

**AFNOR CERTIFICATION VALIDATION STUDY
OF SIMPLE METHOD FOR SALMONELLA
(SMS[®]) TEST FOR THE DETECTION OF
SALMONELLA SPP IN FOOD, FEED AND
ENVIRONMENTAL SAMPLES**

SYNTHESIS REPORT

SIMPLE METHOD FOR SALMONELLA (SMS[®]) TEST - S.R.(V1) - 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: Simple Method for Salmonella (SMS[®]) test 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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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 SMS[®] test is designed to detect *Salmonella* spp. The test kit permits the presumptive detection of the pathogen target after 24 hours of enrichment when present in sample. The protocol of the method is showed in figure 1.

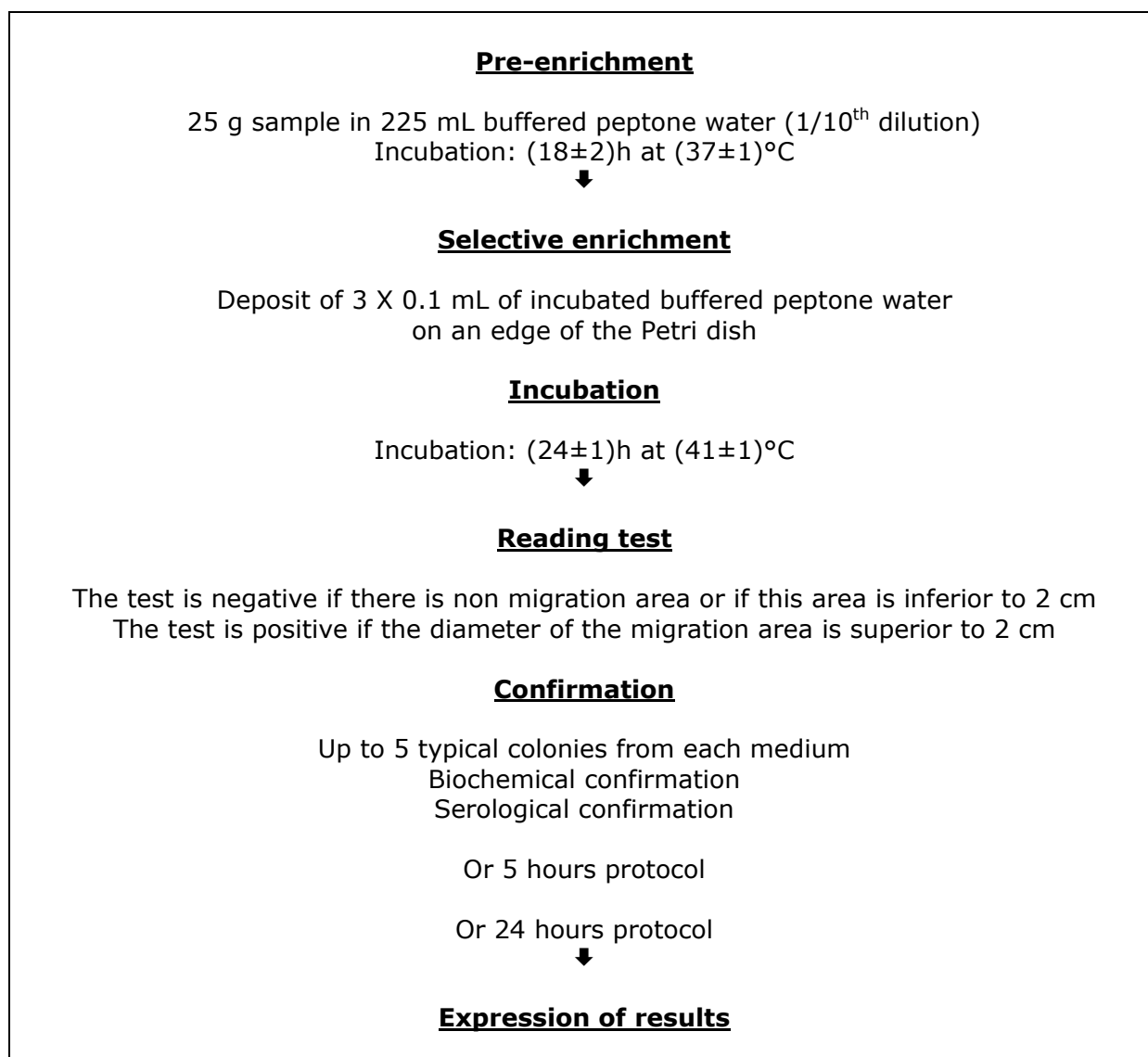


Figure 1: alternative method protocol

