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AFNOR Validation of Alternative Methods FOOD MICROBIOLOGY

SUMMARY REPORT

*(Method comparison and Inter-laboratory studies
according to the NF EN ISO 16140 standard)*

ISO 16140 validation study of the MicroSEQ® *Salmonella* spp method for the detection of *Salmonella* spp in food and feed samples

Qualitative methods

This report includes 48 pages, with 4 annexes.

Only copies including the totality of this report are authorised.

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

MicroSEQ Salmonella (Version 0)

November 30, 2010

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Quality Assurance documents related to this study can be consulted upon request by APPLIED BIOSYSTEMS.

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

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- ✓ **Expert Laboratory :** ADRIA Développement
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- ✓ **Studied method :** **Validation of the MicroSEQ® *Salmonella* spp method for detection of *Salmonella* spp**

- ✓ **Validation standard :** NF EN ISO 16140 (October 2003) : Food microbiology – Protocol for the validation of alternative methods

- ✓ **Reference methods[♦] :** NF EN ISO 6579 (2002): Horizontal method for the detection of *Salmonella* spp.

- ✓ **Products categories :** Food and feed products

[♦] Analyses performed according to the COFRAC accreditation

1 AIM OF THE STUDY

The validation study of MicroSEQ[®] *Salmonella* method for the detection of *Salmonella* in food and feed samples were performed according to the ISO 16140 protocol.

Five criteria were evaluated during the validation study:

method comparison study:

- practicability,
- inclusivity and the exclusivity,
- relative detection limit,
- relative accuracy, relative sensitivity and relative specificity.

interlaboratory study.

2 INTRODUCTION

2.1 Alternative method protocol

Principle

The MicroSEQ[®] *Salmonella* spp. Test is based on the real time PCR technology with TaqMan probes.

Protocol (see annex 1)

- Preenrichment step according to **the ISO 6887 standard**.
- Extraction using PrepSEQ[™] Rapid Spin Sample Preparation Kit
- Real time PCR detection
- Confirmation by the protocol described in the reference method, or by performing a second enrichment step in RVS (0,1 mL BPW in 10 mL RVS) for 24 h ± 3 h at 41,5°C ± 1°C and streaking onto a selective agar.

In order to provide sufficient practicability to the users, all positive BPW enriched cultures used in the accuracy study were stored for 72 hours at 2-8°C and tested again in the alternative test method.

□ **Confirmation protocol**

Two confirmation protocols were tested during the study:

- Tests described in the ISO method,
- Subcultures in RVS broth, streak on XLD agar and latex tests directly on isolated colonies.

2.2 Application

Foodstuffs and feedstuffs

2.3 Reference method

The reference method used is the ISO 6579 standard: Horizontal method for the detection of *Salmonella* spp (Cf. Annex 2).

3 METHODS COMPARISON STUDY

3.1 Relative accuracy, relative specificity and relative sensitivity

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

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.

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).

3.1.1 *Number and nature of samples*

358 samples were analysed. The distribution by food category and type is summarised below:

Category	Type	Positive samples	Negative samples	Total
Meat products	Poultry	11	15	26
	Pork	9	34	43
	Beef and others	10	9	19
	Total	30	58	88
Dairy products	Milk and fermented milk	10	16	26
	Cheeses	10	16	26
	Dessert, milk powder, ice creams	13	13	26
	Total	33	45	78
Egg products	Egg powders	8	5	13
	Liquid egg products	7	11	18
	Egg based products	16	14	30
	Total	31	30	61
Seafood and vegetables	Fresh, raw, frozen	12	12	24
	Cooked products	10	10	20
	Composite foods	10	12	22
	Total	32	34	66
Feeding stuffs products	Raw	11	8	19
	Dried	15	14	29
	Heated	7	10	17
	Total	33	32	65
Total		159	199	358

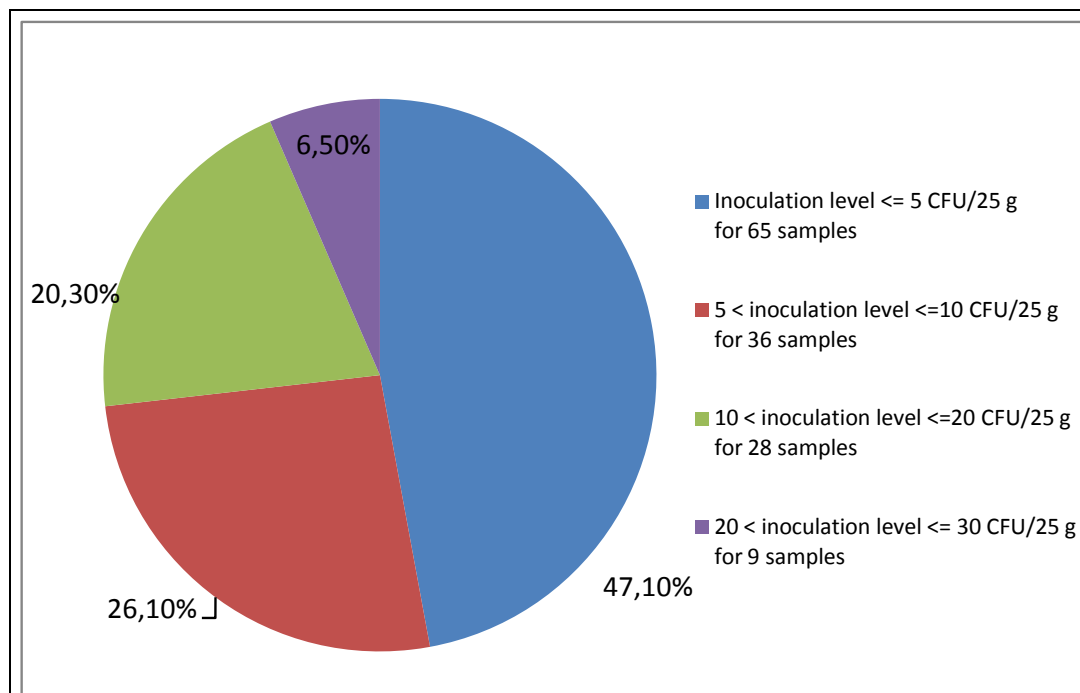
3.1.2 *Artificial contamination of samples*

Artificial contaminations were done by spiking and cross-contamination. For sample spiking, strains were stressed using different treatments, and the stress intensity was evaluated by comparing enumeration done onto selective (XLD plate) and non selective agars (TSYE plate).

Globally, 159 positive samples, artificially or naturally contaminated, were analysed during the accuracy study.

A total of 138 samples were artificially contaminated, of which 118 gave a positive result.

The artificial contaminations were performed with 32 different strains from 20 different serotypes. The contamination levels were distributed as followed:



Overall, 25,8 % of the samples were naturally contaminated.

3.1.3 Confirmation protocols

The positive samples were confirmed by the ISO 6579 method tests. Further testing was carried out when presumptive deviations were observed between both methods. Several RVS tubes and MKTTn were inoculated and several streakings were performed on different selective media.

3.1.4 Test results

Raw data per category are given in annex 3.

Table 1 – Paired results of the reference and alternative methods

Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) PA = 154	Positive deviation (R-/A+) PD = 1
Alternative method negative (A-)	Negative deviation (A-/R+) ND = 4	Negative agreement (A-/R-) NA = 199 (PPNC = 8)

PPNC: positive presumptive non confirmed samples

Results per category of sample

Table 2 – 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 = 58 (PPNC = 1)

Table 3 – Dairy products

Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) PA = 31	Positive deviation (R-/A+) PD = 1
Alternative method negative (A-)	Negative deviation (A-/R+) ND = 1	Negative agreement (A-/R-) NA = 45 (PPNC = 4)

Table 4 – Egg products

Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) PA = 31	Positive deviation (R-/A+) PD = 0
Alternative method negative (A-)	Negative deviation (A-/R+) ND = 0	Negative agreement (A-/R-) NA = 30 (PPNC = 1)

Table 5 – Seafood and vegetable products

Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) PA = 31	Positive deviation (R-/A+) PD = 0
Alternative method negative (A-)	Negative deviation (A-/R+) ND = 1	Negative agreement (A-/R-) NA = 34 (PPNC = 1)

Table 6 – Feedstuffs

Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) PA = 31	Positive deviation (R-/A+) PD = 0
Alternative method negative (A-)	Negative deviation (A-/R+) ND = 2	Negative agreement (A-/R-) NA = 32 (PPNC = 1)

Table 7 – Calculation of relative accuracy (AC), relative sensitivity (SE) and relative specificity (SP)

Category	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	58	0	0	88	100,0	30	100,0	58	100,0
Dairy products	31	45	1	1	78	97,4	32	96,9	46	97,8
Egg products	31	30	0	0	61	100,0	31	100,0	30	100,0
Seafood and vegetales	31	34	1	0	66	98,5	32	96,9	34	100,0
Feedstuffs	31	32	2	0	65	96,9	33	93,9	32	100,0
All products	154	199	4	1	358	98,6	158	97,5	200	99,5

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

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

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

The alternative method percentage values are:

- relative accuracy : **AC = 98,6**
- relative specificity : **SP = 99,5**
- relative sensitivity : **SE = 97,5**

Sensitivity of both methods, when the positive deviations of the alternative method are considered, is presented below:

Alternative method = 97,5

Reference method = 99,4

3.1.6 Analysis of discordants

Four negative deviations were observed:

- sample n° 1987: raw milk, artificially contaminated with *Salmonella* Lagos (5.8 CFU/25 mL) contaminated sample.
For this sample, the first PCR run gave a negative result; two other PCR were done. The RapidFinder™ Express software concludes to a negative result using the stringent threshold setting. The relatively high Ct values (36,2 and 36,1) were obtained when SDS software was applied, suggesting the sample was contaminated at a low level. The observed results are probably due to a sampling heterogeneity enhanced by a low contamination level.
- sample n° 2405: vegetable terrine artificially contaminated sample by *Salmonella* Typhimurium Ad 1249 at a low level (1.2 CFU/25 g).
Three PCR testings were run and gave one negative result (and two positive results with late Ct (37,0; 35,6 and 35,1). The observed results are probably due to a sampling heterogeneity enhanced by a low contamination level.
- sample n° 2410: raw meat for feedstuff, artificially contaminated sample by *Salmonella* Dublin at a low level (2,2 CFU/25 g)
Four PCR tests were done for this sample; three negative and one positive results were observed with late Ct value (36,1, 35,9 and 36,4). The Ct

value did not meet the threshold of RapidFinder™ Express to be interpreted positive. After 72 h storage at 4°C, a positive PCR result was observed. The observed results are probably due to a sampling heterogeneity enhanced by a low contamination level.

