

**AFNOR CERTIFICATION VALIDATION STUDY  
ADIAFOOD SALMONELLA SPP  
TEST SYSTEM**

SYNTHESIS REPORT

ADIAFOOD SALMONELLA TEST SYSTEM - S.R.(V0)  
OCTOBER 2010



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

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**Annex 1:** selectivity strains list

**Annex 2:** accordance calculations

**Annex 3:** concordance calculations

## **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 6579 (2002). 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.

<p><b><u>STEP 1: PRE-ENRICHMENT</u></b>  X g (mL) sample + 9X mL BPW  Incubation at (37±1)°C for 16 to 20 hours  Make a 1 mL aliquot of the suspension and centrifuge for 2 min at (500 ±100)g  Transfer 500 µL of the supernatant and centrifuge for 5 min at (4000±500)g</p> <p><b><u>STEP 2: DNA EXTRACTION</u></b>  Dilute the bottom in 100 µL of EX2 solution  Add the 100 µL in microplate extraction  Perform extraction in thermocycler</p> <p><b><u>STEP 3: DNA AMPLIFICATION</u></b>  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  Place the detection microplate in the thermocycler and begin the detection</p> <p><b><u>STEP 4: RESULTS</u></b>  Results appear automatically at the end of the detection (+ or -)  With inhibited samples, make another assay with DNA extracts diluted at 1/10<sup>th</sup> or make a washing of the concerned wells</p> <p><b><u>STEP 5: CONFIRMATION OF PRESUMED POSITIVE RESULTS</u></b>  Transfer the remaining 500 µL of the first centrifugation (step 1) in 10 mL of RVS broth, then incubate at (41.5±1)°C during (24±2)h and isolate the enriched RVS broth on SALSA medium and incubate at (37±1)°C for 22 to 26 hours</p> <p>*: Ability to use barrettes unit</p>
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**Figure 1:** alternative method protocol

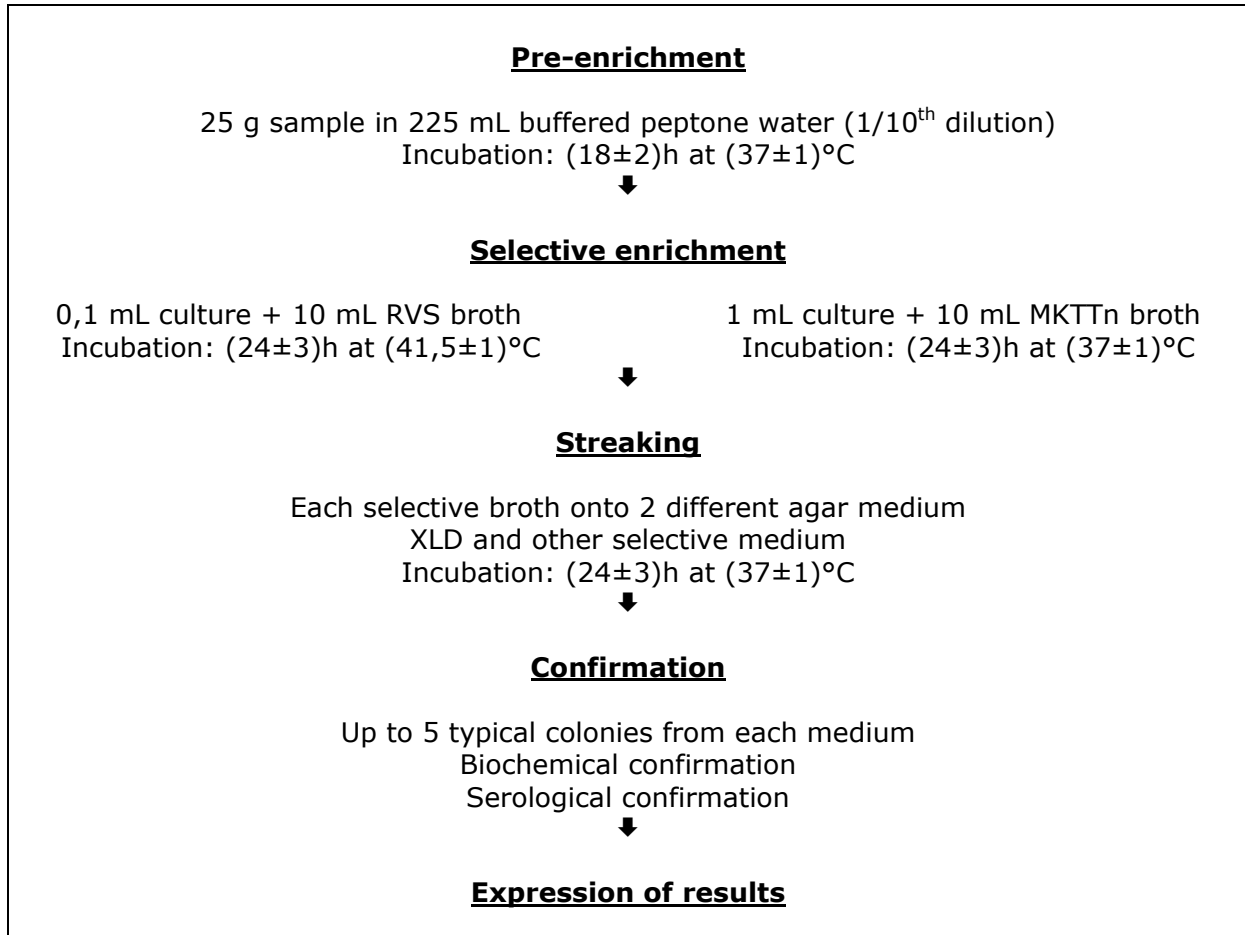
During the detection step, the target pathogen DNA is amplified and detected using specific primers and molecular beacons, the key components of the AES CHEMUNEX technology. Molecular beacons probes consist in a unique sequence probe that allows the identification of the pathogen with a high level of 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 NF EN ISO 6579 (2002) standard: Horizontal method for the detection of *Salmonella* spp has been applied. The protocol of this method is presented 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 368 samples was analysed. Types of products are indicated in table 1.

