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NF VALIDATION
Validation of alternative analytical methods
Application in food microbiology

Summary report

Validation study according to the EN ISO 16140-2:2016

iQ-Check *E. coli* O157:H7
(Certificate number: BRD 07/15 - 06/08)
for the detection of *Escherichia coli O157:H7*
in raw meats

Qualitative method

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This report consists of 69 pages, including 7 appendices.
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Competencies of the laboratory are certified by COFRAC accreditation for the analyses marked with the symbol◆.

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Quality Assurance documents related to this study can be consulted upon request from **BIO-RAD**.

The technical protocol and the result interpretation were carried out according to the EN ISO 16140-2:2016 and the AFNOR technical rules (PR Revision 7).

Validation protocols	☒ ISO 16140-1 (2016): Microbiology of the food chain - Method validation — *Part 1: Vocabulary* ☒ ISO 16140-2(2016): Microbiology of the food chain - Method validation — *Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method* ☒ AFNOR technical rules (PR Revision 7)
Reference method♦	☒ ISO 16654 (May 2001): Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Escherichia coli* O157 ☒ ISO 16654 (May 2011): Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Escherichia coli* O157 - Amendment 1 (March 2017): annex B: result of inter-laboratory studies
Alternative method	**iQ-Check *E. coli* O157:H7**
Scope	**Raw meats** *(25 g test portion and 375 g test portion)*
Certification organism	AFNOR Certification (http://nf-validation.afnor.org/)

♦ Analyses performed according to the COFRAC accreditation

1 INTRODUCTION

The iQ-Check *E. coli* O157:H7 method was validated on the 30th of June 2008 (certificate number BRD 07/15 - 06/08) for detection of *Escherichia coli* O157:H7 in raw beef meats according to the ISO 16140 (2003).

A summary of the extension and renewal studies is given in the table below:

June 2008	Initial validation study ▪ Raw beef meats (25 g test portion): BPW 8-24 h at 41,5°C
September 2008	Extension study for a new enrichment protocol: BPW for 21 h ± 1 h at 37°C
January 2009	Extension study ▪ New version of the Opticon Monitor™ software
February 2010	Extension study: ▪ Use of deepwell plates for lysis step ▪ Use of the CFX96 Deep Well system with the CFX Manager™ software ▪ Use of the Mini Opticon™ with the CFX Manager™
March 2012	Extension: ▪ Use of the CFX96 Deep Well system and the CFX Manager IDE V1.2 software
May 2012	Renewal
March 2013	Extension: ▪ Use of the CFX Manager™ Software IDE version 2.0 and the iQ-Check Prep
October 2013	Extension: ▪ Use of the CFX Manager™ Software IDE version 2.1 and the iQ-Check Prep
November 2014	Extension: ▪ Use of the CFX Manager™ Software IDE version 2.2 and the iQ-Check Prep v2
March 2016	Renewal
May 2018	Extension: ▪ Use of the CFX Manager™ Software IDE version 3.0 and the iQ-Check Prep v3

October 2020	Renewal and extension study ▪ **Only the protocol initially validated was kept for the renewal study with an extension of the scope to raw meats for two tests portions (25 g and 375 g) and a restriction of the incubation range from 8 - 24 h to 8 - 16 h for 25 g test portion.** ▪ The extension also concerns the use of a new Application Protocol File (APF). The classical iQ-Check APF (Application Protocol File) corresponds to a 1h50 min PCR run. Some optimization (reduction of the number of cycles + time reduction of some steps) led to release the APF Fast for iQ-Check methods to reduce the PCR run time down to 1h10 min without impact on the performances (sensitivity and specificity).
December 2022	Extension for the use of the CFX Opus Deepwell (the data corresponding to this study are available from AFNOR Certification)

2 METHOD PROTOCOLS

2.1 Alternative method

The flow diagram of the alternative method is provided in **Appendix 1**.

The iQ-Check *E. coli* O157:H7 kit is a test based on gene amplification and detection by real-time PCR. Ready-to-use PCR reagents contain oligonucleotides (primers and probes) specific *E. coli* O157:H7, as well as DNA polymerase and nucleotides. Detection and data analysis are optimized for use with a Bio-Rad real-time PCR instrument, such as the CFX96™ or the CFX96 Deepwell™ systems.

PCR is a powerful technique used to generate many copies of target DNA. During the PCR reaction, several cycles of heating and cooling allow DNA denaturation, by heat, followed by primers binding to the target region. The DNA polymerase then uses these primers and deoxynucleotide triphosphates (dNTPs) to extend the DNA, creating copies of the target DNA. These copies are called amplicons.

This test allows the detection of *E. coli* O157:H7 in raw meats previously enriched by culture in pre-warmed Buffered peptone water. It includes the following 4 main steps:

- Enrichment;
- DNA extraction;
- Real-time PCR;
- Data analysis & interpretation.

2.1.1 Protocols

Two protocols are available (See **Table 1**).

Table 1 - Protocols

Category	Protocol	Test portion	Enrichment	Extraction	PCR
Raw meats (excluding poultry meats)	①	25 g	Pre-warmed BPW (41.5°C) 8 - 16 h at 41.5°C ± 1°C d 1/10	Easy II 100 µl Tube or Deep Well plate	APF Classic or APF Fast on 5 µl DNA extract
	②	375 g	Pre-warmed BPW (41.5°C) 10 - 18 h at 41.5°C ± 1°C d 1/4	Easy II 100 µl Tube or Deep Well plate	APF Fast on 5 µl DNA extract

The PCR is performed on 5 µl DNA extract. Two APF files are available:

- APF Classic (1h50 PCR run) for protocol ①
- APF Fast (1h10 PCR run) for protocols ① & ②.

