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Oxoid Ltd,
part of Thermo Fisher Scientific
Wade Road, Basingstoke,
Hampshire, RG24 8PW, UK

NF VALIDATION
Validation of alternative analytical methods
Application in food microbiology

Summary report

EN ISO 16140 validation study of the TaqMan® *Salmonella enterica* method for *Salmonella* detection in all human food products and animal feeding stuffs

Qualitative method

This document includes 53 pages, with 7 appendixes.
Only copies including the totality of this document are authorised.

Competences of the laboratory are certified by COFRAC accreditation for the analyses marked with symbol◆.

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ADRIA DEVELOPPEMENT
Creac'h Gwen - F. 29196 QUIMPER Cedex - Tél. (33) 02.98.10.18.18 - Fax (33) 02.98.10.18.08
E-mail : adria.developpement@adria.tm.fr - Site web : http://www.adria.tm.fr
ASSOCIATION LOI DE 1901 - N° SIRET 306 964 271 00036 - N° EXISTENCE 532900006329 - N°TVA FR4530696427100036

Before comment

Quality Assurance documents related to this study can be consulted upon request from OXOID Ltd, part of THERMO FISHER SCIENTIFIC.

The technical protocol and the result interpretation were realised according to the EN ISO 16140 and the AFNOR technical rules.

✓ **Company:**	**OXOID Ltd** Part of THERMO FISHER SCIENTIFIC Wade Road - Basingstoke Hampshire RG24 8PW - England, UK
✓ **Expert Laboratory**:	**ADRIA Développement** ZA Creac'h Gwen F-29196 QUIMPER Cedex
✓ **Studied method**:	**TaqMan® *Salmonella enterica* method for the detection of *Salmonella spp.***
✓ **Validation standard:**	ISO 16140 (October 2003): Food microbiology – Protocol for the validation of alternative methods
✓ **Reference method♦**:	ISO 6579 (2002): Horizontal method for the detection of *Salmonella* spp.
✓ **Scope:**	**All human food products and animal feeding stuffs**
✓ **Alternative method with the following validated thermocyclers:**	7000, 7300, 7500, 7500 FAST, 7700 and STEP ONE.
✓ **Certification organism:**	AFNOR Certification

♦ Analysis performed according to the COFRAC accreditation

1 INTRODUCTION

1.1 Date of the initial validation and renewal studies

TaqMan® *Salmonella* was validated for food and feed products on the 28th of September 2007 (certificate number ABI 29/01—0907). The method was renewed in July 2011 and May 2015.

1.2 Alternative method

This test includes (i) a first enrichment in Buffered Peptone Water (BPW) (18 ± 2 h at 37°C ± 1°C), (ii) a DNA extraction, (iii) real time PCR to amplify a target from the *S. enterica* genome (See **Appendix 1**).

According to the AFNOR technical rules, the positive results are confirmed by performing the ISO 6579 standard or another alternative method, which is not based on the PCR principle.

The BPW enrichment storage at 4°C during 72 h was also tested in order to improve the practicability of the protocol.

1.3 Reference method♦

The reference method is the ISO 6579: Horizontal method for the detection of *Salmonella* spp (See **Appendix 2**).

♦ Analysis performed according to the COFRAC accreditation

2 INITIAL VALIDATION STUDY AND RENEWAL STUDY: RESULTS

2.1 Method comparison study

2.1.1 Relative accuracy, relative specificity and relative sensitivity

The accuracy is the closeness of agreement between a test result and the accepted reference value.

The relative specificity is defined as the degree to which a method is affected (or not) by the other components present in a multi-component sample; that is, it is the ability of the method to measure exactly a given analyte, or its amount, within the sample without interference from non-target components such as matrix effect or background noise.

The relative sensitivity is defined as the ability of the alternative method to detect two different amounts of analyte measured by the reference method within a given matrix over the whole measurement range; that is, it is the minimal quantity variation (increase of the analyte concentration x) which gives a significant variation of the measured signal (response y).

- **Number and nature of samples**

Five categories were studied. The distribution per category and per type is shown Table 1.

Table 1 – Distribution per category and tested type

Category	Type	Positives (number)	Negatives (number)	Total (number)
Meat products	Poultry	17	11	28
	Raw meat (pork, beef)	8	14	22
	Delicatessen	5	18	23
	Total	**30**	**43**	**73**
Dairy products	Raw milk	9	16	25
	Raw milk cheese	11	11	22
	Milk powder, ice cream, others	11	8	19
	Total	**31**	**35**	**66**
Seafood and vegetable products	Frozen vegetables	11	10	21
	Raw fish	12	8	20
	Various samples	10	12	22
	Total	**33**	**30**	**63**
Egg products	Raw egg	15	10	25
	Mayonnaise	7	8	15
	Egg powder, egg-based products	9	16	25
	Total	**31**	**34**	**65**
Feedstuffs	Pet food	11	12	23
	Raw meat	10	12	22
	Low moisture products	9	12	21
	Total	**30**	**36**	**66**
TOTAL		**155**	**178**	**333**

333 samples were analysed, with 155 positive and 178 negative products.

Artificial contamination of samples

Artificial contaminations were realised by spiking and cross-contamination. For sample spiking, strains were stressed using various injury protocols. The injury efficiency was evaluated by enumeration differences between a non selective and a selective Agar, i.e. TSYEA and XLD medium.

129 samples were artificially contaminated, 95 were positive. **38.7 % of the samples were naturally contaminated.**

The artificial contaminations are given in **Appendix 3**.

Confirmation protocols

The positive samples were confirmed by the ISO 6579 method tests. Further test were done to confirm the presumptive positive deviations: subcultures in RVS tubes, and streaking on several selective media.

Test results

Raw data per category are presented in **Appendix 4**.

Table 2 – Paired results of reference and alternative methods
All categories

Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) **PA = 150**	Positive deviation (R-/A+) **PD = 1**
Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 4**	Negative agreement (A-/R-) **NA = 178***

* 21 non-confirmed positive results were obtained by the alternative method.

Results per category of sample

Table 3 – Meat products

Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) **PA = 30**	Positive deviation (R-/A+) **PD = 0**
Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 0**	Negative agreement (A-/R-) **NA = 43**

Table 4 – Dairy products

Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) **PA = 29**	Positive deviation (R-/A+) **PD = 0**
Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 2**	Negative agreement (A-/R-) **NA = 35**

Table 5 – Seafood and vegetable products

Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) **PA = 32**	Positive deviation (R-/A+) **PD = 0**
Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 1**	Negative agreement (A-/R-) **NA = 30**

Table 6 – Egg products

Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) **PA = 30**	Positive deviation (R-/A+) **PD = 1**
Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 0**	Negative agreement (A-/R-) **NA = 34**

Table 7 – Feedstuffs

Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) **PA = 29**	Positive deviation (R-/A+) **PD = 0**
Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 1**	Negative agreement (A-/R-) **NA = 36**

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

Table 8 – Calculation of relative accuracy (AC), relative sensitivity (SE) and relative specificity (SP)

Matrix	PA	NA	ND	PD	N	Relative accuracy AC (%) [100x(PA+NA])/N]	N+ PA + ND	Relative sensitivity SE (%) [100xPA]/N+]	N- NA + PD	Relative specificity SP (%) [100xNA]/N-]
Meat products	**30**	**43**	**0**	**0**	**73**	100.0	30	100.0	43	100.0
Milk products	**29**	**35**	**2**	**0**	**66**	97.0	31	93.5	35	100.0
Seafood products and vegetables	**32**	**30**	**1**	**0**	**63**	98.4	33	97.0	30	100.0
Egg products	**30**	**34**	**0**	**1**	**65**	98.5	30	100.0	35	97.1
Feedstuff	**29**	**36**	**1**	**0**	**66**	98.5	30	96.7	36	100.0
TOTAL	**150**	**178**	**4**	**1**	**333**	**98.5**	**154**	**97.4**	**179**	**99.4**

PA = positive agreement (R+/A+)
NA =negative agreement (R-/A-)
PD =positive deviation (R-/A+)
ND = negative deviation (A-/R+)

The alternative method percentage values are:

- *Relative accuracy:* ***AC =98.5 %***
- *Relative specificity:* ***SP = 99.4 %***
- *Relative sensitivity:* ***SE = 97.4 %***

Sensitivities of the two methods, considering the alternative method positive deviations, are presented below:

- Alternative method = 97.4 %
- Reference method = 99.4 %

Analysis of discordants

The following table shows a breakdown of the 5 discordant samples:

Negative deviations: 4 samples	
Category	**Sample**
Milk products (2)	
Sample n° 784 (Tiramisu)	Artificially contaminated sample with *Salmonella arizonae* Ad 451.
Sample n° 1089 (Raw milk)	Artificially contaminated sample with *Salmonella* Montevideo 604. Characteristic colonies were isolated from RVS broth. The alternative method shows a positive result after BPW storage at 4°C for 72 h.
Seafood and vegetables products (1)	
Sample n° 950 (Courgettes slices)	Artificially contaminated sample with *Salmonella salamae* Ad 593 (sub-group II).
Feedstuffs (1)	
Sample n° 915 (Food for dog)	Artificially contaminated sample with *Salmonella livingstone* F104.

Positive deviation: 1 sample	
Category	**Sample**
Egg products (1)	
Sample n° 52 (Raw egg product)	Naturally contaminated sample. No characteristic colony was observed by the reference method. The confirmation was done on RAPID'*Salmonella* agar.

Y = PD + ND = 1 + 4 = 5
Since 5 deviations are observed, no statistical test can be applied.

The compared methods, i.e. the TaqMan *Salmonella* method and the ISO 6579 standard, are considered as equivalent.

BPW storage at 4°C

The enrichment broths of the alternative method positive samples were stored at 4°C for 72 h and analysed again. Two result modifications were observed:

Samples	First alternative method results	Results after storage at 4°C
983 (Pet meat)	Positive agreement	Negative deviation
1089 (Raw milk) 950 (Courgettes slices) 915 (Dog food)	Negative deviation	Positive agreement

One positive agreement changes for one negative deviation. Three negative deviations change for three positive agreements.

2.1.2 Relative detection level

The relative detection level is the smallest number of culturable microorganisms that can be detected in the sample in 50% of occasions by the alternative and reference methods.

Matrices

The objective of this study is (i) to determine the target species minimal quantity that can be detected in food matrices, (ii) to compare both method detection limits.

Detection limits were defined by analysing the different matrix/strain pairs:

- Ground beef inoculated with *Salmonella* Infantis 14,
- Raw fish inoculated with *Salmonella* Saintpaul F31,
- Raw egg inoculated with *Salmonella* Enteritidis 2532,
- Raw milk inoculated with *Salmonella* Typhimurium 305,
- Dog food inoculated with *Salmonella* Agona A00V038.

Four levels were tested, with six replicates.

Contamination protocol

Contaminations and enumerations were realised according to the AFNOR technical rules

Samples were analysed by both methods, and total flora was enumerated.

Results

Detection levels are presented Table 9.

Table 9 – Relative detection level results

Strain / matrix pairs	Relative detection level (UFC / 25 g or 25 ml) according to Spearman-Kärber test[1]	
	Reference method	Alternative method
Ground beef / *Salmonella* Infantis 14	0.434 [0.284; 0.662]	0.496 [0.310; 0.794]
Raw milk / *Salmonella* Typhimurium 305	0.447 [0.120 ; 1.666]	0.447 [0.120 ; 1.666]
Raw fish / *Salmonella* Saintpaul F31	0.525 [0.178 ; 1.550]	0.698 [0.379 ; 1.286]
Egg product / *Salmonella* Enteritidis 2532	0.545 [0.185 ; 1.608]	0.545 [0.185 ; 1.608]
Dog food / *Salmonella* Agona A00V038	0.346 [0.112 ; 1.068]	0.308 [0.100 ; 0.953]

The alternative and the standard methods show similar detection levels.