Category	Type	Nombre de positifs*	Nombre de négatifs	Total
Meat products	Raw poultry meat	13	8	21
	Raw beef meat	5	2	7
	Others meats	4	4	8
	Delicatessen	4	11	15
	Meat dishes	4	7	11
	<b>Total</b>	<b>30</b>	<b>32</b>	<b>62</b>
Dairy products	Raw milk cheese	9	11	20
	Raw milk	5	3	8
	Pasteurized milk cheese	9	9	18
	Milk	5	4	9
	Yoghurts	1	1	2
	Other dairy product	1	3	4
	<b>Total</b>	<b>30</b>	<b>31</b>	<b>61</b>
Sea food and vegetable products	Fruits et vegetables	23	22	45
	Juice	2	2	4
	Seafoods	0	1	1
	Smoked fish	6	7	13
	<b>Total</b>	<b>31</b>	<b>32</b>	<b>63</b>
Other products	Eggs and egg products	11	15	26
	Pastries	12	13	25
	Others	7	3	10
	<b>Total</b>	<b>30</b>	<b>31</b>	<b>61</b>
Food stuff products	Dog food	8	4	12
	Cat food	2	6	8
	Bird food	6	5	11
	Other	14	15	29
	<b>Total</b>	<b>30</b>	<b>30</b>	<b>60</b>
Environmental samples	Process water	16	13	29
	Swabs	13	16	29
	Sponge	1	2	3
	<b>Total</b>	<b>30</b>	<b>31</b>	<b>61</b>
<b>Total</b>		<b>181</b>	<b>187</b>	<b>368</b>

**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 –XLD-).

46 naturally contaminated samples were analysed. 74,6 % 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 500 µL remaining of the first centrifugation (step 1) in 10 mL of RVS broth, then incubate at (41.5±1) °C during (24±2) h and isolate the enriched RVS broth on SALSA medium and incubate at (37±1) °C for 22 to 26 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.

