



***Validation of the  
iQ-Check™ Listeria spp. method***

***Summary report***

Comparative and interlaboratory studies according to the  
EN ISO 16140 reference

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## 1 Introduction

### 1.1 Validation references

The iQ-Check™ *Listeria* spp. method was fully validated (comparative study of the methods and collaborative study) according to the EN ISO 16140: 2003 reference.

### 1.2 Protocol and principle of the alternative method

#### 1.2.1 Principle

The iQ-Check *Listeria* spp. test is based on gene amplification and detections using the PCR technique in real time. It uses primers and a probe for DNA specific of *Listeria*. After the enrichment step in the specific LSB broth, bacterial lysis releases bacterial DNA. The amplification and detection steps are then performed in a thermal cycler. At the end of the reaction, fluorescence is emitted and measured directly by the thermal cycler. The software associated with the instrument analyzes the results, which are displayed in the form of curves to be interpreted.

#### 1.2.2 Protocols

A specific broth, LSB broth, has been developed by Bio-Rad to provide better revivification of *Listeria* and the use of a simplified lysis protocol eliminating the first centrifugation step.

The different **analytical steps** are the following (protocol in Appendix A):

- Enrichment  
**24 hours +/- 2 hours** at **30 °C** in LSB broth for the standard lysis protocol  
**25 hours +/- 1 hour** at **30 °C** in LSB broth for the easy lysis protocol

- Lysis of bacteria to release the bacterial DNA  
Two bacterial lysis protocols are possible:

- 1) Standard lysis protocol
- 2) Easy lysis protocol

- Amplification – detection

- Confirmations

Samples that are positive after the iQ-Check™ *Listeria* spp. test are confirmed:

- 1) According to the tests of standardized methods, after isolation from the enrichment broth on selective agars such as *Listeria* agar according to Ottaviani and Agosti or PALCAM or Oxford agars.
- 2) By isolation on RAPID<sup>®</sup> *Listeria* spp. agar or RAPID<sup>®</sup> *L.mono* agar.

A confirmation option 2 is proposed: if typical colonies are present on these agars, the iQ-Check™ result will be considered as being confirmed.

Storing the LSB broths for at least 72 hours at 2–8°C before performing the iQ check™ *Listeria* spp. test was also verified: the tests were performed on positive samples from the relative accuracy, relative specificity and relative sensitivity study.

### 1.3 Requested field of application

The protocol described above can be applied to all human food products, as well as to environmental samples.

### 1.4 Reference method

The validation study was performed in relation to the reference method: “NF EN ISO 11290-1/A1: 2005 -Horizontal method for the detection of *Listeria monocytogenes*”, identifying all species of *Listeria* which might develop on the selective agars.

## 2 Comparative study

### 2.1 Relative accuracy, relative specificity and relative sensitivity

The objective of this study is to compare the two methods:

- the reference method, NF EN ISO 11290-1: 2005,
  - the iQ-Check™ *Listeria* spp. method (Bio-Rad),
- on uncontaminated samples and samples contaminated with *Listeria*.

#### 2.1.1 Number and nature of the samples

A minimum of 60 products per category were analyzed, with approximately 50% of positive products and 50% negative products per category, and a minimum of 30 positive samples per category.

Categories	Types	Positive*	Negative	Total
Meat products	raw	26	11	37
	raw and prepared, ready to cook	29	6	35
	sausage meats, prepared dishes, etc.	11	13	24
	<b>Total</b>	<b>66</b>	<b>30</b>	<b>96</b>
Dairy products	cow's milk cheeses	14	12	26
	goat's or sheep's milk cheeses	9	8	17
	desserts, powdered milk, raw milk	16	10	26
	<b>Total</b>	<b>39</b>	<b>30</b>	<b>69</b>
Fish products	fresh fish filets and shellfish	14	10	24
	smoked fish	11	9	20
	fish-based prepared dishes	10	11	21
	<b>Total</b>	<b>35</b>	<b>30</b>	<b>65</b>
Vegetable products	frozen	12	7	19
	fresh or ready prepared	14	14	28
	prepared cooked vegetables	11	10	21
	<b>Total</b>	<b>37</b>	<b>31</b>	<b>68</b>
Environment	miscellaneous waters	6	6	12
	surface samples	19	20	39
	residues	10	4	14
	<b>Total</b>	<b>35</b>	<b>30</b>	<b>65</b>
<b>TOTAL</b>		<b>212</b>	<b>151</b>	<b>363</b>

\* include positive results with either method

## 2.1.2 Artificial contamination of samples and percentage

The following contamination method was used: contamination with a strain of *Listeria* having undergone a combination of heating and cooling or freezing cycles.

Stress was evaluated by calculating the difference in Log between the enumeration on TSAYE agar and the enumeration on PALCAM agar. This difference needed to be at least 0.5 log.

Nine strains of *Listeria monocytogenes* of different origins and serotypes were used to carry out these artificial contaminations.

In all, 30 positive results out of 212 positive *Listeria* results were obtained following the artificial contaminations, i.e. 14%.

## 2.1.3 Test results

Results obtained for the 363 samples analyzed can be summarized as follows.

Results are identical with the two lysis protocols tested:

- 1) LSB broth incubated for 22 hours, followed by the standard lysis protocol
- 2) LSB broth incubated for 24 hours, followed by the easy lysis protocol

	<b>Positive reference method (R+)</b>	<b>Negative reference method (R-)</b>	<b>Total</b>
<b>Positive alternative method (A+)</b>	Positive agreement (A+/R+) <b>PA = 177</b>	Positive deviation (R-/A+) <b>PD = 22</b>	199
<b>Negative alternative method (A-)</b>	Negative deviation (A-/R+) <b>ND = 13<sup>(1)</sup></b>	Negative agreement (A-/R-) <b>NA = 151<sup>(2)</sup></b>	164
<b>Total</b>	190	173	<b>363</b>

Legend:

A+ = confirmed positives

A- = immediate negatives **and** negatives after confirmation when presumptive positives

(1) : including 2 samples presumed positive with the alternative method, negative after confirmation.

(2) : including 8 samples presumed positive with the alternative method with the standard lysis protocol, negative after confirmation and including 6 samples presumed positive with the alternative method with the easy lysis protocol, negative after confirmation.

The same results tables, but for each sample category, can be found in Appendix B.

## 2.1.4 Calculation of relative accuracy (AC), relative specificity (SP) and relative sensitivity (SE)

All of these results are used to calculate relative accuracy, relative sensitivity and relative specificity for each category and for all of the categories, according to the equations in the EN ISO 16140 standard.

Category	PA	NA	ND	PD	Sum N	Relative accuracy AC (%) [100x(PA+NA)]/N	N+ PA + ND	Relative sensitivity SE (%) [100xPA]/N+	N- NA + PD	Relative specificity SP (%) [100xNA]/N-
Meat products	52	30	5	9	96	85.4	57	91.2	39	76.9
Dairy products	33	30	3	3	69	91.3	36	91.7	33	90.9
Fish	29	30	0	6	65	90.8	29	100	36	83.3
Vegetables	31	31	3	3	68	91.2	34	91.2	34	91.2
Environment	32	30	2	1	65	95.4	34	94.1	31	96.8
<b>TOTAL</b>	<b>177</b>	<b>151</b>	<b>13</b>	<b>22</b>	<b>363</b>	<b>90.4</b>	<b>190</b>	<b>93.2</b>	<b>173</b>	<b>87.3</b>

These results are used to calculate the following three criteria for the alternative method:

<i>relative accuracy: AC</i>	<b>90.4%</b>
<i>relative specificity: SP</i>	<b>87.3%</b>
<i>relative sensitivity: SE</i>	<b>93.2%</b>

According to the following calculations required by the Technical Committee, **sensitivity** was recalculated taking into account all confirmed positives (including additional positives with the alternative method):

	<b>Alternative method</b>	<b>Reference method</b>
<i>Standard lysis protocol and easy lysis protocol</i>	$(PA + PD) / (PA + PD + ND) = \mathbf{93.9\%}$	$(PA + ND) / (PA + PD + ND) = \mathbf{89.6\%}$

## 2.1.5 Analysis of discordances

### 2.1.4.1 Statistical test

According to Appendix F of the NF EN ISO 16140 standard, the number of discordant results above which a statistical test must be used in order to compare both methods is 6.

This statistical test was implemented for the iQ Check™ *Listeria* spp. method, since the number of discordant results was 35, whatever lysis protocol used.

