

**AFNOR CERTIFICATION VALIDATION STUDY
OF LUMIPROBE 24 *SALMONELLA* TEST FOR
THE DETECTION OF *SALMONELLA* SPP IN
FOOD AND FEED SAMPLES**

SYNTHESIS REPORT

LUMIPROBE 24 SALMONELLA TEST - S.R.(V0) - AUGUST 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: Lumiprobe 24 *Salmonella* test for the detection of *Salmonella spp*

Scope of validation: food and feed samples

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

SUMMARY

1. Introduction	4
1.1. Validation referential	4
1.2. Alternative method	4
1.3. Scope of application	5
1.4. Reference method (*)	5
1.5. Validation history	5
2. Methods comparison study	6
2.1. Relative accuracy, relative specificity and relative sensitivity	6
2.1.1. Number and nature of samples	6
2.1.2. Artificial contamination of samples	7
2.1.3. Confirmation protocol	7
2.1.4. Results	7
2.1.5. Calculation of relative accuracy (AC), relative specificity (SP) and relative sensitivity (SE)	8
2.1.6. Analysis of discordant results	8
2.2. Relative detection level	9
2.2.1. Matrices	9
2.2.2. Spiking protocol	9
2.2.3. Results	10
2.3. Inclusivity / exclusivity (selectivity)	10
2.3.1. Test protocols	10
2.3.2. Results	11
2.3.3. Conclusion	11
3. Collaborative study	12
3.1. Collaborative study implementation	12
3.1.1. Participating laboratories	12
3.1.2. <i>Salmonella spp</i> absence in the matrix	12
3.1.3. Strain stability in the matrix	12
3.1.4. Samples preparation and spiking	12
3.1.5. Samples labelling	13
3.1.6. Samples shipping	13
3.1.7. Samples reception and analysis	13
3.2. Results	13
3.2.1. Temperature and state of the samples	13
3.2.2. Total viable counts	14
3.2.3. Expert laboratory results	14
3.2.4. Participating laboratories results	14
3.2.5. Specificity (SP) and sensitivity (SE) calculations	15
3.2.6. Relative accuracy calculations	15
3.2.7. Discordant results analysis	16
3.3. Interpretation	16
3.3.1. Accordance	16
3.3.2. Concordance	16
3.3.3. Concordance odds ratio	16
3.3.4. AC, SP, SE comparison	17
4. Practicability	18
5. Conclusion	20

Annexes

- Annex 1:** accordance calculations
Annex 2: 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 Lumiprobe 24 *Salmonella* test 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.