Category	Response	Reference method <sup>(*)</sup> positive (R+)	Reference method <sup>(*)</sup> négative (R-)
Meat products	Alternative method positive (A+)	PA=28	PD=1
	Alternative method négative (A-)	ND=1 including 1 PPND	NA=32 including 4 PPNA
Dairy products	Alternative method positive (A+)	PA=29	PD=0
	Alternative method négative (A-)	ND=1 including 0 PPND	NA=31 including 4 PPNA
Sea food and vegetable products	Alternative method positive (A+)	PA=31	PD=0
	Alternative method négative (A-)	ND=0 including 0 PPND	NA=32 including 3 PPNA
Other products	Alternative method positive (A+)	PA=30	PD=0
	Alternative method négative (A-)	ND=0 including 0 PPND	NA=31 including 0 PPNA
Food stuff products	Alternative method positive (A+)	PA=30	PD=0
	Alternative method négative (A-)	ND=0 including 0 PPND	NA=30 including 1 PPNA
Environ-mental samples	Alternative method positive (A+)	PA=30	PD=0
	Alternative method négative (A-)	ND=0 including 0 PPND	NA=31 including 1 PPNA
All products	Alternative method positive (A+)	PA=178	PD=1
	Alternative method négative (A-)	ND=2 including 1 PPND	NA=187 including 13 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	Exactitude relative AC [(PA+NA)/N]	N+ PA+ND	Sensibilité relative SE [PA/N+]	N- NA+PD	Spécificité relative SP [NA/N-]
Meat products	28	32	1	1	62	96,8%	29	96,6%	33	97,0%
Dairy products	29	31	1	0	61	98,4%	30	96,7%	31	100%
Sea food and vegetable products	31	32	0	0	63	100%	31	100%	32	100%
Other products	30	31	0	0	61	100%	30	100%	31	100%
Food stuff products	30	30	0	0	60	100%	30	100%	30	100%
Environmental samples	30	31	0	0	61	100%	30	100%	31	100%
All products	178	187	2	1	368	99,2%	180	98,9%	188	99,5%

**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
<b>Relative accuracy</b>	99,2%
<b>Relative sensitivity</b>	98,9%
<b>Relative specificity</b>	99,5%

**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)
<b>Sensitivity</b>	98,9%	99,4%

**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,  $Y = 1 + 2 = 3$ , with  $Y < 6$  so, the two methods are considered as equivalent.

- Negative deviations

-Sample numbers: 0075 and 0084:

A positive result is obtained by the reference method whereas a negative result is obtained by the alternative method. However the isolation of the BPW on selective agar medium didn't allow finding typical colonies. The strain isolated from the reference method was tested with alternative method and the result was positive. This result is due growth of the *Salmonella* strain who could be inhibited by the annex flora of the matrix.

- Positive deviations

-Sample numbers: 0090:

A positive result is obtained by the alternative medium whereas a negative result is obtained by the reference method. The result of the alternative method was confirmed using biochemical and serological tests.

## **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.

<b>Matrix</b>	<b>Strain</b>	<b>ISHA code</b>	<b>Origin</b>
Minced meat	S. Typhimurium	SAL.1.133	Minced meat
Raw milk	S. Newport	SAL.1.98	Raw milk cheese
Fish	S. Virchow	SAL.1.155	CIP 105355
Raw egg	S. Enteritidis	SAL.1.48	Egg product
Rodent food	S. Infantis	SAL.1.69	Horse food
Process water	S. Yoruba	SAL.1.162	Environmental sample

**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 1/2 and 1/4 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
S. Typhimurium	Minced meat	0,717 [ 0,465 ; 1,104 ]	0,717 [ 0,465 ; 1,104 ]
S. Newport	Raw milk	0,351 [ 0,155 ; 0,794 ]	0,351 [ 0,155 ; 0,794 ]
S. Virchow	Fish	0,743 [ 0,398 ; 1,387 ]	0,743 [ 0,398 ; 1,387 ]
S. Enteritidis	Raw egg	0,631 [ 0,338 ; 1,178 ]	0,631 [ 0,338 ; 1,178 ]
S. Infantis	Rodent food	0,660 [ 0,389 ; 1,120 ]	0,660 [ 0,389 ; 1,120 ]
S. Yoruba	Process water	0,741 [ 0,378 ; 1,452 ]	0,741 [ 0,378 ; 1,452 ]

**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
S. Typhimurium	Minced meat	0,7 [ 0, 5 ; 1,1 ]	0,7 [ 0, 5 ; 1,1 ]
S. Newport	Raw milk	0,4 [ 0,2 ; 0,8 ]	0,4 [ 0,2 ; 0,8 ]
S. Virchow	Fish	0,7 [ 0,4 ; 1, 4 ]	0,7 [ 0,4 ; 1, 4 ]
S. Enteritidis	Raw egg	0,6 [ 0,3 ; 1,2 ]	0,6 [ 0,3 ; 1,2 ]
S. Infantis	Rodent food	0,7 [ 0,4 ; 1,1 ]	0,7 [ 0,4 ; 1,1 ]
S. Yoruba	Process water	0,7 [ 0,4 ; 1,5 ]	0,7 [ 0,4 ; 1,5 ]

**Table 8:** relative detection level (1 significant number)

The alternative and the reference method show similar detection levels. The detection limit obtained with the both method is comprised between 0.2 and 1.5 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, 51 strains of *Salmonella* spp and 32 non-target strains were tested. A list of the strains figures in annex 1.