*Reminder: the two methods use different enrichment broths and the percentage of naturally contaminated samples is high (86%), factors which strongly influence the number of discordant results.*

When the number of discordant results is greater than 22, the McNemar test with  $\chi^2$  distribution is used for one degree of freedom.

It is a question of determining  $d = |PD - ND|$  and comparing  $d$  with a minimum  $d$  value defined for each number of discordant results. The two methods will be considered to be different if  $d \geq$  minimum  $d$  (Appendix F).

	<b>Number of discordant results</b>	<b>minimum d</b>	<b>d</b>	<b>Conclusion</b>
	35	12	$22 - 13 = 9$	Equivalence

The alternative method is equivalent to the reference method.

## 2.1.6 Comments on inhibitions

- Five inhibited results after the iQ Check™ *Listeria* spp. test using pure DNA were observed with the standard lysis protocol:
  - 2 meat products, negative;
  - 2 dairy products, one negative and one positive;
  - 1 negative fish product.
- One inhibited result after the iQ Check™ *Listeria* spp. test using pure DNA was observed with the easy lysis protocol, for a positive environmental sample.

All of these inhibitions were removed by diluting the DNA 1:10 before performing the test and the results all complied with those expected (notably no result became false negative).

## 2.1.7 Comments on the confirmation protocol

LSB broth isolation on RAPID' *Listeria* spp. and RAPID'L. *mono* agars always allowed *Listeria* detection (except for the particular case of false positive results which did not contain *Listeria*).

In one case (fresh salmon brochettes), *Listeria monocytogenes* was not found on RAPID'L. *mono* agar, even after subculturing from the LSB broth in Fraser broth. On the other hand, *Listeria monocytogenes* was found on the RAPID' *Listeria* spp. agar.

Among the positive samples, certain samples were not positive with the reference method for the same strains as those identified with the alternative method:

- 13 samples were only positive with the reference method for one of the two strains found with the alternative method and among these 13 samples, *Listeria monocytogenes* was not found in 6 cases.
- 7 samples were only positive with the alternative method for one of the two strains found with the reference method and among these 7 samples, *Listeria monocytogenes* was found in 6 cases.
- 2 samples were positive with the alternative method for *Listeria monocytogenes*, whereas they were positive with the reference method for another *Listeria*.

## 2.1.8 Comments on storing the LSB broths at 2-8 °C for 72 hours

The LSB broths were stored for 72 hours at 2-8 °C and a second iQ Check™ *Listeria* spp. test was performed.

Some results were different:

### False positive results:

- one meat product and three fish products, negative, became false positives.

### Additional positive results:

- one negative result for a sample of smoked salmon, which was concordant as negative, became an additional positive for *Listeria innocua*.

### Inhibitions:

- one iQ Check™ *Listeria* spp. sample was inhibited, whereas the result was positive in undiluted DNA when the test was performed directly after LSB broth incubation,
- the raw milk sample was no longer inhibited with the standard lysis protocol.

Overall, the results are comparable to those obtained when the test is carried out directly after incubation. It should be noted that no false negative results were obtained following LSB broth storage.

## 2.2 Relative detection level of the iQ-Check™ *Listeria* spp. method and the reference method

The purpose of these tests is to determine the level of contamination for which less than 50% of the results obtained are positive and the level for which more than 50% of the results obtained are positive.

Parallel studies of different 'food matrix-strain' pairs were carried out with the reference method and the iQ-Check™ *Listeria* spp. method, for five categories.

Artificial contaminations were performed in compliance with the requirements of the EN ISO 16140 standard and the microbiology technical committee.

The detection levels, calculated according to the Spearman–Kärber method\* (LOD<sub>50</sub>), obtained for each "matrix-strain" combination were the following:

Matrix	Strain	Relative detection level of the reference method (CFU / 25 g or 25 ml)	Relative detection level of the alternative method (CFU / 25 g or 25 ml)
home-made rilette	<i>L. welshimeri</i>	0.4 [0.2 – 0.6]	0.3 [0.2 – 0.5]
raw milk	<i>L. monocytogenes</i> 1/2b	0.7 [0.4 – 1.3]	0.9 [0.6 – 1.5]
ready prepared raw vegetable mix	<i>L. seeligeri</i>	0.6 [0.3 – 0.9]	0.7 [0.3 – 1.4]
smoked salmon	<i>L. monocytogenes</i> 1/2a	0.6 [0.3 – 1.1]	0.6 [0.3 – 1.2]
process water	<i>L. innocua</i> 1/2c	0.6 [0.4 – 1.1]	0.6 [0.3 – 1.1]

The results with iQ-Check are identical whatever the lysis protocol used.

## Conclusion

The detection level obtained for the alternative method with the LSB broth is between 0.2 and 1.5 cells per 25 grams vs. 0.2 and 1.3 cells per 25 grams for the reference method.

\* "Hitchins A. Proposed Use of a 50% Limit of Detection Value in Defining Uncertainty Limits in the Validation of Presence-Absence Microbial Detection Methods, Draft 10th December, 2003".

## 2.3 Inclusivity / exclusivity

The inclusivity and exclusivity of the method are defined by the analysis of 50 positive strains and 30 negative strains, respectively.

### 2.3.1 Assay protocols

#### 2.3.1.1 Protocol for inclusivity

For each *Listeria* strain, a nutrient broth culture was prepared.

LSB broth was then inoculated with approximately 10 *Listeria* per 225 ml and incubated at 30°C. The easy lysis protocol and the iQ-Check™ *Listeria* spp. test were then carried out.

#### 2.3.1.2 Protocol for exclusivity

The different negative strains were cultivated in a nutrient broth for 24 hours at 30°C, inoculated in 10 ml of nutrient broth to obtain levels of approximately 10<sup>5</sup> cells per ml, then incubated for 24 hours at 30°C before carrying out the easy lysis protocol and the iQ-Check™ *Listeria* spp. test.

### 2.3.2 Results and conclusion

The results can be found in Appendix C.

All 50 strains of *Listeria monocytogenes* and 34 strains of *Listeria* other than *monocytogenes* were detected. The iQ-Check™ *Listeria* spp. test is specific for *Listeria*.

All 32 strains of other genera gave negative results with the iQ Check™ *Listeria* spp. test. No cross-reactions were observed.

## 3 Interlaboratory study

The purpose of the collaborative study is to determine the variability of the results obtained in different laboratories analyzing identical samples.

Participating laboratories performed the analysis according to the iQ-Check™ *Listeria* spp. method (LSB broth and easy lysis protocol) and according to the reference method.

### 3.1 Implementation

- Number participating laboratories

Thirteen laboratories received samples.

- Matrix used

The “pasteurized milk” matrix was used to carry out the interlaboratory study.

Natural flora was present in the matrix at approximately 10 cells per ml.

- Strain used

The strain used for the contaminations was a strain of *Listeria innocua* isolated from “raw milk cheese”.

- Number of samples per laboratory

Forty-eight samples per laboratory were prepared, divided into 3 levels, with 8 samples per level.

## 3.2 Control of the experimental parameters

### 3.2.1 Contamination rates obtained after artificial contamination

The contamination rates obtained in the matrix and the accuracy estimates are presented in the table below:

Level	Samples	Targeted theoretical rate (b/25ml)	Real rate (b/25ml)	Estimation of the lower contamination threshold per 25 ml of sample	Estimation of the upper contamination threshold per 25ml of sample
Level 0	1-2-3-10 11-12-19-20	0	0		
Low level	4-5-6-13 14-15-21-22	3	3.7	0.8	10.7
High level	7-8-9-16 17-18-23-24	30	34	24	47

### 3.2.2 Temperature problems detected during transport, reception temperature and reception times

The reception temperature obtained by the laboratories varied from 2.1°C to 4.7°C for the laboratories which received their samples on D+1.

These met the requirements (between 0°C and 8°C) for 11 laboratories.

The temperature curves obtained after exploiting the temperature indicator data showed that the temperatures were stable during transport and were between 0.1°C and 4.7°C for the laboratories which received their samples on D+1.

### 3.2.3 Results and conclusion: description of possible problems encountered and reason for excluding laboratories

Eleven laboratories received their samples the day after they were shipped.

Two laboratories received their samples 48 hours after they were shipped. The reception temperature exceeded 8°C. These laboratories performed the analyses, but their results were not included in the calculations.

Another laboratory had announced a reception temperature exceeding 15°C. The analysis of the temperature during transport and upon reception showed a temperature between 0.1°C and 3.6°C. Their results were included.