- sample n° 2411: raw meat for feedstuff, artificially contaminated sample by *Salmonella* Infantis at a low level (1,4 CFU/25 g)

Three PCR testings were run and gave three negative results with late Ct value (36,1, 36,9 and 39,1). The Ct value did not meet the threshold of RapidFinder™ Express to be called positive. The observed results are probably due to a sampling heterogeneity enhanced by a low contamination level.

One positive deviation was observed:

- sample n° 1985: raw milk artificially contaminated by *Salmonella* Dublin (11,4 CFU/25 g). The positive PCR result was confirmed after inoculation on MSR/V and streaking on XLD.

$$Y = PD + ND = 1 + 4 = 5$$

Y < 6, no statistical test can be applied.

The alternative and the reference methods are concluded equivalent.

3.1.7 Confirmations

Two confirmation protocols were applied during this study:

- confirmations by the tests described in the reference method,
- confirmations by subculture in RVS broth, streaking on XLD agar combined with latex test on characteristic colonies.

For four samples (1968, 1978, 1979, 2551), no characteristic colony was observed on XLD (from RVS broth).

For nine samples (1061, 1238, 1414, 1975, 1976, 1980, 2565, 1965, 1985), the tests described in the reference method did not lead to direct confirmation results: several RVS, MKTTn broths and MSR/V plates were then inoculated. The presence of *Salmonella* was only confirmed for the sample n° 1985, using MSR/V and streaks on XLD plates.

3.1.8 BPW storage at 2-8°C

The positive samples by the alternative method were stored at 2-8°C for 72 h and analysed a second time. Two result modifications were observed:

Samples	First alternative method results	Results after storage at 4°C
2410	Negative deviation	Positive agreement
2405	Negative deviation	Positive agreement

3.2 Relative detection level

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

3.2.1 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 results.

Detection limits have been defined by analysing the different matrix/strain pairs. Four levels were tested. Six replicates of each combination were prepared.

The following matrices were tested:

- ground beef inoculated with *Salmonella* Infantis 128,
- raw milk inoculated with *Salmonella* Montevideo 510,
- raw egg product contaminated with *Salmonella* Enteritidis 657,
- mushrooms contaminated with *Salmonella* Virchow F276,
- dog biscuits contaminated with *Salmonella* Derby 630.

3.2.2 Contamination protocol

Contaminations and enumerations were realised according to the AFNOR technical rules (protocol for low level inoculations). The contamination levels are presented below:

- level 1: 0 UFC/g or /ml
- level 2: level necessary to obtain 0 to 50% positives,
- level 3: level necessary to obtain 50 to 75% positives,
- level 4: level necessary to obtain de 100% positives.

The samples were analysed by both methods, and the background microflora were enumerated.

3.2.3 Results

Detection levels are presented in Table 8.

Table 8 – Relative detection level results

Strain / matrix pairs	Relative detection level (CFU / 25 g ou 25 ml) according to Spearman-Kärber test ¹	
	Reference method	Alternative method
Ground beef / <i>Salmonella</i> Infantis 128	0,4 [0,1 ; 1,0]	0,4 [0,1 ; 1,0]
Raw milk / <i>Salmonella</i> Montevideo 510	0,2 [0,1 ; 0,6]	0,2 [0,1 ; 0,6]
Raw egg product / <i>Salmonella</i> Enteritidis 657	0,3 [0,1 ; 1,4]	0,3 [0,1 ; 1,4]
Mushrooms / <i>Salmonella</i> Virchow F276	0,3 [0,1 ; 0,8]	0,3 [0,1 ; 0,8]
Dog biscuits / <i>Salmonella</i> Derby 630	0,4 [0,1 ; 1,5]	0,4 [0,1 ; 1,5]

3.2.4 Conclusion

The alternative and the standard methods show similar detection levels, which are comprised between 0,1 to 1,5 log CFU/25 g.

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".

3.3 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.

3.3.1 Test protocols

- **Protocol for inclusivity**: 54 *Salmonella* strains were tested. Cultures were performed in BHI medium at 37°C. Dilutions were done in BPW in order to inoculate between 10 to 100 cells/225 ml BPW. The alternative method protocol was then performed (BPW incubation time: 16 h).
- **Protocol for exclusivity**: 33 negative strains were tested. Cultures were performed in BHI, incubated at 37°C. Dilutions were done in order to inoculate 10^5 cell/ml BPW. The alternative method protocol was then performed (BPW incubation time: 20 h)

3.3.2 Results

Raw data are given in Annex 4.

The 55 target strains showed positive results.

The 32 tested non-target strains showed negative results. One *Salmonella bongori* (Ad 599) gave a positive PCR result when inoculated at a high level (10^5 cells/225 ml), but gave finally a negative result when inoculated at a low level (6 cells/225 ml).

3.4 Practicability

MicroSEQ[®] *Salmonella* method practicability was evaluated according to the AFNOR criteria relative to preliminary study.

✓ *Mode of packaging of test components*

The components needed for the analyses are:

- lysis buffer: lysis buffer is available in a pouch. Each pouch contains one flask,
- MicroSEQ[®] *Salmonella* spp. detection kit: the kit contains reagents for 96 reactions,
- Pathogen negative control: 1 tube,

✓ *Volume of reagents*

The package insert specifies the reagent volumes:

- MicroSEQ[®] *Salmonella* spp detection kit: 96 tests
- Pathogen negative control: 1,5 ml

✓ *Storage conditions of components and shelf-life of unopened products*

The storage conditions are given on the package:

- MicroSEQ[®] *Salmonella* spp. detection kit: 2 to 8°C, protect from light
- Pathogen negative control: 2 to 8°C.

The shelf life is given on the package:

MicroSEQ[®] *Salmonella* spp. detection kit: 18 months after manufacturing
Negative control: 18 months after manufacturing

✓ *Utilisation modalities after initial use*

All the reagents must be stored at 2 – 8°C after initial use.

✓ *Specific equipment of premises required*

- Applied Biosystems 7500 Fast Real-Time PCR system
- Consumables:
 - * Rapid Spin sample preparation kit tubes
 - * PrepSEQ™ Rapid Spin sample preparation kit
 - * Aerosol-resistant pipette tips (200, 1 000 µl)
 - * Disposable gloves
 - * Pipettors (200, 1 000 µl)
 - * MicroAmp Fast 8 – tube strip 0,1 ml
 - * MicroAmp Optical 8 – capstrip
- Reagents
 - * Nuclease free water

✓ *Reagents ready to use or for reconstitution*

All the reagents are ready to use.

✓ *Training period for operator with no experience of the method*

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

- ✓ *Handling time and flexibility as a function of number of samples to be analysed (in minutes)*

	Reference method ISO 6579	Alternative method MicroSEQ® <i>Salmonella</i> method
	24 samples	24 samples
Sampling	60	60
Stomach	36	36
Extraction		60
PCR		15
RVS and MKTTn sub-cultures	45	
Streaking on selective agar plates	75	
Reading	30	
Total (negative sample)	246	171
Total / negative sample	10,3	7,1
RVS and MKTTn sub-cultures		45 ⁽¹⁾ 25 ⁽²⁾
Streaking on selective agar plates		75 ⁽¹⁾ 20 ⁽²⁾
Reading		30 ⁽¹⁾ 15 ⁽²⁾
Strike on nutritive agar	20	20
Confirmation tests	120	120 ⁽¹⁾ 15 ⁽²⁾
Total (positive sample)	386	461 ⁽¹⁾ 266 ⁽²⁾
Total / positive sample	16	19,2 ⁽¹⁾ 11,1 ⁽²⁾

(1) Confirmation by the tests described in the reference method

(2) Confirmation by subculture in RVS followed by streaking on XLD plate

For negative sample analysis, MicroSEQ® *Salmonella* method requires less time than the reference method .

For positive sample analysis, MicroSEQ® *Salmonella* method require less time than the reference method only if the short confirmation protocol is used.

✓ *Time required for results*

- Negative samples

	ISO 6579 reference method	MicroSEQ® <i>Salmonella</i> method
Pre-enrichment	J0	J0
Enrichment	J1	
Extraction, negative result		J1
Streak	J2	
Reading	J3	

- Positive samples

	ISO 6579 reference method	MicroSEQ® <i>Salmonella</i> method
Pre-enrichment	J0	J0
Enrichment	J1	J1
Extraction, PCR		J1
Streak	J2	J2
Reading	J3	J3
Latex test		J3 ⁽³⁾
Confirmation tests	J4 to J6	J4 to J6 ⁽⁴⁾

⁽³⁾ Confirmation by the tests described in the reference method

⁽⁴⁾ Confirmation by subculture in RVS followed by streaking on XLD plate

Negative results are available within a day using MicroSEQ® *Salmonella* method.

✓ *Operator qualifications*

Technician qualified in microbiology and molecular biology.

✓ *Stages in common with reference method*

Pre-enrichment is common to both methods.

✓ *Traceability of analytical results*

All data obtained with the *Salmonella* method are traced over time by computer archive means.

✓ *Maintenance by laboratory*

Perform the background calibrations and optical calibration every twelve months.

4 INTERLABORATORY STUDY ORGANISATION AND RESULTS

4.1 Study organisation

Collaborators number

Samples were sent to 15 laboratories.

Matrix and strain used

The study was carried out with ground beef samples contaminated by *Salmonella* Typhimurium A00C060.