#### **2.3.1. Test protocols**

- **Inclusivity**

Each *Salmonella* strain was cultivated twice before inoculation in BPW (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 (Trypticase Soy Broth) with a level of contamination expected to occur in the food matrices (about 10<sup>5</sup> 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 51 *Salmonella* 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 thirteen participating laboratories.

##### 3.1.2. *Salmonella* spp absence in the matrix

Before spiking, the absence of *Salmonella* spp 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  $(4\pm 2)^{\circ}\text{C}$ . The strain used was *Salmonella* Infantis (ISHA code: SAL.1.163) isolated from milk.

The two methods were performed. 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 and by the alternative method. The results are summarized in table 9.

<b>Day</b>	<b>Alternative method</b>	<b>Reference method</b>
D0	Presence in 25 mL	Presence in 25 mL
D+1	Presence in 25 mL	Presence in 25 mL
D+2	Presence in 25 mL	Presence in 25 mL
D+3	Presence in 25 mL	Presence in 25 mL

**Table 9:** results of the stability study of the strain SAL.1.163 in supplemented pasteurized milk

The results show that the *Salmonella* strain used is stable for 3 days at  $(4\pm 2)^{\circ}\text{C}$  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 24 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.

<b>Matrix</b>	<b>Total viable count (CFU/mL)</b>	<b>Target level (cells / 25 mL)</b>	<b>Real level (cells / 25 mL)</b>	<b>Confidence interval</b>
Pasteurized milk	$8,5.10^4$	0	0	0
		3	4	[1 ; 7 ]
		30	32	[22 ; 44 ]

**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 M (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	3/4/8/9/10/12/14/19
L1	1/5/6/7/16/17/20/21
L2	2/11/13/15/18/22/23/24

**Table 11:** sample code by contamination level

### 3.1.6. Samples shipping

The samples were shipped in a coolbox the 31<sup>st</sup> of May 2010.

### 3.1.7. Samples reception and analysis

The coolboxes were received the 1st of June 2010 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	5.2	Correct
B	6.6	Correct
C	<b>10.4</b>	Correct
D	3.3	Correct
E	4.9	Correct
F	5.0	Correct
G	3.0	Correct
H	4.1	Correct
I	<b>10.0</b>	Correct
J	3.8	Correct
K	3.8	Correct
L	4.7	Correct
M	6.6	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 C for which it was at 10.4°C and laboratory I for which it was at 10.0°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
Temperature (°C)	Mean	4.20	2.40	1.73	3.20	1.13	2.35	1.54	0.23	0.90	1.73	1.83	2.64	1.39
	SD	0.77	0.60	0.34	0.70	0.49	0.49	0.61	0.78	0.76	0.32	0.42	0.49	0.26

**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 0.23 and 4.2°C.

### 3.2.2. Total viable counts

For the whole laboratories, the total viable counts at 30°C vary between  $5.5 \times 10^3$  and  $> 3.0 \times 10^5$  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	6/8	6/8
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 2 samples 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 *Salmonella* 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

Laboratory	Contamination level		
	L0	L1	L2
A	0/8	7/8	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	8/8	8/8
G	0/8	8/8	8/8
H	0/8	8/8	8/8
I	<b>0/8</b>	<b>8/8</b>	<b>8/8</b>
J	0/8	8/8	8/8
K	0/8	8/8	8/8
L	0/8	7/8	8/8
M	0/8	<b>7/8</b>	8/8

**Table 15:** alternative method positive results for all laboratories

- Reference method results

Laboratory	Contamination level		
	L0	L1	L2
A	0/8	7/8	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	8/8	8/8
G	0/8	8/8	8/8
H	0/8	8/8	8/8
I	<b>0/8</b>	<b>8/8</b>	<b>8/8</b>
J	0/8	8/8	8/8
K	0/8	8/8	8/8
L	0/8	7/8	8/8
M	0/8	8/8	8/8

**Table 16:** reference method positive results for all laboratories

- Results analysis

The laboratories A, D and L presented a negative result by the alternative method and by the reference method for a low level contaminated sample. Due to the low level of contamination of this sample (3 CFU/25 mL), no *Salmonella* cell may have been inoculated in the matrix.

The laboratory M presented a negative result by the alternative method and positive by the reference method for a high level contaminated sample. A second PCR analysis of the extract gave a negative result and the isolation BPW on selective media showed the absence of typical colonies.