Among the 13 participating laboratories, it was therefore possible to analyze the results of 11 of them, given the transport conditions.

### 3.3 Analysis results

#### 3.3.1 Results obtained by the collaborating laboratories

The positive results after confirmation obtained by the collaborating laboratories are presented in the following tables:

##### Positive results obtained with the reference method

Laboratories	Levels of contamination					
	L0		L1		L2	
	Obtained	Nbr. of samples	Obtained	Nbr. of samples	Obtained	Nbr. of samples
Laboratory A	0	8	8	8	8	8
Laboratory B	0	8	6	8	8	8
Laboratory C	0	8	8	8	8	8
Laboratory D	0	8	8	8	8	8
Laboratory E	0	8	7	8	8	8
Laboratory F	0	8	8	8	8	8
Laboratory G	0	8	8	8	8	8
Laboratory H	0	8	8	8	8	8
Laboratory I	0	8	8	8	8	8
Laboratory J	0	8	8	8	8	8
Laboratory K	0	8	8	8	8	8
Laboratory L	0	8	7	8	8	8
Laboratory M	0	8	8	8	8	8

##### Positive results obtained with the alternative method

Laboratories	Levels of contamination					
	L0		L1		L2	
	Obtained	Nbr. of samples	Obtained	Nbr. of samples	Obtained	Nbr. of samples
Laboratory A	0	8	8	8	8	8
Laboratory B	0	8	6	8	8	8
Laboratory C	0	8	8	8	8	8
Laboratory D	0	8	8	8	8	8
Laboratory E	0	8	8	8	8	8
Laboratory F	0	8	8	8	8	8
Laboratory G	0	8	8	8	8	8
Laboratory H	0	8	8	8	8	8
Laboratory I	0	8	8	8	8	8
Laboratory J	0	8	8	8	8	8
Laboratory K	0	8	7	8	8	8
Laboratory L	0	8	8	8	8	8
Laboratory M	0	8	8	8	8	8

Gray: laboratories which did not receive the samples by the deadline.

#### 3.3.2 Comments and conclusion (discordance in relation to expected results, exclusions, etc...)

Among the results from the 11 laboratories accepted, the results for the reference method and the alternative method were **concordant** for 8 laboratories: they found the 8 uncontaminated samples negative and the 16 contaminated samples positive.

Two **laboratories** found one of the samples contaminated at a low level negative with the reference method and positive with the alternative method for one laboratory, and inversely for the other laboratory.

These results are nonetheless coherent since the bottles used for the reference method were different from those used for the alternative method, as the first enrichment medium are different for the two methods.

A third laboratory obtained an unconfirmed positive result for an uncontaminated sample at a Ct value higher than that obtained for the contaminated samples. It therefore retested the DNA extract, which turned out to be negative.

One last **laboratory** found 6 of the 8 uncontaminated samples positive with the iQ Check™ *Listeria* spp. test. The laboratory performed the extractions and tests on the uncontaminated samples a second time at the request of the expert laboratory and the results obtained were all positive, as well as the negative control. It should be noted that the LSB broth isolations did not detect any *Listeria*, and consequently the results after confirmation complied with those expected.

Nonetheless, given the difficulties encountered in implementing the alternative method, these results were **excluded**.

Lastly, the results of the two laboratories which had received their samples 48 hours after shipment were not included in the calculations below, but it can be noted that they were consistent with all of the other laboratory results:

- one laboratory obtained two negative results on uncontaminated samples with each of the methods and two unconfirmed positive results;
- the other laboratory found one of the samples contaminated at a low level negative only with the reference method. Their other results complied with the results expected.

Levels	Total number of samples	Number of samples analyzed	Number of results used*	Number of negative results		Number of positive results	
				Reference	Alternative	Reference	Alternative
0	104	104	80	80	80	0	0
1	104	104	80	1	1	79	79
2	104	104	80	0	0	80	80

\* two laboratories did not receive their samples within the deadline and one laboratory was excluded after handling problems.

## 3.4 Calculations

### 3.4.1 Calculation of the specificity (SP) and sensitivity (SE) percentages for the two methods

**For level L0**, it is required that the percentage of specificity be calculated for each method:

$$SP = \{1 - (FP/N_-)\} \times 100$$

where FP, number of false positives  
N<sub>-</sub>, total number of all L0 tests

**For levels L1 and L2**, it is required that the percentage of sensitivity be calculated for each method:

$$SE = (TP/N_+) \times 100$$

where TP, number of true positives  
N<sub>+</sub>, total number of L1 or L2 tests

The results are presented in the table below:

	Reference method		Alternative method	
		LCL*		LCL*
Level L0	<b>SP = 100%</b>	98%	<b>SP = 100%</b>	98%
Level L1	<b>SE = 98.8%</b>	96%	<b>SE = 98.8%</b>	96%
Level L2	<b>SE = 100%</b>	98%	<b>SE = 100%</b>	98%
Levels L1 and L2	<b>SE = 99.4%</b>	97%	<b>SE = 99.4%</b>	97%

\* LCL: low critical limit

### 3.4.2 Calculation of relative accuracy (AC), expressed in percentage

Relative accuracy is calculated using the following equation:

$$AC = \{(PA + NA) / N\} \times 100$$

where PA, number of positive agreements  
NA, number of negative agreements

	Positive reference method (R+)	Negative reference method (R-)	Total
Positive alternative method (A+)	Positive agreement (A+/R+) PA = 158	Positive deviation (R-/A+) PD = 1	<b>(N+) = 159</b>
Negative alternative method (A-)	Negative deviation (A-/R+) ND = 1*	Negative agreement (A-/R-) NA = 80**	<b>(N-) = 81</b>
Total	<b>(N+) = 159</b>	<b>(N-) = 81</b>	<b>N = 240</b>

\* including no unconfirmed positive result

\*\* including 1 unconfirmed positive result

In this study, relative accuracy is 99.2%.

### 3.4.3 Study of discordant results

According to Appendix F of the EN ISO 16140 standard, the number of discordant results above which a statistical test must be used in order to compare both methods is 6. This statistical test was therefore not applied, since 2 discordances between the two methods were observed.

## 3.5 Interpretation

### 3.5.1 Comparison of the relative accuracy (AC), specificity (SP) and sensitivity (SE) values

The values obtained in the two parts of the validation study are presented in the table below:

	Interlaboratory study	Preliminary study (protocol used in the interlaboratory study)
Relative accuracy (AC)	<b>99.2%</b>	<b>90.4%</b>
Sensitivity (SE)	<b>98.8%</b>	<b>93.2%</b>
Specificity (SP)	<b>100%</b>	<b>87.3%</b>

The values obtained from the collaborative study are higher than those obtained in the preliminary study for relative accuracy and specificity, which is coherent since a single artificially contaminated matrix was studied. The results obtained in the comparative study can be explained by the fact that the two methods use a different enrichment broth and the percentage of naturally contaminated samples tested is high (86%).

The AFNOR Technical Committee requires that the sensitivity of the two methods be recalculated taking into account all of the confirmed positives (samples truly positive):

	Alternative method	Reference method
sensitivity	$(PA + PD) / (PA + PD + ND) = \mathbf{99.4\%}$	$(PA + ND) / (PA + PD + ND) = \mathbf{99.4\%}$

### 3.5.2 Accordance (DA)

The accordance is the percentage of chance of finding the same result for two identical test runs analyzed in the same laboratory under conditions of repeatability, i.e. a single operator using the same equipment and the same reagents within the shortest time interval possible.

To calculate the accordance, the probability of having two identical samples give the same result must be calculated for each participating laboratory, then the average of the probabilities for all of the laboratories is determined.

The different tables used to deduce the accordance are given in Appendix D and the accordance for each of the methods at each level are presented in the table below:

	Reference method	Alternative method
Level L0	DA = 100%	DA = 100%
Level L1	DA = 98%	DA = 98%
Level L2	DA = 100%	DA = 100%

### 3.5.3 Concordance

Concordance is the percentage of chance of finding the same result for two identical samples analyzed in two different laboratories.

It is thus a matter of calculating the percentage of all of the pairs giving the same results out of all of the possible pairs of results.