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

2.1.3 Inclusivity / exclusivity

Inclusivity is the ability of the alternative method to detect the target analyte from a wide range of strains.

Exclusivity is the lack of interference from a relevant range of non-target strains of the alternative method.

- **Test protocols**

- **Protocol for inclusivity**: *Salmonella* strains cultures were performed in BHI medium at 37°C. Dilutions were done in BPW in order to inoculate 10 cells/225 ml BPW. The alternative method protocol was performed.

- **Protocol for exclusivity**: negative strains cultures were done in BHI, incubated at 37°C. Dilutions were realised in order to inoculate 10^5 cell/ml BPW. The alternative method protocol was realised.

- **Results**

Raw data are given in **Appendix 5**.

The 69 target strains show positive results. The 36 non-target strains show negative results.

2.2 Practicability

TaqMan® *Salmonella* method practicability was evaluated according to the AFNOR criteria relative to method comparison study.

- **Packaging**

The information is available on the packaging and the kit insert. TaqMan® *Salmonella enterica* detection kit is available for 100 reactions.

Volume of reagents

The kit insert provides the following informations:

- 2 x environmental Master Mix Volume 750 µl
- 10 x *Salmonella enterica* assay Mix Volume 300 µl
- Negative control water Volume 1 ml

Storage conditions and shelf-life

The storage conditions are given on the packaging.
The shelf-life is 12 months.

Storage after initial use

The environmental master mix and the negative control water must be stored at 2 - 8°C after initial use. The *Salmonella enterica* assay mix must be placed at -15°C to -25°C.

Specific equipment

Product	Quantity for 100 tests
Microcentrifuge	1
Heat bloc (120°C)	1
Sterile microcentrifuge tubes with attached screw-cap lid	2
Pipettors 20, 200, 1000	2 EA
Pipet tips, aerosol resistant	/
Water Nuclease Free	/
DNA et RNA away	/
Disposable gloves	/

Reagents

PCR mix shall be prepared just before the test.

Training

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

Workflow study *(in minutes)*

	Reference method ISO 6579♦		Alternative method TaqMan® *Salmonella*	
	24 samples	36 samples	24 samples	36 samples
Sampling	30	45	30	45
Stomach	30	40	30	40
Extraction			75	115
Dilution - PCR			27	36
RVS and MKTTn sub-cultures	30	45		
Streaking on selective agar plates	50	75		
Reading	48	72		
Total (negative sample)	188	277	162	236
Total / negative sample	7.8	7.7	6.8	6.6
RVX and MKTTn sub-cultures			30	45
Streaking on selective agar			50	75
Reading			48	72
Streaking on nutritive agar	12	18	12	18
Confirmation tests	120	180	120	180
Total (positive sample)	320	475	422	626
Total / positive sample	13.3	13.2	17.6	17.4

Time to results

- Negative samples

	Reference method ISO 6579♦	Alternative method TaqMan® *Salmonella*
Pre-enrichment	Day 0	Day 0
Enrichment	Day 1	
Extraction, negative result		Day 1
Streaking	Day 2	
Reading	Day 3	

- Positive samples

	Reference method ISO 6579♦	Alternative method TaqMan® *Salmonella*
Pre-enrichment	Day 0	Day 0
Enrichment	Day 1	Day 1
Extraction, PCR		Day 1
Streaking	Day 2	Day 2
Reading	Day 3	Day 3
Confirmation tests	Day 4 to Day 6	Day 4 to Day 6

Negative results are available within one day using TaqMan® *Salmonella* method.

♦ Analyses performed according to the COFRAC accreditation

- **<u>Technician background</u>**

Technician qualified in food microbiology and molecular biology.

- **<u>Steps in common with reference method</u>**

Pre-enrichment step

- **<u>Traceability of all among the analysed data</u>**

Traceability fits with CFR 21 part 11.

- **<u>Maintenance</u>**

Perform the background and optical calibrations every six months

2.3 Inter-laboratory study

2.3.1 Study organisation

- **<u>Collaborators number</u>**

14 collaborators were involved in the interlaboratory study.

- **<u>Matrix and strain used</u>**

Pasteurised milk inoculated with *Salmonella typhimurium* 305 isolated from milk.

- **<u>Number of samples per laboratory</u>**

8 flasks were prepared per level for each laboratory, i.e. 24 flasks per collaborator lab. Two non-codified samples were sent to the collaborators in order to (i) enumerate the aerobic mesophilic flora in milk, (ii) to trace the temperature during the logistic conditions

Samples preparation and contamination

Milk flasks were individually inoculated for each level and each laboratory. Each flask contained 25 ml of milk.

- *Contamination suspension preparation*

Two suspensions (125 cells/ml and 25 cells/ml) were prepared with an overnight culture in BHI broth at 35°C, in accordance with the AFNOR technical rules.

- *Samples contamination protocol*

The low contamination level was realised with 200 µl of the 25 cells/ml suspension, the high level inoculation was performed with 200 µl of the 125 cells/ml suspension.

After inoculation, the samples were homogenised, sealed and stored at 4°C before expedition.

The inoculation levels were:

- 0 CFU/25 ml,
- 1 – 10 CFU/25 ml,
- 5 – 50 CFU/25 ml

Expedition conditions

The collaborative study instructions were sent on June 4th, 2007. The samples preparation and expedition were realised on June11th 2007.

2.3.2 Experimental parameters control

- **Contamination level before inoculation, levels obtained after samples artificial contamination**

- *Before inoculation*

A *Salmonella* detection using the ISO 6579 method was performed on five milk samples (25 ml) before the samples inoculation. All the results were negative.

- *Contamination levels*

The contamination levels and the confidence intervals are:

Table 10

Level	Samples	Theoretical target level (b/25 ml)	Real level (b/25 ml sample)	Low limit / 25 ml sample	High limit / 25 ml sample
Level 0	1 - 5 - 6 - 12 - 15 - 7 - 20 - 22	/	/	/	/
Low level	2 - 3 - 8 - 11 - 13 - 16 - 21 - 23	5	5.4	4.7	6.2
High level	4 - 7 - 9 - 10 - 14 - 18 - 19 - 24	25	25.6	22.2	29.4

- *Samples stability*

Enumeration was performed on 5 ml of milk. The results are:

Table 11

Day	Reference method (research)			CFU/25 ml (XLD)		
	Flask 1	Flask 2	Flask 3	Flask 1	Flask 2	Flask 3
Day 0	+	+	+	30	15	45
Day 1	+	+	+	20	20	15
Day 2	+	+	+	55	50	30

No evolution was observed.

Logistic conditions

Temperature conditions are given in Table 12.

Table 12 – Samples temperatures at receipt

Laboratories	Temperature measured by the probe (°C)	Temperature measured at receipt (°C)	Receipt delay
A	3.50	3.6	Day 1
B	*Bust*	6.5	
C	0.50	6.5	
D	0.00	5.0	
E	0.00	6.2	
G	3.00	3.7	
H	0.50	7.3	
I	3.50	3.0	
J	3.00	4.8	
L	0.50	2.9	
M	4.00	5.7	
N	*HS*	1.9	
O	4.00	3.8	
P	0.50	9.1	

The logistic temperatures vary from - 0.5°C to 5.0°C.

Lab P temperature at receipt is 9.1°C, but this result is infirmed by the sensor record.

Conclusion

No problem was encountered during the transport or at receipt.

2.3.3 Results analysis

- **Aerobic mesophilic flora enumeration**

Depending on the lab results, the enumeration levels varied from < 1 to 3 CFU/ml.

- **Expert lab results**

Negative results were observed by both methods for two low inoculated samples. The agreement between both methods is 100 %.

- **Collaborator labs results**

14 labs have participated to the study. Different automates were used: 7300, 7500, 7700 and 7000 Applied Biosystems real time PCR systems

Three labs did not performed the standard protocol:

- Lab C used the Selenite Cystine broth instead of the MKTTn broth,
- Lab E only performed the RVS broth enrichment,
- Lab G only performed a Selenite Cystine broth enrichment.

Cross-contaminations or discordant results between both methods were encountered by lab P. Results were discarded from the statistical analysis.

Several positive results were observed for the non-contaminated samples in the Lab L. The same pipetter was used by the Lab L for the reference method and the alternative method. According to the assurance quality requirements, a specific area has to be defined to prepare the PCR mix. *Salmonella* sp. was further detected on the pipetter, confirming the origin of the observed cross-contaminations.

Finally, 10 other lab results were integrated to the statistical analysis: labs A, B, D, H, I, J, L, M, N, O. Some of these collaborators encountered problems due to cross contaminations. Non-contaminated samples showed (i) alternative method presumptive positive results with negative confirmation tests (Labs D, H, L & M), (ii) reference method positive results (Lab B, D & H).

3 samples which were contaminated at low level, were not detected by both methods in the lab J.

2.3.4 *Results interpretation*

Both methods specificity and sensitivity

For the L0 level and for each method, specificity percentages are calculated according to:

$$SP = \left[1 - \left(\frac{FP}{N-}\right) x\,100\%\right]$$

with: N- = total number of all L0 essays
FP = number of false positive

For each contamination level and each method, the sensitivity percentages are calculated according to:

$$SE = \frac{TP}{N+} x\,100\%$$

with :N+ = total number of all L1 or L2 essays
TP = number of true positive

Results are reported in the table 13.

Table 13

Level	Reference method		Alternative method	
	SP/SE %	LCL%	SP/SE %	LCL%
L0	SP = 95.0	89	SP = 99	98
L1	SE = 96.3	93	SE = 96.3	93
L2	SE = 100	98	SE = 100	98
L1+L2	SE = 98.1	93	SE =98.1	93

Relative accuracy (AC)

Results for all levels are given in table 14:

Table 14 - Paired results of the alternative and reference methods

Alternative method	Reference method		Total
	+	-	
+	PA = 158	PD = 0	158
-	ND = 3	NA = 79 *	82
Total	N+ = 161	N- = 79	N =240

* with 9 non-confirmed presumptive positive results by the alternative method.

Relative accuracy AC) (in %) is calculated according to:
$$AC = \frac{(PA + NA)}{N} x\,100\%$$

with : N = number of samples analysed
PA = number of positive agreement
NA = number of negative agreement

The alternative method accuracy values with regard to the reference method are:

Table 15

Level	AC %	LCL %
L0	96.3	93.0
L1	100.0	98.0
L2	100.0	98.0
L1 + L2	100.0	98.0
Total	**98.8**	**96.0**

- **Discordant results**

Positive results were observed for non-inoculated samples analysis: this is probably induced by cross-contaminations during the standard protocol (labs B, D & H) or the alternative protocol (labs D, H, L & M) realisation.

2.3.5 Interpretation

- **Relative accuracy, specificity and sensitivity comparison values**

The values obtained for the two parts of the validation study (method comparison and inter-laboratory studies) are reported in table 16.