Final analysis was consequently conducted using data supplied by twelve 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,  $SP = [1 - (FP/N-)] \times 100\%$ ,      N-: total number of L0 tests  
FP: number of false positive

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

Specificity / sensitivity	Alternative method	LCL	Reference method	LCL
<b>SP (level L0)</b>	100%	98%	100%	98%
<b>SE (level L1)</b>	95,8%	93%	96,9%	93%
<b>SE (level L2)</b>	100%	98%	100%	98%
<b>SE (level L1+L2)</b>	97,9%	96%	98,4%	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=96	96
	<b>Total</b>	0	96	96
L1	AM+	PA=92	PD=0	92
	AM-	ND=1	NA=3	4
	<b>Total</b>	93	3	96
L2	AM+	PA=96	PD=0	96
	AM-	ND=0	NA=0	0
	<b>Total</b>	96	0	96
L0+L1+L2	AM+	PA=188	PD=0	188
	AM-	ND=1	NA=99	100
	<b>Total</b>	189	99	288

**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:

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

Level	Relative accuracy (AC)	LCL (Low Critical Value)
<b>L0</b>	100%	98,0%
<b>L1</b>	99,0%	98,0%
<b>L2</b>	100%	98,0%
<b>L1+L2</b>	99,5%	98,0%
<b>Total</b>	99,7%	98,0%

**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,  $Y = 0 + 1 = 1$ , with  $Y < 6$  so, the two methods are considered as equivalent.

## 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
<b>L0</b>	100%	100%
<b>L1</b>	92,7%	94,5%
<b>L2</b>	100%	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
<b>L0</b>	100%	100%
<b>L1</b>	92,0%	93,9%
<b>L2</b>	100%	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:  

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

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	92,7	92,0	1,1	94,5	93,9	1,1
L2	100	100	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	99,2%	99,7%
SP	99,5%	100%
SE	98,9%	97,9%

**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)
99,5%	100%

**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

<b>COMPOSANT</b>	<b>DESCRIPTION</b>	<b>Format ou volume</b>
<b>Detection microplates or barrettes</b>	Microplates / barrettes for the real-time PCR detection	ADIF1303 : 5 microplates - 88 tests ADIF6103 : 12 barrettes - 8 tests
<b>Control barrettes</b>	Control barrettes specific of the pathogen	ADIF8103 : 8 control barrettes
<b>DT-1</b>	solution used for the reconstitution of lyophilised DT-2	2.2 mL
<b>DT-2</b>	Lyophilised compound for the detection solution	/
<b>EX-1</b>	solution used for the reconstitution of lyophilised EX-2	10 mL
<b>EX-2</b>	Lyophilised compound for the extraction solution	/
<b>Optical quality PCR corks</b>	Flat corks	/
<b>User guide</b>	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 enclosure
- *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 alcohol 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
Dilution - Suspension	3	30	3	30
Sampling	1	20	/	/
2 <sup>nd</sup> enrichment	/	/	1	16
Extraction	6	15	/	/
Amplification	5	12	/	/
PCR reading	0.1	3	/	/
Isolation on XLD and another selective medium	/	/	2	20
1 <sup>st</sup> reading of the Petri dishes	/	/	0.5	6
2 <sup>nd</sup> reading of the Petri dishes	/	/	0.5	6
<b>Total</b>	<b>15.1</b>	<b>80</b>	<b>7</b>	<b>78</b>

Steps- Manipulation time - Positive samples	Time (minutes)			
	Alternative method		Reference method	
	1 analyse	20 analyses	1 analyse	20 analyses
Dilution - Suspension	3	30	3	30
Sampling	1	20	/	/
2 <sup>nd</sup> enrichment	/	/	1	16
Extraction	6	15	/	/
Amplification	5	12	/	/
PCR reading	0.1	3	/	/
Isolation on XLD and another selective medium	/	/	2	20
1 <sup>st</sup> reading of the Petri dishes	/	/	1	12
2 <sup>nd</sup> reading of the Petri dishes	/	/	1	12
PCR Confirmation - RVS enrichment	1	16	/	/
PCR Confirmation- SALSA isolation	0.5	8	/	/
SALSA reading	0.5	4	/	/
Biochemical confirmation	/	/	3	40
<b>Total</b>	<b>17.1</b>	<b>108</b>	<b>11</b>	<b>130</b>

9- Time required for results

<b>Steps –Time for negative results</b>	<b>Alternative method</b>	<b>Reference method</b>
Dilution - Suspension	D0	D0
Extraction	D1	/
Amplification	D1	/
PCR reading	D1	/
2 <sup>nd</sup> enrichment	/	D1
Isolation on XLD and another selective medium	/	D2
Reading of the Petri dishes	/	D3