The tables of results used in determining these calculations can be found in Appendix E and the percentages of concordance for each method at each level are presented in the table below:

	Reference method	Alternative method
Level L0	Concordance = 100%	Concordance = 100%
Level L1	Concordance = 97.5%	Concordance = 97.5%
Level L2	Concordance = 100%	Concordance = 100%

### 3.5.4 Odds ratio (COR)

This is calculated using the following equation:

$$\text{COR} = \frac{\text{accordance} \times (100 - \text{concordance})}{\text{concordance} \times (100 - \text{accordance})}$$

The Odds ratio for each of the methods and at each level is presented in the table below:

	Reference method	Alternative method
Level L0	COR = 1.00	COR = 1.00
Level L1	COR = 1.15	COR = 1.15
Level L2	COR = 1.00	COR = 1.00

An Odds ratio value of 1.00 means that the accordance and concordance are equal.

The higher the Odds ratio, the greater the predominance of interlaboratory variations.

## 4 Practicability

Practicability is studied in relation to the 13 criteria defined by the technical committee by comparing the reference method with the iQ-Check™ *Listeria* spp method (Bio-Rad).

The criteria defined by AFNOR are indicated below:

<p>1. <i>Packaging of the test components</i> (cf. instruction manual)</p> <p>2. <i>Volume of reagents</i> (cf. instruction manual and bottle packaging)</p>	<p>The kit contains the quantity of reagents necessary for 96 tests:</p> <ul style="list-style-type: none"> <li>- one bottle of lysis reagent (20 ml)</li> <li>- one bottle of lysis beads</li> <li>- one tube of fluorescent probes (0.55 ml)</li> <li>- two tubes of amplification solution (2 x 2.2 ml)</li> <li>- one tube of negative PCR control (0.5 ml)</li> <li>- one tube of positive PCR control (0.25 ml)</li> </ul>
<p>3. <i>Storage condition for the components</i> (cf. instruction manual) – <i>Shelf-life of unopened products</i> (cf. instruction manual)</p>	<p>The kit should be stored between +2°C and +8°C.</p> <p>Each reagent stored between +2°C and +8°C can be used until the expiry date indicated on the tube.</p> <p>The kit is valid for approximately 1 year.</p>
<p>4. <i>Directions for use after initial use</i> (cf. instruction manual)</p>	<p>Each reagent should be stored between +2°C and +8°C.</p> <p>The reaction mixture obtained by mixing the amplification solution and the fluorescent probes can be stored for 1h at 2 – 8°C after preparation.</p> <p>The reconstituted lysis reagent (lysis buffer + lysis beads) can be stored for 6 months at 2 – 8°C.</p>
<p>5. <i>Special equipment or premises required</i> (cf. instruction manual)</p>	<p>Equipment required:</p> <ul style="list-style-type: none"> <li>- 30°C incubator</li> <li>- Cell disruptor</li> <li>- Heating block (95-100°C) for 1.5 ml conical tubes</li> <li>- Laboratory centrifuge (10,000 to 12,000 g max.)</li> <li>- iCycler thermal cycler with iCycler iQ® optical module, similar system such as Chromo4™ system</li> </ul> <p>Premises:</p> <p>4 distinct work <b>areas</b> are recommended: one for the extraction of nucleic acids, one reserved for the preparation of the reaction mixture, one for the distribution of the extracted DNA in PCR plate and a fourth one dedicated to amplification and detection.</p>
<p>6. <i>Reagents ready for use or to be reconstituted</i> (cf. instruction manual)</p>	<p>All of the reagents are ready for use, except for:</p> <ul style="list-style-type: none"> <li>- the reaction mixture, which is obtained by mixing the amplification solution and the fluorescent probes;</li> <li>- the lysis reagent, to which lysis beads are added.</li> </ul>
<p>7. <i>Training time for an operator unfamiliar with the method</i></p>	<p>Two days are needed for an operator trained in traditional microbiology and PCR techniques.</p> <p>For technicians without training in the PCR technique, 4 to 5 days of initial training would be necessary.</p> <p>Occasional contact with Bio-Rad is recommended for interpreting the results.</p>

8. Actual handling time – Flexibility of the method with respect to the number of samples to be analyzed

Steps	Average time for a sample (min)		Average time for 48 samples (min)	
	Standard	iQ-Check™	Standard	iQ-Check™
Preparation, weighing, dilution in the enrichment broth and grinding	7	7	120	120
Transfer from half Fraser to Fraser	1		30	
Performing the iQ-Check™ test: - centrifugations - lysis - transfer into the PCR tube - software programming		Standard lysis 10		Standard lysis 120
Isolation on selective agars from half Fraser and Fraser	2	Easy lysis 7	40	Easy lysis 80
Reading the agars and selection of colonies for identification	2		20	
Interpretation of the results		1		10
<b>Total</b>	12 (0h12)	18 (0h18)	210 (3h30)	210 (3h30)

The advantage of the alternative method particularly lies in the possibility of sorting negative samples from suspicious samples, thus reducing the number of confirmations.

For positive samples, it is necessary to add the time required for confirmations.

For example, the confirmation of 5 colonies with the reference method tests can be evaluated in approximately 21 minutes, without preparation of the media.

For the alternative method using confirmation option 2, confirmations take less time: *RAPID'Listeria* spp. or *RAPID'L. mono* agar isolation usually is sufficient to confirm the results of the iQ-Check™ *Listeria* spp. test for the presence of the *Listeria* genus.

9. Time required to obtain results

negative samples:

Steps	Time required NF EN ISO 11290-1/A1 reference method	Time required iQ-Check™ <i>Listeria</i> spp. method
Preparation of the enrichment broth	D0	D0
Inoculation of the Fraser broth	D1	/
Performing the iQ-Check™ <i>Listeria</i> spp test		D1
Isolation of 10 µl on selective agars	D1 and D3	
<b>Obtaining negative results</b> - if there are no typical colonies - if there is confirmation of non- <i>monocytogenes Listeria</i> - if the test is positive and the confirmation negative	<b>D5</b> D9 to D11	<b>D1</b> D2* to D11**

\* if isolation on *RAPID'L. mono* or *RAPID'Listeria* spp.

\*\* if confirmation by conventional tests described in NF EN ISO 11290-1/A1

positive samples:

<b>Steps</b>	<b>Time required NF EN ISO 11290-1/A1 reference method</b>	<b>Time required iQ-Check™ <i>Listeria</i> spp. method</b>
Preparation of the enrichment broth	D0	D0
Inoculation of Fraser broth	D1	/
Performing the iQ-Check™ <i>Listeria</i> spp. test Isolation on selective agars	D1 and D3	D1 D1
Confirmation tests: <u>Genus</u> - Isolation on TSAYE - Gram, catalase - Reading RAPID <sup>®</sup> <i>Listeria</i> spp or RAPID <sup>®</sup> <i>L. mono</i>	D2 to D5 D3 to D6	D2 D2 D2
<u>Species</u> - CAMP test, hemolysis, TSBYE broth - Use of carbohydrates	D3 to D6 D4 to D7	D3 D4
<b>Obtaining positive results</b> <u>Genus</u> <u>Species</u> - after confirmation with the reference method tests - if miniaturized tests - for <i>Listeria monocytogenes</i> , iQ-Check™ <i>L. monocytogenes</i> test	<b>D3</b>  <b>D9 to D11</b> <b>D4 to D7</b>	<b>D2</b>  <b>D9</b> <b>D3 to D4</b> <b>D1 to D2</b>

10. Operator qualifications	The user should be trained in good laboratory practices for food microbiology and molecular biology.
11. Steps in common with the reference method	None
12. Traceability of the analytical results	All of the results are saved in the form of computer files. The results can be used in spreadsheets or LIMS.
13. Maintenance by the laboratory	It is recommended that the alignment of the software mask be verified at regular 6-month intervals, and that it be realigned when the iCycler iQ thermal cycler is moved.  A maintenance contract and a customer telephone hotline are available to users of the iCycler iQ, Chromo4 or other Bio-Rad systems.

## 5 Conclusion

The validation study was carried out in accordance with the standard EN ISO 16140: 2003.

**The comparative study** of the methods provided results for:

- relative accuracy, relative specificity and relative sensitivity;
- relative detection level;
- inclusivity and exclusivity.

Two lysis protocols were tested after enrichment in LSB broth on all of the food categories and environmental samples: a standard lysis protocol and an easy lysis protocol.

The results are identical for both of the lysis protocols.

The performances of the iQ-Check™ *Listeria* spp. method are equivalent to those of the reference method, NF EN ISO 11290-1/A1: 2005. They were determined by analyzing 363 samples distributed in five product categories.

**The relative accuracy obtained** was 90.4%, **relative sensitivity** was 93.2% and **relative specificity** was 87.3%, according to the calculations required by the EN ISO 16140 standard.

Thirty-five discordant results were obtained: 22 additional positive results and 13 false negative results.

