	Probability of pair of positives	Probability of negative	Probability of pair of negatives	Probability of pair of same results
C	8	1,000	1,000	0,000	0,000	1,000
D	8	1,000	1,000	0,000	0,000	1,000
E	8	1,000	1,000	0,000	0,000	1,000
F	8	1,000	1,000	0,000	0,000	1,000
G	8	1,000	1,000	0,000	0,000	1,000
H	8	1,000	1,000	0,000	0,000	1,000
I	8	1,000	1,000	0,000	0,000	1,000
J	8	1,000	1,000	0,000	0,000	1,000
K	8	1,000	1,000	0,000	0,000	1,000
L	8	1,000	1,000	0,000	0,000	1,000
M	8	1,000	1,000	0,000	0,000	1,000
N	8	1,000	1,000	0,000	0,000	1,000
Mean						100,0%

Reference method Number of replicates: 8

Level L0

Laboratory	Number of positive	Probability of positive	Probability of pair of positives	Probability of negative	Probability of pair of negatives	Probability of pair of same results
C	0	0,000	0,000	1,000	1,000	1,000
D	0	0,000	0,000	1,000	1,000	1,000
E	0	0,000	0,000	1,000	1,000	1,000
F	0	0,000	0,000	1,000	1,000	1,000
G	0	0,000	0,000	1,000	1,000	1,000
H	0	0,000	0,000	1,000	1,000	1,000
I	0	0,000	0,000	1,000	1,000	1,000
J	0	0,000	0,000	1,000	1,000	1,000
K	0	0,000	0,000	1,000	1,000	1,000
L	0	0,000	0,000	1,000	1,000	1,000
M	0	0,000	0,000	1,000	1,000	1,000
N	0	0,000	0,000	1,000	1,000	1,000
Mean						100,0%

Level L1

Laboratory	Number of positive	Probability of positive	Probability of pair of positives	Probability of negative	Probability of pair of negatives	Probability of pair of same results
C	8	1,000	1,000	0,000	0,000	1,000
D	7	0,875	0,766	0,125	0,016	0,781
E	8	1,000	1,000	0,000	0,000	1,000
F	7	0,875	0,766	0,125	0,016	0,781
G	8	1,000	1,000	0,000	0,000	1,000
H	7	0,875	0,766	0,125	0,016	0,781
I	8	1,000	1,000	0,000	0,000	1,000
J	8	1,000	1,000	0,000	0,000	1,000
K	7	0,875	0,766	0,125	0,016	0,781
L	8	1,000	1,000	0,000	0,000	1,000
M	7	0,875	0,766	0,125	0,016	0,781
N	8	1,000	1,000	0,000	0,000	1,000
Mean						90,9%

Level L2

Laboratory	Number of positive	Probability of positive	Probability of pair of positives	Probability of negative	Probability of pair of negatives	Probability of pair of same results
C	8	1,000	1,000	0,000	0,000	1,000
D	8	1,000	1,000	0,000	0,000	1,000
E	8	1,000	1,000	0,000	0,000	1,000
F	8	1,000	1,000	0,000	0,000	1,000
G	8	1,000	1,000	0,000	0,000	1,000
H	8	1,000	1,000	0,000	0,000	1,000
I	8	1,000	1,000	0,000	0,000	1,000
J	8	1,000	1,000	0,000	0,000	1,000
K	8	1,000	1,000	0,000	0,000	1,000
L	8	1,000	1,000	0,000	0,000	1,000
M	8	1,000	1,000	0,000	0,000	1,000
N	8	1,000	1,000	0,000	0,000	1,000
Mean						100,0%

Annex 3 - Concordance calculation

Alternative method

Level L0

Laboratory	Number of negative	Between-lab pairings with the same results	Total between-lab pairings
A	8	768	768
C	8	768	768
D	8	768	768
E	8	768	768
F	8	768	768
G	8	768	768
H	8	768	768
I	8	768	768
J	8	768	768
K	8	768	768
L	8	768	768
M	8	768	768
N	8	768	768
Total		9984	9984
Concordance		100,0%	

Level L1

Laboratory	Number of positive	Between-lab pairings with the same results	Total between-lab pairings
A	8	736	768
C	7	651	768
D	8	736	768
E	8	736	768
F	8	736	768
G	8	736	768
H	7	651	768
I	7	651	768
J	8	736	768
K	8	736	768
L	8	736	768
M	7	651	768
N	8	736	768
Total		9228	9984
Concordance		92,4%	

Level L2

Laboratory	Number of positive	Between-lab pairings with the same results	Total between-lab pairings
A	7	672	768
C	8	760	768
D	8	760	768
E	8	760	768
F	8	760	768
G	8	760	768
H	8	760	768
I	8	760	768
J	8	760	768
K	8	760	768
L	8	760	768
M	8	760	768
N	8	760	768
Total		9792	9984
Concordance		98,1%	

Reference method

Level L0

Laboratory	Number of negative	Between-lab pairings with the same results	Total between-lab pairings
A	8	768	768
C	8	768	768
D	8	768	768
E	8	768	768
F	8	768	768
G	8	768	768
H	8	768	768
I	8	768	768
J	8	768	768
K	8	768	768
L	8	768	768
M	8	768	768
N	8	768	768
Total		9984	9984
Concordance		100,0%	

Level L1

Laboratory	Number of positive	Between-lab pairings with the same results	Total between-lab pairings
A	8	728	768
C	8	728	768
D	7	644	768
E	8	728	768
F	7	644	768
G	8	728	768
H	7	644	768
I	8	728	768
J	8	728	768
K	7	644	768
L	8	728	768
M	7	644	768
N	8	728	768
Total		9044	9216
Concordance		98,1%	

Level L2

Laboratory	Number of positive	Between-lab pairings with the same results	Total between-lab pairings
A	8	768	768
C	8	768	768
D	8	768	768
E	8	768	768
F	8	768	768
G	8	768	768
H	8	768	768
I	8	768	768
J	8	768	768
K	8	768	768
L	8	768	768
M	8	768	768
N	8	768	768
Total		9216	9216
Concordance		100,0%	