Tableau 16 - Alternative method values calculated during the comparative and inter-laboratory studies

	Inter-laboratory study	Method comparison study
Relative accuracy (AC)	98.8	98.5
Sensitivity (SE)	98.1	97.4
Specificity (SP)	98.1	99.4

Accordance (DA)

Accordance values (See **Appendix 6** for the raw data) for both methods are:

Level	Reference method (DA)	Alternative method (DA)
L0	92 %	98 %
L1	95 %	95 %
L2	100 %	100 %

Concordance

Both methods concordance values (See **Appendix 7** for the raw data) are:

Level	Reference method	Alternative method
L0	90.3 %	93.4 %
L1	92.5 %	92.5 %
L2	100 %	100 %

Concordance odds Ratio (COR)

The odds ratio value is determined according to:

$$COR = \frac{Accordance\ x\,(100 - agreement)}{Agreement\ x(100 - accordance)}$$

Both method odds ratio values are:

Level	Reference method (COR)	Alternative method (COR)
L0	1.24	3.46
L1	1.54	1.54
L2	1.00	1.00

2.4 Conclusion

The **methods comparison study conclusions** are:

- ❒ The TaqMan® *Salmonella* method shows a satisfying relative accuracy, relative specificity and relative sensitivity study,
- ❒ The TaqMan® *Salmonella* method and the ISO 6579 standard method show similar limit detection levels.
- ❒ The TaqMan® *Salmonella* method is selective and specific.
- ❒ Negative results are available within one day using TaqMan® *Salmonella* method.

The **inter-laboratory study conclusions** are:

- ❒ Alternative method and reference method show similar performances (accordance, concordance, odds ratio).

Appendix 1 – Flow diagram of the TaqMan® *Salmonella enterica* method

25 g of sample + 225 ml BPW
Incubation 18 h ± 2h at 37°C
↓
Pipet 1 ml of BPW
Spin 3 min à 16 000 g
↓
Aspirate and discard the supernatant
Add 100µl PrepMan™ reagent
Vortex de 10 s
Heat 10 min à 100°C
↓
Spin 3 min, 16 000 g
↓
Transfer 50 µl of supernatant
in a clean microcentrifuge tube
↓
DNA extract dilution (1/10)

Mix 12 µl of supernatant
with 18 µl of PCR reagent
↓
Real time PCR
↓
Results interpretation
↓
Confirmation

Appendix 2 – Reference method
NF EN ISO 6579: 2002: Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

25g of sample + 225 ml BPW

↓

Incubation
18 h ± 2 h at 37°C ± 1°C

↙	↘
0,1 ml BPW 10 ml RVS Incubation 24 h ± 3 h at 41.5°C ± 1°C	1 ml BPW 10 ml MKTTn Incubation 24 h ± 3 h at 37°C ± 1°C

↘ ↙

Streak on XLD and a second medium
Incubation 24 h ± 3 h at 37°C ± 1°C

↓

Streak 1 characteristic colony
onto nutritive agar
(Take 4 other colonies if
the first one is negative)

↓

Incubation 24 h ± 3 h at 37°C ± 1°C

↓

Biochemical and serological confirmation

Appendix 3 – Artificial contamination

TT : thermal treatment

N°Sample	Product	Inoculations					Result
		Strain		Injury protocol	Stress Log TSYEA-XLD	Inoculation level	
		Reference	Origin				
49	**Milk powder**	RAEMA (inter-laboratory study)					+
50	**Milk powder**	RAEMA (interl-aboratory study)					+
51	**Milk powder**	RAEMA (inter-laboratory study)					+
307	**Red pepper**	*Salmonella virchow F276*	Curry	-20°C;TT 30 min 58°C	0.2	43	+
308	**Green peas**	*Salmonella virchow F276*	Curry	-20°C;TT 30 min 58°C	0.2	43	+
309	**Salsify**	*Salmonella virchow F276*	Curry	-20°C;TT 30 min 58°C	0.2	43	+
310	**Tomato wheat sead**	*Salmonella virchow F276*	Curry	-20°C;TT 30 min 58°C	0.2	43	+
311	**Smoked salmon**	*Salmonella sainpaul F31*	Pilchard filet	-20°C;TT 30 min 58°C	0.7	27	+
312	**Seafood cocktail**	*Salmonella sainpaul F31*	Pilchard filet	-20°C;TT 30 min 58°C	0.7	27	+
313	**Grenadier filet**	*Salmonella sainpaul F31*	Pilchard filet	-20°C;TT 30 min 58°C	0.7	27	+
314	**Whiting filet**	*Salmonella sainpaul F31*	Pilchard filet	-20°C;TT 30 min 58°C	0.7	27	+
315	**Infant milk powder**	*Salmonella newington 26*	Milk product	TT 30 min 58°C	0.41	27	+
316	**Infant milk powder**	*Salmonella newington 26*	Milk product	TT 30 min 58°C	0.41	4	+
317	**Milk powder**	*Salmonella newington 26*	Milk product	TT 30 min 58°C	0.41	4	+
318	**Cream-puff**	*Salmonella newington 26*	Milk product	TT 30 min 58°C	0.41	4	+
687	**Raw milk**	*Salmonella montevideo 510*	Raw milk	-80°C	0.6	0.6	-
688	**Raw milk**	*Salmonella montevideo 510*	Raw milk	-80°C	0.6	0.6	-
689	**Raw milk**	*Salmonella montevideo 510*	Raw milk	-80°C	0.6	0.6	-
690	**Raw milk**	*Salmonella montevideo 510*	Raw milk	-80°C	0.6	0.6	-
691	**Mayonnaise**	*Salmonella mbandaka 81*	Egg product	TT 30min 60°C	0.5	1	-
692	**Mayonnaise**	*Salmonella mbandaka 81*	Egg product	TT 30min 60°C	0.5	1	-

TT : thermal treatment

N°Sample	Product	Inoculations					Result
		Strain		Injury protocol	Stress Log TSYEA-XLD	Inoculation level	
		Reference	Origin				
693	**Mashed mushrooms**	*Salmonella brandenburg Ad351*	Seafood cocktail	-80°C	0.3	1	-
694	**Mashed asparagus**	*Salmonella brandenburg Ad352*	Seafood cocktail	-80°C	0.3	1	-
784	**Tiramisu**	*Salmonella arizonae Ad451*	Ewe milk	-80°C	0.52	2.6	+
788	**Rice and vegetables**	*Salmonella indiana 2*	Fish flour	-80°C	0.59	2.8	+
789	**Rice and vegetables**	*Salmonella indiana 2*	Fish flour	-80°C	0.59	2.8	+
794	**Vanilla ice cream**	*Salmonella infantis 401b*	Raw milk	TT 55°C 30 min	0.3	3.7	+
795	**Raw milk cheese (Tomme de Savoie)**	*Salmonella infantis 401b*	Raw milk	TT 55°C 30 min	0.3	3.7	+
796	**Raw milk**	*Salmonella infantis 401b*	Raw milk	TT 55°C 30 min	0.3	3.7	+
797	**Raw milk cheese (Comté)**	*Salmonella infantis 401b*	Raw milk	TT 55°C 30 min	0.3	3.7	+
RA145	**Milk powder**	RAEMA (interlaboratory study)					-
RA356	**Milk powder**						-
RA552	**Milk powder**						+
RA763	**Milk powder**						-
RA831	**Milk powder**						-
866	**Raw milk cheese**	*Salmonella dublin 15706*	Cheese	TT 55°C 10 min	1.3	1.4	-
867	**Raw milk cheese**	*Salmonella dublin 15706*	Cheese	TT 55°C 10 min	1.3	1.4	-
868	**Raw milk cheese**	*Salmonella dublin 15706*	Cheese	TT 55°C 10 min	1.3	1.4	-
869	**Raw milk cheese**	*Salmonella dublin 15706*	Cheese	TT 55°C 10 min	1.3	1.4	-
897	**Ground beef**	Cross contamination with meat					+
898	**Ground beef**	Cross contamination with meat					+
905	**Goat cheese**	*Salmonella newington 26*	Milk product	TT 55°C 10 min	1.11	3.8	+
906	**Raw milk Raclette**	*Salmonella newington 26*	Milk product	TT 55°C 10 min	1.11	3.8	+

TT : thermal treatment

N°Sample	Product	Inoculations					Result
		Strain		Injury protocol	Stress Log TSYEA-XLD	Inoculation level	
		Reference	Origin				
907	**Emmental**	*Salmonella newington 26*	Milk product	TT 55°C 10 min	1.11	3.8	+
908	**Comté**	*Salmonella infantis 401b*	Raw milk	TT 55°C 10 min	1.24	2.2	+
909	**St Nectaire**	*Salmonella infantis 401b*	Raw milk	TT 55°C 10 min	1.24	2.2	+
910	**Raw milk**	*Salmonella newington 26*	Milk product	TT 55°C 10 min	1.11	3.8	+
911	**Raw milk**	*Salmonella newington 26*	Milk product	TT 55°C 10 min	1.11	3.8	+
912	**Raw milk**	*Salmonella infantis 401b*	Raw milk	TT 55°C 10 min	1.24	2.2	+
913	**Raw milk**	*Salmonella infantis 401b*	Raw milk	TT 55°C 10 min	1.24	2.2	+
914	**Raw milk**	*Salmonella infantis 401b*	Raw milk	TT 55°C 10 min	1.24	2.2	+
915	**Dog food**	*Salmonella livingstone F104*	Feed stuff	TT 55°C 10 min	1.11	2.4	+
916	**Dog food**	*Salmonella livingstone F104*	Feed stuff	TT 55°C 10 min	1.11	2.4	+
917	**Meat for pet**	*Salmonella infantis 179*	Feed stuff	TT 55°C 10 min	1	3	+
918	**Sausage for dog**	*Salmonella infantis 179*	Feed stuff	TT 55°C 10 min	1	3	+
919	**Pâté for cat**	*Salmonella infantis 179*	Feed stuff	TT 55°C 10 min	1	3	+
920	**Food for cattle**	*Salmonella livingstone F104*	Feed stuff	TT 55°C 10 min	1.11	2.4	-
921	**Food for cattle**	*Salmonella livingstone F104*	Feed stuff	TT 55°C 10 min	1.11	2.4	+
922	**Food for cattle**	*Salmonella livingstone F104*	Feed stuff	TT 55°C 10 min	1.11	2.4	+
923	**Food for cattle**	*Salmonella infantis 179*	Feed stuff	TT 55°C 10 min	1	3	+
924	**Food for cattle**	*Salmonella infantis 179*	Feed stuff	TT 55°C 10 min	1	3	+
936	**Raw milk Raclette**	*Salmonella montevideo 510*	Raw milk	TT 55°C 10 min	>1.00	1.6	+
937	**Raw milk comté**	*Salmonella montevideo 510*	Raw milk	TT 55°C 10 min	>1.00	1.6	+
938	**Raw milk cheese**	*Salmonella montevideo 510*	Raw milk	TT 55°C 10 min	>1.00	1.6	+
939	**Goat cheees**	*Salmonella montevideo 510*	Raw milk	TT 55°C 10 min	>1.00	1.6	+
947	**Grated carots**	*Salmonella sub-group II Ad593*	Seeds	TT 55°C 10 min	0.84	2.2	+

