

**AFNOR CERTIFICATION VALIDATION STUDY  
RAPIDCHEK™ SELECT™ SALMONELLA TEST  
SYSTEM FOR THE DETECTION OF  
SALMONELLA SPP IN FOOD, FEED AND  
ENVIRONMENTAL SAMPLES**

SYNTHESIS REPORT

RAPIDCHEK™ SELECT™ SALMONELLA TEST SYSTEM - S.R.(V0) - JUNE 2010



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Validation standard: NF EN ISO 16140 (October 2003): Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods

Alternative method: RapidChek™ SELECT™ *Salmonella* test system for the detection of *Salmonella spp*

Scope of validation: food, feed and environmental samples

Reference method (\*): Horizontal method for the detection of *Salmonella spp* – NF EN ISO 6579 (2002)

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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 NF EN ISO 6579 (2002). It consists in a preliminary study and a collaborative study.

### 1.2. Alternative method

The RapidChek™ SELECT™ *Salmonella* Lateral Flow Test Kit is designed to detect *Salmonella spp.* The test kit permits the presumptive detection of the target pathogen after 24 hours of enrichment when present in sample. The protocol of the method is showed in figure 1.

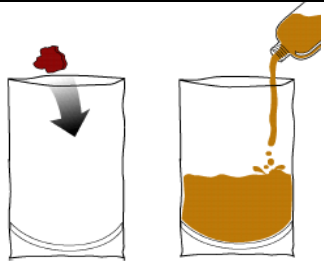
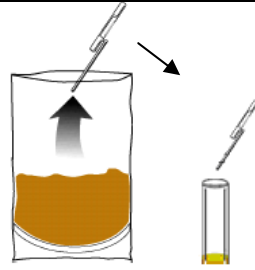

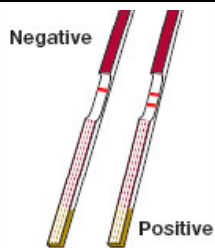
<b>Sample enrichment</b>	25 g of sample + 225 mL RapidChek SELECT <i>Salmonella</i> supplemented Primary Medium prewarmed at 41,5°C	
	Incubation at (41,5±1) °C for 16-22 hours	
	Transfer 0,1 mL of enriched broth to a plastic tube containing 1 mL of RapidChek SELECT Secondary Medium (pre-warmed at 41,5°C)	
Incubation at (41,5±1)°C for 6-8 hours		
<b>RapidChek™ SELECT™ Salmonella Test</b>	Insert the strip into the tube Let the strip develop for 10 min	
<b>Result interpretation</b>	One red line = negative result Two red lines = positive result  (A control line indicates that the strip is functioning properly)	
<b>Confirmation of presumptive positive sample</b>	Streaking from RapidChek SELECT Secondary medium to selective agar plates followed by confirmation tests described in NF EN ISO 6579 (2002) standard	

Figure 1: alternative method protocol

- **Principle of the assay**

The test uses novel, highly selective enrichment technologies coupled to immunochemical detection of *Salmonella*. The immunoassay test uses a lateral flow test strip in a double antibody sandwich format. A specific *Salmonella* antibody that is immobilized on the

surface of the test strip membrane comprises the "test line". A second antibody reagent that also recognizes *Salmonella* is labelled with colloidal gold and is contained within a reagent pad upstream from (below) the test line.

As the sample moves by capillary action from the filter pad into the antibody-gold pad, the antibody-gold reagent specifically binds *Salmonella* (if present in the sample) and moves with the liquid sample into the membrane. The sample passes through the test line where the immobilized *Salmonella* antibody captures the antibody-gold-antigen complex, causing the formation of an antibody-antigen "sandwich" and the development of red colour at the test line. Antibody-antigen sandwiches are not formed in the absence of *Salmonella*, resulting in no red colour development at the test line.

Reagents immobilized at the control line capture excess gold reagent passing through the test line. The presence of red colour at the control line indicates that the test strip flowed correctly. Therefore, the presence of only one line (control line) on the membrane indicates a negative sample and the presence of two lines indicates a positive sample.