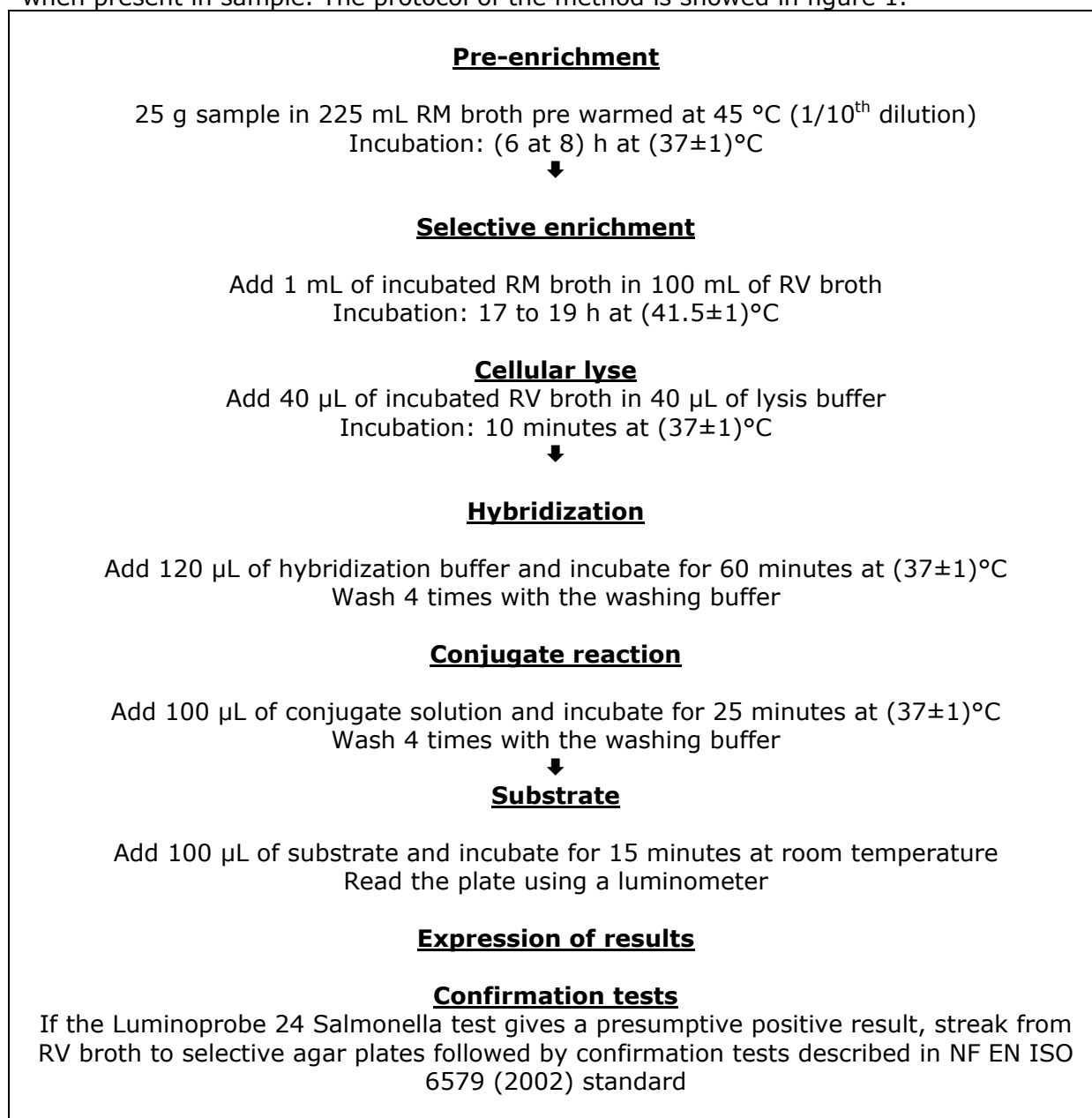


Figure 1: alternative method protocol

- **Principle of the assay**

The Lumiprobe 24 *Salmonella* is a rapid test for *Salmonella* spp detection in foodstuffs. The principle of this method is based on hybridization of DNA probes and RNAr after pre enrichment and selective enrichment steps.

If RLU value > limit value ⇒ positive result

If RLU value < limit value ⇒ negative result

Limit value = [RLU of negative control] X 2

- **Other validation**

The LUMIPROBE 24 *Salmonella* test obtained the NORDVAL validation in 2003 standard C C/ISO IN 12824, under the reference 2003-20-5408 00008.

1.3. Scope of application

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

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.