TT : thermal treatment

N°Sample	Product	Inoculations					Result
		Strain		Injury protocol	Stress Log TSYEA-XLD	Inoculation level	
		Reference	Origin				
948	**Salads**	*Salmonella sub-group II Ad593*	Seeds	TT 55°C 10 min	0.84	2.2	+
949	**Shallots**	*Salmonella sub-group II Ad593*	Seeds	TT 55°C 10 min	0.84	2.2	+
950	**Courgettes slices**	*Salmonella sub-group II Ad593*	Seeds	TT 55°C 10 min	0.84	2.2	+
954	**Salmon terrine for cat**	*Salmonella agona A00V038*	Feedstuff	-20°C	0.43	2	+
955	**Terrine for dog**	*Salmonella agona A00V038*	Feedstuff	-20°C	0.43	2	+
956	**Hamster mix seeds**	*Salmonella agona A00V038*	Feedstuff	-20°C	0.43	2	+
957	**Rabbit mix seeds**	*Salmonella agona A00V038*	Feedstuff	-20°C	0.43	2	-
962	**Whiting filet**	*Salmonella virchow F276*	Spice(curry)	-20°C	1	59	+
963	**Salmon**	*Salmonella virchow F276*	Spice(curry)	-20°C	1	59	+
964	**Pollack**	*Salmonella virchow F276*	Spice(curry)	-20°C	1	59	+
965	**Salmon**	*Salmonella virchow F276*	Spice(curry)	-20°C	1	59	+
966	**Onions**	*Salmonella indiana 2*	Fish flour	-20°C	0.51	32	+
967	**Minced vegetables**	*Salmonella indiana 2*	Fish flour	-20°C	0.51	32	+
968	**Carots slices**	*Salmonella indiana 2*	Fish flour	-20°C	0.51	32	+
969	**Tomato blocks**	*Salmonella indiana 2*	Fish flour	-20°C	0.51	32	+
978	**Andouillette**	*Salmonella bredeney 4873*	Delicatessen	TT 55°C 10 min	2.6	0.2	-
979	**Head pâté**	*Salmonella bredeney 4873*	Delicatessen	TT 55°C 10 min	2.6	0.2	-
980	**Ground beef**	*Salmonella bredeney 4873*	Delicatessen	TT 55°C 10 min	2.6	0.2	-
981	**Ground beef**	*Salmonella bredeney 4873*	Delicatessen	TT 55°C 10 min	2.6	0.2	+
982	**Raw meat for pet**	*Salmonella livingstone F105*	Feed stuff	TT 55°C 10 min	1.2	0.4	-
983	**Raw meat for pet**	*Salmonella livingstone F105*	Feed stuff	TT 55°C 10 min	1.2	0.4	+
984	**Croquettes for dog**	*Salmonella livingstone F105*	Feed stuff	TT 55°C 10 min	1.2	0.4	-
985	**Beef croquettes for dog**	*Salmonella livingstone F105*	Feed stuff	TT 55°C 10 min	1.2	0.4	-

TT : thermal treatment

N°Sample	Product	Inoculations					Result
		Strain		Injury protocol	Stress Log TSYEA-XLD	Inoculation level	
		Reference	Origin				
992	**Pâté**	*Salmonella bredeney 434*	Head pork paté	TT 55°C 10 min	>2.7	4	-
993	**Garlic sausage**	*Salmonella bredeney 434*	Head pork paté	TT 55°C 10 min	>2.7	4	-
994	**West Indian pudding**	*Salmonella bredeney 434*	Head pork paté	TT 55°C 10 min	>2.7	4	-
995	**Brioche with sausage**	*Salmonella bredeney 434*	Head pork paté	TT 55°C 10 min	>2.7	4	-
996	**Roast chicken legs**	*Salmonella bredeney 141*	Pork	-20°C	3.7	0.4	+
997	**Ground ham**	*Salmonella bredeney 141*	Pork	-20°C	3.7	0.4	+
998	**Sauté (pork)**	*Salmonella bredeney 141*	Pork	-20°C	3.7	0.4	+
999	**Sauté (pork)**	*Salmonella bredeney 141*	Pork	-20°C	3.7	0.4	+
1000	**Ground beef**	*Salmonella sainpaul F31*	Pilchard filet	-20°C	0.4	0.6	+
1001	**Ground beef**	*Salmonella sainpaul F31*	Pilchard filet	-20°C	0.4	0.6	+
1002	**Raw milk**	*Salmonella dublin Ad531*	Cheese	-20°C	0.6	2	-
1003	**Raw milk**	*Salmonella dublin Ad531*	Cheese	-20°C	0.6	2	-
1004	**Raw milk**	*Salmonella dublin Ad531*	Cheese	-20°C	0.6	2	-
1005	**Raw milk**	*Salmonella dublin Ad531*	Cheese	-20°C	0.6	2	-
1022	**Horse feed**	*Salmonella braenderup* F286	Feed stuff	TT 55°C 10 min	>0.7	4.6	+
1023	**Horse feed**	*Salmonella braenderup* F286	Feed stuff	TT 55°C 10 min	>0.7	4.6	+
1024	**Cattle feed**	*Salmonella braenderup* F286	Feed stuff	TT 55°C 10 min	>0.7	4.6	+
1025	**Cattle feed**	*Salmonella braenderup* F286	Feed stuff	TT 55°C 10 min	>0.7	4.6	+
1026	**Cattle feed**	*Salmonella derby* 630	Feedstuff	TT 55°C 10 min	1.1	2.2	+
1027	**Horse feed**	*Salmonella derby* 630	Feedstuff	TT 55°C 10 min	1.1	2.2	-
1028	**Croquettes for cat**	*Salmonella derby* 630	Feedstuff	TT 55°C 10 min	1.1	2.2	+
1029	**Croquettes mix for cat**	*Salmonella derby* 630	Feedstuff	TT 55°C 10 min	1.1	2.2	+
1040	**Minced spinash**	*Salmonella brandenburg* Ad351	Seafood cocktail	TT 55°C 10 min	>0.2	4.4	+

TT : thermal treatment

N°Sample	Product	Inoculations					Result
		Strain		Injury protocol	Stress Log TSYEA-XLD	Inoculation level	
		Reference	Origin				
1041	**Brussel sprouts**	*Salmonella typhimurium* 305	Paella	TT 55°C 10 min	>0.9	4.6	+
1042	**Smoked salmon**	*Salmonella brandenburg* Ad351	Seafood cocktail	TT 55°C 10 min	>0.2	4.4	+
1043	**Tuna rillettes**	*Salmonella brandenburg* Ad351	Seafood cocktail	TT 55°C 10 min	>0.2	4.4	+
1044	**Pangasin filets**	*Salmonella brandenburg* Ad351	Seafood cocktail	TT 55°C 10 min	>0.2	4.4	+
1045	**Grenadier filet**	*Salmonella brandenburg* Ad351	Seafood cocktail	TT 55°C 10 min	>0.2	4.4	+
1046	**Preparation for salad**	*Salmonella typhimurium* 305	Paella	TT 55°C 10 min	>0.9	4.6	+
1047	**Remoulade celery**	*Salmonella typhimurium* 305	Paella	TT 55°C 10 min	>0.9	4.6	+
1048	**Tabbouleh**	*Salmonella typhimurium* 305	Paella	TT 55°C 10 min	>0.9	4.6	+
1049	**Piedmontese salad**	*Salmonella typhimurium* 305	Paella	TT 55°C 10 min	>0.9	4.6	+
1079	**Bacon straw**	Cross contamination with white meat					+
1082	**Raw milk**	*Salmonella montevideo* 606	Raw milk	-20°C	0.6	2	+
1083	**Horse feed**	Cross contamination with dehydrated poultry proteins					-
1088	**Raw milk**	*Salmonella infantis* F401b	Cheese	TT 55°C 30 min	>1.8	1	+
1089	**Raw milk**	*Salmonella montevideo* 604	Raw milk	TT 55°C 30 min	>1.1	1.2	+
1090	**Raw milk**	*Salmonella infantis* F401b	Cheese	TT 55°C 30 min	>1.8	1	+
1091	**Dry sausage**	*Salmonella dublin* Ad528	Pastry	TT 55°C 10 min	>1.00	1.6	+
1092	**Cattle feed**	*Salmonella livingstone* F105	Feedstuff	TT 55°C 30 min	1.3	0.4	+

Appendix 4 – Relative accuracy: raw data

+/-: doubtful colonies

MEAT PRODUCTS														
N°	Product	Reference method ISO 6579◆					TaqMan *Salmonella* method			Agreement	TaqMan *Salmonella* method 72H storage at 4°C			Agreement
		Characteristic colonies				Result	PCR result	Confirmation result	Final result		PCR result	Confirmation result	Final result	
		RVS		MKTTn										
		XLD	SMID2	XLD	SMID2									
1	Ready to eat food with veal and olive	-	-	-	-	-	-	/	-	=				
2	Poultry	+	+	+	+	+	+	+	+	=	+	+	+	=
3	Poultry	+	+	+	+	+	+	+	+	=	+	+	+	=
4	Hen leg	-	-	+	+	+	+	+	+	=	+	+	+	=
5	Hen leg	-	-	-	-	-	-	/	-	=				
37	Veal olive	-	-	-	-	-	-	/	-	=				
38	Poultry	+	+	+	+	+	+	+	+	=	+	+	+	=
39	Poultry	-	-	-	-	-	-	/	-	=				
40	Poultry	+	+	+	-	+	+	+	+	=	+	+	+	=
41	Poultry	-	-	-	-	-	-	/	-	=				
42	Pork mat	-	-	-	-	-	-	/	-	=				
43	Poultry	-	-	-	-	-	-	/	-	=				
44	Poultry	+	+	+	+	+	+	+	+	=	+	+	+	=
45	Poultry	+	-	+ Citrobacter youngae	-	-	-	/	-	=				
103	Ground veal	-	-	-	-	-	-	/	-	=				
104	Veal sausage	-	-	-	-	-	-	/	-	=				
105	Ground veal	-	-	-	-	-	-	/	-	=				
106	Sausage	-	-	-	-	-	-	/	-	=				
107	Poultry	-	-	+ Citrobacter youngae	-	-	-	/	-	=				
108	Poultry	-	+ Citrobacter youngae	+ Citrobacter youngae	-	-	-	/	-	=				
109	Ground veal	-	-	-	-	-	-	/	-	=				

◆ Analysis performed according to the COFRAC accreditation

+/-: doubtful colonies

MEAT PRODUCTS														
N°	**Product**	**Reference method ISO 6579◆**					**TaqMan *Salmonella* method**			**Agreement**	**TaqMan *Salmonella* method 72H storage at 4°C**			**Agreement**
		Characteristic colonies				Result	PCR result	Confirmation result	Final result		PCR result	Confirmation result	Final result	
		RVS		MKTTn										
		XLD	SMID2	XLD	SMID2									
110	Ready to eat with veal and olive	-	-	-	-	-	-	/	-	=				
111	Raw meat	+	+	+	+	+	+	+	+	=	+	+	+	=
119	Poultry	+	+	+	+	+	+	+	+	=	+	+	+	=
120	Raw veal meat	+	+	+	-	+	+	+	+	=	+	+	+	=
121	Raw meat	+	+	+	-	+	+	+	+	=	+	+	+	=
122	Raw meat	+	+	+	+	+	+	+	+	=	+	+	+	=
123	Poultry	+ Citrobacter youngae	-	+ Citrobacter youngae	-	-	+/-/-/+	-	-	=	+	-	-	=
124	Poultry	-	-	+ Citrobacter youngae	-	-	-	/	-	=				
125	Poultry	-	-	+	+	+	+	+	+	=	+	+	+	=
126	Poultry	-	-	+	+	+	+	+	+	=	+	+	+	=
127	Dry sausage	-	-	-	-	-	-	/	-	=				
813	Poultry	+	+	+	+	+	+	+	+	=	+	+	+	=
814	Raw meat	-	-	+	+	+	+	+	+	=	+	+	+	=
815	Poultry	+	-	+	+	+	+	+	+	=	+	+	+	=
816	Poultry	-	-	-	-	-	-	/	-	=				
817	Poultry	-	-	-	-	-	-	/	-	=				
818	Ground beef	-	-	-	-	-	-	/	-	=				
819	Ground beef	-	-	-	-	-	-	/	-	=				
820	Poultry	+	+	+	+	+	+	+	+	=	+	+	+	=
821	Ground beef	-	-	-	-	-	-	/	-	=				
822	Poultry	+	-	+	+	+	+	+	+	=	+	+	+	=
897	Ground beef	+	+	+	+	+	+	+	+	=	+	+	+	=
898	Ground beef	+	+	+	+	+	+	+	+	=	+	+	+	=
899	Ground beef	-	-	-	-	-	-	/	-	=				
900	Ground beef	-	-	-	-	-	-	/	-	=				