- **Other validation**

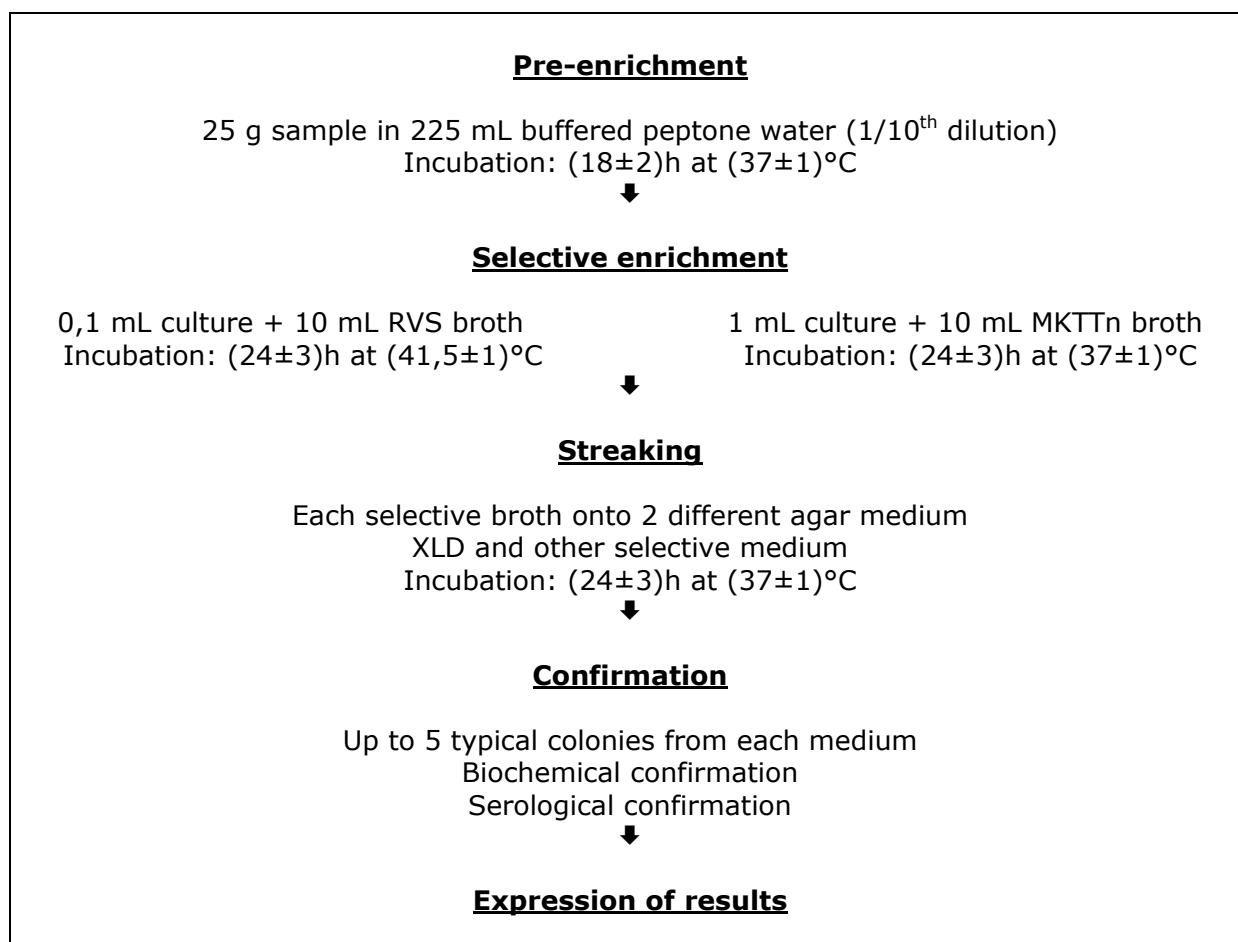
The RapidChek™ SELECT™ *Salmonella* Test System has been AOAC certified in 2006 (License number 080601).

### **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. Methods comparison 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 and 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 and vegetable products, egg products, feedstuffs and environmental samples.

A number of 380 samples was analysed. Types of products are indicated in table 1.

<b>Category</b>	<b>Type</b>	<b>Number of positive<sup>a</sup></b>	<b>Number of negative</b>	<b>Total</b>
<b>Meat products</b>	Raw poultry	11	9	<b>20</b>
	Raw beef meat	13	8	<b>21</b>
	Variety meat	2	0	<b>2</b>
	Delicatessen	4	8	<b>12</b>
	Other raw meat	0	4	<b>4</b>
	Cooked meat	1	6	<b>7</b>
	<b>Total</b>	<b>31</b>	<b>35</b>	<b>66</b>
<b>Dairy products</b>	Raw milk cheese	11	9	<b>20</b>
	Pasteurized milk cheese	4	6	<b>10</b>
	Milk	5	8	<b>13</b>
	Yoghourts	10	7	<b>17</b>
	Other dairy products	1	4	<b>5</b>
	<b>Total</b>	<b>31</b>	<b>34</b>	<b>65</b>
<b>Seafood and vegetable products</b>	Raw and cooked fruit and vegetables	13	21	<b>34</b>
	Frozen fruit and vegetables	6	2	<b>8</b>
	Fresh seafood	7	7	<b>14</b>
	Smoked/frozen/cooked seafood	5	3	<b>8</b>
	<b>Total</b>	<b>31</b>	<b>33</b>	<b>64</b>
<b>Egg products</b>	Eggs and derived	17	9	<b>26</b>
	Varied products	13	21	<b>34</b>
	<b>Total</b>	<b>30</b>	<b>30</b>	<b>60</b>
<b>Feedstuffs</b>	Dog food	8	4	<b>12</b>
	Cat food	17	17	<b>34</b>
	Other pet food	8	9	<b>17</b>
	<b>Total</b>	<b>33</b>	<b>30</b>	<b>63</b>
<b>Environmental samples</b>	Process waters	5	10	<b>15</b>
	Sampling swabs	25	22	<b>47</b>
	<b>Total</b>	<b>30</b>	<b>32</b>	<b>62</b>

**Table 1:** nature and number of analysed samples (a=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 –ASAP-).

Among positive results, samples spiking was realised on 136 samples so 26.9% of naturally contaminated samples were analysed.