Samples

Samples were inoculated and sent on Monday 5 July 2010, as described below:

- 24 blinded samples for *Salmonella* detection by the MicroSEQ *Salmonella* method and by the reference method (ISO 6579:2002),
- 1 ground beef sample for the aerobic mesophilic flora enumeration by ISO 4833 method,
- 1 water flask labelled "Temperature Control" with a temperature probe, which records the temperature variations during transportation.

The analyses were started on Wednesday 7 July 2010.

Inoculation

The targeted inoculation levels were:

- Level 0: 0 CFU/g,
- Level 1: 5 CFU/g,
- Level 2: 25 CFU/g.

At least, each laboratory received 24 samples of 25 g, i.e. 8 samples per inoculation level and method.

Labelling and shipping

Blinded samples (code is only known by the expert laboratory) were placed in isothermal boxes, which contained cooling blocks, and express-shipped to the different laboratories.

A temperature control flask containing temperature probe was added to the package in order to register the temperature profile during the transport, package delivery.

Samples were shipped in 24 h to 48 h to the different laboratories. Sample temperature had to stay lower or equal to 8,4°C during transport, and between 0°C – 8,4°C at arrival.

Analyses

Collaborative study laboratories and the expert laboratory carried out the analyses with the alternative and reference methods at day 2.

Expedition conditions

The collaborative study instructions were sent on June 21, 2010.

4.2 Experimental parameters control

4.2.1 Contamination level before inoculation, levels obtained after the artificial contaminations of the samples

Before inoculation

In order to detect Salmonella, the 6579 method was performed on five ground beef samples (25 g) before the samples inoculation. All the results were negative.

Sample stability

Sample stability was performed by inoculating the matrix at 25 CFU/25 g and 5 CFU/25 g. Enumerations were performed on 2,5 g of ground beef samples for the high contamination level and detection analyses were performed for the low contamination level. *Triplicata* were analysed, and the results were the following:

Day	Reference method (research)			CFU/25 g (XLD)			Aerobic mesophilic flora (CFU/g)
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	
Day 0	+	+	+	< 10	30	30	1,5.10 ⁴
Day 1	+	+	+	20	20	40	3,4.10 ⁴
Day 2	+	+	+	30	30	10	1,6.10 ⁵

No evolution was observed during storage at 4°C.

Contamination levels

The contamination levels and the confidence intervals were:

Level	Samples	Theoretical target level (b/25 g)	True level (b/25 g sample)	Low limit / 25 g sample	High limit / 25 g sample
Level 0	3 – 4 – 8 – 11 – 13 – 18 – 21 – 24	0	/	/	/
Low level	1 – 6 – 7 – 12 – 14 – 16 – 20 – 23	5	3,8	3,3	4,4
High level	2 – 5 – 9 – 10 – 15 – 17 – 19 – 22	25	20,3	17,6	23,3

4.2.2 Logistic conditions

Temperature conditions are given below:

Sample temperatures at receipt

Laboratories	Temperature measured by the sensor (°C)	Temperature measured at receipt (°C)	Receipt delay	Day of analysis
A	5,0	6,3	Day 1 – 11h00	Day 2
B	5,0	6,8	Day 1 – 11h45	Day 2
C	4,0	6,5	Day 1 – 10h30	Day 2
D	4,5	9,4	Day 1 – 10h45	Day 2
E	4,0	3,7	Day 1 – 10h40	Day 2
F	2,5	5,3	Day 1 – 09h10	Day 2
G	4,0	7,8	Day 1 – 13h45	Day 2
H	5,0	6,3	Day 1 – 11h45	Day 2
I	4,5	2,0	Day 1 – 08h00	Day 1
J	4,0	6,7	Day 1 – 09h45	Day 2
K	4,0	8,2	Day 1 – 10h30	Day 2
L	24,0	25,0	Day 2 – 17h15	Day 2
M	5,0	<i>Not measured</i>	Day 1 – 10h30	Day 2
N	5,0	11,2	Day 1 – 10h55	Day 2
O	6,5	8,0	Day 1 – 12h00	Day 2

4.2.3 Conclusion

No problem was encountered during the transport or at receipt, except for two Labs: Lab L and Lab N.

4.3 Results analysis

4.3.1 *Aerobic mesophilic flora enumeration*

Four Labs did not realise this analysis.

Depending on the Lab results, the enumeration levels varied from $3,7 \cdot 10^3$ to $2,0 \cdot 10^5$ CFU/g.

4.3.2 *Expert lab results*

All the inoculated samples gave a positive result. All the results were in agreement between the reference and the alternative methods.

4.3.3 *Collaborator lab results*

The results of 13 Labs were finally taken into account for the interpretation.

4.4 Results interpretation

Lab N found: one non-inoculated sample positive (N21) by both methods and one negative sample (N7) of low inoculation level by both methods. This Lab has repeated the extraction and PCR analyses on these two samples: the results were confirmed. Strain transfer was done and molecular fingerprinting was done with Pulse Field Gel Electrophoresis (PFGE). This analysis clearly confirmed that the samples N21 and N7 were inverted, i.e. the PFGE pattern of the strain from the sample N21 fits with the PFGE pattern of the inoculated strain.

The Lab E found some characteristic colonies on the selective media from the sample E1, but was not able to confirm the *Salmonella enterica* species identification. The isolates were sent to the expert Lab; the observed characteristic colonies clearly belong to *Salmonella enterica* species according to the ISO 6579 confirmatory test results.

4.4.1 Specificity and sensitivity for each method

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

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

with :N- = total number of all L0 assays

FP = number of false positive results

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

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

with :N+ = total number of all L1 or L2 assays

TP = number of true positive results

Results are reported in the following table:

Level	Reference method		Alternative method	
	SP/SE %	LCL%	SP/SE %	LCL%
L0	SP = 99,0	98	SP = 99,0	98
L1	SE = 99,0	98	SE = 99,0	98
L2	SE = 100,0	98	SE = 100,0	98
L1+L2	SE = 99,5	98	SE = 99,5	98

4.4.2 Relative accuracy (AC)

Results for all levels are given below:

Table 9 - Paired results of the alternative and reference methods

Alternative method	Reference method		Total
	+	-	
+	PA = 208	PD = 0	208
-	ND = 0	NA = 104	104
Total	N+ = 208	N- = 104	N = 312

Relative accuracy (AC) (in %) is calculated according to:

$$AC = \frac{(PA + NA)}{N} \times 100\%$$

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 10

Level	AC %	LCL %
L0	100	98
L1	100	98
L2	100	98
L1 + L2	100	98
Total	100	98

4.4.3 Discordant results

No discordant result was observed between the reference and the alternative methods.

4.5 Interpretation

4.5.1 Comparison of the relative accuracy, specificity and sensibility values

The values obtained for the two parts of the validation study (comparative and inter-laboratory studies) are reported in table 11.

Tableau 11 - Alternative method values calculated during the comparative and inter-laboratory studies

	Interlaboratory study	Methods comparative study
Relative accuracy (AC)	100	98,6
Sensibility (SE)	100	97,5
Specificity (SP)	100	99,5

4.5.2 Accordance (DA)

Accordance values for both methods are:

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

4.5.3 Concordance

Both methods concordance values are:

Level	Reference method	Alternative method
L0	98	98
L1	98	98
L2	100	100

4.5.4 Odds Ratio (COR)

The odds ratio value is determined according to:

$$COR = \frac{\text{Accordance} \times (100 - \text{agreement})}{\text{Agreement} \times (100 - \text{accordance})}$$

Both method odds ratio values are:

Level	Reference method (COR)	Alternative method (COR)
L0	1,00	1,00
L1	1,00	1,00
L2	1,00	1,00

5 CONCLUSION

The methods comparative study conclusions are:

- The MicroSEQ[®] *Salmonella* method shows satisfying relative accuracy, specificity and sensitivity.
- The detection limits of the MicroSEQ[®] *Salmonella* method and ISO 6579 method are similar.
- MicroSEQ[®] *Salmonella* method is selective and specific.
- Negative results are available within a day using the MicroSEQ[®] *Salmonella* method.

The interlaboratory study conclusions are:

- The alternative method and reference method show equivalent performances (accordance, concordance, odds ratio).

Annex 1 – Alternative method

25 g sample + 225 ml BPW (Buffered Peptoned Water)



16 - 20 h at 37°C ± 1°C



Extraction



Insert a spin column into a labelled tube



Load 750 µl of sample onto the spin column and cap the column



Load the tube into the microcentrifuge



Microcentrifuge for 3 min at max speed
(12,000 to 16,000 g)



Remove the tube from the microcentrifuge,
then discard the used spin column



Aspirate and discard the supernatant



Add 50 µl of lysis buffer to the pellet



Resuspend by pipetting up and down,
or vortex until the pellet is resuspended



Cap the tube, then incubate at 95°C for 10 min



Allow the sample to cool for
2 min at room temperature



Add 250 µl of water



Microcentrifuge for 1 min at max speed (12,000 to 16,000 g)