The positive samples with the alternative method having been confirmed positive samples, the sensitivities were recalculated in relation to all of the positive results:

- 93.9% sensitivity for the alternative method;
- 89.6% sensitivity for the reference method.

The **relative detection level** of the iQ-Check™ *Listeria* spp. method and the reference method was evaluated by artificial contaminations of five different products representative of the five categories tested.

The level of detection obtained for the alternative method with the LSB broth was between 0.2 and 1.5 cells per 25 grams vs. 0.2 and 1.3 cells per 25 grams for the reference method.

The results of the **inclusivity** study were satisfactory, since the 84 strains of *Listeria* tested were all detected. In the **exclusivity** study, no cross reactions were observed with the 34 strains of other genera tested.

The results of the **interlaboratory study** obtained for all 10 laboratories accepted showed that the alternative method and the reference method have equivalent relative accuracy, specificity and sensitivity values, similar to those obtained during the preliminary study.

The variability of the alternative method (accordance, concordance, Odds ratio) was comparable to that of the reference method.

The advantage of this method particularly lies in the possibility of sorting negative samples from suspicious samples, thus reducing number of confirmations. Furthermore, handling times are reduced compared with the reference method if sample series are used.

The times required for obtaining negative results are also of interest: 24 hours for a negative result and starting at 48 hours for a positive result if it is confirmed with RAPID' *Listeria* spp. or RAPID' *L. mono* agar vs. 5 to 11 days for the reference method.

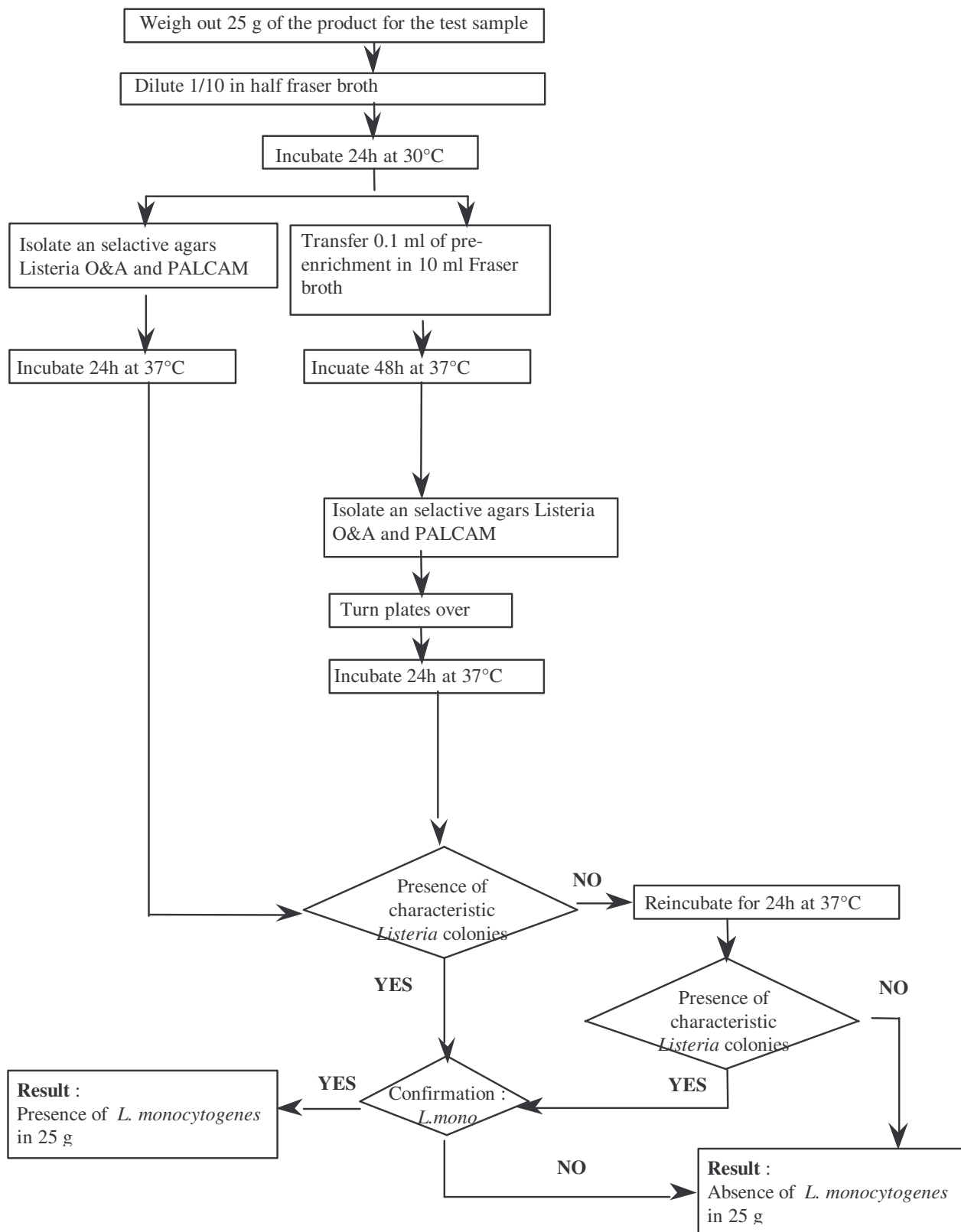
Given these results, the iQ-Check™ *Listeria* spp. method (Bio-Rad) was validated in May 2007 under number BRD 07/13 – 05/07.