+/-: doubtful colonies

MEAT PRODUCTS														
N°	Product	Reference method ISO 6579◆					TaqMan *Salmonella* method			Agreement	TaqMan *Salmonella* method 72H storage at 4°C			Agreement
		Characteristic colonies				Result	PCR result	Confirmation result	Final result		PCR result	Confirmation result	Final result	
		RVS		MKTTn										
		XLD	SMID2	XLD	SMID2									
901	Duck pâté	+	+	+	+	+	+	+	+	=	+	+	+	=
902	Cured meat	-	-	-st	-st	-	-	/	-	=				
903	Liver pâté	-st	-st	-st	-st	-	-	/	-	=				
904	Pâté	-st	-st	-st	-st	-	-	/	-	=				
944	Mortadelle	-st	-st	-st	-st	-	-	/	-	=				
945	Salami	-st	-st	-st	-st	-	-	/	-	=				
946	Garlic sausage	-st	-st	-st	-st	-	-	/	-	=				
974	Andouille	-st	-st	-st	-st	-	-	/	-	=				
975	Chorizo	-st	-st	-st	-st	-	-	/	-	=				
976	Montbéliard sausages	-	-	-st	-	-	-	/	-	=				
977	White pudding	-	-	-	-	-	-	/	-	=				
978	Andouillette	-	-	-st	-st	-	+/+/+	-	-	=	+	-		
979	Pâté	-	-	-	-st	-	-	/	-	=				
980	Ground beef	-	-	-	-	-	-	/	-	=				
981	Ground beef	+	+	+	+	+	+	+	+	=	+	+	+	=
992	Pâté	-st	-st	-st	-st	-	-	/	-					
993	Garlic sausage	-st	-st	-st	st	-	-	/	-					
994	West Indian pudding	-	-	-	-st	-	+/-/-	-	-	=	-	-	-	=
995	Brioche with sausage	-st	-st	-st	-st	-	-	/	-	=				
996	Poultry	+	+	+	+	+	+	+	+	=	+	+	+	=
997	Ham	+	+	+	+	+	+	+	+	=	+	+	+	=
998	Ready to eat food with pork	+	+	+	+	+	+	+	+	=	+	+	+	=
999	Ready to eat food with pork	+	+	+	+	+	+	+	+	=	+	+	+	=
1000	Ground beef	+	+	+	+	+	+	+	+	=	+	+	+	=
1001	Ground beef	+	+	+	+	+	+	+	+	=	+	+	+	=
1079	Bacon	+	+	+	+	+	+	+	+	=	+	+	+	=
1091	Dried sausage	+	+	+	+	+	+	+	+	=	+	+	+	=

+/-: doubtful colonies
* : final dilution of DNA extract 1/50

DAIRY PRODUCTS														
N°	**Product**	**Reference method ISO 6579◆**					**TaqMan *Salmonella* method**			**Agreement**	**TaqMan *Salmonella* method 72H storage at 4°C**			**Agreement**
		Characteristic colonies				Result	PCR result	Confirmation result	Final result		PCR result	Confirmation result	Final result	
		RVS		MKTTn										
		XLD	SMID2	XLD	SMID2									
49	**Milk powder**	+	+	+	+	+	+	+	+	=	+	+	+	=
50	**Milk powder**	+	+	+	+	+	+	+	+	=	+	+	+	=
51	**Milk powder**	+	+	+	+	+	+	+	+	=	+	+	+	=
55	Raw milk	-	-	-	-	-	-	/	-	=				
56	Raw milk	-	-	-	-	-	-	/	-	=				
57	Raw milk	-	-	-	-	-	-	/	-	=				
58	Ewe raw milk	-	-	-	+/-	-	-	/	-	=				
147	Raw milk	-	-	-	-	-	-	/	-	=				
148	Raw milk cheese	-	-	-	-	-	-	/	-	=				
149	Fresh cream	-st	-st	-st	-st	-	-	/	-	=				
315	**Infant milk powder**	+	+	+	+	+	+	+	+	=	+	+	+	=
316	**Infant milk powder**	+	+	+	+	+	+	+	+	=	+	+	+	=
317	**Milk powder**	+	+	+	+	+	+	+	+	=	+	+	+	=
318	**Custard**	+	+	+	+	+	+	+	+	=	+	+	+	=
687	**Raw milk**	-	-	-	-	-	+/-/-	-	-	=	-	-	-	=
688	**Raw milk**	-	-	-	-	-	-	/	-	=				
689	**Raw milk**	-	-	+/- Citrobacter koseri	-	-	-	/	-	=				
690	**Raw milk**	-	-	-	-	-	-	/	-	=				
784	**Tiramisu**	+	+	+	+	+	-	/	-	ND	-	/	-	ND
794	**Vanilla ice cream**	+	+	+	+	+	+	+	+	=	+	+	+	=
795	**Raw milk cheese(Tomme de Savoie)**	+	+	+	+	+	+	+	+	=	+	+	+	=
796	**Raw milk**	+	+	+	+	+	+	+	+	=	+	+	+	=

◆ Analysis performed according to the COFRAC accreditation

+/-: doubtful colonies
* : final dilution of DNA extract 1/50

DAIRY PRODUCTS														
N°	**Product**	**Reference method ISO 6579◆**					**TaqMan *Salmonella* method**			**Agreement**	**TaqMan *Salmonella* method 72H storage at 4°C**			**Agreement**
		Characteristic colonies				Result	PCR result	Confirmation result	Final result		PCR result	Confirmation result	Final result	
		RVS		MKTTn										
		XLD	SMID2	XLD	SMID2									
797	**Raw milk cheese(comté)**	+	+	+	+	+	+	+	+	=	+	+	+	=
798	Vanilla ice cream	-	-	-	-	-	-	/	-	=				
RA145	**Milk powder**	-	-	-	-	-	-	/	-	=				
RA356	**Milk powder**	-	-	-	-	-	-	/	-	=				
RA552	**Milk powder**	+	+	+	+	+	+	+	+	=	+	+	+	=
RA763	**Milk powder**	-	-	-	-	-	-	/	-	=				
RA831	**Milk powder**	-	-	-	-	-	-	/	-	=				
866	Raw milk cheese	-	-	-	-	-	-	/	-	=				
867	Raw milk cheese	-	-	-	-	-	-	/	-	=				
868	Raw milk cheese	-	-	-	-	-	-	/	-	=				
869	Raw milk cheese	-	-	-	-	-	-	/	-	=				
887	Chocolate cream	-st	-st	-st	-st	-	-*	/	-	=				
905	Goat cheese	+	+	+	+	+	+	+	+	=	+	+	+	=
906	Raw milk Raclette	+	+	+	+	+	+	+	+	=	+	+	+	=
907	Emmental	+	+	+	+	+	+	+	+	=	+	+	+	=
908	Comté	+	+	+	+	+	+	+	+	=	+	+	+	=
909	St Nectaire	+	+	+	+	+	+	+	+	=	+	+	+	=
910	Raw milk	+	+	+	+	+	+	+	+	=	+	+	+	=
911	Raw milk	+	+	+	+	+	+	+	+	=	+	+	+	=
912	Raw milk	+	+	+	+	+	+	+	+	=	+	+	+	=
913	Raw milk	+	+	+	+	+	+	+	+	=	+	+	+	=
914	Raw milk	+	+	+	+	+	+	+	+	=	+	+	+	=
936	Raw milk Raclette	+	+	+	+	+	+	+	+	=	+	+	+	=
937	Raw milk comté	+	+	+	+	+	+	+	+	=	+	+	+	
938	Raw milk cheese	+	+	+	+	+	+	+	+	=	+	+	+	=

+/-: doubtful colonies
* : final dilution of DNA extract 1/50

DAIRY PRODUCTS														
N°	**Product**	**Reference method ISO 6579◆**					**TaqMan *Salmonella* method**			**Agreement**	**TaqMan *Salmonella* method 72H storage at 4°C**			**Agreement**
		Characteristic colonies				Result	PCR result	Confirmation result	Final result		PCR result	Confirmation result	Final result	
		RVS		MKTTn										
		XLD	SMID2	XLD	SMID2									
939	Goat cheees	+	+	+	+	+	+	+	+	=	+	+	+	=
951	Vanilla ice cream	-st	-st	-st	-st	-	-	/	-	=				
952	Chocolate ice cream	-st	-st	-st	-st	-	-	/	-	=				
970	Raw milk	-	-	-	-	-	-	/	-	=				
971	Raw milk	-	-	-	-	-	+/-/-	-	-	=	-	-	-	=
972	Goat cheese with herbs	-	-	-	-	-	-	/	-	=				
973	Raw milk camembert	-	-	-	-	-	-	/	-	=				
988	Cheese (Rocamadour)	-	-	-	-	-	+/-/-	-	-	=	-	-	-	=
989	Cheese (Picodon)	-	-	-	-	-	-	/	-	=				
990	Raw milk cheese(Crottin de chavignol)	-	-	-	-	-	-	/	-	=				
991	Raw milk cheese(Tomme des Pyrénées)	-	-	-	-	-	-	/	-	=				
1002	Raw milk	+(NC sur GN)	-	-	-	-	+/-/+	-	-	=	+	-	-	=
1003	Raw milk	-	-	-	-	-	+/-/+	-	-	=	+	-	-	=
1004	Raw milk	-	-	-	-	-	+/+/+	-	-	=	-/-/-	-	-	=
1005	Raw milk	-	-	-	-	-	-	/	-	=				
1082	Raw milk	+	+	+	+	+	+	+	+	=	+	+	+	=
1088	Raw milk	+	+	-	-	+	+	+	+	=	+	+	+	=
1089	Raw milk	+	+	-	-	+	-	/	-	ND	+	+	+	=
1090	Raw milk	+	+	-	+	+	-	+	+	=	+	+	+	=