Proceed with PCR or store the tube at -20°C for up to 1 week

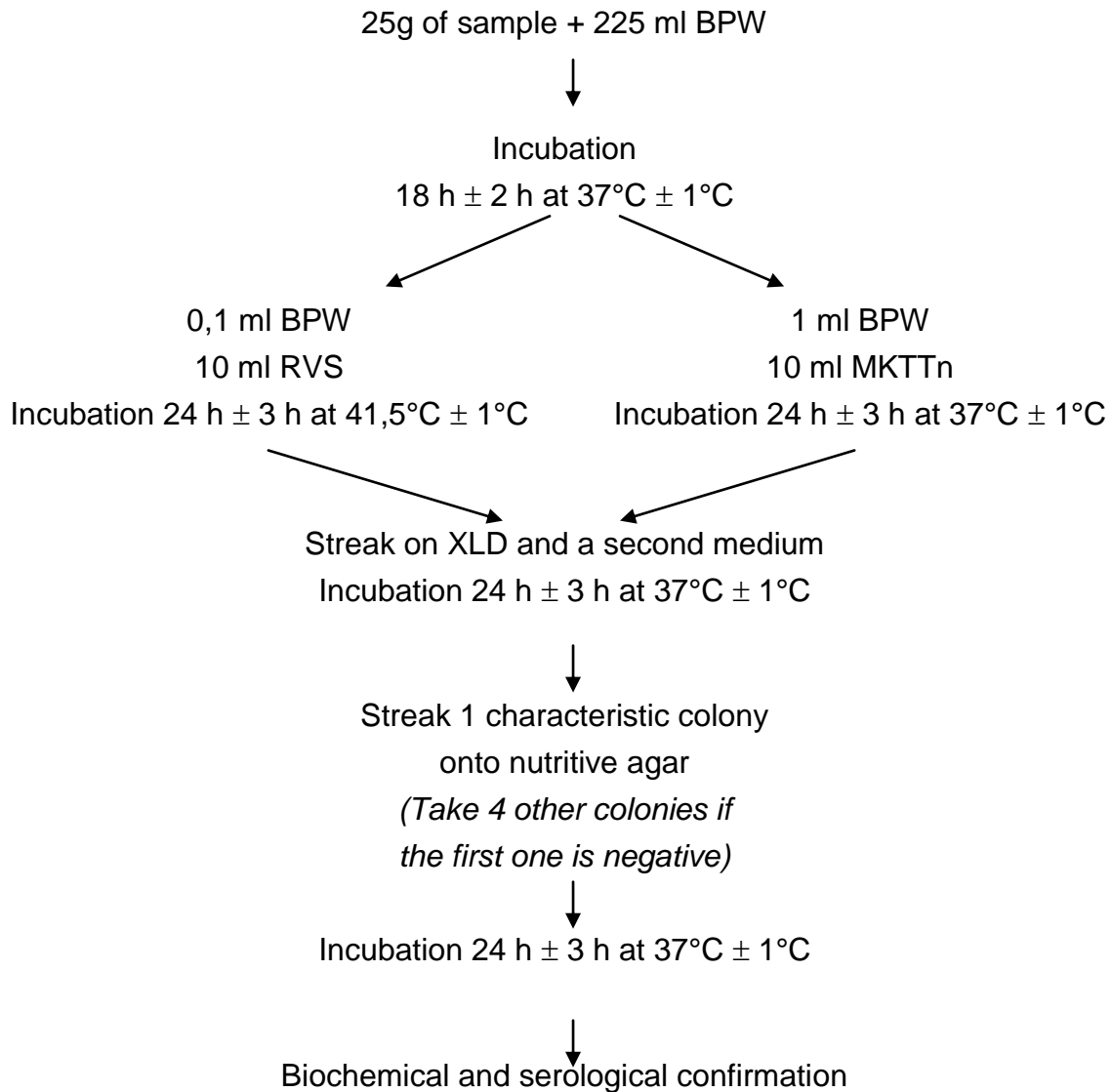
For samples with high
lipid content
(PrepSEQ™ Rapid
Spin Extra Clean
Protocol)
Transfer the lysate to
a clean 1,5 ml tube.



Confirmation:

- Option 1: by the protocol described in the reference method
- Option 2: subculture in RVS broth and streaking onto selective agar

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



Annex 3 – Relative accuracy : raw data

ni: non isolated colonies

NC: non characteristic colonies on nutrient agar

+/-:doubtful colonies

i:inhibition

in bold typing: artificially contaminated samples

MEAT PRODUCTS																	
N° Sample	Product	ISO 6579 method [♦]					MicroSEQ® <i>Salmonella</i> method										
		RVS broth		MKTTn broth		Result	PCR result	Confirmation (Reference method)	Confirmation (RVS/XLD/Latex)	Final result Ref.conf.	Agreement Ref.conf.	Final result RVS/XLD	Agreement RVS/XLD	Storage BPW 72H 4°C			
		XLD	Chromagar <i>Salmonella</i>	XLD	Chromagar <i>Salmonella</i>									PCR	Confirmation	Final result	Agreement
951	Pork meat	-	-	-	-	-	-	-	-	-	=	-	=				
952	Mechanically deboned poultry meat	-	-	-	-	-	-	-	-	-	=	-	=				
953	Mechanically deboned poultry meat	-	-	-	-	-	-	-	-	-	=	-	=				
954	Hen meat pieces	-	-	-	-	-	-	-	-	-	=	-	=				
1011	Pork "crépine"	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1013	Pork-rind	-	-	-	-	-	-	-	-	-	=	-	=				
1015	Sausage meat	-	-	-	-	-	-	-	-	-	=	-	=				
1017	Pork breast	-	-	-	-	-	-	-	-	-	=	-	=				
1019	Pork tongue	-	-	-	-	-	-	-	-	-	=	-	=				
1021	Pork breast	-	-	-	-	-	-	-	-	-	=	-	=				
1023	Thin flank of beef	-	-	-	-	-	-	-	-	-	=	-	=				
1025	Knuckle of pork	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1027	Deboned breast	-	-	-	-	-	-	-	-	-	=	-	=				
1029	Turkey tournedos	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1031	Ground beef (Bolognaise)	-	-	-	-	-	-	-	-	-	=	-	=				
1033	Smoked raw sausage	-	-	-	-	-	-	-	-	-	=	-	=				
1067	Mechanically deboned poultry meat	-	-	-	-	-	-	-	-	-	=	-	=				
1068	Ground beef (Bolognaise)	-	-	-	-	-	-	-	-	-	=	-	=				
1069	Sausage meat	-	-	-	-	-	-	-	-	-	=	-	=				
1070	Marinated beef(faux filet)	-	-	-	-	-	-	-	-	-	=	-	=				
1071	Maxi steak	-	-	-	-	-	-	-	-	-	=	-	=				

♦ Analyses performed according to the COFRAC accreditation

MEAT PRODUCTS																	
N° Sample	Product	ISO 6579 method♦					MicroSEQ® <i>Salmonella</i> method										
		RVS broth		MKTn broth		Result	PCR result	Confirmation (Reference method)	Confirmation (RVS/XLD/Latex)	Final result Ref.conf.	Agreement Ref.conf.	Final result RVS/XLD	Agreement RVS/XLD	Storage BPW 72H 4°C			
		XLD	Chromagar <i>Salmonella</i>	XLD	Chromagar <i>Salmonella</i>									PCR	Confirmation	Final result	Agreement
1072	Hen meat pieces	-	-	-	-	-	-	-	-	-	=	-	=				
1073	Meat based stuffing	+(citrobacter freundii)	-	-	-	-	-	-	-	-	=	-	=				
1074	Hen pieces with skin	+(NC)	-	+(NC)	-	-	-	-	-	-	=	-	=				
1075	Pork meat (Crêpinette)	-	-	-	-	-	-	-	-	-	=	-	=				
1076	Dried sausage	-	-	-	-	-	-	-	-	-	=	-	=				
1077	Cooked sausage	+/(NC)	-	+/(NC)	-	-	-	-	-	-	=	-	=				
1094	Pork tongue	-	-	-	-	-	-	-	-	-	=	-	=				
1095	Pork rind	-	-	-	-	-	-	-	-	-	=	-	=				
1096	Pork meat caul (crêpine)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1097	Pork rind	-	-	-	-	-	-	-	-	-	=	-	=				
1098	Pork breast	-	-	-	-	-	-	-	-	-	=	-	=				
1099	Pork breast	-	-	-	-	-	-	-	-	-	=	-	=				
1100	Pork meat caul (crêpine)	-	-	-	-	-	-	-	-	-	=	-	=				
1101	Ground beef (Bolognaise)	-	-	-	-	-	-	-	-	-	=	-	=				
1102	Sausages with herbs	-	-	-	-	-	-	-	-	-	=	-	=				
1103	Sausage meat	-	-	-	-	-	-	-	-	-	=	-	=				
1104	Mechanically deboned poultry meat	-	-	-	-	-	-	-	-	-	=	-	=				
1105	Minced beef and pork based meat balls	-	-	-	-	-	-	-	-	-	=	-	=				
1106	Ground beef	-	-	-	-	-	-	-	-	-	=	-	=				
1113	Pork shoulder	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1114	Basquaise chicken preparation	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1115	Ready to eat food with lamb (Navarin)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1116	Couscous	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1117	Ready to eat food with veal (Italian dressing)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1128	Chicken nem	-	-	-	-	-	-	-	-	-	=	-	=				
1129	Friand (pufpastry)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1130	Dried sausage	-	-	-	-	-	-	-	-	-	=	-	=				
1131	Pork-rind	-	-	-	-	-	-	-	-	-	=	-	=				
1132	Pork tongue	-	-	-	-	-	-	-	-	-	=	-	=				
1133	Pork carcass	-	-	-	-	-	-	-	-	-	=	-	=				

MEAT PRODUCTS																	
N° Sample	Product	ISO 6579 method♦					MicroSEQ® <i>Salmonella</i> method										
		RVS broth		MKTn broth		Result	PCR result	Confirmation (Reference method)	Confirmation (RVS/XLD/Latex)	Final result Ref.conf.	Agreement Ref.conf.	Final result RVS/XLD	Agreement RVS/XLD	Storage BPW 72H 4°C			
		XLD	Chromagar <i>Salmonella</i>	XLD	Chromagar <i>Salmonella</i>									PCR	Confirmation	Final result	Agreement
1134	Pork carcass	-	-	-	-	-	-	-	-	-	=	-	=				
1135	Hen	-	-	-	-	-	-	-	-	-	=	-	=				
1136	Hen	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1137	Pork tongue	-	-	-	-	-	-	-	-	-	=	-	=				
1194	Pork meat caul (crêpine)	-	-	-	-	-	-	-	-	-	=	-	=				
1195	Pork meat caul (crêpine)	-	-	-	-	-	-	-	-	-	=	-	=				
1196	Pork tongue	-	-	-	-	-	-	-	-	-	=	-	=				
1197	Pork tongue	-	-	-	-	-	-	-	-	-	=	-	=				
1206	Hachis parmentier	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1207	Bolognaise pasta	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1208	Moussaka	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1213	Cured duck filet	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1214	Ground beef	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1215	Frozen ground beef with onions	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1216	Meat balls	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1226	Sausages (Fricadelles)	-	-	-	-	-	-	-	-	-	=	-	=				
1227	Sausages (Fricadelles)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1228	Veal paupiette	-	-	-	-	-	-	-	-	-	=	-	=				
1229	Veal paupiette	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1230	Asian ready to eat food with poultry	-	-	-	-	-	-	-	-	-	=	-	=				
1231	Asian ready to eat meal (poultry)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1232	Basquaise chicken preparation	-	-	-	-	-	-	-	-	-	=	-	=				
1233	Basquaise chicken preparation	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1241	Carcass	-	-	-	-	-	-	-	-	-	=	-	=				
1965	Sausages	-	+ni/(ox+)	+(Citrobacter youngae)	-	-	+/-	-	-	-(PPNC)	=	-	=	-/-	-	-	
1966	Sausages with herbs	+ni/+	+ni/(ox+)	+	+	+	+	+	+	+	=	+	=	+	+	+	
1967	Pork ribs	-	-	-	-	-	-	-	-	-	=	-	=				
1968	Pork liver	-	-	+	+	+	+	+	-	+	=	-	ND	+	+	+	
1969	Pork shoulder	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1970	Poultry neck skin	-	+(ox+)	-	-	-	-	-	-	-	=	-	=				