The confirmation of positive PCR results is performed as follows:

- By streaking 10 µl enriched sample onto CT-SMAC (18-24 h at 37°C) or RAPID'*E.coli* O157:H7 (24h ± 2h at 37°C);
- By proceeding to an IMS step before streaking 50 µl of immunobeads suspension onto CT-SMAC or RAPID'*E.coli* O157:H7.

The typical colonies (1 to 3) are confirmed by O157 and H7 latex tests after subculture onto non-selective agar.

It is possible to store the enrichment broths for 72 h at 5°C ± 3°C for protocol 2 only (375g test portion).

2.1.2 Restriction

There is no restriction for use.

2.2 Reference method◆

The reference methods are the ISO 16654 method (2011): Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Escherichia coli* O157 and the ISO 16654/A1 (March 2017): Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Escherichia coli* O157 - Amendment 1: annex B: result of inter-laboratory studies (See **Appendix 2**).

2.3 Study design

The study is **an unpaired study design** as the reference and the alternative methods have different enrichment procedures.

3 INITIAL VALIDATION, EXTENSION/RENEWAL STUDIES: RESULTS

3.1 Method Comparison Study

The method comparison study is a study performed by the expert laboratory to compare the alternative method with the reference method.

The study was carried out on a diversity of samples and strains representative of agri-food products. This does not constitute an exhaustive list of the different matrices included in the scope.
For any comment on the alternative method, please contact AFNOR Certification at http://nf-validation.afnor.org/contact-2/.

3.1.1 Sensitivity study

The sensitivity (SE) is the ability of the method to detect the analyte by either the reference or alternative method.

◆ Analysis performed according to the COFRAC accreditation

3.1.1.1 Number and nature of samples

For the initial validation study performed in June 2008, on the categories and protocol concerned by the renewal study, 67 samples were tested providing 36 positive and 31 negative results, 4 samples inoculated between 5 and 10 CFU and 3 samples inoculated at 12.6 CFU have been excluded for the renewal study. All these samples were in positive agreement.

115 samples have been tested in 2020 providing 69 positive and 46 negative results.

For the study performed in 2008, the APF Classic was applied. For all the samples tested in 2020, only the APF Fast was used.

Taking into account all the studies, 175 samples were tested providing 98 positive and 77 negative results. The distribution per tested category, type and APF is given in **Table 2**.

Table 2 – Distribution per tested category, type and APF

Category		Protocol	Type		Positive samples	Negative samples	Total
1	Raw meat 25 g	8 h Easy II APF Classic	a	Chilled	17	17	34
			b	Frozen	4	1	5
			c	Seasoned or marinated	8	13	21
				Total	29	31	60
		8 h Easy II APF FAST	a	Chilled	15	3	18
			b	Frozen	5	10	15
			c	Seasoned or marinated	18	3	21
				Total	38	16	54
				Total	67	47	114
	Raw meat 375 g	10 h Easy II APF FAST	a	Chilled	11	9	20
			b	Frozen	9	11	20
			c	Seasoned or marinated	11	10	21
				Total	31	30	61
Total					**98**	**77**	**175**

3.1.1.2 Artificial contamination of samples

Naturally contaminated samples were preferentially analyzed. Artificial contaminations were carried out by spiking or seeding protocol. When spiking, the strains were stressed using various injury protocols (heat treatment, cold storage, salt stress). The injury efficiency was evaluated by comparing enumeration results onto selective and non-selective agars (respectively CT-SMAC and TSA).

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

107 samples were artificially contaminated, using 31 different strains. 97 gave a positive result.

The repartition of the positive samples per inoculation protocol and inoculation level is given in **Table 3**.

Table 3 - Repartition of positive naturally and artificially contaminated samples per inoculation level

Protocol/ Test portion		Naturally contaminated samples	Artificially contaminated samples						Total positive samples
			Spiking protocol			Seeding protocol			
			≤ 5 CFU/ sample	5<x≤ 10 CFU/sample	10<x≤ 30 CFU/sample	≤ 3 CFU/ sample	3<x≤ 10 CFU/ sample	10<x≤ 30 CFU/sample	
① 25 g	Number of samples	1	21	7	0	33	5	0	67
	%	1,5%	31,3%	10,4%	0,0%	49,3%	7,5%	0,0%	100,0%
② 375 g	Number of samples	0	0	0	0	26	5	0	31
	%	0,0%	0,0%	0,0%	0,0%	83,9%	16,1%	0,0%	100,0%
Total	**Number of samples**	**1**	**21**	**7**	**0**	**59**	**10**	**0**	**98**
	%	**1,0%**	**21,4%**	**7,1%**	**0,0%**	**60,2%**	**10,2%**	**0,0%**	**100,0%**

Only one naturally contaminated sample was found positive: sample 3127 (Raw beef meat) tested in 2008.
Combining the spiking and the seeding inoculation protocols, 17.3 % of the samples were contaminated between 3 and 10 CFU.

1 % of the samples was naturally contaminated.

3.1.1.3 Protocols applied during the validation study

Incubation times

For each protocol, the minimum incubation time was applied:

- Raw meat (25 g): 8 h at 41.5°C;
- Raw meat (375 g): 10 h at 41.5°C.

Lysis step

The Deep Well plates were used for the study (Easy II protocol).

PCR

The data obtained for the validation performed in 2008, the PCR was run using the APF Classic. For this extension study (2020), only the APF Fast was used.

Confirmation protocols

The following protocols were tested:

- Direct streaking onto CT-SMAC and RAPID'*E. coli* O157:H7;
- IMS and streaking onto CT-SMAC and RAPID'*E. coli* O157:H7.