- **Principle of the assay**

SMS[®] is a simple and economical rapid test for *Salmonella* spp detection in foodstuffs. The principle of this method is based on the motility of *Salmonella* and its Lysine decarboxylation activity. *Salmonella* spp produce migration areas associated with a bend of the colour of the media. Incubation temperature of the media (41°C) gives more selectivity at the SMS[®] method.

The SMS test doesn't detect the non motile *Salmonella* spp.

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.

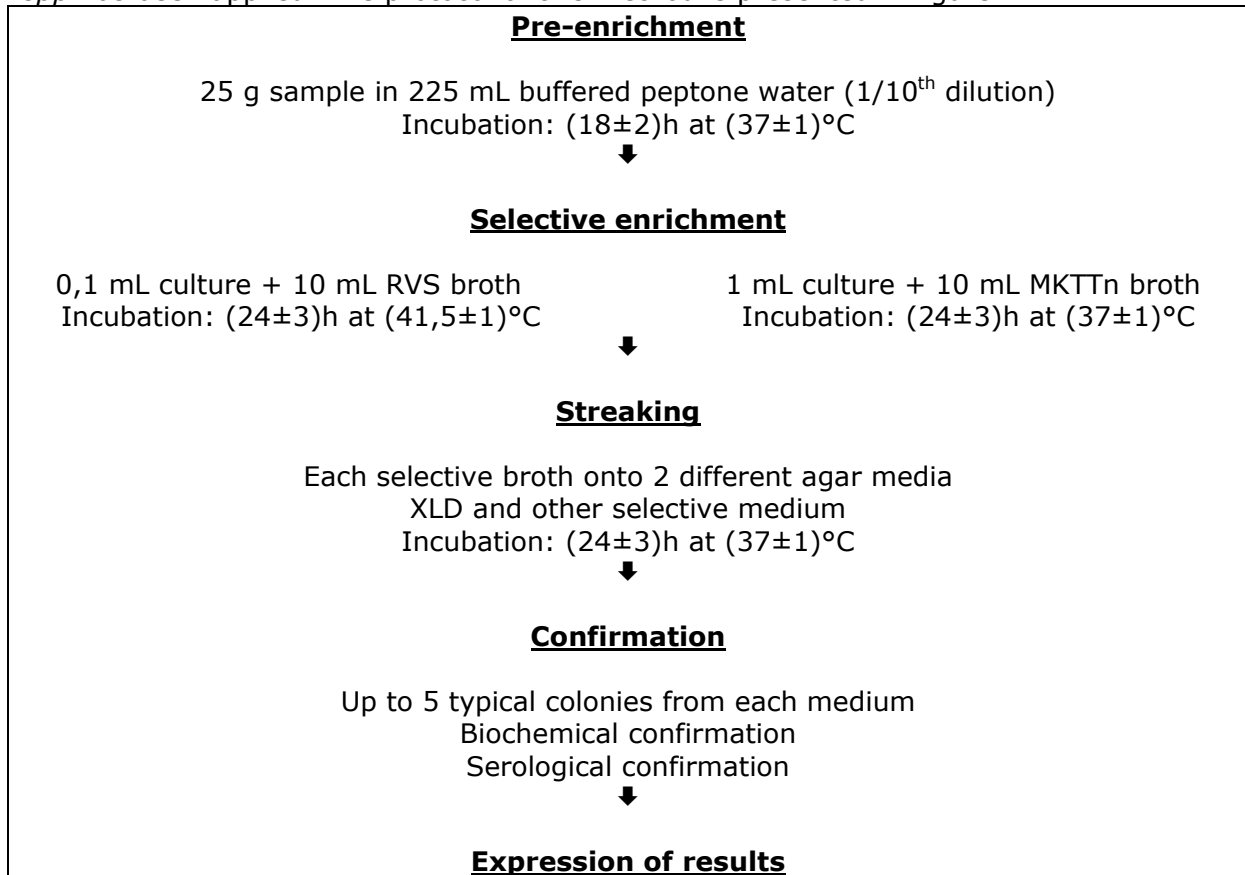


Figure 2: reference method protocol

1.5. Validation history

The initial validation was performed in 2004 according to the ISO 16140 referential. In 2008, the renewal study was made according to the same referential.