+/-: doubtful colonies
* : final dilution of DNA extract 1/50

SEAFOOD AND VEGETABLES PRODUCTS														
N°	Product	Reference method ISO 6579◆					TaqMan *Salmonella* method			Agreement	TaqMan *Salmonella* method 72H storage at 4°C			Agreement
		Characteristic colonies				Result	PCR result	Confirmation result	Final result		PCR result	Confirmation result	Final result	
		RVS		MKTTn										
		XLD	SMID2	XLD	SMID2									
6	Cod	-	-	-	-	-	-	/	-	=				
7	Hake filet	-	-	+ Citrobacter youngae	-	-	-	/	-	=				
36	Piedmontese salad	-	-	-	-	-	-	/	-	=				
307	**Red pepper**	+	+	+	+	+	+	+	+	=				
308	**Green peas**	+	+	+	+	+	+	+	+	=	+	+	+	=
309	**Salsify**	+	+	+	+	+	+	+	+	=	+	+	+	=
310	**Ready to eat food with tomatoes**	+	+	+	+	+	+	+	+	=				
311	**Smoked salmon**	+	+	+	+	+	+	+	+	=	+	+	+	=
312	**Seafood cocktail**	+	+	+	+	+	+	+	+	=	+	+	+	=
313	**Raw fish**	+	+	+	+	+	+	+	+	=	+	+	+	=
314	**Raw fish**	+	+	+	+	+	+	+	+	=	+	+	+	=
693	**Mushrooms**	-	-	-st	-	-	+/+/-	-	-	=	+/-/+	-	-	=
694	**Asparagus**	-	-	-st	-	-	+/-/-	-	-	=	-	/	-	=
788	**Rice and vegetables mix**	+	+	+	+	+	+	+	+	=	+	+	+	=
789	**Rice and vegetables mix**	+	+	+	+	+	+	+	+	=	+	+	+	=
872	Gurnard	-	-	-	-	-	-	/	-	=				
873	Pollack	-	-	-	-	-	-	/	-	=				
874	Smoked salmon	-	-	-	-	-	-	/	-	=				
875	Dab	-	-	-	-	-	-	/	-	=				
876	Salmon pieces	-	-	-	-	-	-	/	-	=				
877	Ready to eat hake	-	-	-	-	-	-	/	-	=				
878	Turnip	-	-	-	-	-	+/-/-	-	-	=	-	/	-	=

◆ Analysis performed according to the COFRAC accreditation

+/-: doubtful colonies
* : final dilution of DNA extract 1/50

SEAFOOD AND VEGETABLES PRODUCTS														
N°	**Product**	**Reference method ISO 6579◆**					**TaqMan *Salmonella* method**			**Agreement**	**TaqMan *Salmonella* method 72H storage at 4°C**			**Agreement**
		Characteristic colonies				Result	PCR result	Confirmation result	Final result		PCR result	Confirmation result	Final result	
		RVS		MKTTn										
		XLD	SMID2	XLD	SMID2									
879	Courgettes	-	-	-	-	-	-	/	-	=				
880	Broccolis	-	-	-	-	-	-	/	-	=				
881	Red pepper	-	-	-	-	-	-	/	-	=				
882	Green beans	-	-	-	-	-	-	/	-	=				
883	Red pepper	-	-	-st	-st	-	-	/	-	=				
884	Yellow pepper	-	-	-	-	-	-	/	-	=				
885	Strawberries	-st	-st	-st	-st	-	-*	/	-	=				
886	Dry grapes	-st	-st	-st	-st	-	-*	/	-	=				
888	Peach purée	-st	-st	-st	-st	-	-	/	-	=				
889	Ripes purée	-st	-st	-st	-st	-	-*	/	-	=				
890	Cooked beans	-	-	-st	-st	-	-	/	-	=				
891	Mango coulis	-st	-st	-st	-st	-	-	/	-	=				
892	Tomato blocks	-	-	-	-	-	-	/	-	=				
893	Lemon butter sauce	+	+	+	+	+	+	+	+	=	+	+	+	=
894	Basilic tomato sauce	-st	-st	-st	-st	-	-	/	-	=				
895	Croissant dough	-	-	-	-	-	-	/	-	=				
896	Bread dough	-	-	-	-	-	-	/	-	=				
947	Carrots	+	+	+	+	+	+	+	+	=	+	+	+	=
948	Salads	+	+	+	+	+	+	+	+	=	+	+	+	=
949	Shallots	+	+	+	+	+	+	+	+	=	+	+	+	=
950	Courgettes	+	+	+	+	+	-	/	-	ND	+	+	+	=
962	Raw fish	+	+	+	+	+	+	+	+	=	+	+	+	=
963	Salmon	+	+	+	+	+	+	+	+	=	+	+	+	=
964	Pollack	+	+	+	+	+	+	+	+	=	+*	+	+	=
965	Salmon	+	+	+	+	+	+	+	+	=	+	+	+	=

+/-: doubtful colonies
* : final dilution of DNA extract 1/50

SEAFOOD AND VEGETABLES PRODUCTS														
N°	**Product**	**Reference method ISO 6579◆**					**TaqMan *Salmonella* method**			**Agreement**	**TaqMan *Salmonella* method 72H storage at 4°C**			**Agreement**
		Characteristic colonies				Result	PCR result	Confirmation result	Final result		PCR result	Confirmation result	Final result	
		RVS		MKTTn										
		XLD	SMID2	XLD	SMID2									
966	Onions	+	+	+	+	+	+	+	+	=	+	+	+	=
967	Minced vegetables	+	+	+	+	+	+	+	+	=	+	+	+	=
968	Carrots	+	+	+	+	+	+	+	+	=	+	+	+	=
969	Tomato	+	+	+	+	+	+	+	+	=	+	+	+	=
1040	Spinash	+	+	+	+	+	+	+	+	=	+	+	+	=
1041	Brussels sprouts	+	+	+	+	+	+	+	+	=	+	+	+	=
1042	Smoked salmon	+	+	+	+	+	+	+	+	=	+	+	+	=
1043	Tuna rillettes	+	+	+	+	+	+	+	+	=	+	+	+	=
1044	Pangasin filets	+	+	+	+	+	+	+	+	=	+	+	+	=
1045	Grenadier filet	+	+	+	+	+	+	+	+	=	+	+	+	=
1046	Preparation for salad	+	+	+	+	+	+	+	+	=	+	+	+	=
1047	Remoulade celery	+	+	+	+	+	+	+	+	=	+	+	+	=
1048	Tabbouleh	+	+	+	+	+	+	+	+	=	+	+	+	=
1049	Piemontaise salad	+	+	+	+	+	+	+	+	=	+	+	+	=
1080	Salmon kebab	-	-	-	-	-	-	/	-	=				
1081	Scallops terrine	-	-	-st	-st	-	-	/	-	=				

+/-: doubtful colonies

* : final dilution of DNA extract 1/50

EGG PRODUCTS														
N°	Product	Reference method ISO 6579◆					TaqMan *Salmonella* method			Agreement	TaqMan *Salmonella* method 72H storage at 4°C			Agreement
		Characteristic colonies				Result	PCR result	Confirmation result	Final result		PCR result	Confirmation result	Final result	
		RVS		MKTTn										
		XLD	SMID2	XLD	SMID2									
8	Raw egg product	+ 10 col red	-	+	+	+	+	+	+	=	+	+	+	=
9	Raw egg product	-	-	+	-	+	+	+	+	=	+	+	+	=
10	Raw egg product	+	-	+	-	+	+	+	+	=	+	+	+	=
52	Raw egg product	-	-	-	-	-	+	+	+	PD	+	+	+	PD
53	Raw egg product	-	-	+	-	+	+	+	+	=	+	+	+	=
54	Raw egg product	-	-	+	+	+	+	+	+	=	+	+	+	=
150	Egg product	+	+	+	-	+	+	+	+	=	+	+	+	=
151	Egg product	+	+	+	-	+	+	+	+	=	+	+	+	=
152	Egg product	+	+	+	-	+	+	+	+	=	+	+	+	=
153	Egg product	+	+	+	-	+	+	+	+	=	+	+	+	=
154	Egg product	+	+	+	-	+	+	+	+	=	+	+	+	=
155	Custard	-st	-st	-st	-st	-	-	/	-	=				
156	Custard	-st	-st	-st	-st	-	-	/	-	=				
157	Custard	-	-	-st	-st	-	-	/	-	=				
158	Custard	-st	-st	-st	-st	-	-	/	-	=				
159	Mayonnaise	+ 1col	+	-	-	+	+	+	+	=	+	+	+	=
160	Mayonnaise	+	+	+/- red	+	+	+	+	+	=	+	+	+	=
161	Mayonnaise	-	+ 1col	+1col Citrobacter youngae	-	+	+	+	+	=	+	+	+	=
162	Custard	-	-	-	-	-	-	/	-	=				
163	Custard	-	-	+/- red Providencia rettgeri	-	-	-	/	-	=				
164	Custard	-st	-st	-st	-st	-	+/-/-/-	-	-	=	-	/	-	=
691	**Mayonnaise**	-st	-	-st	-	-	+/+/+	-	-	=	-	/	-	=

◆ Analysis performed according to the COFRAC accreditation

+/-: doubtful colonies

* : final dilution of DNA extract 1/50

EGG PRODUCTS														
N°	Product	Reference method ISO 6579◆					TaqMan *Salmonella* method			Agreement	TaqMan *Salmonella* method 72H storage at 4°C			Agreement
		Characteristic colonies				Result	PCR result	Confirmation result	Final result		PCR result	Confirmation result	Final result	
		RVS		MKTTn										
		XLD	SMID2	XLD	SMID2									
692	**Mayonnaise**	-st	-	-st	-	-	-	/	-	=				
799	Custard	-	-	-	-	-	-	/	-	=				
800	Custard	-	-	-	-	-	-	/	-	=				
801	Custard	-	-	-	-	-	-	/	-	=				
802	Mayonnaise	+	-	+	-	+	+	+	+	=	+	+	+	=
803	Mayonnaise	+	+	+	+	+	+	+	+	=	+	+	+	=
804	Mayonnaise	+	-	+	-	+	+	+	+	=	+	+	+	=
805	Mayonnaise	+	+	+	+	+	+	+	+	=	+	+	+	=
806	Egg product	-	-	+	-	+	+	+	+	=	+	+	+	=
807	Egg product	-	-	+	-	+	+	+	+	=	+	+	+	=
808	Egg product	+	-	+	-	+	+	+	+	=	+	+	+	=
809	Egg product	+	-	+	-	+	+	+	+	=	+	+	+	=
810	Custard	+	-	-	-	+	+	+	+	=	+	+	+	=
811	Custard	-	-	-	-	-	-	/	-	=				
812	Custard	-	-	-	-	-	-	/	-	=				
870	Egg white powder	-	-	-	-	-	-	/	-	=				
871	Egg yolk powder	-	-	-	-	-	+/+/+	-	-	=	+	-	-	=
925	Egg product	-st	-st	-st	-st	-	-	/	-	=				
926	Egg product	-st	-st	-st	-st	-	-	/	-	=				
927	Egg product	-st	-st	-st	-st	-	-	/	-	=				
928	Egg product	-st	-st	-st	-st	-	-	/	-	=				
940	Mayonnaise	-st	-st	-st	-st	-	-	/	-	=				
941	Mayonnaise	-st	-st	-st	-st	-	-	/	-	=				
942	Mayonnaise	-st	-st	-st	-st	-	-	/	-	=				
943	Mayonnaise	-st	-st	-st	-st	-	-	/	-	=				
986	Mayonnaise	-st	-st	-st	-st	-	-	/	-	=				