<b>Steps –Time for positive results</b>	<b>Alternative method</b>	<b>Reference method</b>
Dilution - Suspension	D0	D0
Extraction	D1	/
Amplification	D1	/
PCR reading	D1	/
2 <sup>nd</sup> enrichment	/	D1
PCR Confirmation – RVS enrichment	D1	/
PCR Confirmation– SALSA isolation	D2	/
Isolation on XLD and another selective medium	/	D2
Reading of the Petri dishes	D3	D3
Purification	/	D3
Biochemical confirmation	/	D5

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 *Salmonella* spp test for the detection of *Salmonella* spp are comparable to those of the standard NF EN ISO 6579 (2002).

This study concerned 368 samples of five categories of products (meat, dairy, seafood, vegetable, food stuffs and environmental products).

Values obtained for the 3 criteria are the following:

- relative accuracy: 99.2%
- relative sensitivity: 98.9%
- relative specificity: 99.5 %

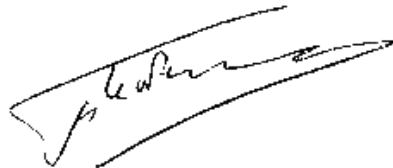
Three discordant results were observed (1PD and 2 ND°).

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 two methods between 0.2 and 1.5 CFU in 25 g for all categories.

The specificity of the method is satisfactory.

Concerning the interlaboratory study, the results obtained for the 12 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 29<sup>th</sup> of October 2010  
François Le Nestour  
*Research engineer*

## **Annex 1 : selectivity**

### **Inclusivity list**

Code ISHA	Microorganism	Origin (French designation)
SAL.1.5	<i>Salmonella</i> Anatum	Sésame décortiqué
SAL.1.7	<i>Salmonella</i> Arizonae (48 : z4, z23 :-)	Canard
SAL.1.8	<i>Salmonella</i> Arizonae (18 : z4, z23 :-)	Canard
SAL.1.10	<i>Salmonella</i> Braenderup	Env. atelier (alim. humaine)
SAL.1.17	<i>Salmonella</i> Brandenburg	Canard
SAL.1.21	<i>Salmonella</i> Bredeney	Blanc de poulet cru
SAL.1.23	<i>Salmonella</i> Cerro	Farine de lapin
SAL.1.29	<i>Salmonella</i> Derby	Echine de porc
SAL.1.38	<i>Salmonella</i> Derby	Chiffonnette salaisonerie
SAL.1.40	<i>Salmonella</i> Diarizonae	Semoule de blé
SAL.1.42	<i>Salmonella</i> Diarizonae	Boue station épuration
SAL.1.43	<i>Salmonella</i> Dublin	Lait
SAL.1.44	<i>Salmonella</i> Dublin	Quality management UK
SAL.1.47	<i>Salmonella</i> Enteritidis	Poulet
SAL.1.52	<i>Salmonella</i> Enteritidis	Chiffonnette pâtisserie
SAL.1.170	<i>Salmonella</i> Gallinarum	Env. élevage de pintade
SAL.1.171	<i>Salmonella</i> Gallinarum	Elevage de poules
SAL.1.57	<i>Salmonella</i> Hadar	Escalope de volaille
SAL.1.60	<i>Salmonella</i> Havana	Env. atelier (alim. humaine)
SAL.1.61	<i>Salmonella</i> Heidelberg	Viande de volaille
SAL.1.64	<i>Salmonella</i> Indiana	Filet de bœuf
SAL.1.69	<i>Salmonella</i> Infantis	Farine de viande
SAL.1.163	<i>Salmonella</i> Infantis	Lait (alim. humaine)
SAL.1.169	<i>Salmonella</i> Kedougou	Couenne de porc
SAL.1.76	<i>Salmonella</i> Kottbus	Sauté de dinde cru
SAL.1.78	<i>Salmonella</i> Livingstone	Environnement atelier de production
SAL.1.83	<i>Salmonella</i> London	Abattoir de volaille (alim. humaine)
SAL.1.84	<i>Salmonella</i> Manhattan	Bovin
SAL.1.85	<i>Salmonella</i> Mbandaka	Pintadeau
SAL.1.91	<i>Salmonella</i> Montevideo	Tartare pur bœuf
SAL.1.97	<i>Salmonella</i> Napoli	Canard
SAL.1.98	<i>Salmonella</i> Newport	Fromage au lait cru
SAL 1.101	<i>Salmonella</i> Orion	Canard
SAL.1.102	<i>Salmonella</i> Paratyphi A	CIP 55 39
SAL.1.104	<i>Salmonella</i> Paratyphi A	CIP A 220
SAL.1.110	<i>Salmonella</i> Paratyphi B	Filet de poulet cru
SAL.1.111	<i>Salmonella</i> Paratyphi B	Paupiette de lapin cuite
SAL.1.112	<i>Salmonella</i> Paratyphi C	CIP 55.108
SAL.1.114	<i>Salmonella</i> Poona	Environnement atelier (alim. animale)
SAL.1.115	<i>Salmonella</i> Regent	Manchon de canard
SAL.1.116	<i>Salmonella</i> Rissen	Environnement atelier de production
SAL.1.120	<i>Salmonella</i> Saint-Paul	Viande surgelée
SAL.1.121	<i>Salmonella salamae</i>	Lait cru
SAL 1.122	<i>Salmonella</i> Schwarzengrund	Sauté de porc cru
SAL.1.126	<i>Salmonella</i> Senftenberg	Tourteau de soja (alim. animale)
SAL.1.129	<i>Salmonella</i> Typhi	CIP 54 136
SAL.1.131	<i>Salmonella</i> Typhimurium	CIP 104115
SAL.1.147	<i>Salmonella</i> Typhimurium	Cordon bleu surgelé
SAL.1.155	<i>Salmonella</i> Virchow	CIP 105355
SAL.1.158	<i>Salmonella</i> Virchow	11337 (intox)
SAL.1.181	<i>Salmonella bongori</i>	Environnement industriel