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 388 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	10	10	20
	Raw beef meat	19	19	38
	Delicatessen	1	3	4
	Total	30	32	62
Dairy products	Raw milk cheese	16	10	26
	Pasteurized milk cheese	9	21	30
	Other dairy products	5	0	5
	Total	30	31	61
Seafood and vegetable products	Raw and cooked fruit and vegetables	8	5	13
	Fresh seafood	12	22	34
	Smoked/frozen/cooked seafood	10	4	14
	Total	30	31	61
Egg products	Eggs and derived	27	10	37
	Varied products	3	21	24
	Total	30	31	61
Feedstuffs	Dog food	17	18	35
	Cat food	17	17	34
	Other pet food	6	5	11
	Total	40	40	80
Environmental samples	Process waters	17	4	21
	Sampling swabs	13	29	42
	Total	30	33	63

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 138 samples so 25.45% of naturally contaminated samples were analysed.

2.1.3. Confirmation protocol

For alternative method, confirmation of presumed positive samples was performed from SMS[®] media. 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.

In 2007, an extension study was performed to add new protocols of confirmation. 150 target strains (*Salmonella* spp) and 105 non target strains were tested. Two protocols were proposed:

- 1 – Five hours protocol using latex test,
- 2 – 24 hours protocol using SALSA medium and latex test.

The results showed good concordance with the two protocols.

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 ^(*) 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=32 including 0 PPNA
Dairy products	Alternative method positive (A+)	PA=28	PD=2
	Alternative method negative (A-)	ND=0 including 0 PPND	NA=31 including 0 PPNA
Seafood and vegetable products	Alternative method positive (A+)	PA=29	PD=1
	Alternative method negative (A-)	ND=0 including 0 PPND	NA=31 including 0 PPNA
Egg products	Alternative method positive (A+)	PA=30	PD=0
	Alternative method negative (A-)	ND=0 including 0 PPND	NA=31 including 0 PPNA
Feedstuffs	Alternative method positive (A+)	PA=40	PD=0
	Alternative method negative (A-)	ND=0 including 0 PPND	NA=40 including 0 PPNA
Environmental samples	Alternative method positive (A+)	PA=30	PD=0
	Alternative method negative (A-)	ND=0 including 0 PPND	NA=33 including 0 PPNA
All products	Alternative method positive (A+)	PA=186	PD=3
	Alternative method negative (A-)	ND=1 including 0 PPND	NA=198 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	32	1	0	62	98%	30	97%	32	100%
Dairy products	28	31	0	2	61	97%	28	100%	33	94%
Seafood and vegetable products	29	31	0	1	61	98%	29	100%	32	97%
Egg products	30	31	0	0	61	100%	30	100%	31	100%
Feedstuffs	40	40	0	0	80	100%	40	100%	40	100%
Environmental samples	30	33	0	0	63	100%	30	100%	33	100%
All products	186	198	1	3	388	99%	187	99%	201	99%

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	98.9 %
Relative sensitivity	93.5 %
Relative specificity	98.5 %

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)
Sensitivity	99.5 %	98.4 %

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. This analysis is presented in table 6.

	Alternative method
Y = PD + ND	Y = 3 + 1 = 4
Conclusion Y < 6	No statistical test applied

Table 6: statistical analysis of discordant results

The SMS[®] test and the reference method NF EN ISO 6579 can be statistically considered as equivalent.

- **Negative deviations**

For one sample, the reference method gave a positive result while the alternative method result was negative. The confirmation tests showed the presence of *S. Brandenburg*.

- **Positive deviations**

For three samples, the alternative method gave a positive result while the reference method result was negative. All these results were confirmed by serological and biochemical tests. *S. Enteritidis* (sea product) and *S. Dublin* (dairy product) were observed.

Remark: for the positive results, MUCAP test (confirmation test) was applied and the results were positive.