# APPENDICES

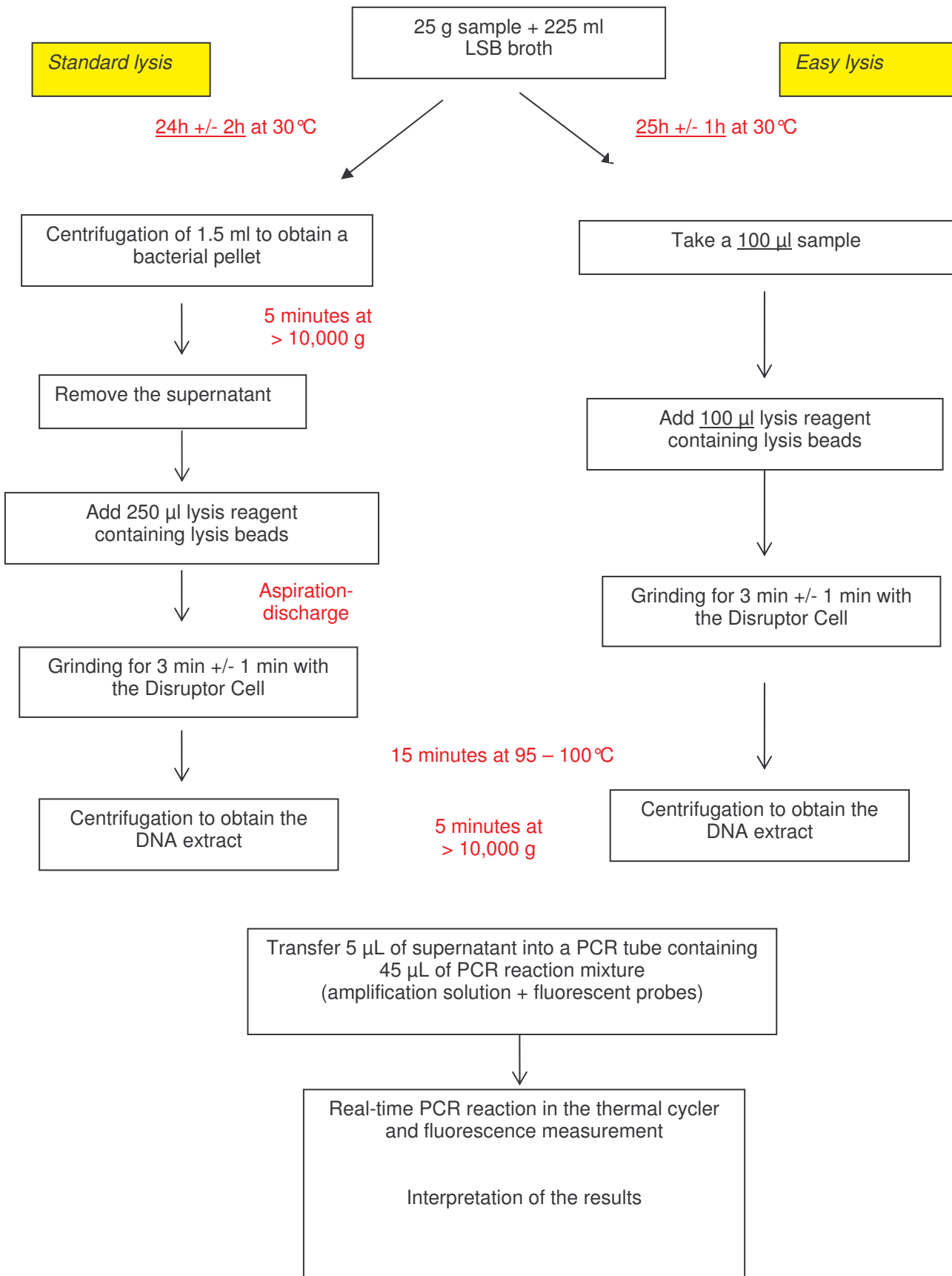
# APPENDIX A

## ANALYTICAL PROTOCOLS

# NF EN ISO 11290-1: 2005 STANDARD



# iQ-Check™ *Listeria* spp. METHOD



## APPENDIX B

### METHODS COMPARATIVE STUDY - SUMMARY OF THE RESULTS BY CATEGORY

# TABLE OF RESULTS BY SAMPLE CATEGORY

<u>meat products (96)</u>	<b>Positive reference method (R+)</b>	<b>Negative reference method (R-)</b>
Positive alternative method (A+)	Positive agreement (A+/R+) <b>PA = 52</b>	Positive deviation (R-/A+) <b>PD = 9</b>
Negative alternative method (A-)	Negative deviation (A-/R+) <b>ND = 5</b>	Negative agreement (A-/R-) <b>NA = 30</b>

<u>dairy products (69)</u>	Positive reference method (R+)	Negative reference method (R-)
Positive alternative method (A+)	Positive agreement (A+/R+) <b>PA = 33</b>	Positive deviation (R-/A+) <b>PD = 3</b>
Negative alternative method (A-)	Negative deviation (A-/R+) <b>ND = 3</b>	Negative agreement (A-/R-) <b>NA = 30</b>

<u>fish products (65)</u>	Positive reference method (R+)	Negative reference method (R-)
Positive alternative method (A+)	Positive agreement (A+/R+) <b>PA = 29</b>	Positive deviation (R-/A+) <b>PD = 6</b>
Negative alternative method (A-)	Negative deviation (A-/R+) <b>ND = 0</b>	Negative agreement (A-/R-) <b>NA = 30</b>

<u>vegetables (68)</u>	Positive reference method (R+)	Negative reference method (R-)
Positive alternative method (A+)	Positive agreement (A+/R+) <b>PA = 31</b>	Positive deviation (R-/A+) <b>PD = 3</b>
Negative alternative method (A-)	Negative deviation (A-/R+) <b>ND = 3</b>	Negative agreement (A-/R-) <b>NA = 31</b>

<u>environmental samples (64)</u>	Positive reference method (R+)	Negative reference method (R-)
Positive alternative method (A+)	Positive agreement (A+/R+) <b>PA = 32</b>	Positive deviation (R-/A+) <b>PD = 1</b>
Negative alternative method (A-)	Negative deviation (A-/R+) <b>ND = 2</b>	Negative agreement (A-/R-) <b>NA = 30</b>

## APPENDIX C

### SPECIFICITY STUDY - TABLES OF RESULTS

## Inclusivity

Reference	Strain	Origin	Level of inoculation in 225 mL of LSB broth	Easy Lysis protocol following LSB		
				Ct C.int	Ct FAM	Result
L5	<i>Listeria monocytogenes</i> 1/2a	Smoked salmon pieces	13.0	N/A	21.67	+
L6	<i>Listeria monocytogenes</i> 1/2a	Pizza	12.0	N/A	21.71	+
L7	<i>Listeria monocytogenes</i> 1/2a	Munster crust	11.0	31.76	26.35	+
L9	<i>Listeria monocytogenes</i> 1/2a	Munster crust	6.0	N/A	25.17	+
L10	<i>Listeria monocytogenes</i> 1/2a	Rillettes	6.0	N/A	24.29	+
L11	<i>Listeria monocytogenes</i> 1/2a	Munster crust	12.0	N/A	21.82	+
L12	<i>Listeria monocytogenes</i> 1/2a	Smoked salmon	11.0	32.73	25.48	+
L13	<i>Listeria monocytogenes</i> 1/2b	Pig ear	7.0	30.80	32.80	+
L14	<i>Listeria monocytogenes</i> 1/2c	Raw ground beef	5.0	N/A	17.49	+
L15	<i>Listeria monocytogenes</i> 1/2c	Beef MP	9.0	N/A	20.17	+
L16	<i>Listeria monocytogenes</i> 1/2c	Raw ground beef	7.0	N/A	16.88	+
L17	<i>Listeria monocytogenes</i> 1/2c	Breast	6.0	N/A	18.73	+
L18	<i>Listeria monocytogenes</i> 1/2c	Munster crust	7.0	N/A	19.41	+
L20	<i>Listeria monocytogenes</i> 1/2	Smoked salmon shavings	5.0	N/A	22.16	+
L25	<i>Listeria monocytogenes</i> 1/2	Hen	10.0	N/A	21.19	+
L28	<i>Listeria monocytogenes</i> 1/2c	Surface sponge	6.0	N/A	20.25	+
L32	<i>Listeria monocytogenes</i> 4b	Munster	6.0	N/A	20.60	+
L37	<i>Listeria monocytogenes</i> 1/2b	Raw milk Maroille cheese	8.0	N/A	16.79	+
L39	<i>Listeria monocytogenes</i>	Salami ham	6.0	N/A	17.49	+
L40	<i>Listeria monocytogenes</i> 1/2a	Farm munster	8.0	N/A	18.61	+
L42	<i>Listeria monocytogenes</i> 1/2a	Chicken breast	6.0	N/A	21.91	+
L43	<i>Listeria monocytogenes</i> 1/2a	Raw ground beef	11.0	N/A	22.90	+
L44	<i>Listeria monocytogenes</i> 1/2a	Salami	5.0	N/A	23.41	+
L45	<i>Listeria monocytogenes</i> 1/2a	Rabbit terrine	8.0	N/A	24.06	+
L47	<i>Listeria monocytogenes</i> 1/2a	Sauted apples	6.0	N/A	23.89	+
L48	<i>Listeria monocytogenes</i> 1/2b	Pig tongue	11.0	N/A	22.14	+
L49	<i>Listeria monocytogenes</i> 1/2b	Chicken liver cream	8.0	N/A	22.78	+
L51	<i>Listeria monocytogenes</i> 1/2b	Cured Germain cheese	5.5	N/A	23.39	+
L53	<i>Listeria monocytogenes</i> 1/2c	Raw ground beef	6.6	N/A	21.65	+
L54	<i>Listeria monocytogenes</i> 1/2c	Beef bourguignon	6.0	N/A	25.44	+
L55	<i>Listeria monocytogenes</i> 3b	SLCC 2540	6.0	30.81	28.29	+
L56	<i>Listeria monocytogenes</i> 3c	SLCC 2479	6.0	31.77	25.79	+
L58	<i>Listeria monocytogenes</i> 4b	Salad	8.0	N/A	22.07	+
L62	<i>Listeria monocytogenes</i> 4e	Reblochon cheese	7.2	N/A	21.62	+
L63	<i>Listeria monocytogenes</i> 4e	Munster	6.5	N/A	19.36	+
L116	<i>Listeria monocytogenes</i> 1/2a	Scallop shell	4.5	N/A	19.12	+
L117	<i>Listeria monocytogenes</i> 1/2c	Montbéliard sausage	5.5	N/A	19.89	+
L119	<i>Listeria monocytogenes</i>	Spinach	6.5	N/A	19.08	+
L121	<i>Listeria monocytogenes</i>	Neufchâtel cheese	6.0	N/A	19.00	+
L123	<i>Listeria monocytogenes</i>	Mozzarella	5.5	N/A	20.09	+
L124	<i>Listeria monocytogenes</i>	Perch filet	5.5	N/A	19.36	+
L128	<i>Listeria monocytogenes</i> 1/2a	Soya cakes	4.5	N/A	18.93	+
L129	<i>Listeria monocytogenes</i> 1/2a	Sauted apples	7.0	N/A	19.71	+
L130	<i>Listeria monocytogenes</i>	Raw ground beef	6.0	N/A	19.82	+
L137	<i>Listeria monocytogenes</i>	Raw milk Coulommier cheese	6.0	N/A	19.57	+
L141	<i>Listeria monocytogenes</i>	Environmental sample	6.0	N/A	22.79	+
L149	<i>Listeria monocytogenes</i>	Environmental sample	5.0	N/A	20.37	+
L152	<i>Listeria monocytogenes</i>	Environmental sample	6.5	N/A	19.81	+
L156	<i>Listeria monocytogenes</i>	Fried potatoes	6.0	N/A	19.20	+
L176	<i>Listeria monocytogenes</i>	Rib steak	4.5	N/A	20.39	+