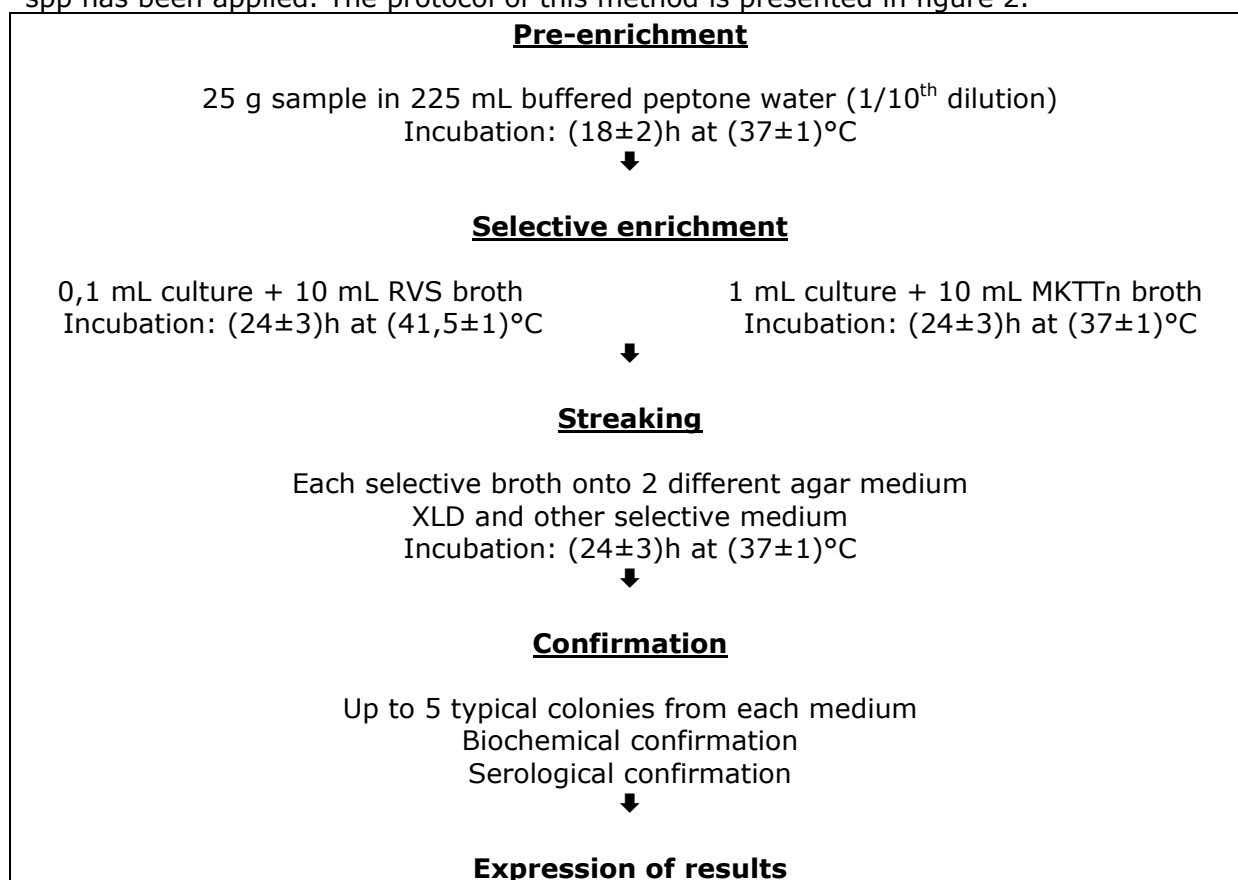


Figure 2: reference method protocol

1.5. Validation history

The initial validation was established in 2000 for egg products category only. The extension study was performed for other categories (dairy products, meat products and vegetable products) in 2002. These studies were performed according to the AFNOR protocol (rev 07). In 2005 the Lumiprobe 24 *Salmonella* test was revalidated for all products' categories according to the ISO 16140 referential (only the preliminary study). The interlaboratory study was performed according to the ISO referential in 2009.

2. Methods comparison study

The following characteristics are studied during the preliminary study performed in 2005:

- 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 and feedstuffs.

A number of 300 samples was analysed. Types of products are indicated in table 1.

Category	Type	Number of positive^a	Number of negative	Total
Meat products	Raw poultry	12	10	22
	Raw beef meat	4	14	18
	Delicatessen	4	1	5
	Other raw meat	1		1
	Cooked meat	9	5	14
	Total		30	30
Dairy products	Raw milk cheese	10	9	19
	Pasteurized milk cheese	7	13	20
	Milk	5	6	11
	Yoghourts	2	2	4
	Other dairy products	6		6
	Total		30	30
Seafood and vegetable products	Raw and cooked fruit and vegetables	11	2	13
	Fresh seafood	13	15	28
	Smoked/frozen/cooked seafood	6	13	19
	Total	30	30	60
Egg products	Eggs and derived	16	17	33
	Varied products	14	13	27
	Total	30	30	60
Feedstuffs	Dog food	5	3	8
	Cat food	8	9	17
	Other pet food	17	18	35
	Total	30	30	60

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

2.1.2. Artificial contamination of samples

Artificial contaminations of food samples were 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-).

Among positive results, samples spiking was realised on 110 samples so 26.66% of naturally contaminated samples were analysed.

2.1.3. Confirmation protocol

For alternative method, confirmation of presumed positive samples was performed from RV broth. Plating out on XLD and HEKTOEN 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 from results of 2005 and 2008 studies.

Category	Response	Reference method ^(*) positive (R+)	Reference method ^(*) negative (R-)
Meat products	Alternative method positive (A+)	PA=29	PD=0
	Alternative method negative (A-)	ND=1 including 0 PPND	NA=30 including 0 PPNA
Dairy products	Alternative method positive (A+)	PA=34	PD=3
	Alternative method negative (A-)	ND=4 including 0 PPND	NA=53 including 0 PPNA
Seafood and vegetable products	Alternative method positive (A+)	PA=30	PD=0
	Alternative method negative (A-)	ND=0 including 0 PPND	NA=30 including 0 PPNA
Egg products	Alternative method positive (A+)	PA=30	PD=0
	Alternative method negative (A-)	ND=0 including 0 PPND	NA=30 including 0 PPNA
Feedstuffs	Alternative method positive (A+)	PA=28	PD=0
	Alternative method negative (A-)	ND=2 including 0 PPND	NA=30 including 0 PPNA
All products	Alternative method positive (A+)	PA=151	PD=3
	Alternative method negative (A-)	ND=7 including 0 PPND	NA=173 including 0 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	29	30	1	0	60	98	30	97	30	100
Dairy products	34	53	4	3	94	93	38	89	56	95
Seafood and vegetable products	30	30	0	0	60	100	30	100	30	100
Egg products	30	30	0	0	60	100	30	100	30	100
Feedstuffs	28	30	2	0	60	97	30	93	30	100
All products	151	173	7	3	334	97	158	96	176	98