+/-: doubtful colonies

* : final dilution of DNA extract 1/50

EGG PRODUCTS														
N°	**Product**	**Reference method ISO 6579◆**					**TaqMan *Salmonella* method**			**Agreement**	**TaqMan *Salmonella* method 72H storage at 4°C**			**Agreement**
		Characteristic colonies				Result	PCR result	Confirmation result	Final result		PCR result	Confirmation result	Final result	
		RVS		MKTTn										
		XLD	SMID2	XLD	SMID2									
987	Mayonnaise	-st	-st	-st	-st	-	-	/	-	=				
1006	Custard	+	+	+	+	+	+	+	+	=	+	+	+	=
1007	Custard	+	+	-	-	+	+	+	+	=	+	+	+	=
1008	Custard	+	+	+	+	+	+	+	+	=	+	+	+	=
1009	Pastry and custard	-	-	-	-	-	-*	/	-	=				
1010	Pastry and custard	+	+	-	-	+	+	+	+	=	+	+	+	=
1011	Pastry and custard	+	+	+	+	+	+	+	+	=	+	+	+	=
1012	Egg powder	+	-	+	-	+	+	+	+	=	+	+	+	=
1013	Egg powder	+	-	+	+	+	+	+	+	=	+	+	+	=
1014	White egg powder	-st	-st	-st	-st	-	-	/	-	=				
1015	White egg powder	+	+	+	+	+	+	+	+	=	+	+	+	=
1016	Egg product	-st	-st	-st	-st	-	-	/	-	=				
1017	Egg product	-st	-st	-st	-st	-	-	/	-	=				
1018	Egg product	-st	-st	-st	-st	-	-	/	-	=				
1019	Egg product	-st	-st	-st	-st	-	+/+/+	-	-	=	+	-	-	=
1020	Egg product	-st	-st	-st	-st	-	+/+/+	-	-	=	+	-	-	=
1021	Egg product	-st	-st	-st	-st	-	+/+/+	-	-	=	+	-	-	=

* : final dilution of DNA extract 1/50
+/-: doubtful colonies

FEEDSTUFFS														
N°	**Product**	**Reference method ISO 6579◆**					**TaqMan *Salmonella* method**			**Agreement**	**TaqMan *Salmonella* method 72H storage at 4°C**			**Agreement**
		Characteristic colonies				Result	PCR result	Confirmation result	Final result		PCR result	Confirmation result	Final result	
		RVS		MKTTn										
		XLD	SMID2	XLD	SMID2									
35	Dehydrated poultry proteins	+	+	+	+	+	+	+	+	=	+	+	+	=
46	Dehydrated poultry proteins	-	-	-	-	-	-	/	-	=				
47	Dehydrated poultry proteins	+	+	+	+	+	+	+	+	=	+	+	+	=
48	Dehydrated poultry proteins	+	+	+	+	+	+	+	+	=	+	+	+	=
112	Dehydrated poultry proteins	-	-	-	-	-	-	/	-	=				
113	Dehydrated poultry proteins	+	+	-	+	+	+	+	+	=	+	+	+	=
114	Dehydrated poultry proteins	-	-	-	-	-	-	/	-	=				
115	Dehydrated poultry proteins	+	+	+	+	+	+	+	+	=				
116	Dehydrated poultry proteins	-	-	-	-	-	-	/	-	=				
117	Dehydrated poultry proteins	+	+	+	+	+	+	+	+	=	+	+	+	=
118	Dehydrated poultry proteins	-	-	-	-	-	-	/	-	=				
859	Pet food for cat	-	-	-	-	-	-	/	-	=				
860	Seeds for birds	-	-	-	-	-	-	/	-	=				
861	Feeding stuffs (cow)	-	-	-	-	-	-	/	-	=				
862	Feeding stuffs (pig)	-	-	-	-	-	-	/	-	=				
863	Feeding stuffs (young bovine)	-	-	-	-	-	-	/	-	=				
864	Feeding stuffs (cow)	-	-	-	-	-	-	/	-	=				
865	Creals	-	-	-	-	-	-	/	-	=				
915	Pet food	+	+	+	+	+	-	/	-	ND	+	+	+	=
916	Pet food	+	+	+	+	+	+	+	+	=	+	+	+	=
917	Raw meat	+	+	+	+	+	+	+	+	=	+	+	+	=
918	Sausage for dog	+	+	+	+	+	+	+	+	=	+	+	+	=
919	Pâté for cat	+	+	+	+	+	+	+	+	=	+	+	+	=

◆ Analysis performed according to the COFRAC accreditation

* : final dilution of DNA extract 1/50
+/-: doubtful colonies

FEEDSTUFFS														
N°	Product	Reference method ISO 6579◆					TaqMan *Salmonella* method			Agreement	TaqMan *Salmonella* method 72H storage at 4°C			Agreement
		Characteristic colonies				Result	PCR result	Confirmation result	Final result		PCR result	Confirmation result	Final result	
		RVS		MKTTn										
		XLD	SMID2	XLD	SMID2									
920	Feeding stuffs (cow)	-st	-st	-st	-st	-	-	/	-	=				
921	Feeding stuffs (cow)	+	+	+	+	+	+	+	+	=	+	+	+	=
922	Feeding stuffs (bovine)	+	+	+	+	+	+	+	+	=	+	+	+	=
923	Feeding stuffs (cow)	+	+	+	+	+	+	+	+	=	+	+	+	=
924	Feeding stuffs (pigelet)	+	+	+	+	+	+	+	+	=	+	+	+	=
931	Dehydrated poultry proteins	+	+	+	+	+	+	+	+	=	+	+	+	=
932	Dehydrated poultry proteins	-	-	-	-	-	-	/	-	=				
933	Low moisture product	+	+	+	+	+	+	+	+	=	+	+	+	=
934	Low moisture product	-st	-st	-st	-st	-	+/-/+	-	-	=	+	-	-	=
935	Low moisture product	-	-	-st	-st	-	-	/	-	=				
953	Low moisture product)	-	-	-	-	-	-	/	-	=				
954	Salmon terrine for cat	+	+	+	+	+	+*	+	+	=	+	+	+	=
955	Terrine for dog	+	+	+	+	+	+	+	+	=	+	+	+	=
956	Mix seeds for hamster	+	+	+	+	+	+	+	+	=	+	+	+	=
957	Mix seeds for rabbit	-	-	-	-	-	-	/	-	=				
958	Raw meat for pet	-	-	-	-	-	-	/	-	=				
959	Raw meat for pet	-	-	-	-	-	-	/	-	=				
960	Raw meat for pet	-	-	-	-	-	-	/	-	=				
961	Raw meat for pet	-	-	-	-	-	+/-/-	-	-	=	-	/	-	=
982	Raw meat for pet	-	-	-	-	-	-	/	-	=				
983	Raw meat for pet	+	+	+	+	+	+	+	+	=	-	-	-	ND
984	Croquettes for dog	-st	-st	-st	-st	-	-	/	-	=				
985	Beef croquettes for dog	-st	-st	-st	-st	-	-	/	-	=				
1022	Feed for horses	+	+	+	+	+	+	+	+	=	+	+	+	=
1023	Feed for horses	+	+	+	+	+	+	+	+	=	+	+	+	=

* : final dilution of DNA extract 1/50
+/-: doubtful colonies

FEEDSTUFFS														
N°	**Product**	**Reference method ISO 6579◆**					**TaqMan *Salmonella* method**			**Agreement**	**TaqMan *Salmonella* method 72H storage at 4°C**			**Agreement**
		Characteristic colonies				Result	PCR result	Confirmation result	Final result		PCR result	Confirmation result	Final result	
		RVS		MKTTn										
		XLD	SMID2	XLD	SMID2									
1024	Feed for cow	+	+	+	+	+	+	+	+	=	+	+	+	=
1025	Feed for pork	+	+	+	+	+	+	+	+	=	+	+	+	=
1026	Feed for cow	+	+	+	+	+	+	+	+	=	+	+	+	=
1027	Feed for horses	-	-	-st	-st	-	-	/	-	=				
1028	Croquettes for cat	+	+	+	+	+	+	+	+	=	+	+	+	=
1029	Croquettes mix for cat	+	+	+	+	+	+	+	+	=	+	+	+	=
1030	Dehydrated poultry proteins	-st	-	-st	-st	-	-	/	-	=				
1031	Dehydrated poultry proteins	-st	-st	-st	-st	-	-	/	-	=				
1032	Feed for horses	-st	-	-st	-st	-	-	/	-	=				
1033	Feed for horses	-	-	-	-st	-	-	/	-	=				
1034	Feed for horses	-	-	-	-st	-	-	/	-	=				
1035	Feed for pork	-st	-st	-st	-st	-	-	/	-	=				
1036	Croquettes for cat	-st	-	-st	-st	-	-	/	-	=				
1037	Croquettes for kitten	-st	-st	-st	-st	-	-	/	-	=				
1038	Low moisture product	-	-	-	-	-	+/+/+	-	-	=	+	-	-	=
1039	Low moisture product	+	+	+	+	+	+	+	+	=	+	+	+	=
1083	Feed for horses	-	-	-	-	-	+/-/+	-	-	=	+	-	-	=
1092	Feed for bovines	+	+	+	+	+	+	+	+	=	+	+	+	=

Appendix 5 – Inclusivity and exclusivity: raw data

	NEGATIVE STRAINS			
	Strain	**Reference**	**Origin**	**TaqMan result**
1.	*Citrobacter diversus*	adria 140	Raw milk	-
2.	*Citrobacter koseri*	CIP 8294T (ATCC 27156)		-
3.	*Citrobacter freundii*	adria 23	Sausages	-
4.	*Citrobacter freundii*	59		-
5.	*Citrobacter freundii*	adria 175	Duck meat	-
6.	*Escherichia coli*	adria 2B	Sausages	-
7.	*Escherichia coli*	adria 6	Sausages	-
8.	*Escherichia coli*	adria 19	Carrots	-
9.	*Escherichia coli*	CIP 54117		-
10.	*Enterobacter aerogenes*	CIP 6086T (ATCC 13048)		-
11.	*Enterobacter agglomerans*	adria 11	Cheese	-
12.	*Enterobacter cloacae*	adria 10	Raw milk	-
13.	*Enterobacter cloacae*	adria 128	Ground beef	-
14.	*Enterobacter sakazakii*	adria 95	Cheese	-
15.	*Enterobacter sakazakii*	adria D7	Poultry	-
16.	*Hafnia alvei*	adria 167	Sausages	-
17.	*Hafnia alvei*	adria 168	Duck meat	-
18.	*Klebsiella oxytoca*	57		-
19.	*Klebsiella oxytoca*	42		-
20.	*Klebsiella pneumoniae*	CIP 8291T (ATCC 13883)		-
21.	*Klebsiella pneumoniae*	28		-
22.	*Proteus mirabilis*	adria 54	Poultry	-
23.	*Proteus mirabilis*	55		-
24.	*Proteus vulgaris*	56		-
25.	*Serratia liquefaciens*	8	Egg product	-
26.	*Serratia proteomaculans*	A00C056	Ham	-
27.	*Shigella sonnei*	CIP 51.1		-
28.	*Shigella sonnei*	CIP 8249T (ATCC 29930)		-
29.	*Yersinia enterocolotica*	adria 32	Bacon	-
30.	*Yersinia enterocolotica*	CIP 8027T (ATCC 9610)		-
31.	*Bacillus cereus*	11	RTE food	-
32.	*Bacillus pumilus*	24	Chicken	-
33.	*Bacillus firmus*	Ad145	Vegetables	-
34.	*Enterococcus durans*	Ad149	Ham	-
35.	*Enterococcus faecalis*	Ad175	Egg product	-
36.	*Salmonella bongori (sub-group V)*	Ad598	Environmental sample	-