### 2.1.3. Confirmation protocol

For alternative method, confirmation of presumed positive samples was performed from RapidChek™ SELECT™ *Salmonella* Secondary Medium broth. Plating out on XLD and ASAP agar was realised. After incubation of the media in adequate conditions, typical colonies were isolated on nutrient agar and analysed by biochemical and serological tests.

### 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> negative (R-)
Meat products	Alternative method positive (A+)	PA=25	PD=2
	Alternative method negative (A-)	ND=4 including 0 PPND	NA=35 including 2 PPNA
Dairy products	Alternative method positive (A+)	PA=26	PD=3
	Alternative method negative (A-)	ND=2 including 1 PPND	NA=34 including 1 PPNA
Seafood and vegetable products	Alternative method positive (A+)	PA=29	PD=0
	Alternative method negative (A-)	ND=2 including 1 PPND	NA=33 including 1 PPNA
Egg products	Alternative method positive (A+)	PA=28	PD=1
	Alternative method negative (A-)	ND=1 including 0 PPND	NA=30 including 7 PPNA
Feedstuffs	Alternative method positive (A+)	PA=31	PD=0
	Alternative method negative (A-)	ND=2 including 0 PPND	NA=30 including 3 PPNA
Environmental samples	Alternative method positive (A+)	PA=29	PD=0
	Alternative method negative (A-)	ND=1 including 1 PPND	NA=32 including 8 PPNA
All products	Alternative method positive (A+)	PA=171	PD=6
	Alternative method negative (A-)	ND=12 including 3 PPND	NA=194 including 22 PPNA

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

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

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

Category	PA	NA	ND	PD	N	Relative accuracy AC [(PA+NA)/N]	N+ PA+ND	Relative sensitivity SE [PA/N+]	N- NA+PD	Relative specificity SP [NA/N-]
Meat products	25	35	4	2	66	90.9%	29	86.2%	37	94.6%
Dairy products	26	34	2	3	65	92.3%	28	92.9%	37	91.9%
Seafood and vegetable products	29	33	2	0	64	96.9%	31	93.5%	33	100.0%
Egg products	28	30	1	1	60	96.7%	29	96.6%	31	96.8%
Feedstuffs	31	30	2	0	63	96.8%	33	93.9%	30	100.0%
Environmental samples	29	32	1	0	62	98.4%	30	96.7%	32	100.0%
All products	168	194	12	6	380	95.3%	180	93.3%	200	97.0%

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

Criteria values in percent are shown in table 4.

	Alternative method
Relative accuracy	95.3 %
Relative sensitivity	93.3 %
Relative specificity	97.0 %

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

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

	Alternative method (PA+PD)/(PA+PD+ND)	Reference method (PA+ND)/(PA+PD+ND)
Sensitivity	93.5 %	96.8 %

**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. This analysis is presented in table 6.

	Alternative method
Y = PD + ND	Y = 12 + 6 = 18
m	6
M (for 15 ≤ Y ≤ 16)	4
Conclusion	m > M the methods are not different at α = 0.05

**Table 6:** statistical analysis of discordant results

The RapidChek™ SELECT™ *Salmonella* Test system and the reference method NF EN ISO 6579 can be statistically considered as equivalent.

- **Negative deviations**

For six samples, the reference method gave a positive result while the alternative method result was negative. However the streaking of the RapidChek Primary Medium on selective agar media showed the absence of typical colonies. As the first step of the methods (the enrichment broth) is different, it is possible that no cell of *Salmonella* was present in the test portion analysed with the alternative method.

For three samples, the reference method gave a positive result while the alternative method result was negative (false positive results). However the streaking of the RapidChek Primary Medium gave typical colonies on one or both selective agar media. Two hypotheses seem possible: a lack of sensitivity of the test in a low burden enrichment broth or a lack of selectivity towards a *Salmonella* strain.

For three samples, the reference method and the alternative method gave positive results (presumed positive, negative deviation results). However the streaking of the RapidChek Primary Medium on selective agar media showed the absence of typical colonies. As the first step of the methods (the enrichment broth) is different, it is possible that no cell of *Salmonella* was present in the test portion analysed with the alternative method. The positive reaction was probably obtained either by a cross-reaction or by a reaction with a very low burden of *Salmonella*.

- **Positive deviations**

For six samples, the alternative method gave a positive result while the reference method result was negative. As the first step of the methods (the enrichment broth) is different, it is possible that no cell of *Salmonella* was present in the test portion analysed with the reference method.