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	97.0 %
Relative sensitivity	95.6 %
Relative specificity	98.3 %

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	95.6 %	98.1 %

Table 5: sensitivity of both methods including all confirmed positive

2.1.6. Analysis of discordant results

Discordant results are examined according to annex F of NF EN ISO 16140 standard, with Y as the number of discordant results and m as the smallest of the two values of PD and ND. This analysis is presented in table 6.

	Alternative method
Y = PD + ND	Y = 3 + 7 = 10
m	3
M	1
Conclusion	m > M the methods are not different at $\alpha = 0.05$

Table 6: statistical analysis of discordant results

The Lumiprobe 24 *Salmonella* test and the reference method NF EN ISO 6579 can be statistically considered as equivalent.

- **Negative deviations**

For seven samples (4 dairy products, 2 feedstuffs and 1 meat product), the reference method gave a positive result while the alternative method result was negative.

- **Positive deviations**

For one sample, the alternative method gave a positive result while the reference method result was negative. The result was confirmed by serological and biochemical tests.

In 2009, a complimentary study was performed and 34 dairy products (raw milk and raw cheese milk) were analysed. The results show a concordance between both methods for 32 samples and discordance for 2 samples with 2 positive deviations.

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

Five 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.

Matrix	Strain	ISHA code	Origin
Minced meat	<i>S. Typhimurium</i>	S 15	Raw minced meat
Raw milk	<i>S. Dublin</i>	S 59	Raw milk
Raw fish	<i>S. Enteritidis</i>	S 63	Mussels
Egg	<i>S. Enteritidis</i>	S 38	Egg product
Horse food	<i>S. spp</i>	S 65	Feedstuffs

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	1.320 [0.997 ; 1.761]	1.550 [1.010 ; 2.380]
<i>S. Dublin</i>	Raw milk	1.930 [1.097 ; 3.425]	8.178 [5.595 ; 11.953]
<i>S. Enteritidis</i>	Raw fish	0.984 [0.557 ; 1.737]	0.984 [0.557 ; 1.737]
<i>S. Enteritidis</i>	Egg	0.764 [0.474 ; 1.227]	0.608 [0.361 ; 1.025]
<i>S. spp</i>	Horse food	0.990 [0.560 ; 1.740]	0.645 [0.383 ; 1.086]

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	1.3 [1.0 ; 1.8]	1.6 [1.0 ; 2.4]
<i>S. Dublin</i>	Raw milk	1.9 [1.1 ; 3.4]	8.2 [5.6 ; 11.9]
<i>S. Enteritidis</i>	Raw fish	1.0 [0.6 ; 1.7]	1.0 [0.6 ; 1.7]
<i>S. Enteritidis</i>	Egg	0.8 [0.5 ; 1.2]	0.6 [0.4 ; 1.0]
<i>S. spp</i>	Horse food	1.0 [0.6 ; 1.7]	0.6 [0.4 ; 1.1]

Table 8: relative detection level (1 significant number)

The detection limit obtained with the reference method is comprised between 0.5 and 3.4 cells per 25 g and the detection limit obtained with alternative method is comprised between 0.4 and 11.9 cells per 25 g.

A complementary study was conducted in May 2009 to evaluate the relative detection level of two *Salmonella* strains (*S. Dublin* and *S. Newport* isolated from dairy products) in two different raw milks. The results are given in the following tables.

		Relative detection level according to Spearman-Kärber method (cells in 25 mL)	
Strain	Matrix	Reference method(*)	Alternative method
<i>S. Dublin</i>	Lait cru	0.945 [0.709 ; 1.261]	0.847 [0.605 ; 1.186]
<i>S. Newport</i>	Lait cru	0.618 [0.423 ; 0.750]	0.618 [0.463 ; 0.824]

		Relative detection level according to Spearman-Kärber method (cells in 25 mL)	
Strain	Matrix	Reference method(*)	Alternative method
<i>S. Dublin</i>	Lait cru	0.9 [0.7 ; 1.3]	0.8 [0.6 ; 1.2]
<i>S. Newport</i>	Lait cru	0.6 [0.4 ; 0.8]	0.6 [0.5 ; 0.8]

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.