	Sub-species	Strain	Reference	Origin	TaqMan result
		POSITIVE STRAINS			
1.	*Enterica (sub-group I)*	*Salmonella Anatum*	A00E007	Dusts (dairy)	+
2.		*Salmonella Blockley*	Ad923	Poultry	+
3.		*Salmonella Bovis morbificans*	adria 132	Bacon	+
4.		*Salmonella Bovis morbificans*	adria 6629	Sausages	+
5.		*Salmonella Brandenburg*	adria 499	Toulouse sausages	+
6.		*Salmonella Branderup*	adria 111	Pork meat	+
7.		*Salmonella Bredeney*	adria 141	Sausage meat	+
8.		*Salmonella Bredeney*	adria 464	Pork pate	+
9.		*Salmonella Cerro*	Ad689	Dehydrated proteins	+
10.		*Salmonella Cremieu*	Ad 230	Meat	+
11.		*Salmonella Derby*	adria 374	Sausages	+
12.		*Salmonella Dublin*	Ad 531	Raw cheese milk	+
13.		*Salmonella Enteritidis*	adria 657	Egg product	+
14.		*Salmonella Enteritidis*	adria 2532	Ham	+
15.		*Salmonella Enteritidis*	10	White egg powder	+
16.		*Salmonella Gallinarum*	1	Poultry	+
17.		*Salmonella Gallinarum*	2	Poultry	+
18.		*Salmonella Hadar*	35	Poultry	+
19.		*Salmonella Hadar*	adria 24871	White chicken meat	+
20.		*Salmonella Havana*	Ad930	Poultry	+
21.		*Salmonella Heidelberg*	36	Food	+
22.		*Salmonella Heidelberg*	adria 285	Tomato forcemeat	+
23.		*Salmonella Heidelberg*	adria 24876	White chicken meat	+
24.		*Salmonella Indiana*	adria 2	Fish flour	+
25.		*Salmonella Infantis*	adria 14	Egg product	+
26.		*Salmonella Infantis*	adria 401B	Raw milk	+
27.		*Salmonella Infantis*	128	Ground beef	+
28.		*Salmonella Kedougou*	Ad929	Slaughterhouse	+
29.		*Salmonella Kottbus*	1	Poultry	+
30.		*Salmonella Livingstone*	E1	White egg powder	+
31.		*Salmonella London*	adria 326	Cooked pork shoulder	+
32.		*Salmonella Manhattan*	900	Dusts (dairy industry)	+
33.		*Salmonella Mbandaka*	adria 81	Egg product	+
34.		*Salmonella Monteivideo*	Ad912	Raw milk	+
35.		*Salmonella Napoli*	Ad928	Clinical	+
36.		*Salmonella Newport*	adria 540	Toulouse sausages	+
37.		*Salmonella Newport*	adria 586	Beef carcass	+
38.		*Salmonella panama*	adria 8	Ground beef	+
39.		*Salmonella panama*	adria 882	Sausages with herbs	+

	Sub-species	POSITIVE STRAINS Strain	Reference	Origin	TaqMan result
40.		*Salmonella paratyphi A*	ATCC 11511		+
41.		*Salmonella paratyphi A*	ATCC 9150		+
42.		*Salmonella paratyphi B*	Ad 301	Human	+
43.		*Salmonella paratyphi C*	ATCC 13428		+
44.		*Salmonella Regent*	328	Duck meat	+
45.		*Salmonella Rissen*	39	Poultry	+
46.		*Salmonella Seftenberg*	1	Food	+
47.		*Salmonella Saintpaul*	631	Poultry	+
48.		*Salmonella Tennessee*	A00E006	Dusts (dairy)	+
49.		*Salmonella typhi*	Ad 302	Human	+
50.		*Salmonella Typhimurium*	adria 193	Sausages	+
51.		*Salmonella Typhimurium*	adria 206	Pasteurised egg product	+
52.		*Salmonella Typhimurium*	adria 305	Paella	+
53.		*Salmonella Typhimurium*	adria 528	Brine	+
54.		*Salmonella Typhimurium SI 1,4,[5],12:-:- (Non mobile)*	Ad1333	Tiramisu	+
55.		*Salmonella Typhimurium SI 1,4,[5],12:i:- (monophasic)*	Ad1334	Pork product	+
56.		*Salmonella Typhimurium SI 1,4,[5],12:-:1,2 (monophasic)*	Ad1335	Poultry	+
57.		*Salmonella Virchow*	F276	Curry	+
58.		*Salmonella Virchow*	CIP 105355		+
59.		*Salmonella Worthington*	adria 3506	Terrine	+
60.	*salamae (sub-group II)*	*Salmonella salamae*	Ad592	Kangaroo meat	+
61.		*Salmonella salamae*	Ad593	Seed	+
62.		*Salmonella arizonae 51;z4:z23*	CIP 5523		+
63.	*arizoane (sub-group IIIa)*	*Salmonella arizonae*	Ad 450	Ewe milk	+
64.		*Salmonella diarizonae 47:IV:z35*	Ad 478	Clams	+
65.		*Salmonella diarizonae 38:IC:z35*	Ad594	Frog	+
66.	*diarizonae (sub-group IIIb)*	*Salmonella diarizonae 61:i:z53*	Ad595	Cheese	+
67.	*houtanae (sub-group IV)*	*Salmonella houtanae*	Ad596	Milk product	+
68.		*Salmonella houtanae*	Ad597	RTE food	+
69.	*indica (sub-group VI)*	*Salmonella indica*	Ad600	Environmental sample	+

Appendix 6 – Accordance

Reference method

Level L0							
Laboratory	Positive results number obtained	Positive results probability	Positive pairs probability	Negative results number obtained	Negative results probability	Positive pairs probability	Identical results pairs probability
A	0	0	0	8	1	1	1
B	1	0.125	0.015625	7	0.875	0.765625	0.78125
D	2	0.25	0.0625	6	0.75	0.5625	0.625
H	1	0.125	0.015625	7	0.875	0.765625	0.78125
I	0	0	0	8	1	1	1
J	0	0	0	8	1	1	1
L	0	0	0	8	1	1	1
M	0	0	0	8	1	1	1
N	0	0	0	8	1	1	1
O	0	0	0	8	1	1	1
						Average	0.919
						Accord degree	92%

Level L1							
Laboratory	Positive results number obtained	Positive results probability	Positive pairs probability	Negative results number obtained	Negative results probability	Positive pairs probability	Identical results pairs probability
A	8	1	1	0	0	0	1
B	8	1	1	0	0	0	1
D	8	1	1	0	0	0	1
H	8	1	1	0	0	0	1
I	8	1	1	0	0	0	1
J	5	0.625	0.390625	3	0.375	0.140625	0.53125
L	8	1	1	0	0	0	1
M	8	1	1	0	0	0	1
N	8	1	1	0	0	0	1
O	8	1	1	0	0	0	1
						Average	0.953
						Accord degree	95%

Level L2							
Laboratory	Positive results number obtained	Positive results probability	Positive pairs probability	Negative results number obtained	Negative results probability	Positive pairs probability	Identical results pairs probability
A	8	1	1	0	0	0	1
B	8	1	1	0	0	0	1
D	8	1	1	0	0	0	1
H	8	1	1	0	0	0	1
I	8	1	1	0	0	0	1
J	8	1	1	0	0	0	1
L	8	1	1	0	0	0	1
M	8	1	1	0	0	0	1
N	8	1	1	0	0	0	1
O	8	1	1	0	0	0	1
						Average	1
						Accord degree	100%

Alternative method

Level L0							
Laboratory	Positive results number obtained	Positive results probability	Positive pairs probability	Negative results number obtained	Negative results probability	Positive pairs probability	Identical results pairs probability
A	0	0	0	8	1	1	1
B	1	0.125	0.015625	7	0.875	0.765625	0.78125
D	0	0	0	8	1	1	1
H	0	0	0	8	1	1	1
I	0	0	0	8	1	1	1
J	0	0	0	8	1	1	1
L	0	0	0	8	1	1	1
M	0	0	0	8	1	1	1
N	0	0	0	8	1	1	1
O	0	0	0	8	1	1	1
						Average	0.978125
						Accord degree	98%

Level L1							
Laboratory	Positive results number obtained	Positive results probability	Positive pairs probability	Negative results number obtained	Negative results probability	Positive pairs probability	Identical results pairs probability
A	8	1	1	0	0	0	1
B	8	1	1	0	0	0	1
D	8	1	1	0	0	0	1
H	8	1	1	0	0	0	1
I	8	1	1	0	0	0	1
J	5	0.625	0.390625	3	0.375	0.140625	0.53125
L	8	1	1	0	0	0	1
M	8	1	1	0	0	0	1
N	8	1	1	0	0	0	1
O	8	1	1	0	0	0	1
						Average	0.953
						Accord degree	95%

Level L2							
Laboratory	Positive results number obtained	Positive results probability	Positive pairs probability	Negative results number obtained	Negative results probability	Positive pairs probability	Identical results pairs probability
A	8	1	1	0	0	0	1
B	8	1	1	0	0	0	1
D	8	1	1	0	0	0	1
H	8	1	1	0	0	0	1
I	8	1	1	0	0	0	1
J	8	1	1	0	0	0	1
L	8	1	1	0	0	0	1
M	8	1	1	0	0	0	1
N	8	1	1	0	0	0	1
O	8	1	1	0	0	0	1
						Average	1
						Accord degree	100%

Appendix 7 – Concordance

Reference method

Level LO

Laboratories number 10

Number of negative results per laboratory: 8

Laboratory	Number of negative results	Interlaboratories pairs with the same result	Total interlaboratories pairs number
A	8	544	576
B	7	486	576
D	6	424	576
H	7	486	576
I	8	544	576
J	8	544	576
L	8	544	576
M	8	544	576
N	8	544	576
O	8	544	576
Total		5 204	5 760
Concordance		90.3%	

Total + 4

Total - 76

Level L1

Laboratories number 10

Number of positive results per laboratory: 8

Laboratory	Number of positive results	Interlaboratories pairs with the same result	Total interlaboratories pairs number
A	8	552	576
B	8	552	576
D	8	552	576
H	8	552	576
I	8	552	576
J	5	360	576
L	8	552	576
M	8	552	576
N	8	552	576
O	8	552	576
Total		5 328	5 760
Concordance		92.5%	

Total + 77

Total - 3

Level L2

Laboratories number 10

Number of positive results per laboratory: 8

Laboratory	Number of positive results	Interlaboratories pairs with the same result	Total interlaboratories pairs number
A	8	576	576
B	8	576	576
D	8	576	576
H	8	576	576
I	8	576	576
J	8	576	576
L	8	576	576
M	8	576	576
N	8	576	576
O	8	576	576
Total		5 760	5 760
Concordance		100.0%	

Total + 80

Total - 0

Alternative method

Level LO

Laboratories number 10

Number of negative results per laboratory: 8

Laboratory	Number of negative results	Interlaboratories pairs with the same result	Total interlaboratories pairs number
A	8	544	576
B	7	486	576
C	8	544	576
H	8	544	576
I	8	544	576
J	8	544	576
L	8	544	576
M	8	544	576
N	8	544	576
O	8	544	576
Total		5 382	5 760
Concordance		93.4%	

Total + 1

Total - 79

Level L1

Laboratories number 10

Number of positive results per laboratory: 8

Laboratory	Number of positive results	Interlaboratories pairs with the same result	Total interlaboratories pairs number
A	8	552	576
B	8	552	576
C	8	552	576
H	8	552	576
I	8	552	576
J	5	360	576
L	8	552	576
M	8	552	576
N	8	552	576
O	8	552	576
Total		5 328	5 760
Concordance		92.5%	

Total + 77

Total - 3

Level L2

Laboratories number 10

Number of positive results per laboratory: 8

Laboratory	Number of positive results	Interlaboratories pairs with the same result	Total interlaboratories pairs number
A	8	576	576
B	8	576	576
C	8	576	576
H	8	576	576
I	8	576	576
J	8	576	576
L	8	576	576
M	8	576	576
N	8	576	576
O	8	576	576
Total		5 760	5 760
Concordance		100.0%	

Total + 80

Total - 0