MEAT PRODUCTS																	
N° Sample	Product	ISO 6579 method♦					MicroSEQ® <i>Salmonella</i> method										
		RVS broth		MKTTn broth		Result	PCR result	Confirmation (Reference method)	Confirmation (RVS/XLD/Latex)	Final result Ref.conf.	Agreement Ref.conf.	Final result RVS/XLD	Agreement RVS/XLD	Storage BPW 72H 4°C			
		XLD	Chromagar <i>Salmonella</i>	XLD	Chromagar <i>Salmonella</i>									PCR	Confirmation	Final result	Agreement
1971	Poultry neck skin	+ni/+	+(ox+)	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1972	Cockerel	-	+(ox+)	-	-	-	+/(36,2)/ -/(38,1)	-	-	-	=	-	=	-	-	-	=
2157	Turkey roast	-	-	-	-	-	-	-	-	-	=	-	=				
2250	Cooked turkey meat	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2251	Cooked chicken meat pieces	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2252	Turkey ham	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2253	White chicken with herbs	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=

ni: non isolated colonies NC: non characteristic colonies on nutrient agar +/-:doubtful colonies i:inhibition in bold typing: artificially contaminated samples

DAIRY PRODUCTS																	
N° Sample	Product	ISO 6579 method♦					MicroSEQ® <i>Salmonella</i> method										
		RVS broth		MKTn broth		Result	PCR result	Confirmation (Reference method)	Confirmation (RVS/XLD/Latex)	Final result Ref.conf.	Agreement Ref.conf.	Final result RVS/XLD	Agreement RVS/XLD	Storage BPW 72H 4°C			
		XLD	Chromagar <i>Salmonella</i>	XLD	Chromagar <i>Salmonella</i>									PCR	Confirmation	Final result	Agreement
950	Raw cow milk cheese	-	-	-	-	-	-	-	-	-	=	-	=				
991	Cow raw milk	-	-	-	-	-	-	-	-	-	=	-	=				
993	Ewe raw milk	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
995	Ewe raw milk	-	-	-	-	-	-	-	-	-	=	-	=				
997	Raw milk cheese	-	-	-	-	-	-	-	-	-	=	-	=				
999	Ewe raw milk cheese	-	-	-	-	-	-	-	-	-	=	-	=				
1001	Goat raw milk	-	-	-	-	-	i/-	-	i/-	-	=	-	=				
1057	Ewe raw milk N°4	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1058	Ewe raw milk N°6	-	-	-	-	-	-	-	-	-	=	-	=				
1059	Ewe raw milk N°14	-	-	-	-	-	-	-	-	-	=	-	=				
1060	Ewe raw milk N°13	-	+/-	-	-	+	+	+	+	+	=	+	=	+	+	+	=
1061	Raw cow milk cheese	-	-	-	-	-	i+/i/i	-	-	-(PPNC)	=	-	=	+/i/i	-	-	=
1062	Raw cow milk cheese	-	-	-	-	-	-	-	-	-	=	-	=				
1063	Cow raw milk	-	-	-	-	-	-	-	-	-	=	-	=				
1064	Raw milk N°11	-	-	-	-	-	-	-	-	-	=	-	=				
1065	Raw milk N°13	-	-	-	-	-	-	-	-	-	=	-	=				
1066	Raw milk cheese	-	-	-	-	-	-	-	-	-	=	-	=				
1090	Milk powder	-	-	-	-	-	-	-	-	-	=	-	=				
1091	Milk powder	-	-	-	-	-	-	-	-	-	=	-	=				
1092	Milk powder	-	-	-	-	-	-	-	-	-	=	-	=				
1093	Milk powder	-	-	-	-	-	-	-	-	-	=	-	=				
1210	Ice cream (mint-chocolate)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1211	Vanilla ice cream	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=

♦ Analyses performed according to the COFRAC accreditation

DAIRY PRODUCTS																	
N° Sample	Product	ISO 6579 method♦					MicroSEQ® Salmonella method										
		RVS broth		MKTn broth		Result	PCR result	Confirmation (Reference method)	Confirmation (RVS/XLD/Latex)	Final result Ref.conf.	Agreement Ref.conf.	Final result RVS/XLD	Agreement RVS/XLD	Storage BPW 72H 4°C			
		XLD	Chromagar Salmonella	XLD	Chromagar Salmonella									PCR	Confirmation	Final result	Agreement
1212	Coffee ice cream	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1378	Raw milk cheese (Morbier)	-	-	-	-	-	-	-	-	-	=	-	=				
1379	Milk cheese (Gouda)	-	-	-	-	-	-	-	-	-	=	-	=				
1381	Raw milk cheese (Saint Nectair)	-	-	-	-	-	-	-	-	-	=	-	=				
1396	Raw milk cheese (Brie de Meaux)	-	-	-	-	-	i/-	-	-	-	=	-	=				
1973	Milk powder(RAEMA)	-	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1974	Milk powder(RAEMA)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1975	Milk powder(RAEMA)	-	-	-	-	-	+(35,09)/ +(34,9)/ +(34,2)	-	-	-	=	-	=	+/-	-	-	=
1976	Milk powder(RAEMA)	-	-	-	-	-	+(34,2)/-/+	-	-	-	=	-	=	+(28,4)/ +/(27,3)	-	-	=
1977	Raw milk cheese	-	-	-	-	-	-	-	-	-	=	-	=				
1978	Raw milk cheese	-	-	+	-	+	+	+	-	+	=	-	ND	+	+	+	=
1979	Raw milk cheese	-	-	+	-	+	+	+	-	+	=	-	ND	+	+	+	=
1980	Raw milk cheese	-	-	-	-	-	+/+/(34,8)	-	-	-	=	-	=	-	-	-	=
1985	Raw milk	-	-	-	-	-	+	(+ MSRV/ XLD)	-	+	PD	+	=	+	(+ MSRV/XLD)	+	PD
1986	Raw milk cheese(Rocamadour)	-	-	+	-	+	+	+	+	+	=	+	=	+	+	+	=
1987	Raw milk	+ni/+	-	+	+	+	-(0)/-(36,2)/ -(36,1)	+	+	+	ND	-	ND	-(37,1)/ -(37,3)/ -(38,3)	+	-	ND
1988	Raw milk cheese (Saint Nectaire)	+ni/+	-	+ni/+	+	+	+	+	+	+	=	+	=	+	+	+	=
2257	Raw milk cheese(Tomme de Savoie)	-	-	-	-	-	-	-	-	-	=	-	=				
2258	Raw milk cheese(Morbier)	-	-	-	-	-	-	-	-	-	=	-	=				
2259	Raw milk cheese(Salers)	+ni/+	-	+	-	+	+	+	+	+	=	+	=	+	+	+	=
2260	Raw milk cheese(Raclette)	-	-	-	-	-	-	-	-	-	=	-	=				
2364	Raw milk	-	-	-	-	-	-	-	-	-	=	-	=				
2365	Cream	-	-	-	-	-	-	-	-	-	=	-	=				
2366	Lait ribot	-	-	-	-	-	-	-	-	-	=	-	=				
2367	Gros lait	-	-	-	-	-	-	-	-	-	=	-	=				
2368	Cream	-	-	-	-	-	-	-	-	-	=	-	=				
2369	Raw milk	-	-	-	-	-	-	-	-	-	=	-	=				
2370	Ice cream (Pistachio)	-	-	-	-	-	-	-	-	-	=	-	=				

DAIRY PRODUCTS																	
N° Sample	Product	ISO 6579 method♦					MicroSEQ® <i>Salmonella</i> method										
		RVS broth		MKTn broth		Result	PCR result	Confirmation (Reference method)	Confirmation (RVS/XLD/Latex)	Final result Ref.conf.	Agreement Ref.conf.	Final result RVS/XLD	Agreement RVS/XLD	Storage BPW 72H 4°C			
		XLD	Chromagar <i>Salmonella</i>	XLD	Chromagar <i>Salmonella</i>									PCR	Confirmation	Final result	Agreement
2371	Ice cream(nougat)	-	-	-	-	-	-	-	-	-	=	-	=				
2372	Milk rice	-	-	-	-	-	-	-	-	-	=	-	=				
2373	Tiramisu	-	-	-	-	-	-	-	-	-	=	-	=				
2374	Chantilly	-	-	-	-	-	-	-	-	-	=	-	=				
2375	Chantilly	-	-	-	-	-	-	-	-	-	=	-	=				
2376	Tiramisu	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2377	Chantilly	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2378	Milk rice	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2379	Cheese(Brie)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2380	Cheese(Rustique)	-	-	-	-	-	-	-	-	-	=	-	=				
2381	Cheese	-	-	-	-	-	-	-	-	-	=	-	=				
2382	Ice cream (Pistachio)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2383	Ice cream(nougat)	-	-	-	+/- (Acinetobacter)	-	-	-	-	-	=	-	=				
2549	Raw milk	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2550	Raw milk	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2551	Raw milk	-	+	+	+	+	+	+	-	+	=	-	ND	+	+	+	
2552	Raw milk cheese(Tomme des Avis)	+ni/+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2553	Raw milk cheese(Morbier)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2554	Raw milk cheese(Saint Nectaire)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2555	Raw milk cheese(Comté)	-	-	-	-	-	-	-	-	-	=	-	=	-			
2556	Raw milk cheese(Bethmale)	-	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2568	Raw milk	+ni/+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2569	Raw milk	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2613	Crème brûlée	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2614	Ile flottante	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2615	English cream	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2618	English cream	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	

ni: non isolated colonies NC: non characteristic colonies on nutrient agar +/-:doubtful colonies i:inhibition in bold typing: artificially contaminated samples