Experimentations were made in 2000, 2002, 2005 and 2008.

2.3.1. Test protocols

• Inclusivity

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

- **Exclusivity**

Each non-target strain was cultivated twice before inoculation in growth medium with a level of contamination expected to occur in the food matrices (about 10^5 CFU/mL). After 24 hours of incubation, the Lumiprobe 24 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

- 2000 and 2002 studies:

50 Salmonella strains tested were detected by the test.

30 non target strains were tested. 27 were non detected and 3 strains gave a cross-reaction: 2 *Citrobacter diversus* and 1 *Enterobacter sakazakii*. These cross reactions did not take place with tests with food matrices (egg). 7 other *E. sakazakii* strains gave a negative result.

2 *Citrobacter freundii* and 1 *Enterobacter agglomerans* strains gave a positive result with the test in growth medium. There were not detected with the complete protocol of the alternative broth.

- 2005 complementary study:

6 Salmonella strains were tested and detected (1 Typhi, 2 Paratyphi A, 2 Paratyphi B, 1 Paratyphi C).

- 2008 complementary study:

24 Salmonella strains were tested. 3 *S. Gallinarum* strains were not detected, because the detection threshold wasn't reach with these strains whose growth is slower than other *Salmonella* strains.

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 twelve 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.

The strain stability in the pasteurized milk matrix was evaluated for 4 days at $(4\pm 2)^{\circ}\text{C}$. The strain used was *Salmonella* Enteritidis, 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.

Day	Alternative method	Reference method (*)
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 pasteurized milk

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

3.1.4. Samples preparation and spiking

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

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

The matrix was distributed at 25 mL in sterile vials. Every vial was individually spiked and homogenized. Eight samples per level, per laboratory and per method were prepared. Each laboratory received 48 samples to analyse, 1 sample to quantify the endogenous microflora and 1 water sample containing a temperature probe.

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

Matrix	TVC (CFU/mL)	Target level (cells/25 mL)	Real level (cells/25 mL)	Confidence interval
Pasteurized milk	< 10	0	0	0
		3	2.8	[0 ; 7]
		30	26.2	[17 ; 37]

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

Table 11: sample code by contamination level

3.1.6. Samples shipping

The samples were shipped in a coolbox on the 24th of November 2008. The transport has been entrusted to Chronopost International.

3.1.7. Samples reception and analysis

The coolboxes were received the 25th of November for all laboratories.

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 26th of November 2008 by eleven 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	2.7	Correct
B	2.2	Correct
C	1.4	Correct
D	2.3	Correct
E	3.9	Correct
F	1.8	Correct
G	1.6	Correct
H	2.7	Correct
I	1.3	Correct
J	3.4	Correct
K	2.2	Correct
L	11.5*	Correct

Table 12: temperature and state of the samples upon reception

The thermal profiles analysis indicates for all laboratories mean temperatures comprises between 0.5 and 7.5°C, except for the laboratory L (11.5°C) but the temperature indicated by the probe was correct.

3.2.2. Total viable counts

For the whole laboratories, the total viable counts at 30°C is comprised between < 10 CFU/mL and 500 000 CFU/mL.

3.2.3. Expert laboratory results

The results obtained by the expert laboratory are summarized in table 13.

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

Table 13: 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 14 and 15.

- Alternative method results

Laboratory	Contamination level		
	L0	L1	L2
A	0/8	7/8	8/8
B	0/8	7/8	8/8
C	0/8	7/8	8/8
D	0/8	2/8	7/8
E	0/8	7/8	8/8
F	0/8	7/8	8/8
G	0/8	5/8	8/8
H	0/8	7/8	8/8
I	0/8	8/8	8/8
J	0/8	7/8	8/8
K	0/8	6/8	7/8
L	0/8	8/8	8/8

Table 14: 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
D	0/8	7/8	8/8
E	0/8	8/8	8/8
F	0/8	7/8	8/8
G	0/8	7/8	8/8
H	0/8	8/8	8/8
I	1/8	5/8	7/8
J	0/8	8/8	8/8
K	0/8	7/8	8/8
L	0/8	8/8	8/8

Table 15: reference method positive results for all laboratories

- Results analysis

Results are consistent with those expected for all laboratories, except for the laboratories H and J. For the laboratory H, there is a swish between samples (level L0 and level L2) during analysis. For the laboratory D, a problem occurred during the hybridization reaction which lowered the sensitivity of the kit. This lab was excluded from the statistical analysis of the results with the agreement of the Technical Committee.