### **Exclusivity list**

Code	Microorganism	Origin (French designation)
CIT.1.1	<i>Citrobacter freundii</i>	CIP 53.62
CIT.1.2	<i>Citrobacter freundii</i>	ATCC 8090
CIT.2.4	<i>Citrobacter koseri</i>	Effluent secondaire
CIT.2.1	<i>Citrobacter koseri</i>	CIP 72.11
CIT.2.2	<i>Citrobacter diversus</i>	CIP 82.87 T
CIT.2.3	<i>Citrobacter diversus</i>	CIP 82.94
ENTB.1.1	<i>Enterobacter aerogenes</i>	Industrie laitière
ENTB.2.1	<i>Enterobacter cloacae</i>	Eaux usagées
ENTB.3.1	<i>Enterobacter sakazakii</i>	Poudre de lait
ENTB.3.2	<i>Enterobacter sakazakii</i>	CIP 57.33
ESC.1.6	<i>Escherichia coli</i>	Ravioli poulet
ESC.1.3	<i>Escherichia coli</i>	Industrie laitière
ESC.2.1	<i>Escherichia hermanii</i>	CIP 103176
ESC.3.1	<i>Escherichia vulneris</i>	CIP 103177T
HAF.1.1	<i>Hafnia alvei</i>	Taboulé
HAF.1.2	<i>Hafnia alvei</i>	CNRZ 713
KLE.1.1	<i>Klebsiella oxytoca</i>	Salade soja
KLE.2.1	<i>Klebsiella pneumoniae</i>	Pâtisserie
PAN.1.1	<i>Pantoea agglomerans</i>	A181
PRO.1.1	<i>Proteus mirabilis</i>	CIP 103181
PRO.2.1	<i>Proteus vulgaris</i>	Environnement industrie
PSE.1.2	<i>Pseudomonas aeruginosa</i>	Omelette gruyère
PSE.2.2	<i>Pseudomonas fluorescens</i>	CIP102127
SER.1.1	<i>Serratia ficaria</i>	CIP 79.23
SER.2.1	<i>Serratia fonticola</i>	CIP 103580
SER.3.1	<i>Serratia marcescens</i>	Environnement industrie
SHI.1.1	<i>Shigella flexneri</i>	CIP 82.48T
SHI.2.1	<i>Shigella sonnei</i>	ATCC 9290
PROV.1.1	<i>Providencia stuartii</i>	HPA RM
YER 1.1	<i>Yersinia enterocolitica</i>	CIP 80.27
KLU.1.1	<i>Kluyvera spp</i>	Eau
RAH.1.1	<i>Rahnella aquatilis</i>	CIP 78.65

## Annex 2 - Accordance

**Alternative method**

Number of replicates:

8

Level L0

Laboratory	Number of positive	Probability of positive	Probability of paired positive	Probability of negative	Probability of paired negative	Probability of identical paired results
A	0	0,000	0,000	1,000	1,000	1,000
B	0	0,000	0,000	1,000	1,000	1,000
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
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
Mean						100,0%