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
Minced meat	<i>S. Typhimurium</i>
Raw milk	<i>S. Dublin</i>
Raw fish	<i>S. Virchow</i>
Egg	<i>S. Enteritidis</i>
Process water	<i>S. Typhimurium</i>

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	0.460 [0.315 ; 0.673]	0.460 [0.315 ; 0.673]
<i>S. Dublin</i>	Raw milk	0.500 [0.362 ; 0.703]	0.500 [0.362 ; 0.703]
<i>S. Virchow</i>	Raw fish	0.250 [0.151 ; 0.428]	0.250 [0.151 ; 0.428]
<i>S. Enteritidis</i>	Egg	0.360 [0.261 ; 0.506]	0.360 [0.261 ; 0.506]
<i>S. Typhimurium</i>	Process water	0.460 [0.315 ; 0.673]	0.460 [0.315 ; 0.673]

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	0.5 [0.3 ; 0.7]	0.5 [0.3 ; 0.7]
<i>S. Dublin</i>	Raw milk	0.5 [0.4 ; 0.7]	0.5 [0.4 ; 0.7]
<i>S. Virchow</i>	Raw fish	0.3 [0.2 ; 0.4]	0.3 [0.2 ; 0.4]
<i>S. Enteritidis</i>	Egg	0.4 [0.3 ; 0.5]	0.4 [0.3 ; 0.5]
<i>S. Typhimurium</i>	Process water	0.5 [0.3 ; 0.7]	0.5 [0.3 ; 0.7]

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 methods is comprised between 0.2 and 0.7 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, 53 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 buffered peptone water (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 10^5 CFU/mL). After 24 hours of incubation, the SMS[®] 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**

53 *Salmonella* strains were tested:

The serovars Gallinarum and Paratyphi A gave negative results. These serovars are not detected by SMS[®] test because they are non motile (Gallinarum) or with out lysine decarboxylase activity (Paratyphi A). One *S. Infantis* strain and one *S. paratyphi C* strain gave negative results. However 3 other strains of *S. Infantis* gave positive results.

- **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 was observed with non-target strains. However all serovars (non motile and decarboxylase lyse negative) are not detected.

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 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 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.

Matrix	TVC (CFU/mL)	Target level (cells/25 mL)	Real level (cells/25 mL)	Confidence interval
Pasteurized milk	< 10	0	0	0
		3	3	[0 ; 7]
		30	31	[21 ; 42]

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

Table 11: sample code by contamination level

3.1.6. Samples shipping

The samples were shipped in a coolbox on the 30th of March 2004. The transport has been entrusted to Chronopost International.

3.1.7. Samples reception and analysis

The coolboxes were received the 31st of March 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 31th of March 2004 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	1.0	Correct
B	4.7	Correct
C	4.4	Correct
D	3.6	Correct
E	6.5	Correct
F	3.5	Correct
G	3.8	Correct
H	2.9	Correct
I	4.9	Correct
J	4.0	Correct
K	2.9	Correct
L	3.8	Correct
M	4.6	Correct
N	3.0	Correct

Table 12: temperature and state of the samples upon reception

The thermal profiles analysis indicates for all laboratories mean temperatures comprises between 0.48 and 5.18°C.

3.2.2. Total viable counts

For the whole laboratories, the total viable counts at 30°C was < 10 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	8/8	8/8
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	8/8	8/8
B	0/8	8/8	8/8
C	0/8	8/8	8/8
D	-	-	-
E	0/8	7/8	8/8
F	0/8	8/8	8/8
G	0/8	8/8	8/8
H	0/8	7/8	8/8
I	0/8	8/8	8/8
J	0/8	7/8	8/8
K	0/8	8/8	8/8
L	0/8	7/8	8/8
M	0/8	7/8	8/8
N	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	-	-	-
E	0/8	7/8	8/8
F	0/8	8/8	8/8
G	0/8	8/8	8/8
H	0/8	7/8	8/8
I	0/8	8/8	8/8
J	0/8	7/8	8/8
K	0/8	8/8	8/8
L	0/8	7/8	8/8
M	0/8	7/8	8/8
N	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 laboratory D which found only 6 positive on 8 at level L1 and L2 with the alternative method. This laboratory made mistakes in labelling and switched the results.