Final analysis was consequently conducted using data 11 participants.

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

The specificity and sensitivity calculations of both methods are presented in table 16, 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
SP (level L0)	100%	98%	99%	98%
SE (level L1)	86%	79%	93%	89%
SE (level L2)	99%	98%	99%	98%
SE (level L1+L2)	93%	89%	96%	93%

Table 16: 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 17.

Level	Alternative method	Reference method		
		RM+	RM-	Total
L0	AM+	PA=0	PD=0	0
	AM-	ND=1	NA=87	88
	Total	1	87	88
L1	AM+	PA=70	PD=6	76
	AM-	ND=12	NA=0	12
	Total	82	6	88
L2	AM+	PA=86	PD=1	87
	AM-	ND=1	NA=0	1
	Total	87	1	88
L0+L1+L2	AM+	PA=156	PD=7	163
	AM-	ND=14	NA=87	101
	Total	170	94	264

Table 17: 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 18 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)
L0	90%	84%
L1	80%	71%
L2	98%	96%
L1+L2	89%	84%
Total	92%	88%

Table 18: 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: $Y = PD + ND$.

	Alternative method
Y = PD + ND	$Y = 7 + 14 = 21$
m	7
M	5
Conclusion	$m > M$ the methods are not different at $\alpha = 0.05$

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 19. Calculations of accordance by level and method are presented in annex 2.

Level	Alternative method	Reference method
L0	100%	98%
L1	78%	90%
L2	98%	98%

Table 19: 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 20. Calculations of concordance by level and method are presented in annex 3.

Level	Alternative method	Reference method
L0	100%	98%
L1	78%	87%
L2	98%	98%

Table 20: concordance by level and method

3.3.3. Concordance odds ratio

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

It is therefore helpful to calculate the concordance odds ratio (COR) defined as follows:
 $COR = \frac{\text{accordance} \times (100 - \text{concordance})}{\text{concordance} \times (100 - \text{accordance})}$.

Values of COR for both methods are shown in table 21.

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	98%	98%	1,0
L1	78%	78%	1,0	90%	87%	1,3
L2	98%	98%	1,0	100%	100%	1,0

Table 21: COR values for each method by contamination level

3.3.4. AC, SP, SE comparison

Table 22 summarizes the values obtained for AC, SP and SE parameters for the preliminary study and the interlaboratory study.

Parameter	Preliminary study	Interlaboratory study
AC	97 %	92%
SP	99 %	100%
SE	95 %	93%

Table 22: AC, SP and SE comparison between preliminary and interlaboratory study

The values obtained during the preliminary study are better than those obtained during the collaborative study, probably because of the results obtained by some laboratories.

The sensitivity of both methods is recalculated in table 23 by including all confirmed positive results.

Alternative method (PA+PD)/(PA+PD+ND)	Reference method (PA+ND)/(PA+PD+ND)
92%	96%

Table 23: 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 Lumiprobe 24 Salmonella test kit contains:

2- Volume of reagents

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

4- Modalities after first use

5- Equipment and specific local requirements

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

- Incubator capable of maintaining $41.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$
- Incubator capable of maintaining $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$
- Sample bags
- Balance
- Stomacher machine
- Luminometer

6- Reagents ready to use or for reconstitution

All the reagents are ready to use.

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	34	7	80
Second enrichment	/	/	1	16
SMS test	0.25	10	/	/
Isolation on selective agar media	/	/	1.5	20
Reading	0.25	14	0.8	16
Total	7.5	58	10.3	132

Step	Alternative method		Reference method	
	1 analysis	20 analyses	1 analysis	20 analyses
Sample enrichment	7	34	7	80
Second enrichment	/	/	1	16
Hybridization test	0.25	14	/	/
Isolation on selective agar media	0.8	12	1.5	20
Biochemical confirmation	16	200	16	200
Serological confirmation	3	50	3	50
Total	27.05	310	28.5	366

9- Time required for results

Step	Alternative method	Reference method
Sample enrichment	J0	J0
Selective enrichment (RVS and MKTTn °)	J1	J1
Hybridization test	J1	/
Isolation on selective agar media	/	J2
Reading	/	J3
Total	J1	J3

Step	Alternative method	Reference method
Sample enrichment	J0	J0
Selective enrichment (RVS and MKTTn°)	J1	J1
Hybridization test	J1	/
Isolation on selective agar media	J1	J2
Reading and isolation on non-selective agar	J2	J3
Confirmation test	J3 to J6	J4 to J7
Total	J6	J7

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 Lumiprobe 24 Salmonella test for the detection of *Salmonella spp* are comparable to those of the method NF EN ISO 6579. This study concerned 300 samples of five categories of products (meat, dairy, egg, seafood and vegetable and feedstuffs).