## **2.2. Relative detection level**

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

### **2.2.1. Matrices**

Six couples "matrix-strain" were studied in parallel with the reference method and the alternative method for all categories. 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	<i>S. Typhimurium</i>	SAL.1.133	Raw minced meat
Raw milk	<i>S. Newport</i>	SAL.1.98	Raw milk cheese
Raw fish	<i>S. London</i>	SAL.1.80	Shelled winkle
Egg	<i>S. Enteritidis</i>	SAL.1.48	Egg product
Cat food	<i>S. Infantis</i>	SAL.1.69	Meat meal
Process water	<i>S. Mbandaka</i>	SAL.1.85	Young guinea fowl

**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  $\frac{1}{2}$  and  $\frac{1}{4}$  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
<i>S. Typhimurium</i>	Minced meat	0.879 [0.725 ; 1.065]	1.156 [0.866 ; 1.542]
<i>S. Newport</i>	Raw milk	0.566 [0.424 ; 0.755]	0.666 [0.496 ; 0.882]
<i>S. London</i>	Raw fish	0.687 [0.595 ; 0.794]	0.878 [0.658 ; 1.171]
<i>S. Enteritidis</i>	Egg	0.557 [0.361 ; 0.858]	0.698 [0.453 ; 1.075]
<i>S. Infantis</i>	Cat food	0.892 [0.552 ; 1.442]	1.301 [0.767 ; 2.206]
<i>S. Mbandaka</i>	Process water	0.907 [0.648 ; 1.127]	0.830 [0.593 ; 1.161]

**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
<i>S. Typhimurium</i>	Minced meat	0.9 [0.7 ; 1.1]	1.2 [0.9 ; 1.5]
<i>S. Newport</i>	Raw milk	0.6 [0.4 ; 0.8]	0.7 [0.5 ; 0.9]
<i>S. London</i>	Raw fish	0.7 [0.6 ; 0.8]	0.9 [0.7 ; 1.2]
<i>S. Enteritidis</i>	Eggs product	0.6 [0.4 ; 0.9]	0.7 [0.5 ; 1.1]
<i>S. Infantis</i>	Cat food	0.9 [0.6 ; 1.4]	1.3 [0.8 ; 2.2]
<i>S. Mbandaka</i>	Process water	0.9 [0.6 ; 1.1]	0.8 [0.6 ; 1.2]

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

The alternative and the reference method show similar detection levels. The detection limit obtained with the alternative method is comprised between 0.5 and 2.2 cells per 25 g. The detection limit obtained with the reference method is comprised between 0.4 and 1.4 cells per 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 30 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 RapidCheck™ SELECT™ *Salmonella* Primary Medium (about 1 to 100 CFU/225 mL). The complete protocol of alternative method was applied with the minimum time of incubation (16 hours for Primary Medium and 6 hours for Secondary Medium).

- **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  $10^5$  CFU/mL). After 24 hours of incubation, the RapidChek™ SELECT™ *Salmonella* 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

- **Inclusivity**

51 *Salmonella* strains were tested:

- 43 strains gave a positive result
- 5 strains did not grow in RapidChek Primary Medium
- 3 strains gave a negative result

Complementary tests were performed on the 8 strains which gave an unexpected result (see table in annex). Suspensions of *Salmonella* were inoculated at a level comprised between 30 and 60 CFU in 25 mL of pasteurized milk then diluted at  $1/10^{\text{th}}$  on RapidChek Primary Medium. The test was then carried out.

Among the 5 strains which did not grow in Primary Medium, 4 gave a positive result. Only a strain of *Salmonella* Typhimurium gave a negative result.

Among the 3 strains which gave a negative result, one gave a positive result. A strain of *S. Cerro* and a strain of *S. Arizonae* showed a negative result.

The reference method was positive for the final 3 strains which gave a negative result by the alternative method.

Other tests were performed on these specific serovars. Another strain of *S. Arizonae* was tested for the inclusivity test and gave a positive result (SAL.1.7).

Three other strains of *S. Typhimurium* were tested and all gave a positive result.

A strain of *S. Cerro* from an American research laboratory gave a negative result.

After presentation of these results, the Technical Committee asked the expert laboratory to test more *Salmonella* from group 18. Four strains, provided by the AFSSA (Maisons-Alfort, France), were tested by the alternative method:

- Salmonella* Cerro (6,14,18:z4,z23:-), isolated from meat powder
- Salmonella* Cerro (6,14,18:z4,z23:-), isolated in a dairy industry
- Salmonella* Carnac, isolated from a poultry drinker
- Salmonella* Toulon, isolated from a dog

These strains gave a negative result by alternative method, even with the other protocol explained above (suspension with milk and a higher level of bacteria). The reference method was positive for these four strains.

- **Exclusivity**

30 non-target strains were tested. No cross reaction was observed.

### 2.3.3. Conclusion

The selectivity of the method is satisfactory. No cross reactions were observed with non-target strains. However all serovars from group 18 of *Salmonella* gave a negative result.

### **3. Collaborative study**

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

#### **3.1. Collaborative study implementation**

##### 3.1.1. Participating laboratories

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

##### 3.1.2. *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 (0.5mL 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* Enteritidis (ISHA code: SAL.1.48), a wild strain isolated from an egg product.

The detection of *Salmonella* spp was realized after inoculation of about 10 cells in 25 mL of pasteurized milk. The samples were analysed at D0, D+1, D+2 and D+3 by reference method and 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.1.48 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 48 samples to analyse, 1 sample to quantify the endogenous microflora and 1 water sample containing a temperature probe.

The results of the enumerations of the TVC, the target levels and the real levels of contamination are presented in table 10.