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

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

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

The specificity and sensitivity calculations of both methods are presented in table 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%	100%	98%
SE (level L1)	95%	96%	95%	96%
SE (level L2)	100%	98%	100%	98%
SE (level L1+L2)	98%	98%	98%	98%

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=0	NA=104	104
	Total	0	104	104
L1	AM+	PA=99	PD=0	99
	AM-	ND=0	NA=5	5
	Total	99	5	104
L2	AM+	PA=104	PD=0	104
	AM-	ND=0	NA=0	0
	Total	104	0	104
L0+L1+L2	AM+	PA=203	PD=0	203
	AM-	ND=0	NA=109	109
	Total	203	109	312

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	100%	98%
L1	100%	98%
L2	100%	98%
L1+L2	100%	98%
Total	100%	98%

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: $Y = PD + ND$.

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

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

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%	100%
L1	91%	91%
L2	100%	100%

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:

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

Values of COR for both methods are shown in table 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	100%	100%	1,0
L1	92%	91%	1,14	92%	91%	1,14
L2	100%	100%	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	98.9 %	100%
SP	93.5 %	100%
SE	98.5 %	100%

Table 22: 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 23 by including all confirmed positive results.

Alternative method (PA+PD)/(PA+PD+ND)	Reference method (PA+ND)/(PA+PD+ND)
100%	100%

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 SMS test kit contains:

The SMS media is pre-poured in Petri dishes and ready to use; The MUCAP test is presented in glass bottle.

2- Volume of reagents

-The MUCAP bottle contains 8 mL of product (160 tests).

-

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

The SMS test and MUCAP test should be refrigerated (2-8°C).

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 MUCAP test should be refrigerated (2-8°C) after opening.

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

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
SMS 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
SMS 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
SMS 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 SMS® test for the detection of *Salmonella spp* are comparable to those of the method NF EN ISO 6579. This study concerned 388 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: 98.9%
- relative sensitivity: 93.5%
- relative specificity: 98.5 %

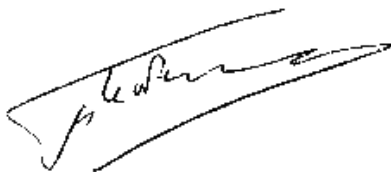
Four discordant results were observed (1 ND and 3 PD).

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 both methods varies from 0.3 to 0.7 CFU /25 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 no motile and decarboxylase negative were not detected by the alternative method at the incubation times specified in the protocol.

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

The study of the practicability of the alternative method shows a simple and easy-to-use method.