EGG PRODUCTS																	
N° Sample	Product	ISO 6579 method♦					MicroSEQ® <i>Salmonella</i> method										
		RVS broth		MKTTn broth		Result	PCR result	Confirmation (Reference method)	Confirmation (RVS/XLD/Latex)	Final result Ref.conf.	Agreement Ref.conf.	Final result RVS/XLD	Agreement RVS/XLD	Storage BPW 72H 4°C			
		XLD	Chromagar <i>Salmonella</i>	XLD	Chromagar <i>Salmonella</i>									PCR	Confirmation	Final result	Agreement
987	Bread pastry	-	-	-	-	-	-	-	-	-	=	-	=				
1003	Egg white	-	-	-	-	-	-	-	-	-	=	-	=				
1005	Egg white	-	-	-	-	-	-	-	-	-	=	-	=				
1007	Egg yolk	-	-	-	-	-	i/-	-	i/-	-	=	-	=				
1009	Egg yolk	-	-	-	-	-	-	-	-	-	=	-	=				
1054	Egg white	-	-	-	-	-	-	-	-	-	=	-	=				
1055	Egg white	-	-	-	-	-	-	-	-	-	=	-	=				
1056	Whole egg product	-	-	-	-	-	-	-	-	-	=	-	=				
1122	Egg based dessert (île flottante)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1123	Egg based cream (vanilla flavor)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1124	Chocolate mousse	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1125	Egg based cream (crème brûlée)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1217	Dessert (Crème brûlée au caramel)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1234	Pastry with custard (gland)	-	-	-	-	-	-	-	-	-	=	-	=				
1235	Pastry with custard (gland)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1236	Pastry with custard (éclair)	-	-	-	-	-	-	-	-	-	=	-	=				
1237	Pastry with custard (éclair)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1238	Egg based cream (crème anglaise)	-	-	-	-	-	+(33)/+/-	-	-	-(PPNC)	=	-	=	-	-	-	=
1239	Egg based cream (crème anglaise)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1402	Whole egg powder	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1403	White egg powder	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1404	Whole egg powder	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=

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EGG PRODUCTS																	
N° Sample	Product	ISO 6579 method♦					MicroSEQ® <i>Salmonella</i> method										
		RVS broth		MKTn broth		Result	PCR result	Confirmation (Reference method)	Confirmation (RVS/XLD/Latex)	Final result Ref.conf.	Agreement Ref.conf.	Final result RVS/XLD	Agreement RVS/XLD	Storage BPW 72H 4°C			
		XLD	Chromagar <i>Salmonella</i>	XLD	Chromagar <i>Salmonella</i>									PCR	Confirmation	Final result	Agreement
1405	White egg powder	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2158	Liquid whole egg	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2159	Liquid whole egg	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2234	Liquid whole egg	-	-	-	-	-	-	-	-	-	=	-	=				
2235	Liquid egg yolk	-	-	-	-	-	i/-	-	-	-	=	-	=				
2236	Liquid whole egg	-	-	-	-	-	-	-	-	-	=	-	=				
2237	Liquid whole egg	-	-	-	-	-	-	-	-	-	=	-	=				
2254	Mayonnaise	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2255	Dressing(Béarnaise)	-	-	-	-	-	-	-	-	-	=	-	=				
2256	Dressing(Hollandaise)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2261	Mayonnaise	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2384	Egg yolk powder	-	-	-	-	-	i/i/-	-	-	-	=	-	=				
2385	Egg yolk powder	-	-	-	-	-	-	-	-	-	=	-	=				
2386	Whole egg powder	-	-	-	-	-	-	-	-	-	=	-	=				
2387	White egg powder	-	-	-	-	-	-	-	-	-	=	-	=				
2388	Mayonnaise	-	-	-	-	-	-	-	-	-	=	-	=				
2389	Mayonnaise	-	-	-	-	-	-	-	-	-	=	-	=				
2390	Salad dressing	-	-	-	-	-	-	-	-	-	=	-	=				
2391	Dressing(Hollandaise)	-	-	-	-	-	-	-	-	-	=	-	=				
2392	Baked custard	-	-	-	-	-	-	-	-	-	=	-	=				
2393	Egg powder	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2394	Egg powder	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2395	Egg powder	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2396	Egg powder	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2570	Liquid whole egg	+ni/+	+ni	+ni/+	+/-	+	+	+	+	+	=	+	=	+	+	+	=
2571	Liquid whole egg	+	+ni/+	+ni/+	+	+	+	+	+	+	=	+	=	+	+	+	=
2572	Liquid whole egg	+	+ni	+ni/+	+	+	+	+	+	+	=	+	=	+	+	+	=
2573	Liquid whole egg	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2574	Liquid whole egg	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2575	Mayonnaise	+	+ni	+ni	+	+	+	+	+	+	=	+	=	+	+	+	=
2576	Mayonnaise	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=

EGG PRODUCTS																	
N° Sample	Product	ISO 6579 method♦					MicroSEQ® <i>Salmonella</i> method										
		RVS broth		MKTTn broth		Result	PCR result	Confirmation (Reference method)	Confirmation (RVS/XLD/Latex)	Final result Ref.conf.	Agreement Ref.conf.	Final result RVS/XLD	Agreement RVS/XLD	Storage BPW 72H 4°C			
		XLD	Chromagar <i>Salmonella</i>	XLD	Chromagar <i>Salmonella</i>									PCR	Confirmation	Final result	Agreement
2577	Mayonnaise	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2578	Mayonnaise	+ni/+	+ni	+ni/-	+	+	+	+	+	+	=	+	=	+	+	+	=
2579	Mayonnaise	+	+ni/+	+ni	+/-	+	+	+	+	+	=	+	=	+	+	+	=
2580	Whole egg powder	-	-	-	-	-	-	-	-	-	=	-	=	-			
2611	Vanilla egg cream	-	-	-	-	-	-	-	-	-	=	-	=				
2612	Chocolate mousse	-	-	-	-	-	-	-	-	-	=	-	=				
2616	Preparation for baked custard and cream	-	-	-	-	-	-	-	-	-	=	-	=				
2617	Preparation for baked custrad	-	-	-	-	-	-	-	-	-	=	-	=				

ni: non isolated colonies NC: non characteristic colonies on nutrient agar +/-:doubtful colonies i:inhibition in bold typing: artificially contaminated samples

SEAFOOD AND VEGETABLES																	
N° Sample	Product	ISO 6579 method♦					MicroSEQ® <i>Salmonella</i> method										
		RVS broth		MKTn broth		Result	PCR result	Confirmation (Reference method)	Confirmation (RVS/XLD/Latex)	Final result Ref.conf.	Agreement Ref.conf.	Final result RVS/XLD	Agreement RVS/XLD	Storage BPW 72H 4°C			
		XLD	Chromagar <i>Salmonella</i>	XLD	Chromagar <i>Salmonella</i>									PCR	Confirmation	Final result	Agreement
949	Ready to eat fish	-	-	-	-	-	-	-	-	-	=	-	=				
955	Spring rolls	+ni/-	-	+/-	+ni/-	-	-	-	-	-	=	-	=				
956	Chinese salad(vegetables)	-	-	-	-	-	-	-	-	-	=	-	=				
989	Ready to eat fish	-	-	-	-	-	-	-	-	-	=	-	=				
1118	Cooked shrimps	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1119	Crayfish tails	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1120	Mussels (marinière)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1121	Salmon terrine	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1126	Croque Monsieur	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1127	Quiche Lorraine	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1198	Frozen minced vegetables	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1199	Frozen asian vegetables	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1200	Frozen vegetables (ratatouille)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1201	Frozen mixed vegetables	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1202	Cooked tuna with vegetables	-	-	-	-	-	-	-	-	-	=	-	=				
1203	Sole filet	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1204	Frozen ready to eat food with fish and vegetables	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1205	Frozen Sole	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1209	Peas with bacon	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1240	Cooked shrimps	-	-	-	+/- (1col:ox+)	-	-	-	-	-	=	-	=				
1377	Frozen asian vegetables	-	-	-	+/- (1col:ox+)	-	-	-	-	-	=	-	=				
1380	Frozen spinach with cream	-	-	-	-	-	-	-	-	-	=	-	=				