<b>Matrix</b>	<b>TVC (CFU/mL)</b>	<b>Target level (cells/25 mL)</b>	<b>Real level (cells/25 mL)</b>	<b>Confidence interval</b>
Supplemented pasteurized milk	4,0.10 <sup>3</sup>	0	0	0
		3	5	[ 1 ; 9 ]
		30	33	[ 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 N (cf. table 11) and a code to identify each sample, only known by the expert laboratory. The samples and the temperature control vials (water sample with a temperature probe) were stored at 4°C before shipping.

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

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

### 3.1.6. Samples shipping

The samples were shipped in a coolbox on Monday the 18<sup>th</sup> of January 2010. The transport has been entrusted to Chronopost International.

### 3.1.7. Samples reception and analysis

The coolboxes were received the 19<sup>th</sup> of January for 7 laboratories and the 20<sup>th</sup> of January for 5 laboratories. The laboratories D and I never received the coolboxes; they are consequently excluded of the results list.

The control temperature was recorded upon receipt of the package and the temperature probe sent to the expert laboratory. The samples were analysed on the 20<sup>th</sup> of January 2010 by twelve participating laboratories.

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	Non determined	Correct
B	5.0	Correct
C	7.1	Correct
E	8.1	Correct
F	7.4	Correct
G	Non determined	Correct
H	3.7	Correct
J	2.7	Correct
K	4.8	Correct
L	3.1	Correct
M	10.8	Correct
N	5.0	Correct

**Table 12:** temperature and state of the samples upon reception

The temperature measurements are inferior to 8.4°C for 9 laboratories. For 2 laboratories (A and G), the temperature was not mentioned and for 1 laboratory (M), the temperature at reception was at 10.8°C. However the temperature probes indicate correct mean temperatures between the shipping and the reception of the coolbox for these 3 laboratories. The analysis of thermal profiles is shown in table 13.

Laboratory		A	B	C	E	F	G	H	J	K	L	M	N
Temperature (°C)	Mean	5.8	2.4	2.9	2.8	2.6	3.0	3.4	2.5	2.6	2.8	2.9	2.5
	Standard deviation	0.8	0.4	0.5	0.6	0.8	0.8	0.9	0.8	0.6	0.5	0.5	0.6

**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 2.4 and 5.8°C.

### 3.2.2. Total viable counts

For the whole laboratories, the total viable counts at 30°C vary between 1800 and 12000 CFU/mL.

### 3.2.3. Expert laboratory results

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

Contamination level	Alternative method	Reference method
L0	0/8	0/8
L1	8/8	8/8
L2	8/8	8/8

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

The results are consistent with those expected.

### 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	4/8	4/8
B	0/8	8/8	8/8
C	0/8	8/8	8/8
E	0/8	8/8	8/8
F	0/8	8/8	8/8
G	0/8	8/8	8/8
H	0/8	8/8	8/8
J	0/8	8/8	8/8
K	0/8	8/8	8/8
L	0/8	8/8	8/8
M	0/8	8/8	8/8
N	0/8	8/8	8/8

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

- Reference method results

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

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

- Results analysis

Results are consistent with those expected for all laboratories, except for the laboratory A which found only 4 positive on 8 at level L1 and L2 with the alternative method. This

laboratory has eliminated all its samples just after the analysis so the expert laboratory could not ask it for further analysis. However, after discussion with this laboratory, it seems that a mistake appeared somewhere in the protocol which lead to the non detection of 8 spiked samples (4 at L1 and 4 at L2).

According to this finding, the expert laboratory proposed to exclude the results of laboratory A of the statistical analysis of the results. This proposition was accepted by the Technical Committee.

Final analysis was consequently conducted using data supplied by eleven 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>	100%	98%	100%	98%
<b>SE (level L2)</b>	100%	98%	100%	98%
<b>SE (level L1+L2)</b>	100%	98%	100%	98%

**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
<b>L0</b>	<b>AM+</b>	PA=0	PD=0	0
	<b>AM-</b>	ND=0	NA=88	88
	<b>Total</b>	0	88	88
<b>L1</b>	<b>AM+</b>	PA=88	PD=0	88
	<b>AM-</b>	ND=0	NA=0	0
	<b>Total</b>	88	0	88
<b>L2</b>	<b>AM+</b>	PA=88	PD=0	88
	<b>AM-</b>	ND=0	NA=0	0
	<b>Total</b>	88	0	88
<b>L0+L1+L2</b>	<b>AM+</b>	PA=176	PD=0	176
	<b>AM-</b>	ND=0	NA=88	88
	<b>Total</b>	176	88	264

**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%
<b>L1</b>	100%	98%
<b>L2</b>	100%	98%
<b>L1+L2</b>	100%	98%
<b>Total</b>	100%	98%

**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,  $Y = 0$ , so  $Y < 6$ , no tests are available. The 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>	100%	100%
<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>	100%	100%
<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	COR	Accordance	Concordance	COR
L0	100	100	1,0	100	100	1,0
L1	100	100	1,0	100	100	1,0
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	95.3%	100%
SP	97.0%	100%
SE	93.3%	100%

**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)
100%	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

The RapidChek™ SELECT™ *Salmonella* test kit contains:

- 50 Test strips in a plastic canister with a moisture indicator card enclosed
- 50 test tubes
- 50 transfer pipettes
- RapidChek SELECT Primary media 500 g
- RapidChek SELECT Primary supplement 250 mL
- RapidChek SELECT Secondary media 10 g

### 2- Volume of reagents

- RapidChek SELECT Primary media 500 g
- RapidChek SELECT Primary supplement 250 mL
- RapidChek SELECT Secondary media 10 g

### 3- Storage conditions of components and shelf-life of unopened products (expiration of not opened products)

The RapidChek SELECT Primary supplement should be refrigerated (2-8°C).