Massy, 2010, the 3rd of July
François Le Nestour
Research Engineer

Annex 1: Selectivity, target and non target strains

Microorganisms & Origin	Results			
	Reference method		Alternative method	
	Result expected	Result obtained	Result expected	Result obtained
<i>S. anatum</i> (salami)	/	/	+	+
<i>S. agona</i> (milk)	/	/	+	+
<i>S. arizonae</i> (salami)	/	/	+	+
<i>S. brandenburg</i> (cooked meat)	/	/	+	+
<i>S. brandenburg</i> (smoked ham)	/	/	+	+
<i>S. brandenburg</i> (pork)	/	/	+	+
<i>S. brandenburg</i> (zucchini gratin)	/	/	+	+
<i>S. bredeney</i> (raw roast turkey)	/	/	+	+
<i>S. derby</i> (loin)	/	/	+	+
<i>S. derby</i> (pork)	/	/	+	+
<i>S. derby</i> (sausage)	/	/	+	+
<i>S. enteritidis</i> (chicken)	/	/	+	+
<i>S. enteritidis</i> (egg product)	/	/	+	+
<i>S. enteritidis</i> (egg product)	/	/	+	+
<i>S. enteritidis</i> (beef tenderloin)	/	/	+	+
<i>S. gallinarum</i> (CIP 57.53)	+	+	-	-
<i>S. gallinarum</i> (CIP A 255)	+	+	-	-
<i>S. hadar</i> (raw chicken)	/	/	+	+
<i>S. hadar</i> (chicken cutlet)	/	/	+	+
<i>S. hadar</i> (Merguez)	/	/	+	+
<i>S. heidelberg</i> (Poultry)	/	/	+	+
<i>S. kottbus</i> (mixed vegetables)	/	/	+	+
<i>S. kottbus</i> (raw fried turkey)	/	/	+	+
<i>S. paratyphi A</i> (CIP 55 39)	+	+	-	-
<i>S. paratyphi A</i> (CIP 55 40)	+	+	-	-
<i>S. paratyphi A</i> (CIP A220)	+	+	-	-
<i>S. paratyphi B</i> (CIP 54 100)	/	/	+	+
<i>S. paratyphi B</i> (SAL 19.1)	/	/	+	+
<i>S. paratyphi B</i> (SAL 19.2)	/	/	+	+
<i>S. paratyphi C</i> (CIP 55 108)	+	+	+	-
<i>S. typhimurium</i> (pork foot)	/	/	+	+
<i>S. typhimurium</i> (Pigeon)	/	/	+	+
<i>S. typhimurium</i> (CIP 104 115)	/	/	+	+
<i>S. typhimurium</i> (CIP 60. 62)	/	/	+	+
<i>S. typhimurium</i> (raw beef)	/	/	+	+
<i>S. typhimurium</i> (cutting table)	/	/	+	+
<i>S. typhi</i> (CIP 54 136)	/	/	+	+
<i>S. infantis</i> (CIP 103 . 549)	+	+	+	-
<i>S. infantis</i> (ATCC 51741)	/	/	+	+
<i>S. infantis</i> (Neo. C1794)	/	/	+	+
<i>S. infantis</i> (Neo. C189.2983)	/	/	+	+
<i>S. saintpaul</i> (raw turkey fillet)	/	/	+	+
<i>S. saintpaul</i> (roast rabbit)	/	/	+	+
<i>S. virchow</i> (CIP 105 . 355)	/	/	+	+
<i>S. virchow</i> (Afssa 11337)	/	/	+	+
<i>S. virchow</i> (Afssa 6838 lac+)	/	/	+	+
<i>S. virchow</i> (souche B afssa)	/	/	+	+
<i>S. montevideo</i> (SAL 17.1)	/	/	+	+
<i>S. montevideo</i> (SAL 17.3)	/	/	+	+
<i>S. montevideo</i> (SAL 17.4)	/	/	+	+
<i>S. montevideo</i> (SAL 17.5)	/	/	+	+
<i>S. montevideo</i> (SAL 17.7)	/	/	+	+
<i>S. schevazengrund</i> (pork)	/	/	+	+
<i>S. senftenberg</i> (CIP 105343)	/	/	+	+

Microorganisms & Origin	Results			
	Reference method		Alternative method	
	Result expected	Result obtained	Result expected	Result obtained
<i>Bacillus cereus</i> (CIP 549)	/	/	-	-
<i>Bacillus cereus</i> (milk)	/	/	-	-
<i>Bacillus circulans</i> (dairy industry)	/	/	-	-
<i>Bacillus subtilis</i> (pudding)	/	/	-	-
<i>Streptococcus faecalis</i> (CIP 58 55)	/	/	-	-
<i>Staphylococcus epidermis</i> (environment)	/	/	-	-
<i>Staphylococcus aureus</i> (ATCC 6538)	/	/	-	-
<i>Escherichia coli</i> (grated carrots)	/	/	-	-
<i>Escherichia coli</i> (ATCC 8739)	/	/	-	-
<i>Escherichia coli</i> (Dairy industry)	/	/	-	-
<i>Escherichia hermanii</i> (CIP 103 176)	/	/	-	-
<i>Enterobacter aerogenes</i> (Dairy industry)	/	/	-	-
<i>Enterobacter aerogenes</i> (CIP 60 86 T)	/	/	-	-
<i>Enterobacter cloacae</i> (CIP 60 85)	/	/	-	-
<i>Enterobacter cloacae</i> (-)	/	/	-	-
<i>Hafnia alvei</i> (Taboulé)	/	/	-	-
<i>Klebsiella pneumoniae</i> (Pastry)	/	/	-	-
<i>Klebsiella oxytoca</i> (soybean salad)	/	/	-	-
<i>Klebsiella pneumoniae</i> (CIP 82 91)	/	/	-	-
<i>Pseudomonas aeruginosa</i> (CIP 100 720)	/	/	-	-
<i>Pseudomonas aeruginosa</i> (ATCC 194 29)	/	/	-	-
<i>Pseudomonas fluorescens</i> (CIP 69 13 T)	/	/	-	-
<i>Pseudomonas fluorescens</i> (CIP 102 127)	/	/	-	-
<i>Citrobacter freundii</i> (ATCC 80 90)	/	/	-	-
<i>Citrobacter koserii</i> (CIP 72 11)	/	/	-	-
<i>Citrobacter freundii</i> (CIP 53 62)	/	/	-	-
<i>Candida albicans</i> (ATCC 102 31)	/	/	-	-
<i>Acinetobacter baumannii</i> (sandwich cheese turkey)	/	/	-	-
<i>Shigella flexneri</i> (CIP 82 48 T)	/	/	-	-
<i>Shigella sonnei</i> (ATCC 92 90)	/	/	-	-