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SEAFOOD AND VEGETABLES																	
N° Sample	Product	ISO 6579 method♦					MicroSEQ® <i>Salmonella</i> method										
		RVS broth		MKTn broth		Result	PCR result	Confirmation (Reference method)	Confirmation (RVS/XLD/Latex)	Final result Ref.conf.	Agreement Ref.conf.	Final result RVS/XLD	Agreement RVS/XLD	Storage BPW 72H 4°C			
		XLD	Chromagar <i>Salmonella</i>	XLD	Chromagar <i>Salmonella</i>									PCR	Confirmation	Final result	Agreement
1382	Frozen mixed vegetables	-	-	-	-	-	-	-	-	-	=	-	=				
1383	Frozen mixed vegetables	-	+/- (ox+)	-	+/- (ox+)	-	-	-	-	-	=	-	=				
1384	Frozen fish	-	-	-	+/- (ox+)	-	-	-	-	-	=	-	=				
1385	Haddock filet	-	-	-	-	-	-	-	-	-	=	-	=				
1386	Whiting filet	-	-	-	-	-	-	-	-	-	=	-	=				
1387	Coalfish filet	-	-	-	-	-	-	-	-	-	=	-	=				
1388	Cauliflower	-	-	-	-	-	-	-	-	-	=	-	=				
1389	Mixed vegetables	-	-	-	-	-	-	-	-	-	=	-	=				
1390	Mixed vegetables	+(Citrobacter)	-	-	-	-	-	-	-	-	=	-	=				
1391	Salmon terrine	-	-	-	-	-	-	-	-	-	=	-	=				
1392	Scallops terrine	-	-	-	-	-	-	-	-	-	=	-	=				
1393	Calmar salad	-	-	-	-	-	-	-	-	-	=	-	=				
1394	Little octopus based salad	-	-	-	-	-	-	-	-	-	=	-	=				
1395	Anchovy fillet with garlic	-	-	-	-	-	-	-	-	-	=	-	=				
1406	Frozen mixed spinach	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1407	Frozen spinach branches	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1408	Beans puree	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1409	Carots puree	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1410	Anchovy fillet with garlic	-	-	-	-	-	-	-	-	-	=	-	=				
1411	Calmar salad	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1412	Little octopus salad	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1413	Vegetable macedoine	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1414	Ready to eat mixed vegetables	-	-	-	-	-	+(35,0)/ +(35,6)/ +(35,6)	-	-	-	=	-	=	+(34,0)/+(34,4)/ +(34,4)	-	-	=
1415	Minced carrots	-	-	-	-	-	-	-	-	-	=	-	=				
1416	Cauliflower	+	+	+	+	+	+(35,5)	+	+	+	=	+	=	+(32,8)	+	+	
1417	Fresh vegetables	-	-	-	+/- (ox+)	-	-	-	-	-	=	-	=				
2397	Sandwich	-	-	-	-	-	-	-	-	-	=	-	=				
2398	Salad	-	+/(Enterobacter sakazakii)	-	-	-	-	-	-	-	=	-	=				
2399	Vegetables terrine	-	-	+/(Citrobacter braakii)	-	-	-	-	-	-	=	-	=				

SEAFOOD AND VEGETABLES																	
N° Sample	Product	ISO 6579 method♦					MicroSEQ® <i>Salmonella</i> method										
		RVS broth		MKTn broth		Result	PCR result	Confirmation (Reference method)	Confirmation (RVS/XLD/Latex)	Final result Ref.conf.	Agreement Ref.conf.	Final result RVS/XLD	Agreement RVS/XLD	Storage BPW 72H 4°C			
		XLD	Chromagar <i>Salmonella</i>	XLD	Chromagar <i>Salmonella</i>									PCR	Confirmation	Final result	Agreement
2400	Vegetables terrine	+/(Citrobacter braakii)	-	+/(Citrobacter braakii)	-	-	-	-	-	-	=	-	=				
2401	Surimi	-	-	-	-	-	-	-	-	-	=	-	=				
2402	Cooked vegetables	-	-	-	-	-	-	-	-	-	=	-	=				
2403	Ready to eat meal	-	-	-	-	-	-	-	-	-	=	-	=				
2404	Cooked vegetables	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2405	Vegetables terrine	+	+	-	-	+	-(37,0) / +(35,6) / +(35,1)	+	+	-	ND	-	ND	+	+(5RVS)	+	=
2406	Carots salad	-	-	-	-	-	-	-	-	-	=	-	=				
2557	Salad(Celeri rémoulade)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2558	Grated carots	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2559	Mini sweet pepper with tuna	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2560	Grecian tomatoes	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2561	Ready to eat meal with vegetables	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2562	Stuffed marrow	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2563	Gratinated vegetables	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2564	Gratinated leeks with goat cheese	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=

ni: non isolated colonies NC: non characteristic colonies on nutrient agar +/-:doubtful colonies i:inhibition in bold typing: artificially contaminated samples

FEEDING STUFFS PRODUCTS																	
N° Sample	Product	ISO 6579 method♦					MicroSEQ® <i>Salmonella</i> method										
		RVS broth		MKTn broth		Result	PCR result	Confirmation (Reference method)	Confirmation (RVS/XLD/Latex)	Final result Ref.conf.	Agreement Ref.conf.	Final result RVS/XLD	Agreement RVS/XLD	Storage BPW 72H 4°C			
		XLD	Chromagar <i>Salmonella</i>	XLD	Chromagar <i>Salmonella</i>									PCR	Confirmation	Final result	Agreement
947	Dehydrated poultry proteins(Feedstuff)	+	+	+	+	+	+	+	+	+	=	+	=	i/+	+	+	=
948	Dehydrated poultry proteins(Feedstuff)	+	+	+	+	+	+	+	+	+	=	+	=	i/+	+	+	=
1035	Fish croquettes(feedstuff)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1037	Fish croquettes(feedstuff)	-	-	-	-	-	-	-	-	-	=	-	=				
1039	Feedstuff powder	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1107	Dog pellet	-	-	-	-	-	-	-	-	-	=	-	=				
1108	Dog pellets	-	-	-	-	-	-	-	-	-	=	-	=				
1109	Dog pellets	-	-	-	-	-	-	-	-	-	=	-	=				
1110	Dog pellets	-	-	-	-	-	-	-	-	-	=	-	=				
1111	Dog pellets	-	-	-	-	-	-	-	-	-	=	-	=				
1112	Dog pellets	-	-	-	-	-	-	-	-	-	=	-	=				
1218	Feedstuff (pellets for dog)	-	-	-	-	-	-	-	-	-	=	-	=				
1219	Feedstuff (pellets for dog)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1220	Feedstuff (poultry based pet food)	-	-	-	-	-	-	-	-	-	=	-	=				
1221	Feedstuff (poultry based pet food)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1222	Feedstuff (rabbit based pet food)	-	-	-	-	-	-	-	-	-	=	-	=				
1223	Feedstuff (rabbit based pet food)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1224	Feed stuff (beef based pellets dogfood)	-	-	-	-	-	-	-	-	-	=	-	=				
1225	Feed stuff (beef based pellets dogfood)	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
1397	Raw meat petfood	-	-	-	+/--(ox+)	-	-	-	-	-	=	-	=				
1398	Raw slaughter petfood	-	-	-	-	-	-	-	-	-	=	-	=				
1399	Horse feedstuff	-	-	-	-	-	-	-	-	-	=	-	=				

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FEEDING STUFFS PRODUCTS																	
N° Sample	Product	ISO 6579 method♦					MicroSEQ® <i>Salmonella</i> method										
		RVS broth		MKTn broth		Result	PCR result	Confirmation (Reference method)	Confirmation (RVS/XLD/Latex)	Final result Ref.conf.	Agreement Ref.conf.	Final result RVS/XLD	Agreement RVS/XLD	Storage BPW 72H 4°C			
		XLD	Chromagar <i>Salmonella</i>	XLD	Chromagar <i>Salmonella</i>									PCR	Confirmation	Final result	Agreement
1400	Horse feedstuff	-	-	-	-	-	-	-	-	-	=	-	=				
1401	Pig sop	-	-	-	-	-	-	-	-	-	=	-	=				
1418	Pork haemoglobin	-	-	-	-	-	-	-	-	-	=	-	=				
1419	Dehydrated poultry proteins	+	+	+	+	+	+	+	+	+	=	+	=	i/+	+	+	
1420	Dehydrated poultry proteins	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1421	Feedstuff(dehydrated)	+	+	+	+	+	+	+	+	+	=	+	=	i/+	+	+	
1422	Dehydrated poultry proteins	-	-	-	-	-	-	-	-	-	=	-	=				
1423	Meat flour (pet food)	-	-	-	-	-	-	-	-	-	=	-	=				
1424	Viscera flour	+	+	+	+	+	+	+	+	+	=	+	=	i/+	+	+	
1981	Bone for pet	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1982	Raw meat for pet	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1983	Balls for pet	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
1984	Big sausage for dog	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2153	Feed stuff powder	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2154	Feed stuff powder	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2155	Feed stuff powder	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2156	Feed stuff powder	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2238	Pork hemoglobin	-	-	-	-	-	-	-	-	-	=	-	=				
2239	Pellets	-	-	-	-	-	-	-	-	-	=	-	=				
2240	Feed stuff powder	-	-	-	-	-	-	-	-	-	=	-	=				
2241	Viscera flour(Feed stuff)	+	+	+	+	+	i/+	+	+	+	=	+	=	i/+	+	+	
2242	Feed stuff powder	+ni/+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2243	Viscera flour(Feed stuff)	-	-	-	-	-	i/-	-	-	-	=	-	=				
2244	Feed stuff powder	-	-	-	-	-	-	-	-	-	=	-	=				
2245	Feed for hen	-	-	-	-	-	-	-	-	-	=	-	=				
2246	Raw pork meat for pet	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2247	Raw pork meat for pet	-	-	-	-	-	-	-	-	-	=	-	=				
2248	Raw pork meat for pet	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	
2249	Raw pork meat for pet	-	-	-	-	-	-	-	-	-	=	-	=				
2407	Granules for chicken	-	-	-	-	-	-	-	-	-	=	-	=				
2408	Granules for pigs	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	