### Inclusivity

Reference	Strain	Origin	Level of inoculation in 225 mL of LSB broth	Easy Lysis protocol following LSB		
				Ct C.int	Ct FAM	Result
L 190	<i>Listeria grayi</i>	Frozen fries	10.0	N/A	18.31	+
L80	<i>Listeria grayi</i>	ATCC 19120	10.0	N/A	18.13	+
L188	<i>Listeria grayi</i>	Environmental sample	11.0	N/A	22.88	+
L 143	<i>Listeria grayi</i>	Frozen fries	9.0	30.48	31.66	+
L108	<i>Listeria innocua</i>	Gorgonzola cheese	10.0	N/A	17.95	+
L113	<i>Listeria innocua</i>	Smoked halibut	10.0	N/A	18.25	+
L64	<i>Listeria innocua</i>	Epoisses cheese	3.0	N/A	18.19	+
L65	<i>Listeria innocua</i>	Epoisses cheese	4.5	N/A	20.23	+
L66	<i>Listeria innocua</i>	Spinach	4.0	N/A	18.46	+
L72	<i>Listeria innocua</i>	Meatballs	4.0	N/A	20.19	+
L78	<i>Listeria innocua</i>	Chicken	3.5	N/A	20.38	+
L175	<i>Listeria innocua</i>	Process water	8.0	N/A	21.52	+
L88	<i>Listeria innocua</i>	Salami	6.0	N/A	21.44	+
L77	<i>Listeria innocua</i> 6a	Sausage	4.0	N/A	23.49	+
L1	<i>Listeria innocua</i> 6a	ATCC 33090	15.0	N/A	18.01	+
L2	<i>Listeria innocua</i> 6b	Raw ground beef	4.5	N/A	19.39	+
L161	<i>Listeria ivanovii</i>	Raw ground beef	4.5	N/A	21.64	+
L153	<i>Listeria ivanovii</i>	Environmental sample	12.0	N/A	19.51	+
L80	<i>Listeria ivanovii</i>	Collection	3.7	N/A	16.83	+
L82	<i>Listeria ivanovii</i>	Bird net	15.0	33.03	26.74	+
L 151	<i>Listeria ivanovii</i>	Raw ground beef	8.0	N/A	24.91	+
L179	<i>Listeria ivanovii</i>	Environmental sample	4.0	31.59	28.45	+
L 154	<i>Listeria ivanovii</i>	Herb sausage	6.0	N/A	24.82	+
L115	<i>Listeria seeligeri</i>	Drain water	10.0	N/A	18.28	+
L84	<i>Listeria seeligeri</i>	Raw ground beef	14.0	N/A	17.17	+
L140	<i>Listeria seeligeri</i>	Frozen fries	6.0	N/A	21.96	+
L83	<i>Listeria seeligeri</i> 1/2b	Tongue	3.0	N/A	18.27	+
L100	<i>Listeria welshimeri</i>	Spread	10.0	N/A	19.09	+
L101	<i>Listeria welshimeri</i>	Ham	10.0	N/A	17.78	+
L 155	<i>Listeria welshimeri</i>	Salmon filet	5.0	N/A	23.48	+
L 174	<i>Listeria welshimeri</i>	Spinach	7.0	N/A	21.26	+
L91	<i>Listeria welshimeri</i>	Salami	5.0	N/A	22.40	+
L89	<i>Listeria welshimeri</i> 6a	Raw ground beef	2.0	30.83	28.10	+
L 90	<i>Listeria welshimeri</i> 6b	Raw ground beef	7.0	N/A	22.72	+

### Exclusivity

Reference	Strain	Origin	Level of inoculation in 225 mL of LSB broth	Easy Lysis protocol following LSB		
				Ct C.int	Ct FAM	Result
BA2	<i>Bacillus cereus</i>	Beets	3.0E+05	33.96	N/A	-
BA 1	<i>Bacillus cereus</i>	Munster cheese crust	5.0E+05	32.04	N/A	-
BA 15	<i>Bacillus cereus</i>	Munster cheese crust	3.0E+05	32.56	N/A	-
BA 19	<i>Bacillus cereus</i>	Environment	3.0E+05	32.68	N/A	-
BA 3	<i>Bacillus cereus</i>	Munster cheese crust	3.6E+05	32.89	N/A	-
BA7	<i>Bacillus coagulans</i>	Smoked salmon	5.0E+05	34.42	N/A	-
BA22	<i>Bacillus pumilus</i>	Pig's ear	6.0E+05	33.94	N/A	-
15	<i>Brochotrix thermosphacta</i>	Raw ground beef	5.0E+00	33.87	N/A	-
38	<i>Corynebacterium variabilis</i>	Beef (raw material)	4.9E+05	34.00	N/A	-
E10	<i>Enterococcus durans</i>	Raw ground beef	6.0E+05	32.38	N/A	-
E8	<i>Enterococcus durans</i>	Breast	6.0E+05	32.71	N/A	-
E1	<i>Enterococcus faecalis</i>	Munster cheese crust	7.0E+00	34.49	N/A	-
E6	<i>Enterococcus faecalis</i>	Smoked salmon shavings	4.0E+05	34.20	N/A	-
E2	<i>Enterococcus faecium</i>	Hen	3.0E+05	34.04	N/A	-
E7	<i>Enterococcus faecium</i>	Surface sponge	6.7E+05	36.75	N/A	-
E9	<i>Enterococcus faecium</i>	Tarama (fish egg salad)	3.0E+05	32.47	N/A	-
L139	<i>Jonesia denitrificans</i>	Raw milk Maroille cheese	1.6E+05	31.07	N/A	-
33	<i>Lactobacillus lactis</i>	Ham salami	2.3E+05	34.21	N/A	-
B1	<i>Lactobacillus casei</i>	Farm munster cheese	2.3E+05	31.79	N/A	-
34	<i>Lactobacillus plantarum</i>	Chicken breast	3.0E+05	33.98	N/A	-
B2	<i>Lactobacillus spp</i>	Raw ground beef	5.0E+05	31.05	N/A	-
Le1	<i>Rhodotorula rubra</i>	Salami	2.0E+05	34.47	N/A	-
ST17	<i>Staphylococcus aureus</i>	Rabbit terrine	2.3E+05	34.08	N/A	-
B3	<i>Streptococcus anginosus</i>	Sauted apples	6.7E+05	32.82	N/A	-
B4	<i>Streptococcus anginosus</i>	Pig's tongue	5.0E+05	31.34	N/A	-
E3	<i>Streptococcus bovis</i>	Chicken liver cream	2.3E+05	34.96	N/A	-
B5	<i>Streptococcus bovis</i>	Cured Germain cheese	2.8E+05	33.62	N/A	-
B6	<i>Streptococcus bovis</i>	Raw ground beef	7.7E+05	30.85	N/A	-
B7	<i>Streptococcus equinus</i>	Beef bourguignon	3.0E+05	32.19	N/A	-
B8	<i>Streptococcus intermedius</i>	Collection 1201	3.0E+05	31.04	N/A	-
B9	<i>Streptococcus salivarius</i>	Collection 1115	6.7E+05	30.83	N/A	-
B10	<i>Streptococcus salivarius</i>	Salad	2.3E+05	32.95	N/A	-

## APPENDIX D

# INTERLABORATORY STUDY - ACCORDANCE

## Accordance calculation

### ALTERNATIVE METHOD

#### Level L0

Laboratory	Expected # of negatives	Actual # of negatives	Probability of negatives	Probability of pairs of negatives	Probability of positive	Probability of pairs of positive	Probability of identical result pairs
Laboratory A	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory C	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory D	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory E	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory F	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory G	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory H	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory I	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory K	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory M	8	8	1.0	1.0	0.0	0.0	1.0
<b>Averag</b>							<b>1.0</b>
<b>Accordance:</b>							<b>100</b>