The typical colonies were confirmed after subculture on non-selective agar (TCS) by O157 and H7 latex test (Remel from OXOID). An Indol test was also applied.

For samples in negative agreement, the BPW was incubated until 18 h before performing IMS and streaking onto selective agar plates.

Enrichment broth storage for 72 h at 5°C ± 3°C

The enrichment broths from positive and discordant samples were tested again after storage for 72 h at 5°C ± 5°C for protocol 2 (375 g test portion).

3.1.1.4 Test results

Raw data per category are given in **Appendix 4**. The results are given in **Table 4**.

Table 4 – Interpretation of sample results between the reference and alternative method (based on the confirmed alternative method)

Category		Protocol	Type		PA	NA*	PD	ND**	PPND	PPNA	Total
1	Raw meat 25 g	8 h Easy II APF Classic	a	Chilled	12	17	2	3	0	0	34
			b	Frozen	3	1	0	1	0	0	5
			c	Seasoned or marinated	6	13	2	0	0	0	21
				Total	21	31	4	4	0	0	60
		8 h Easy II APF FAST	a	Chilled	5	2	5	5	0	1	18
			b	Frozen	3	10	0	2	0	0	15
			c	Seasoned or marinated	11	3	4	3	0	0	21
				Total	19	15	9	10	0	1	54
		Total			40	46	13	14	0	1	114
	Raw meat 375 g	10 h Easy II APF FAST	a	Chilled	5	9	4	2	0	0	20
			b	Frozen	2	11	7	0	0	0	20
			c	Seasoned or marinated	4	10	4	3	0	0	21
				Total	11	30	15	5	0	0	61
Total					**51**	**76**	**28**	**19**	**0**	**1**	**175**

* PPNA not included ** PPND not included

3.1.1.5 Calculation of relative trueness (RT), sensitivity (SE) and false positive ratio (FPR)

The calculations are presented in **Table 5**.

Table 5 – Calculation of the relative trueness (RT), the sensitivity (SE) and the false positive ratio (FPR)

Category		Protocol	Type		PA	NA*	PD	ND**	PPND	PPNA	SE_{alt} %	SE_{ref} %	RT %	FPR %
1	Raw meat 25 g	8h Easy II APF Classic	a	Chilled	12	17	2	3	0	0	82,4	88,2	85,3	0,0
			b	Frozen	3	1	0	1	0	0	75,0	100,0	80,0	0,0
			c	Seasoned or marinated	6	13	2	0	0	0	100,0	75,0	90,5	0,0
				Total	21	31	4	4	0	0	86,2	86,2	86,7	0,0
		8h Easy II APF FAST	a	Chilled	5	2	5	5	0	1	66,7	66,7	44,4	33,3
			b	Frozen	3	10	0	2	0	0	60,0	100,0	86,7	0,0
			c	Seasoned or marinated	11	3	4	3	0	0	83,3	77,8	66,7	0,0
				Total	19	15	9	10	0	1	73,7	76,3	64,8	6,3
		Total			*40*	*46*	*13*	*14*	*0*	*1*	*79,1*	*80,6*	*76,3*	*2,1*
	Raw meat 375 g	10h Easy II APF FAST	a	Chilled	5	9	4	2	0	0	81,8	63,6	70,0	0,0
			b	Frozen	2	11	7	0	0	0	100,0	22,2	65,0	0,0
			c	Seasoned or marinated	4	10	4	3	0	0	72,7	63,6	66,7	0,0
				Total	*11*	*30*	*15*	*5*	*0*	*0*	*83,9*	*51,6*	*67,2*	*0,0*
Total					**51**	**76**	**28**	**19**	**0**	**1**	**80,6**	**71,4**	**73,1**	**1,3**

* PPNA not included

** PPND not included

A summary of the results is given in **Table 6**.

Table 6 - Summary of results

		Raw meats		
		Protocol ① 25 g	Protocol ② 375 g	Combining both tests portions
Sensitivity for the alternative method	$SE_{alt} = \frac{(PA + PD)}{(PA + ND + PD)} \times 100\%$	79.1 %	83.9 %	80.6 %
Sensitivity for the reference method	$SE_{ref} = \frac{(PA + ND)}{(PA + ND + PD)} \; x \; 100\,\%$	80.6 %	51.6 %	91.4 %
Relative trueness	$RT = \frac{(PA + NA)}{N} \times 100\%$	76.3 %	67.2 %	73.1 %
False positive ratio for the alternative method* **FP = PPNA + PPND**	$FPR = \frac{(FP)}{NA} \times 100\%$	2.1 %	0.0 %	1.3 %

**With ND = ND + PPND*
NA = NA + PPNA

3.1.1.6 Analysis of discordant results

The negative deviations are given in **Table 7** and the positive deviations in **Table 8**.

19 negative deviations were observed when combining the two tests portions (25 g and 375 g), 14 for 25 g and 5 for 375 g. For protocol ① (25 g), the presence of *E. coli* O157:H7 was not detected in the enrichment broth, the negative deviations were probably linked to the unpaired study design and the heterogeneity of sampling. For protocol ② (375 g), the presence of *E. coli* O157:H7 was confirmed for one sample (2678), note that for this sample, 3 PCR tests were carried out and one positive result was observed. The contamination level was probably just at the detection limit of the alternative method.

28 positive deviations were observed combining both tests portions, 13 for 25 g and 15 for 375 g.