Level L1

Laboratory	Number of positive	Probability of positive	Probability of paired positive	Probability of negative	Probability of paired negative	Probability of identical paired results
A	7	0,875	0,766	0,125	0,016	0,781
B	8	1,000	1,000	0,000	0,000	1,000
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	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
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	7	0,875	0,766	0,125	0,016	0,781
M	7	0,875	0,766	0,125	0,016	0,781
Mean						92,7%

Level L2

Laboratory	Number of positive	Probability of positive	Probability of paired positive	Probability of negative	Probability of paired negative	Probability of identical paired results
A	8	1,000	1,000	0,000	0,000	1,000
B	8	1,000	1,000	0,000	0,000	1,000
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
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
Mean						100,0%

**Reference method**

Number of replicates:

8

Level L0

Laboratory	Number of positive	Probability of positive	Probability of paired positive	Probability of negative	Probability of paired negative	Probability of identical paired results
A	0	0,000	0,000	1,000	1,000	1,000
B	0	0,000	0,000	1,000	1,000	1,000
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
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
Mean						100,0%

Level L1

Laboratory	Number of positive	Probability of positive	Probability of paired positive	Probability of negative	Probability of paired negative	Probability of identical paired results
A	7	0,875	0,766	0,125	0,016	0,781
B	8	1,000	1,000	0,000	0,000	1,000
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	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
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	7	0,875	0,766	0,125	0,016	0,781
M	8	1,000	1,000	0,000	0,000	1,000
Mean						94,5%

Level L2

Laboratory	Number of positive	Probability of positive	Probability of paired positive	Probability of negative	Probability of paired negative	Probability of identical paired results
A	8	1,000	1,000	0,000	0,000	1,000
B	8	1,000	1,000	0,000	0,000	1,000
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
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
Mean						100,0%

## Annex 3 - Concordance

### Alternative method

Level L0	Laboratory	Number of negative	Interlaboratory pairs with the same results	Total number of interlaboratory pairs
	A	8	704	704
	B	8	704	704
	C	8	704	704
	D	8	704	704
	E	8	704	704
	F	8	704	704
	G	8	704	704
	H	8	704	704
	J	8	704	704
	K	8	704	704
	L	8	704	704
	M	8	704	704
	Total		8448	8448
	Concordance			100,0%

Level L1	Laboratory	Number of positive	Interlaboratory pairs with the same results	Total number of interlaboratory pairs
	A	7	598	704
	B	8	672	704
	C	8	672	704
	D	7	598	704
	E	8	672	704
	F	8	672	704
	G	8	672	704
	H	8	672	704
	J	8	672	704
	K	8	672	704
	L	7	598	704
	M	7	598	704
	Total		7768	8448
	Concordance			92,0%

Level L2	Laboratory	Number of positive	Interlaboratory pairs with the same results	Total number of interlaboratory pairs
	A	8	704	704
	C	8	704	704
	D	8	704	704
	E	8	704	704
	F	8	704	704
	G	8	704	704
	H	8	704	704
	I	8	704	704
	J	8	704	704
	K	8	704	704
	L	8	704	704
	M	8	704	704
	Total		8448	8448
	Concordance			100,0%

**Reference method**

Level L0

Laboratory	Number of negative	Interlaboratory pairs with the same results	Total number of interlaboratory pairs
A	8	704	704
B	8	704	704
C	8	704	704
D	8	704	704
E	8	704	704
F	8	704	704
G	8	704	704
H	8	704	704
J	8	704	704
K	8	704	704
L	8	704	704
M	8	704	704
Total		8448	8448
Concordance		100,0%	

Level L1

Laboratory	Number of positive	Interlaboratory pairs with the same results	Total number of interlaboratory pairs
A	7	604	704
B	8	680	704
C	8	680	704
D	7	604	704
E	8	680	704
F	8	680	704
G	8	680	704
H	8	680	704
J	8	680	704
K	8	680	704
L	7	604	704
M	8	680	704
Total		7932	8448
Concordance		93,9%	

Level L2

Laboratory	Number of positive	Interlaboratory pairs with the same results	Total number of interlaboratory pairs
A	8	704	704
B	8	704	704
C	8	704	704
D	8	704	704
E	8	704	704
F	8	704	704
G	8	704	704
H	8	704	704
J	8	704	704
K	8	704	704
L	8	704	704
M	8	704	704
Total		8448	8448
Concordance		100,0%	