Values obtained for the 3 criteria are the following:

- relative accuracy: 97.0%
- relative sensitivity: 95.6%
- relative specificity: 98.3 %

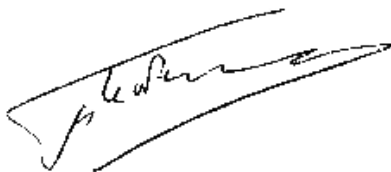
Ten discordant results were observed (7 ND and 3 PD).

The detection limit obtained with the reference methods is comprised between 0.5 and 3.4 cells per 25 g and the detection limit obtained with alternative method is comprised between 0.4 and 11.9 cells per 25 g.

The specificity of the method is satisfactory. No cross reactions were observed with non-target strains. All the tested serovars of *Salmonella* were detected by the alternative method at the incubation times specified in the protocol (except for the Gallinarum serovar, with was detected at high level of contamination).

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 3rd of July
François Le Nestour
Research Engineer

Annex 1: Accordance calculations

Contamination level L0

Alternative method

Laboratory	Number of positive	Probability of positive	Probability of pairs of positive	Probability of négative	Probability of pair of negative	Probability of pair of same results
A	0	0,00	0,00	1,00	1,00	1,00
B	0	0,00	0,00	1,00	1,00	1,00
C	0	0,00	0,00	1,00	1,00	1,00
E	0	0,00	0,00	1,00	1,00	1,00
F	0	0,00	0,00	1,00	1,00	1,00
G	0	0,00	0,00	1,00	1,00	1,00
H	0	0,00	0,00	1,00	1,00	1,00
I	0	0,00	0,00	1,00	1,00	1,00
J	0	0,00	0,00	1,00	1,00	1,00
K	0	0,00	0,00	1,00	1,00	1,00
L	0	0,00	0,00	1,00	1,00	1,00
Mean						1,00

Reference method

Laboratory	Number of positive	Probability of positive	Probability of pairs of positive	Probability of négative	Probability of pair of negative	Probability of pair of same results
A	0	0,00	0,00	1,00	1,00	1,00
B	0	0,00	0,00	1,00	1,00	1,00
C	0	0,00	0,00	1,00	1,00	1,00
E	0	0,00	0,00	1,00	1,00	1,00
F	0	0,00	0,00	1,00	1,00	1,00
G	0	0,00	0,00	1,00	1,00	1,00
H	1	0,13	0,02	0,88	0,77	0,78
I	0	0,00	0,00	1,00	1,00	1,00
J	0	0,00	0,00	1,00	1,00	1,00
K	0	0,00	0,00	1,00	1,00	1,00
L	0	0,00	0,00	1,00	1,00	1,00
Mean						0,98

Contamination level L1

Alternative method

Laboratory	Number of positive	Probability of positive	Probability of pairs of positive	Probability of négative	Probability of pair of negative	Probability of pair of same results
A	7	0,88	0,77	0,13	0,02	0,78
B	7	0,88	0,77	0,13	0,02	0,78
C	7	0,88	0,77	0,13	0,02	0,78
E	7	0,88	0,77	0,13	0,02	0,78
F	7	0,88	0,77	0,13	0,02	0,78
G	5	0,63	0,39	0,38	0,14	0,53
H	7	0,88	0,77	0,13	0,02	0,78
I	8	1,00	1,00	0,00	0,00	1,00
J	7	0,88	0,77	0,13	0,02	0,78
K	6	0,75	0,56	0,25	0,06	0,63
L	8	1,00	1,00	0,00	0,00	1,00
Mean						0,78