According to the ISO 16140-2 (2016), the enrichment broths from samples in negative agreement (NA) were incubated until 24 h and tested using the protocol of the ISO 16654.All the samples gave negative confirmation results. But note that for one sample in NA (2039: frozen ground veal, 375 g), the presence of *E.coli* O157:H7 strain was detected by applying an IMS step prior streaking onto selective agar plates after 10 h incubation time at 41.5°C.For this sample 10 PCR tests were performed, 8 positive and 2 negative results were obtained, showing that the contamination level was just at the detection limit of the alternative method.

Two APF (Classic and Fast) have been tested for 25g test portions, similar results have been observed for both: 4 ND and 4 PD for APF Classic, 10 ND and 9 PD for the APF Fast.

Table 7 - Negative deviations

Year of analysis	Sample N°	Product	Artificial contamination		ISO 16654♦	Alternative method: iQ-Check *E. coli* O157:H7				Agreement	Protocol	Type
						PCR			Confirmation			
			Strain	Inoculation level (CFU/sample)		Ct IC	Ct HEX	PCR result				
2008	**2687**	**Ground beef meat**	*E. coli* O157:H7 Ad485	0,8	+	33,72	-	-	-	**ND**	1	a
2008	**2705**	**Ground beef meat**	*E. coli* O157:H7 A215-16	1,2	+	34,04	-	-	-	**ND**	1	a
2008	**2706**	**Ground beef meat**	*E. coli* O157:H7 A215-16	1,2	+	33,71	-	-	-	**ND**	1	a
2020	**1868**	**Chilled raw pork meat**	*E. coli* O157:H7 Ad1501	1,4	+	33,23	-	-	-	**ND**	1	a
2020	**1869**	**Chilled raw veal meat**	*E. coli* O157:H7 Ad1071	2,8	+	33,65	-	-	-	**ND**	1	a
2020	**2318**	**Chilled raw lamb meat**	*E. coli* O157:H7 Ad555	2,8	+	33,68	-	-	-	**ND**	1	a
2020	**2968**	**Chilled raw lamb meat**	*E. coli* O157:H7 Ad1174	1,8	+	33,33	-	-	-	**ND**	1	a
2020	**2969**	**Chilled raw veal meat**	*E. coli* O157:H7 Ad568	3	+	33,27	-	-	-	**ND**	1	a
2008	**2694**	**Frozen ground beef meat**	*E. coli O157:H7 Ad485*	0,8	+	34,58	-	-	-	**ND**	1	b
2020	**1668**	**Frozen raw lamb meat**	*E. coli* O157:H7 Ad684	4	+	34,16	-	-	-	**ND**	1	b
2020	**1822**	**Frozen raw lamb meat**	*E. coli* O157:H7 Ad589	0,8	+	34,38	-	-	-	**ND**	1	b
2020	**1594**	**Marinated raw pork meat**	*E. coli* O157:H7 Ad1248	1,4	+	33,52	-	-	-	**ND**	1	c
2020	**1879**	**Marinated raw lamb meat**	*E. coli* O157:H7 Ad685	2,8	+	33,31	-	-	-	**ND**	1	c
2020	**2689**	**Seasoned raw beef meat**	*E. coli* O157:H7 Ad976	3,6	+	33,53	-	-	-	**ND**	1	c
2020	**1970**	**Chilled raw pork meat**	*E. coli* O157:H7 Ad933	2,6	+	33,12	-	-	-	**ND**	2	a
2020	**1972**	**Chilled raw lamb meat**	*E. coli* O157:H7 Ad560	1,2	+	33,05	-	-	-	**ND**	2	a
2020	**2678**	**Seasoned raw beef meat**	*E. coli* O157:H7 Ad976	3,6	+	33,80/33,74/33,02	-/37,59/-	-/+/-	+	**ND**	2	c
2020	**2681**	**Seasoned raw pork meat**	*E. coli* O157:H7 Ad562	2,8	+	32,99/33,30/33,05	-/-/37,08	-/-/+	-	**ND**	2	c
2020	**2687**	**Seasoned raw beef meat**	*E. coli* O157:H7 Ad558	3	+	33,66/34,13/33,09	-/41,08/-	-/+/-	-	**ND**	2	c