The RapidChek SELECT Primary and Secondary media and test strips should be stored at room temperature.

The expiration date is shown on the product label. The product has one year shelf life from the date of manufacture under desiccated room temperature conditions.

### 4- Modalities after first use

The RapidChek SELECT media supplement should be refrigerated (2-8°C).

After opening the canister, care should be taken to re-close after use to protect the test strips from moisture.

### 5- Equipment and specific local requirements

Among the required equipment (paragraph materials required but not supplied):

- Incubator capable of maintaining  $41.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$
- Sample bags
- Balance
- Stomacher machine

### 6- Reagents ready to use or for reconstitution

Reconstitution of RapidChek SELECT media.

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

Less than 1 day is required for technicians with microbiology knowledge.

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

Step	Alternative method		Reference method	
	1 analysis	20 analyses	1 analysis	20 analyses
Sample enrichment	7	80	7	80
Second enrichment	0.5	8	1	16
RapidCheck™ SELECT™ <i>Salmonella</i> test	0.25	1	/	/
Isolation on selective agar media	/	/	1.5	20
Reading	0.25	2	0.8	16
Total	8	91	10.3	132

Step	Alternative method		Reference method	
	1 analysis	20 analyses	1 analysis	20 analyses
Sample enrichment	7	80	7	80
Second enrichment	0.5	8	1	16
RapidCheck™ SELECT™ <i>Salmonella</i> test	0.25	1	/	/
Isolation on selective agar media	0.8	12	1.5	20
Biochemical confirmation	16	200	16	200
Serological confirmation	3	50	3	50
Total	27.55	351	28.5	366

#### 9- Time required for results

Step	Alternative method	Reference method
Sample enrichment (BPW or RapidCheck™ SELECT™ Primary Medium)	J0	J0
Selective enrichment (RVS and MKTTn or RapidCheck™ SELECT™ Secondary Medium)	J1	J1
RapidCheck™ SELECT™ <i>Salmonella</i> test	J1	/
Isolation on selective agar media	/	J2
Reading	/	J3
Total	J1	J3

Step	Alternative method	Reference method
Sample enrichment (BPW or RapidCheck™ SELECT™ Primary Medium)	J0	J0
Selective enrichment (RVS and MKTTn or RapidCheck™ SELECT™ Secondary Medium)	J1	J1
RapidCheck™ SELECT™ <i>Salmonella</i> test	J1	/
Isolation on selective agar media	J1	J2
Reading and isolation on non-selective agar	J2	J3
Confirmation test	J3 to J4	J4 to J5
Total	J4	J5

#### 10- Operator qualification

Identical as necessary for the reference method

#### 11- Steps common with the reference method

None.

#### 12- Traceability of analysis results

Usual traceability applied in a laboratory

#### 13- Maintenance by laboratory

None.

## **5. Conclusion**

Concerning the preliminary study, the performances of the RapidChek™ SELECT™ *Salmonella* test system for the detection of *Salmonella spp* are comparable to those of the method NF EN ISO 6579. This study concerned 380 samples of six categories of products (meat, dairy, egg, seafood and vegetable, feedstuffs and environmental products).

Values obtained for the 3 criteria are the following:

- relative accuracy: 95.3%
- relative sensitivity: 93.3%
- relative specificity: 97.0 %

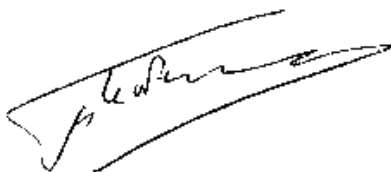
Several discordant results were observed. Mostly of them may be explained because the first culture step of each method differs. Consequently, 2 replicates were prepared for each sample. Because of the low level of artificial contamination, it is possible that no cell of *Salmonella* was present in the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

The relative level of detection of the alternative method and the reference method was evaluated for all categories. The results are comparable because the detection limit of the alternative method varies from 0.5 to 2.2 CFU /25 g and the detection limit of the reference method varies from 0.4 to 1.4 CFU/25 g for all categories.

The specificity of the method is satisfactory. No cross reactions were observed with non-target strains. All the tested serovars of *Salmonella* from group 18 were not detected by the alternative method at the incubation times specified in the protocol.

Concerning the interlaboratory study, the results obtained for the 11 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.