FEEDING STUFFS PRODUCTS																	
N° Sample	Product	ISO 6579 method♦					MicroSEQ® <i>Salmonella</i> method										
		RVS broth		MKTn broth		Result	PCR result	Confirmation (Reference method)	Confirmation (RVS/XLD/Latex)	Final result Ref.conf.	Agreement Ref.conf.	Final result RVS/XLD	Agreement RVS/XLD	Storage BPW 72H 4°C			
		XLD	Chromagar <i>Salmonella</i>	XLD	Chromagar <i>Salmonella</i>									PCR	Confirmation	Final result	Agreement
2410	Raw bovine meat for animals	+	+	+	+	+	-(36,09)/-(35,9)/ +/(36,4)	+	+	-	ND	-	ND	+	+	+	=
2411	Raw meat for animals 1	+	+	-	-	+	-(36,1)/-(36,9)/ -(39,1)	+	+	-	ND	-	ND	-/(0)/-(0)	+	-	ND
2412	Raw meat for animals 2	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2413	Raw meat for animals 3	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2414	Sausage for dog	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2565	Bone for animals	-	-	-	+ni/-	-	+/+/(35,7)	-	-	-(PPNC)	=	-	=	-(37,1)/-(0)/ (37,5)	-	-	=
2566	Bovine meat for animals	-	-	-	-	-	-	-	-	-	=	-	=	-			
2567	Meat for animals	-	-	-	-	-	-	-	-	-	=	-	=	-			
2581	Meat for animals	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2582	Bone for animals	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2583	Bovine meat for animals	+	+	+	+	+	+	+	+	+	=	+	=	+	+	+	=
2584	Pork fat	-	-	-	-	-	-	-	-	-	=	-	=	-			

Annex 4 – Inclusivity and exclusivity: raw data

Inclusivity						
	Strain		Reference	Origin	Inoculation level ufc/225ml	PCR result
1.	<i>Salmonella</i>	Agona	A00V38	Feedstuff	5	+
2.	<i>Salmonella</i>	Anatum	6140	Bœuf Bourguignon	7	+
3.	<i>Salmonella</i>	<i>arizonae</i> SIIIa 51 :24,223 :-	CIP 5523	Turkey	4	+
4.	<i>Salmonella</i>	<i>diarizonae</i> SIIIb 47 :IV :253	Ad478	Clams	9	+
5.	<i>Salmonella</i>	<i>diarizonae</i> SIIIb 38 :IV :253	Ad451	Ewe milk	1	+
6.	<i>Salmonella</i>	<i>arizonae</i>	4851	Turkey	1	+
7.	<i>Salmonella</i>	<i>diarizonae</i> SIIIb 61:-:, 1,5,7		Raw ewe milk	7	+
8.	<i>Salmonella</i>	Blockley	Ad 923	Chicken	5	+
9.	<i>Salmonella</i>	Bovismorbificans	728	Agar	14	+
10.	<i>Salmonella</i>	Braenderup	178	Food	22	+
11.	<i>Salmonella</i>	Brandenburg	Ad 351	Seafood	20	+
12.	<i>Salmonella</i>	Bredeney	396	Ground beef	17	+
13.	<i>Salmonella</i>	Cerro	Ad 689	Dehydrated proteins	2	+
14.	<i>Salmonella</i>	Cremieu	230	Hare	3	+
15.	<i>Salmonella</i>	Derby	Ad 1093	Frozen fish fillet	4	+
16.	<i>Salmonella</i>	<i>diarizonae</i> 38 :lv :253	Ad 453	Raw milk cheese	2	+
17.	<i>Salmonella</i>	Dublin	Ad 528	Pancake	9	+
18.	<i>Salmonella</i>	Enteritidis	Ad 926	Raw veal meat	6	+
19.	<i>Salmonella</i>	Gallinarum	Ad 300	Poultry slaughterhouse	2	+
20.	<i>Salmonella</i>	Give	436	Ground beef	7	+
21.	<i>Salmonella</i>	Hadar	35	Poultry	19	+
22.	<i>Salmonella</i>	Havana	Ad 930	Poultry	18	+
23.	<i>Salmonella</i>	Heidelberg	A00E005	Dairy industry environmental sample	17	+
24.	<i>Salmonella</i>	<i>houtenae</i> (sub-group IV) 43:z4z32	Ad 597	Fish	13	+
25.	<i>Salmonella</i>	Indiana	2	Fish flour	9	+
26.	<i>Salmonella</i>	<i>indica</i> (sub-group VI) 1,26,14,25:a:enx	Ad 600	Environment	1	+
27.	<i>Salmonella</i>	Infantis	12	Ready to eat food	1	+
28.	<i>Salmonella</i>	Kedougou	Ad 929	Environmental sample (Slaughterhouse)	3	+
29.	<i>Salmonella</i>	Kottbus	1	Environmental sample (slaughterhouse)	11	+
30.	<i>Salmonella</i>	Lagos	173	Sausage	13	+
31.	<i>Salmonella</i>	Landau	Ad 499		12	+
32.	<i>Salmonella</i>	Livingstone	E1	Egg white powder	11	+
33.	<i>Salmonella</i>	London	326	Ham	5	+
34.	<i>Salmonella</i>	Manhattan	900	Dairy environmental sample	9	+
35.	<i>Salmonella</i>	Mbandaka	Ad 914	Mayonnaise	9	+
36.	<i>Salmonella</i>	Meleagridis	505	Raw milk	2	+
37.	<i>Salmonella</i>	Montevideo	Ad 912	Raw milk	20	+
38.	<i>Salmonella</i>	Napoli	Ad 928	Bovine	13	+
39.	<i>Salmonella</i>	Newport	540	Toulouse sausage	11	+
40.	<i>Salmonella</i>	Panama	195	Ground beef	14	+
41.	<i>Salmonella</i>	Paratyphi A	ATCC 9150		2	+
42.	<i>Salmonella</i>	Paratyphi B	Ad 301	Clinical	3	+
43.	<i>Salmonella</i>	Paratyphi C	ATCC 13428		1	+
44.	<i>Salmonella</i>	Regent	328	Duck	3	+

Inclusivity						
	Strain		Reference	Origin	Inoculation level ufc/225ml	PCR result
45.	<i>Salmonella</i>	Rissen	39	Poultry	4	+
46.	<i>Salmonella</i>	Saintpaul	F31	Pilchard fillet	6	+
47.	<i>Salmonella</i>	<i>salamae</i> (sub-group II) 42:b:enxz	Ad 593	Cereals	7	+
48.	<i>Salmonella</i>	Senftenberg	Ad 355	Seafood	13	+
49.	<i>Salmonella</i>	Tennessee	A00E006	Dairy industry environmental sample	23	+
50.	<i>Salmonella</i>	Thompson	AER301	Poultry	17	+
51.	<i>Salmonella</i>	Typhi	Ad 302	Clinical	1	+
52.	<i>Salmonella</i>	Typhimurium	305	Paella	10	+
53.	<i>Salmonella</i>	Urbana	Ad 501	?	6	+
54.	<i>Salmonella</i>	Virchow	F276	Curry	8	+

Exclusivity					
	Strain		Origin	Inoculation level ufc/ml	PCR Result
1.	<i>Citrobacter braakii</i>	Ad833	Raw beef meat	5,0.10 ⁵	-
2.	<i>Citrobacter diversus</i>	adria 140	Raw milk	5,3.10 ⁵	-
3.	<i>Citrobacter freundii</i>	adria 23	Raw pork sausage	6,9.10 ⁵	-
4.	<i>Citrobacter freundii</i>	adria 175	Raw duck meat	7,1.10 ⁵	-
5.	<i>Citrobacter koseri</i>	adria 71	Frozen vegetables	9,0.10 ⁵	-
6.	<i>Cronobacter sakazakii</i>	adria 95	Fermented milk	4,1.10 ⁵	-
7.	<i>Enterobacter agglomerans</i>	adria 11	Cheese	5,2.10 ⁵	-
8.	<i>Enterobacter amnigenus</i>	A00C068	Raw poultry meat	4,6.10 ⁵	-
9.	<i>Enterobacter cloacae</i>	adria 10	Raw milk	4,0.10 ⁵	-
10.	<i>Enterobacter intermedius</i>	adria 60	Bean	1,3.10 ⁵	-
11.	<i>Enterobacter kobei</i>	Ad 342	Ham	2,7.10 ⁵	-
12.	<i>Erwinia carotovora</i>	CIP 8283	Potatoes	2,0.10 ³	-
13.	<i>Escherichia coli</i>	adria 19	Grated carrots	3,1.10 ⁵	-
14.	<i>Escherichia hermanii</i>	Ad 461	Dessert	2,8.10 ⁵	-
15.	<i>Escherichia vulneris</i>	adria 127	Raw milk	9,8.10 ⁵	-
16.	<i>Hafnia alvei</i>	adria 167	Raw pork sausage	9,2.10 ⁵	-
17.	<i>Klebsiella oxytoca</i>	57	Food product	3,2.10 ⁵	-
18.	<i>Klebsiella pneumoniae</i>	47	Raw turkey meat	3,9.10 ⁵	-
19.	<i>Kluyvera spp</i>	adria 41	Raw milk	3,0.10 ⁵	-
20.	<i>Morganella morganii</i>	CIP A236	/	6,5.10 ⁵	-
21.	<i>Pantoea agglomerans</i>	adria 86	Frozen vegetables	7,7.10 ⁵	-
22.	<i>Proteus mirabilis</i>	Ad639	Mayonnaise	7,3.10 ⁵	-
23.	<i>Proteus vulgaris</i>	adria 43	Sliced ham	5,8.10 ⁴	-
24.	<i>Providencia rettgeri</i>	adria 112	White liquid egg	3,7.10 ⁵	-
25.	<i>Rhanella aquatilis</i>	adria 69	Molluscs	7,0.10 ⁴	-
26.	<i>Serratia liquefaciens</i>	26	Egg product	2,6.10 ⁵	-
27.	<i>Serratia marcescens</i>	Ad447	Raw milk	6,8.10 ⁵	-
28.	<i>Serratia proteomaculans</i>	A00C056	Ham	9,2.10 ⁴	-
29.	<i>Shigella flexneri</i>	CIP 8248	/	3,2.10 ⁵	-
30.	<i>Shigella sonnei</i>	CIP 8249T (ATCC 29930)	/	3,7.10 ⁵	-
31.	<i>Yersinia enterocolitica</i>	adria 32	Bacon	9,4.10 ⁴	-
32.	<i>Salmonella bongori</i>	Ad599	Turkey breeding	3,2.10 ⁵	+
				4,8.10 ⁵	+
				6 (protocol for inclusivity testing)	-
33.	<i>Salmonella bongori</i>	Ad598	Turkey breeding	2,9.10 ⁵	-
				10 (protocol for inclusivity testing)	-