2008: APF Classic 2020: APF Fast

♦ Analyses performed according to the COFRAC accreditation

Table 8 - Positive deviations

Year of analysis	Sample N°	Product	Artificial contamination		ISO 16654♦	Alternative method: iQ-Check *E. coli* O157:H7				Agreement	Protocol	Type
						PCR			Confirmation			
			Strain	Inoculation level (CFU/sample)		Ct IC	Ct HEX	PCR result				
2008	**2682**	**Ground beef meat**	*E. coli O157:H7* Ad485	0,8	-	33,45	31,67	+	+	**PD**	1	a
2008	**2683**	**Ground beef meat**	*E. coli O157:H7* Ad489	7,8	-	33,73	30,43	+	+	**PD**	1	a
2020	**1870**	**Chilled raw veal meat**	*E. coli* O157:H7 Ad685	2,8	-	32,43	27,39	+	+	**PD**	1	a
2020	**2963**	**Chilled raw pork meat**	*E. coli* O157:H7 Ad925	2,6	-	33,52	24,70	+	+	**PD**	1	a
2020	**2964**	**Chilled raw pork meat**	*E. coli* O157:H7 Ad1174	1,8	-	33,06	35,82	+	+	**PD**	1	a
2020	**2973**	**Chilled raw pork meat**	*E. coli* O157:H7 Ad568	3	-	33,39	33,66	+	+	**PD**	1	a
2020	**2974**	**Chilled raw lamb meat**	*E. coli* O157:H7 Ad924	1,4	-	32,68	30,76	+	+	**PD**	1	a
2008	**2676**	**Seasoned raw beef meat**	*E. coli O157:H7* Ad1513	2,2	-	34,30	33,76	+	+	**PD**	1	c
2008	**2677**	**Seasoned raw beef meat**	*E. coli O157:H7* Ad1513	2,2	-	33,47	34,12	+	+	**PD**	1	c
2020	**1871**	**Seasoned raw lamb meat**	*E. coli* O157:H7 Ad1501	1,4	-	32,45	25,65	+	+	**PD**	1	c
2020	**1872**	**Seasoned raw lamb meat**	*E. coli* O157:H7 Ad1071	2,8	-	32,40	27,96	+	+	**PD**	1	c
2020	**2692**	**Seasoned raw pork meat**	*E. coli* O157:H7 Ad562	2,8	-	33,83	28,90	+	+	**PD**	1	c
2020	**2699**	**Seasoned raw beef meat**	*E. coli* O157:H7 Ad558	3	-	32,73	28,34	+	+	**PD**	1	c
2020	**1963**	**Chilled raw veal meat**	*E. coli* O157:H7 Ad570	2,2	-	32,90	29,75	+	+	**PD**	2	a
2020	**1967**	**Chilled raw beef meat**	*E. coli* O157:H7 Ad570	2,2	-	33,52	33,65	+	+	**PD**	2	a
2020	**1969**	**Chilled raw pork meat**	*E. coli* O157:H7 Ad552	3,6	-	33,92	35,52	+	+	**PD**	2	a
2020	**1971**	**Chilled raw pork meat**	*E. coli* O157:H7 Ad570	2,2	-	32,96	32,12	+	+	**PD**	2	a
2020	**2031**	**Frozen raw veal meat**	*E. coli* O157:H7 Ad1501	1,2	-	35,05	29,19	+	+	**PD**	2	b
2020	**2033**	**Frozen raw lamb meat**	*E. coli* O157:H7 Ad686	3	-	34,79	24,11	+	+	**PD**	2	b
2020	**2034**	**Frozen ground beef meat**	*E. coli* O157:H7 Ad567	1,2	-	33,17	29,24	+	+	**PD**	2	b
2020	**2035**	**Frozen raw beef meat**	*E. coli* O157:H7 Ad1501	1,2	-	33,75	34,25	+	+	**PD**	2	b
2020	**2037**	**Frozen raw pork meat**	*E. coli* O157:H7 Ad567	1,2	-	33,45	31,07	+	+	**PD**	2	b
2020	**2041**	**Frozen raw pork meat**	*E. coli* O157:H7 Ad1071	1,2	-	33,21	36,66	+	+	**PD**	2	b
2020	**2042**	**Frozen raw pork meat**	*E. coli* O157:H7 Ad1501	1,2	-	32,94	26,62	+	+	**PD**	2	b
2020	**2679**	**Seasoned raw beef meat**	*E. coli* O157:H7 Ad562	2,8	-	33,85	33,52	+	+	**PD**	2	c
2020	**2680**	**Seasoned raw pork meat**	*E. coli* O157:H7 Ad562	2,8	-	35,27	38,32	+	+	**PD**	2	c
2020	**2685**	**Marinated raw veal meat**	*E. coli* O157:H7 Ad558	3	-	33,53	38,20	+	+	**PD**	2	c
2020	**2686**	**Seasoned raw lamb meat**	*E. coli* O157:H7 Ad558	3	-	34,17	36,37	+	+	**PD**	2	c

2008: APF Classic 2020: APF Fast

♦ Analyses performed according to the COFRAC accreditation

The analyses of discordant results according to the EN ISO 16140-2:2016 is the following (See **Table 9**):

Table 9 - Analyses of discordant results

Category		Type		N+	ND	PPND	PD	(ND+PPND)-PD	AL
1	Raw meat 25g ①	8h Easy II APF Classic	Chilled	17	3	0	2		
			Frozen	4	1	0	0		
			Seasoned or marinated	8	0	0	2		
			Total	29	4	0	4		
		8h Easy II APF FAST	Chilled	15	5	0	5		
			Frozen	5	2	0	0		
			Seasoned or marinated	18	3	0	4		
			Total	38	10	0	9		
		Total		**67**	**14**	**0**	**13**	1	4
	Raw meat 375g ②	8h Easy II	Chilled	11	2	0	4		
			Frozen	9	0	0	7		
			Seasoned or marinated	11	3	0	4		
			Total	31	5	0	15	-10	3
Total				**98**	**19**	**0**	**28**	**-9**	**5**

For protocol ① and all samples, the AL is based on the number of positive samples as this number is equivalent to two categories (67 samples for protocol ① and 98 for all samples)

The observed values for ((ND+ PPND) – PD) meet the acceptability limit for each individual protocol and for both combined protocols (calculated values ≤ AL).

3.1.1.7 Enrichment broth storage at 5 ± 3 °C for 72 h

The enrichment broth for 375 g test portions were stored for 72 h at 5°C ± 3°C and tested again (PCR and confirmatory tests).

The following changes were observed (See **Table 10**).

Table 10 - Enrichment broth storage

Year of analysis	Sample N°	Product	iQ-Check *E. coli* O157:H7		Category	Type
			Agreement before storage	Agreement after storage		
2020	**2035**	**Frozen raw beef meat**	PD	NA	2	b
2020	**2039**	**Frozen ground veal meat**	NA	PD	2	b
2020	**2676**	**Seasoned raw beef meat**	NA	PD	2	c
2020	**2681**	**Seasoned raw pork meat**	ND	PA	2	c
2020	**2687**	**Seasoned raw beef meat**	ND	PA	2	c

The analyses of discordant become (See **Table 11**).