Massy, 2010, the 3<sup>rd</sup> of June  
François Le Nestour  
Research Engineer

## **ANNEX 1 - SELECTIVITY**

### Exclusivity

ISHA code	Microorganism	Origin
AER.1.2	<i>Aeromonas hydrophila</i>	Smoked salmon
AER.2.1	<i>Aeromonas salmonicida</i>	CIP 63.4
CIT.1.1	<i>Citrobacter freundii</i>	CIP 53.62
CIT.1.2	<i>Citrobacter freundii</i>	ATCC 8090
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>	Dairy product
ENTB.2.1	<i>Enterobacter cloacae</i>	Process water
ENTB.3.1	<i>Enterobacter sakazakii</i>	Milk powder
ENTB.3.2	<i>Enterobacter sakazakii</i>	CIP 57.33
ENTB.3.3	<i>Enterobacter sakazakii</i>	CIP 103581
ESC.1.3	<i>Escherichia coli</i>	Milk industry
ESC.1.26	<i>Escherichia coli</i>	Cress sauce
ESC.2.1	<i>Escherichia hermanii</i>	CIP 103176
ESC.2.2	<i>Escherichia hermanii</i>	/
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>	Soy salad
KLE.2.1	<i>Klebsiella pneumoniae</i>	Pastry
PAN.1.1	<i>Pantoea agglomerans</i>	A181
PRO.1.1	<i>Proteus mirabilis</i>	CIP 103181
PSE.1.2	<i>Pseudomonas aeruginosa</i>	Cheese omelet
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
SHI.1.1	<i>Shigella flexneri</i>	CIP 82.48T
SHI.2.1	<i>Shigella sonnei</i>	ATCC 9290
YER 1.2	<i>Yersinia enterocolitica</i>	ATCC 9610

## Inclusivity

ISHA code	Microorganism	Origin
SAL.1.5	<i>Salmonella</i> Anatum	Sesame
SAL.1.7	<i>Salmonella</i> Arizonae (48 : z4, z23 :-)	Duck
SAL.1.8	<i>Salmonella</i> Arizonae (18 : z4, z23 :-)	Duck
SAL.1.10	<i>Salmonella</i> Braenderup	Environment
SAL.1.17	<i>Salmonella</i> Brandenburg	Duck
SAL.1.21	<i>Salmonella</i> Bredeney	Chicken
SAL.1.23	<i>Salmonella</i> Cerro	Flour of rabbit
SAL.1.29	<i>Salmonella</i> Derby	Pork
SAL.1.38	<i>Salmonella</i> Derby	Swabs
SAL.1.40	<i>Salmonella</i> Diarizonae	Semolina
SAL.1.41	<i>Salmonella</i> Diarizonae	Semolina
SAL.1.42	<i>Salmonella</i> Diarizonae	Waste water
SAL.1.43	<i>Salmonella</i> Dublin	Milk
SAL.1.47	<i>Salmonella</i> Enteritidis	Chicken
SAL.1.52	<i>Salmonella</i> Enteritidis	Pastry swab
SAL.1.170	<i>Salmonella</i> Gallinarum	Poultry industry
SAL.1.171	<i>Salmonella</i> Gallinarum	Chicken industry
SAL.1.57	<i>Salmonella</i> Hadar	Poultry
SAL.1.60	<i>Salmonella</i> Havana	Environment
SAL.1.61	<i>Salmonella</i> Heidelberg	Poultry
SAL.1.64	<i>Salmonella</i> Indiana	Beef
SAL.1.66	<i>Salmonella</i> Infantis	ATCC 51741
SAL.1.69	<i>Salmonella</i> Infantis	Flour of meat
SAL.1.163	<i>Salmonella</i> Infantis	Milk
SAL.1.169	<i>Salmonella</i> Kedougou	Pork
SAL.1.76	<i>Salmonella</i> Kottbus	Turkey
SAL.1.78	<i>Salmonella</i> Livingstone	Environment
SAL.1.83	<i>Salmonella</i> London	Poultry industry
SAL.1.84	<i>Salmonella</i> Manhattan	Bovin industry
SAL.1.85	<i>Salmonella</i> Mbandaka	Poultry
SAL.1.91	<i>Salmonella</i> Montevideo	Beef (tartare)
SAL.1.97	<i>Salmonella</i> Napoli	Duck
SAL.1.98	<i>Salmonella</i> Newport	Raw milk cheese
SAL.1.102	<i>Salmonella</i> Paratyphi A	CIP 55 39

### Inclusivity

ISHA code	Microorganism	Origin
SAL.1.104	<i>Salmonella</i> Paratyphi A	CIP A 220
SAL.1.105	<i>Salmonella</i> Paratyphi A	CIP 55.41
SAL.1.107	<i>Salmonella</i> Paratyphi B	CIP 54 100
SAL.1.110	<i>Salmonella</i> Paratyphi B	Chicken
SAL.1.111	<i>Salmonella</i> Paratyphi B	Rabbit
SAL.1.112	<i>Salmonella</i> Paratyphi C	CIP 55.108
SAL.1.114	<i>Salmonella enterica</i> Poona	Feed industry
SAL.1.115	<i>Salmonella</i> Regent	Duck
SAL.1.116	<i>Salmonella</i> Rissen	Environment
SAL.1.120	<i>Salmonella</i> Saint-Paul	Frozen beef
SAL.1.122	<i>Salmonella enterica</i> Schwarzengrund	Pork sauté
SAL.1.126	<i>Salmonella</i> Senftenberg	Flour of soya
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	Frozen meat
SAL.1.155	<i>Salmonella</i> Virchow	CIP 105355
SAL.1.158	<i>Salmonella</i> Virchow	11337 (intox)