Reference method

Laboratory	Number of positive	Probability of positive	Probability of pairs of positive	Probability of négative	Probability of pair of negative	Probability of pair of same results
A	8	1,00	1,00	0,00	0,00	1,00
B	8	1,00	1,00	0,00	0,00	1,00
C	8	1,00	1,00	0,00	0,00	1,00
E	8	1,00	1,00	0,00	0,00	1,00
F	7	0,88	0,77	0,13	0,02	0,78
G	7	0,88	0,77	0,13	0,02	0,78
H	8	1,00	1,00	0,00	0,00	1,00
I	5	0,63	0,39	0,38	0,14	0,53
J	8	1,00	1,00	0,00	0,00	1,00
K	7	0,88	0,77	0,13	0,02	0,78
L	8	1,00	1	0,00	0	1,00
Mean						0,90

Contamination level L2

Alternative method

Laboratory	Number of positive	Probability of positive	Probability of pairs of positive	Probability of négative	Probability of pair of negative	Probability of pair of same results
A	8	1,00	1,00	0,00	0,00	1,00
B	8	1,00	1,00	0,00	0,00	1,00
C	8	1,00	1,00	0,00	0,00	1,00
E	8	1,00	1,00	0,00	0,00	1,00
F	8	1,00	1,00	0,00	0,00	1,00
G	8	1,00	1,00	0,00	0,00	1,00
H	8	1,00	1,00	0,00	0,00	1,00
I	8	1,00	1,00	0,00	0,00	1,00
J	8	1,00	1,00	0,00	0,00	1,00
K	7	0,88	0,77	0,13	0,02	0,78
L	8	1,00	1,00	0,00	0,00	1,00
Mean						0,98

Reference method

Laboratory	Number of positive	Probability of positive	Probability of pairs of positive	Probability of négative	Probability of pair of negative	Probability of pair of same results
A	8	1,00	1,00	0,00	0,00	1,00
B	8	1,00	1,00	0,00	0,00	1,00
C	8	1,00	1,00	0,00	0,00	1,00
E	8	1,00	1,00	0,00	0,00	1,00
F	8	1,00	1,00	0,00	0,00	1,00
G	8	1,00	1,00	0,00	0,00	1,00
H	7	0,88	0,77	0,13	0,02	0,78
I	8	1,00	1,00	0,00	0,00	1,00
J	8	1,00	1,00	0,00	0,00	1,00
K	8	1,00	1,00	0,00	0,00	1,00
L	8	1,00	1,00	0,00	0,00	1,00
Mean						0,98

Annex 2: Concordance calculations

Contamination level L0

Alternative method

Laboratory	Number of negative	Between-lab pairings with the same results	Total between-lab pairings
A	8	640	640
B	8	640	640
C	8	640	640
E	8	640	640
F	8	640	640
G	8	640	640
H	8	640	640
I	8	640	640
J	8	640	640
K	8	640	640
L	8	640	640
Total		7040	7040
Concordance			100%

Reference method

Laboratory	Number of negative	Between-lab pairings with the same results	Total between-lab pairings
A	8	632	640
B	8	632	640
C	8	632	640
E	8	632	640
F	8	632	640
G	8	632	640
H	7	560	640
I	8	632	640
J	8	632	640
K	8	632	640
L	8	632	640
Total		6880	7040
Concordance			97,7%

Contamination level L1

Alternative method

Laboratory	Number of positive	Between-lab pairings with the same results	Total between-lab pairings
A	7	494	640
B	7	494	640
C	7	494	640
E	7	494	640
F	7	494	640
G	5	382	640
H	7	494	640
I	8	608	640
J	7	494	640
K	6	440	640
L	8	608	640
Total		5496	7040
Concordance		78,1%	

Reference method

Laboratory	Number of positive	Between-lab pairings with the same results	Total between-lab pairings
A	8	592	640
B	8	592	640
C	8	592	640
E	8	592	640
F	7	530	640
G	7	530	640
H	8	592	640
I	5	394	640
J	8	592	640
K	7	530	640
L	8	592	640
Total		6128	7040
Concordance		87,0%	

Contamination level L2Alternative method

Laboratory	Number of positive	Between-lab pairings with the same results	Total between-lab pairings
A	8	632	640
B	8	632	640
C	8	632	640
E	8	632	640
F	8	632	640
G	8	632	640
H	8	632	640
I	8	632	640
J	8	632	640
K	7	560	640
L	8	632	640
Total		6880	7040
Concordance		97,7%	

Reference method

Laboratory	Number of positive	Between-lab pairings with the same results	Total between-lab pairings
A	8	632	640
B	8	632	640
C	8	632	640
E	8	632	640
F	8	632	640
G	8	632	640
H	7	560	640
I	8	632	640
J	8	632	640
K	8	632	640
L	8	632	640
Total		6880	7040
Concordance		97,7%	