Table 11 - Analysis of discordant after storage 72 h at 5 ± 3°C

Category		Type		N+	ND	PPND	PD	(ND+PPND)-PD	AL
1	Raw meat 375g	8h Easy II	Chilled	11	2	0	4		
			Frozen	9	0	0	7		
			Seasoned or marinated	12	1	0	5		
			Total	32	3	0	16	-13	3

The observed values for ((ND+ PPND) – PD) meet the acceptability limit for the raw meat category (375 g test portion) (calculated values ≤ AL).

3.1.1.8 Confirmation

A summary of the differences observed between direct streaking onto CT SMAC and RAPID'*E.coli* plates and IMS before streaking onto agar plates is given in **Table 12**.

Table 12 - Differences observed between the different confirmations procedures

Test portion	Number of positive PCR results	Direct streaking		IMS		All confirmatory tests	PPNC
		CT-SMAC	REC	CT-SMAC	REC		
25 g	54	49 (90,7%)	39 (72,2%)	53 (98,1%)	50 (92,6%)	53	1(sample 1877)
375 g	26	18 (69.2%)	10 (38.5%)	22 (84.6%)	17 (65,4%)	26	0
Total	**80**	**67**	**49**	**75**	**67**	**79**	**1**

Best results were observed when an IMS step was carried out before streaking onto selective agar plates particularly when 375 g test portions were tested (84.6% vs 69,2% when CT-SMAC was used).

For one sample (2679), the latex test performed on the typical colonies was negative using the Remel™ RIM *E. coli* O157:H7 Latex test Remel kit. Two other kits were tested: the Slidex O157 from bioMérieux and the OXOID *Escherichia coli* O157 Latex test. The first one gave a doubtful result and the second one a positive O157 latex test. Note that the same strain was used to inoculate other samples and no problem was encountered with the Remel Latex test.

3.1.1.9 PCR inhibition

208 PCR tests have been performed when combining all the studies; no PCR inhibition has been observed.

3.1.2 Relative level of detection

The relative level of detection is the level of detection at P = 0.50 (LOD_{50}) of the alternative (proprietary) method divided by the level of detection at P = 0.50 (LOD_{50}) of the reference method.

The RLOD is defined as the ratio of the alternative and reference methods:

$$RLOD = \frac{LOD_{Alt.}}{LOD_{Ref.}}$$

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.

3.1.2.1 Experimental design

According to the AFNOR Technical Committee request (perform again the RLOD for 25 g test portion using veal meat), two matrix/strain pairs were tested for this extension and renewal study using the following protocol:

- A negative control: 5 samples,
- A low contamination level providing fractional recovery data, with 20 replicates,
- A high contamination level, with 5 replicates.

A total plate count was performed to estimate the total microbial load on the day of analysis.

2 (matrix/strain) pairs were analyzed by the reference method and by the alternative method (See **Table 13**):

Table 13 - Defined (matrix/strain) pairs for the RLOD determination

Protocol	Test portion	Matrix	Strain	Origin	Inoculation procedure	Test portion	Enrichment protocol	Extraction protocol	PCR
①	25 g	Veal meat	*E. coli* O157:H7 Ad485	Ground beef	Seeding 48 h 3°C ± 2°C	25 g	8 h 41°C	Easy II	APF Fast
②	375 g	Ground beef	*E. coli* O157:H7 Ad2222	Ground beef	Seeding 48 h 3°C ± 2°C	375 g	10 h 41.5°C	Easy II	APF Fast

3.1.2.2 Calculation and interpretation of the RLOD

The raw data are given in **Appendix 5**.

The RLOD calculations were performed using the Excel spreadsheet available at http://standards.iso.org/iso/16140 - RLOD (clause 5-1-4-2 Calculation and interpretation of RLOD) version 15.08.2015. The RLOD are given **Table 14**.

Table 14 – Presentation of RLOD before and after confirmation of the alternative method results

Category	Test portion	Matrix / Strain	RLOD	RLODL	RLODU	b=ln (RLOD)	sd(b)	z-Test statistic	p-value	AL
Raw meat	25 g	Veal meat / *E. coli* O157:H7 Ad485	0,868	0,392	1,926	-0,141	0,398	0,354	1,277	2.5
	375 g	Ground beef / *E. coli* O157:H7 Ad2222	0,410	0,157	1,069	-0,891	0,479	1,860	1,937	
Combined results			**0,618**	**0,339**	**1,127**	**-0,481**	**0,300**	**1,602**	**1,891**	

The $LOD_{50\,\%}$ calculations according to Wilrich & Wilrich POD-LOD calculation program - version 11, 2022.10.12 test are given in **Table 15**.

Table 15 - LOD_{50} results

Category	Test portion	Matrix / Strain	Level of detection at 50% (CFU / sample size) according to Wilrich & Wilrich[1]	
			Reference method	Alternative method
Raw meat	25 g	Veal meat / *E. coli* O157:H7 Ad485	0,6 [0,4;1,1]	0,5 [0,3;1, 0]
	375 g	Ground beef / *E. coli* O157:H7 Ad2222	1,4 [0,8;2,7]	0,7 [0,4;1,2]
Combined results			0,9 [0,6;1,4]	0,6 [0,4;0,9]

3.1.3 Conclusion

The RLOD values (using the confirmed alternative method results) meet the acceptability limit of 2.5 for unpaired studies, for the two matrix/strain pairs tested.

The LOD_{50} varies from 0.6 to 1.4 CFU/test portion for the reference method and from 0.5 to 0.7 CFU/ sample size for the alternative method.