+(\*): weak coloration

### Complementary tests

ISHA code	Microorganism	Origin
SAL.1.133	<i>Salmonella</i> Typhimurium	Raw beef meat
SAL.1.136	<i>Salmonella</i> Typhimurium	Pigeon
SAL.1.137	<i>Salmonella</i> Typhimurium	Pork
SAL.1.110	<i>Salmonella</i> Cerro	American laboratory

## Annex 5 - Accordance calculation

### Alternative method

Number of replicates:

8

Level L0	Laboratory	Number of positive	Probability of positive	Probability of pair of positives	Probability of negative	Probability of pair of negatives	Probability of pair of same results
	B	0	0,000	0,000	1,000	1,000	1,000
	C	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
	N	0	0,000	0,000	1,000	1,000	1,000
	Mean						100,0%

Level L1	Laboratory	Number of positive	Probability of positive	Probability of pair of positives	Probability of negative	Probability of pair of negatives	Probability of pair of same results
	B	8	1,000	1,000	0,000	0,000	1,000
	C	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
	N	8	1,000	1,000	0,000	0,000	1,000
	Mean						100,0%

Level L2	Laboratory	Number of positive	Probability of positive	Probability of pair of positives	Probability of negative	Probability of pair of negatives	Probability of pair of same results
	B	8	1,000	1,000	0,000	0,000	1,000
	C	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
	N	8	1,000	1,000	0,000	0,000	1,000
	Mean						100,0%

**Reference method**

Number of replicates:

8

Level L0

Laboratory	Number of positive	Probability of positive	Probability of pair of positives	Probability of negative	Probability of pair of negatives	Probability of pair of same results
B	0	0,000	0,000	1,000	1,000	1,000
C	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
N	0	0,000	0,000	1,000	1,000	1,000
Mean						100,0%

Level L1

Laboratory	Number of positive	Probability of positive	Probability of pair of positives	Probability of negative	Probability of pair of negatives	Probability of pair of same results
B	8	1,000	1,000	0,000	0,000	1,000
C	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
N	8	1,000	1,000	0,000	0,000	1,000
Mean						100,0%

Level L2

Laboratory	Number of positive	Probability of positive	Probability of pair of positives	Probability of negative	Probability of pair of negatives	Probability of pair of same results
B	8	1,000	1,000	0,000	0,000	1,000
C	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
N	8	1,000	1,000	0,000	0,000	1,000
Mean						100,0%

## Annex 3 - Concordance calculation

### Alternative method

Level L0	Laboratory	Number of negative	Between-lab pairings with the same results	Total between-lab pairings
	B	8	640	640
	C	8	640	640
	E	8	640	640
	F	8	640	640
	G	8	640	640
	H	8	640	640
	J	8	640	640
	K	8	640	640
	L	8	640	640
	M	8	640	640
	N	8	640	640
	<b>Total</b>		<b>7040</b>	<b>7040</b>
	<b>Concordance</b>		<b>100,0%</b>	

Level L1	Laboratory	Number of positive	Between-lab pairings with the same results	Total between-lab pairings
	B	8	640	640
	C	8	640	640
	E	8	640	640
	F	8	640	640
	G	8	640	640
	H	8	640	640
	J	8	640	640
	K	8	640	640
	L	8	640	640
	M	8	640	640
	N	8	640	640
	<b>Total</b>		<b>7040</b>	<b>7040</b>
	<b>Concordance</b>		<b>100,0%</b>	

Level L2	Laboratory	Number of positive	Between-lab pairings with the same results	Total between-lab pairings
	B	8	640	640
	C	8	640	640
	E	8	640	640
	F	8	640	640
	G	8	640	640
	H	8	640	640
	J	8	640	640
	K	8	640	640
	L	8	640	640
	M	8	640	640
	N	8	640	640
	<b>Total</b>		<b>7040</b>	<b>7040</b>
	<b>Concordance</b>		<b>100,0%</b>	

**Reference method**

Level L0	Laboratory	Number of negative	Between-lab pairings with the same results	Total between-lab pairings
	B	8	640	640
	C	8	640	640
	E	8	640	640
	F	8	640	640
	G	8	640	640
	H	8	640	640
	J	8	640	640
	K	8	640	640
	L	8	640	640
	M	8	640	640
	N	8	640	640
	Total		7040	7040
	Concordance			100,0%

Level L1	Laboratory	Number of positive	Between-lab pairings with the same results	Total between-lab pairings
	B	8	640	640
	C	8	640	640
	E	8	640	640
	F	8	640	640
	G	8	640	640
	H	8	640	640
	J	8	640	640
	K	8	640	640
	L	8	640	640
	M	8	640	640
	N	8	640	640
	Total		7040	7040
	Concordance			100,0%

Level L2	Laboratory	Number of positive	Between-lab pairings with the same results	Total between-lab pairings
	B	8	640	640
	C	8	640	640
	E	8	640	640
	F	8	640	640
	G	8	640	640
	H	8	640	640
	J	8	640	640
	K	8	640	640
	L	8	640	640
	M	8	640	640
	N	8	640	640
	Total		7040	7040
	Concordance			100,0%