Annex 2 : accordance

Contamination level L0

Lab.	Number of positive	Probability of positive	Probability of pair of positive	Probability of negative	Probability of pair of negative	Probability Of pair of same results
A	0	0	0	1.00	1.00	1.00
B	0	0	0	1.00	1.00	1.00
C	0	0	0	1.00	1.00	1.00
E	0	0	0	1.00	1.00	1.00
F	0	0	0	1.00	1.00	1.00
G	0	0	0	1.00	1.00	1.00
H	0	0	0	1.00	1.00	1.00
I	0	0	0	1.00	1.00	1.00
J	0	0	0	1.00	1.00	1.00
K	0	0	0	1.00	1.00	1.00
L	0	0	0	1.00	1.00	1.00
M	0	0	0	1.00	1.00	1.00
N	0	0	0	1.00	1.00	1.00
Mean						1.00

Contamination level L1

Lab.	Number of positive	Probability of positive	Probability of pair of positive	Probability of negative	Probability of pair of negative	Probability Of pair of same results
A	8	1.00	1.00	0	0	1.00
B	8	1.00	1.00	0	0	1.00
C	8	1.00	1.00	0	0	1.00
E	7	0.88	0.77	0.10	0.01	0.78
F	8	1.00	1.00	0	0	1.00
G	8	1.00	1.00	0	0	1.00
H	7	0.88	0.77	0.10	0.01	0.78
I	8	1.00	1.00	0	0	1.00
J	7	0.88	0.77	0.10	0.01	0.78
K	8	1.00	1.00	0	0	1.00
L	7	0.88	0.77	0.10	0.01	0.78
M	7	0.88	0.77	0.10	0.01	0.78
N	8	1.00	1.00	0	0	1.00
Moyenne						0.92

Contamination level L2

Lab.	Number of positive	Probability of positive	Probability of pair of positive	Probability of negative	Probability of pair of negative	Probability Of pair of same results
A	8	1.00	1.00	0	0	1.00
B	8	1.00	1.00	0	0	1.00
C	8	1.00	1.00	0	0	1.00
E	8	1.00	1.00	0	0	1.00
F	8	1.00	1.00	0	0	1.00
G	8	1.00	1.00	0	0	1.00
H	8	1.00	1.00	0	0	1.00
I	8	1.00	1.00	0	0	1.00
J	8	1.00	1.00	0	0	1.00
K	8	1.00	1.00	0	0	1.00
L	8	1.00	1.00	0	0	1.00
M	8	1.00	1.00	0	0	1.00
N	8	1.00	1.00	0	0	1.00
Moyenne						1.00

Annex 3 : concordance

Contamination level L0

Lab.	Number of negative	Between-lab pairings with the same results	Total between-lab pairings
A	8	728	768
B	8	728	768
C	8	728	768
E	7	648	768
F	8	728	768
G	8	728	768
H	7	648	768
I	8	728	768
J	7	648	768
K	8	728	768
L	7	648	768
M	7	648	768
N	8	728	768
Total		9064	9984

Contamination level L1

Lab.	Number of positive	Between-lab pairings with the same results	Total between-lab pairings
A	8	728	768
B	8	728	768
C	8	728	768
E	7	648	768
F	8	728	768
G	8	728	768
H	7	648	768
I	8	728	768
J	7	648	768
K	8	728	768
L	7	648	768
M	7	648	768
N	8	728	768
Total		9064	9984

Contamination level L2

Lab.	Number of positive	Between-lab pairings with the same results	Total between-lab pairings
A	8	768	768
B	8	768	768
C	8	768	768
E	8	768	768
F	8	768	768
G	8	768	768
H	8	768	768
I	8	768	768
J	8	768	768
K	8	768	768
L	8	768	768
M	8	768	768
N	8	768	768
Total		9984	9984