3.1.4 Inclusivity / exclusivity

The inclusivity is the ability of the alternative method to detect the target analyte from a wide range of strains. The exclusivity is the lack of interference from a relevant range of non-target strains of the alternative method.

3.1.4.1 Test protocols

The study was performed in June 2008 for the initial validation study.

- **Inclusivity**

The strains were grown in BHI and diluted in order to inoculate 10 to 100 cells/225 ml BPW. The broths were incubated for 8 h at 41.5°C ± 1°C. The protocol of the alternative method was then applied.

[1] Wilrich, C., and P.-Th. Wilrich: Estimation of the POD function and the LOD of a qualitative microbiological measurement method. AOAC International **92** (2009) 1763 - 1772.

- **Exclusivity**

The strains were grown in BHI and diluted in order to inoculate 10^5 CFU/ml of BPW. The broths were incubated for 24 h at 41.5°C ± 1°C. The protocol of the alternative method was then applied.

3.1.4.2 Results

Raw data are given in **Appendix 6**.

- **Inclusivity**

The 50 target strains tested gave positive PCR tests.

- **Exclusivity**

No cross reaction was observed with the 36 non-target strains tested.

The iQ-Check *E. coli* O157:H7 method is specific and selective.

In agreement with the AFNOR Technical Committee, no additional test was required for the extension study (2020).

3.1.5 Practicability

The alternative method practicability was evaluated according to the AFNOR criteria relative to method comparison study.

<table>
<tr><td>Storage conditions, shelf-life and modalities of utilisation after first use</td><td colspan="3">The storage temperature is indicated on the kit and in the instructions on page 2/13; it is 2 - 8°C.
The expiry date is indicated on the kit and on each vial or tube of reagent.
The reagents are used up to exhaustion.</td></tr>
<tr><td rowspan="15">Time to result</td><td colspan="3">Negative samples</td></tr>
<tr><td>Steps</td><td>Reference method</td><td>Alternative method</td></tr>
<tr><td>Sampling</td><td>Day 0</td><td>Day 0</td></tr>
<tr><td>IMS 6 h</td><td>Day 0</td><td></td></tr>
<tr><td>IMS 24 h</td><td>Day 1</td><td></td></tr>
<tr><td>Extraction, PCR</td><td></td><td>Day 0</td></tr>
<tr><td>Negative result</td><td>Day 2</td><td>Day 0</td></tr>
<tr><td colspan="3">Presumptive positive or positive results</td></tr>
<tr><td>Steps</td><td>Reference method</td><td>Alternative method</td></tr>
<tr><td>Sampling</td><td>Day 0</td><td>Day 0</td></tr>
<tr><td>IMS 6 h and streaking onto selective agar plates</td><td>Day 0</td><td></td></tr>
<tr><td>IMS 24 h and streaking onto selective agar plates</td><td>Day 1</td><td></td></tr>
<tr><td>PCR</td><td></td><td>Day 0</td></tr>
<tr><td>Steaking onto selective agar plates</td><td></td><td>Day 0</td></tr>
<tr><td>Streaking onto non-selective agar</td><td>Day 1 - Day 2</td><td>Day 1</td></tr>
<tr><td>Indol test</td><td>Day 2 - Day 3</td><td></td></tr>
<tr><td>Latex test</td><td>Day 3 - Day 4</td><td>Day 1 (O157) - Day 2 (H7)</td></tr>
<tr><td>Common step with the reference method</td><td colspan="3">No common step</td></tr>
</table>

Negative results are obtained the day of initiating the analysis with the alternative method and 1 day is required to obtain positive confirmed results while 2 days is necessary for the reference method to screen the samples and 3 to 4 days to confirm the positive results.

3.2 Inter-Laboratory Study

The inter-laboratory study is a study performed by multiple laboratories testing identical samples at the same time, the results of which are used to estimate alternative-method performance parameters.

3.2.1 Study organisation

The inter-laboratory study was carried out in June 2008. Ground beef was contaminated with *Escherichia coli* O157:H7 ATCC 43888. 14 laboratories participated to the study. Protocol ① was used for the study with an incubation time of 8 h at 41,5°C.

3.2.2 Experimental parameters controls

3.2.2.1 Strain stability and background microflora stability

Strain stability was checked by inoculating the matrix at 5 CFU/25 g and 50 CFU/25 g. Enumerations were performed for the high contamination level and detection analyses were performed for the low contamination level after 24 h and 48 h storage at 5 ± 3°C. *Triplicates* were analyzed; the results are given in **Table 16**.

Table 16 - Sample stability

Day	CFU/25 g (CT-SMAC)			Detection / 25 g		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Day 0	46	34	57	+	+	+
Day 1	34	40	46	+	+	+

No evolution was observed during storage at 5°C ± 3°C.

3.2.2.2 Contamination levels

The contamination levels and the sample codification were the following (see **Table 17**).

Table 17 - Contamination levels

Level	Samples	Theoretical target level (b/25 g)	True level (b/25 g sample)	Low limit / 25 g sample	High limit / 25 g sample
1	3, 4, 8, 11, 14, 16, 21, 22	/	/	/	/
2	2, 7, 10, 12, 17, 18, 20, 24	5	10	8,7	11,6
3	1, 5, 6, 9, 13, 15, 19, 23	25	43	37,3	49

3.2.2.3 Logistic conditions

Temperature conditions are given in **Table 18**.