#### Level L1

Laboratory	Expected # of negatives	Actual # of negatives	Probability of negatives	Probability of pairs of negatives	Probability of positive	Probability of pairs of positive	Probability of identical result pairs
Laboratory A	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory C	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory D	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory E	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory F	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory G	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory H	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory I	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory K	8	7	0.8	0.7	0.1	0.0	0.7
Laboratory M	8	8	1.0	1.0	0.0	0.0	1.0
<b>Averag</b>							<b>0.9</b>
<b>Accordance:</b>							<b>98</b>

#### Level L2

Laboratory	Expected # of negatives	Actual # of negatives	Probability of negatives	Probability of pairs of negatives	Probability of positive	Probability of pairs of positive	Probability of identical result pairs
Laboratory A	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory C	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory D	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory E	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory F	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory G	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory H	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory I	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory K	8	8	1.0	1.0	0.0	0.0	1.0
Laboratory M	8	8	1.0	1.0	0.0	0.0	1.0
<b>Averag</b>							<b>1.0</b>
<b>Accordance:</b>							<b>100</b>

## Accordance calculation

### ALTERNATIVE METHOD

#### Level L0

Laboratory	Expected # of negatives	Actual # of negatives	Probability of negatives	Probability of pairs of negatives	Probability of positives	Probability of pairs de positives	Probability of identical result pairs
Laboratory A	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory C	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory D	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory E	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory F	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory G	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory H	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory I	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory K	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory M	8	8	1.00	1.00	0.00	0.00	1.00
<b>Average:</b>							<b>1.00</b>
<b>Accordance:</b>							<b>100%</b>

#### Level L1

Laboratory	Expected # of positives	Actual # of positives	Probability of positives	Probability of pairs of positives	Probability of negatives	Probability of pairs of negatives	Probability of identical result pairs
Laboratory A	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory C	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory D	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory E	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory F	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory G	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory H	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory I	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory K	8	7	0.88	0.77	0.13	0.02	0.78
Laboratory M	8	8	1.00	1.00	0.00	0.00	1.00
<b>Average:</b>							<b>0.98</b>
<b>Accordance:</b>							<b>98%</b>

#### Level L2

Laboratory	Expected # of positives	Actual # of positives	Probability of positives	Probability of pairs of positives	Probability of negatives	Probability of pairs of negatives	Probability of identical result pairs
Laboratory A	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory C	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory D	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory E	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory F	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory G	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory H	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory I	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory K	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory M	8	8	1.00	1.00	0.00	0.00	1.00
<b>Average:</b>							<b>1.00</b>
<b>Accordance:</b>							<b>100%</b>

## REFERENCE METHOD

### Level L0

Laboratory	Expected # of negatives	Actual # of negatives	Probability of negatives	Probability of pairs of negatives	Probability of positives	Probability of pairs de positives	Probability of identical result pairs
Laboratory A	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory C	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory D	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory E	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory F	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory G	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory H	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory I	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory K	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory M	8	8	1.00	1.00	0.00	0.00	1.00
<b>Average:</b>							<b>1.00</b>
<b>Accordance:</b>							<b>100%</b>

### Level L1

Laboratory	Expected # of positives	Actual # of positives	Probability of positives	Probability of pairs of positives	Probability of negatives	Probability of pairs of negatives	Probability of identical result pairs
Laboratory A	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory C	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory D	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory E	8	7	0.88	0.77	0.13	0.02	0.78
Laboratory F	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory G	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory H	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory I	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory K	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory M	8	8	1.00	1.00	0.00	0.00	1.00
<b>Average:</b>							<b>0.98</b>
<b>Accordance:</b>							<b>98%</b>

### Level L2

Laboratory	Expected # of positives	Actual # of positives	Probability of positives	Probability of pairs of positives	Probability of negatives	Probability of pairs of negatives	Probability of identical result pairs
Laboratory A	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory C	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory D	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory E	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory F	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory G	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory H	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory I	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory K	8	8	1.00	1.00	0.00	0.00	1.00
Laboratory M	8	8	1.00	1.00	0.00	0.00	1.00
<b>Average:</b>							<b>1.00</b>
<b>Accordance:</b>							<b>100%</b>

## APPENDIX E

# INTERLABORATORY STUDY - CONCORDANCE

## Concordance calculation

### ALTERNATIVE METHOD

Number of laboratories 10

Number of negatives by laboratory 8

#### **Level L0**

Laboratory	# of expected negatives	# of actual negatives	Interlaboratory pairs with the same result	Total # of interlaboratory pairs
Laboratory A	8	8	576	576
Laboratory C	8	8	576	576
Laboratory D	8	8	576	576
Laboratory E	8	8	576	576
Laboratory F	8	8	576	576
Laboratory G	8	8	576	576
Laboratory H	8	8	576	576
Laboratory I	8	8	576	576
Laboratory K	8	8	576	576
Laboratory M	8	8	576	576
<b>Total</b>			<b>5760</b>	<b>5760</b>
<b>Concordance</b>	100.00%			

Number of laboratories 10

Number of positives by laboratory 8

#### **Level L1**

Laboratory	# of expected positives	# of actual positives	Interlaboratory pairs with the same result	Total # of interlaboratory pairs
Laboratory A	8	8	568	576
Laboratory C	8	8	568	576
Laboratory D	8	8	568	576
Laboratory E	8	8	568	576
Laboratory F	8	8	568	576
Laboratory G	8	8	568	576
Laboratory H	8	8	568	576
Laboratory I	8	8	568	576
Laboratory K	8	7	504	576
Laboratory M	8	8	568	576
<b>Total</b>			<b>5616</b>	<b>5760</b>
<b>Concordance</b>	97.50%			

Number of laboratories 10

Number of positives by laboratory 8

#### **Level L2**

Laboratory	# of expected positives	# of actual positives	Interlaboratory pairs with the same result	Total # of interlaboratory pairs
Laboratory A	8	8	576	576
Laboratory C	8	8	576	576
Laboratory D	8	8	576	576
Laboratory E	8	8	576	576
Laboratory F	8	8	576	576
Laboratory G	8	8	576	576
Laboratory H	8	8	576	576
Laboratory I	8	8	576	576
Laboratory K	8	8	576	576
Laboratory M	8	8	576	576
<b>Total</b>			<b>5760</b>	<b>5760</b>
<b>Concordance</b>	100.00%			

## REFERENCE METHOD

Number of laboratories 10

Number of negatives by laboratory 8

### Niveau L0

Laboratory	# of expected negatives	# of actual negatives	Interlaboratory pairs with the same result	Total # of interlaboratory pairs
Laboratory A	8	8	576	576
Laboratory C	8	8	576	576
Laboratory D	8	8	576	576
Laboratory E	8	8	576	576
Laboratory F	8	8	576	576
Laboratory G	8	8	576	576
Laboratory H	8	8	576	576
Laboratory I	8	8	576	576
Laboratory K	8	8	576	576
Laboratory M	8	8	576	576
<b>Total</b>			<b>5760</b>	<b>5760</b>
<b>Concordance</b>	100.00%			

Number of laboratories 10

Number of positives by laboratory 8

### Niveau L1

Laboratory	# of expected positives	# of actual positives	Interlaboratory pairs with the same result	Total # of interlaboratory pairs
Laboratory A	8	8	568	576
Laboratory C	8	8	568	576
Laboratory D	8	8	568	576
Laboratory E	8	7	504	576
Laboratory F	8	8	568	576
Laboratory G	8	8	568	576
Laboratory H	8	8	568	576
Laboratory I	8	8	568	576
Laboratory K	8	8	568	576
Laboratory M	8	8	568	576
<b>Total</b>			<b>5616</b>	<b>5760</b>
<b>Concordance</b>	97.50%			

Number of laboratories 10

Number of positives by laboratory 8

### Niveau L2

Laboratory	# of expected positives	# of actual positives	Interlaboratory pairs with the same result	Total # of interlaboratory pairs
Laboratory A	8	8	576	576
Laboratory C	8	8	576	576
Laboratory D	8	8	576	576
Laboratory E	8	8	576	576
Laboratory F	8	8	576	576
Laboratory G	8	8	576	576
Laboratory H	8	8	576	576
Laboratory I	8	8	576	576
Laboratory K	8	8	576	576
Laboratory M	8	8	576	576
<b>Total</b>			<b>5760</b>	<b>5760</b>
<b>Concordance</b>	100.00%			