Table 18 - Sample temperatures at receipt

Collaborators	Temperature measured by the probe (°C)	Temperature measured at receipt (°C)	Receipt date
A	2,5	2,6	Day 1
B	3,0	4,0	Day 1
C	3,5	3,1	Day 1
D	2,0	2,9	Day 1
E	2,5	2,8	Day 1
F	2,5	*Not communicated*	*Analysis not realized*
G	3,0	4,5	Day 1
H	2,5	10,3	Day 1
I	3,0	4,5	Day 1
J	2,0	4,7	Day 1
K	4,0	4,3	Day 1
L	3,0	4,5	Day 1
M	4,0	6,4	Day 1
N	3,5	3,8	Day 1

Laboratory H recorded a temperature at receipt of 10.3°C, the temperature recorded by the thermo probe was 2.5°C.

Laboratory F did not realize the analyses.

No problem was encountered during the transport or at receipt for the 14 collaborators. All the samples were delivered on time and in appropriate conditions. Temperatures during shipment and at receipt were all correct.

3.2.3 Results analysis

Raw data are provided in **Appendix 7**.

3.2.3.1 Expert laboratory results

The results obtained by the expert laboratory are given in **Table 19**.

Table 19 – Results obtained by the expert Lab.

Level	Reference method	Alternative method
L0	0/8	0/8
L1	6/8	8/8
L2	8/8	8/8

3.2.3.2 Results observed by the collaborative laboratories

- ***Aerobic mesophilic flora enumeration***

Depending on the Lab results, the enumeration levels varied from 700 to 9 500 CFU/g.

- ***Escherichia coli O157:H7 detection***

13 collaborators participated to the study. The results obtained are provided in **Table 20** (reference method) and **Table 21** (alternative method).

Table 20 - Positive results by the reference method (ALL the collaborators)

Collaborators	Contamination level		
	L0	*L1*	*L2*
A	0	8	8
B	1	8	8
C	0	8	8
D	0	8	8
E	0	8	8
G	0	8	8
H	0	8	8
I	0	7	8
J	0	6	8
K	3	8	8
L	4	8	8
M	0	8	8
N	0	8	8
Total	**P_0 = 8**	**P_1 =101**	**P_2 =104**

Table 21 - Positive results (before and after confirmation) by the alternative method (ALL the collaborators)

Collaborators	Contamination level								
	L0			*L1*			*L2*		
	PCR result	*Confirmation result*	*Final result*	*PCR result*	*Confirmation result*	*Final result*	*PCR result*	*Confirmation result*	*Final result*
A	0	0	0	8	8	**8**	8	8	**8**
B	6	1	**1**	8	8	**8**	8	8	**8**
C	0	0	0	8	8	**8**	8	8	**8**
D	0	0	0	8	8	**8**	8	8	**8**
E	0	0	0	8	8	**8**	8	8	**8**
G	0	0	0	8	8	**8**	8	8	**8**
H	0	0	0	8	8	**8**	8	8	**8**
I	0	0	0	8	8	**8**	8	8	**8**
J	0	0	0	8	8	**8**	8	8	**8**
K	2	2	**2**	8	8	**8**	8	8	**8**
L	0	0	0	8	8	**8**	8	8	**8**
M	0	0	0	8	8	**8**	8	8	**8**
N	0	0	0	8	8	**8**	8	8	**8**
Total	**P_0 = 8**	**C_0 = 3**	**CP_0 = 3**	**P_1 = 104**	**C_1 = 104**	**CP_1 = 104**	**P_2 = 104**	**C_2 = 104**	**CP_2 = 104**

For the reference method, 2 collaborators obtained positive results for unspiked samples: Lab K for 3 samples and Lab L for 4 samples.

For the alternative method, unspiked samples were found positive for 2 labs: Lab B for 6 samples (PCR results) and lab K for 2 samples.

Cross contamination probably occurred for these 3 collaborators (B, K and L).

.

Some negative H7 latex tests were observed by the collaborators while the O157 latex tests were positive (Laboratories B and G). It was asked to these labs to send their plates to ADRIA to test the H7 latex. Unfortunately, the plates were discarded, and the tests could not be done. It has been considered that the typical colonies observed on the plates correspond to the *E. coli* O157:H7 strain inoculated.

According to the AFNOR Technical rules it is possible to include the results from a collaborator with maximum one cross contamination at Level 0. This rule was applied and data from Laboratories B K and L were not kept for interpretation.

3.2.3.3 Results of the collaborators retained for interpretation

The results obtained with the 10 labs kept for interpretation are presented in **Table 22** (reference method) and **Table 23** (alternative method).

Table 22 - Positive results by the reference method (Without Labs B, K and L)

Collaborators	Contamination level		
	L0	*L1*	*L2*
A	0	8	8
C	0	8	8
D	0	8	8
E	0	8	8
G	0	8	8
H	0	8	8
I	0	7	8
J	0	6	8
M	0	8	8
N	0	8	8
Total	**P_0 =0**	**P_1=77**	**P_2 =80**

Table 23 - Positive results (before and after confirmation) by the alternative method (Without Labs B, K and L)

Collabo-rators	Contamination level								
	L0			*L1*			*L2*		
	PCR result	*Confirmation result*	*Final result*	*PCR result*	*Confirmation result*	*Final result*	*PCR result*	*Confirmation result*	*Final result*
A	0	0	0	8	8	**8**	8	8	**8**
C	0	0	0	8	8	**8**	8	8	**8**
D	0	0	0	8	8	**8**	8	8	**8**
E	0	0	0	8	8	**8**	8	8	**8**
G	0	0	0	8	8	**8**	8	8	**8**
H	0	0	0	8	8	**8**	8	8	**8**
I	0	0	0	8	8	**8**	8	8	**8**
J	0	0	0	8	8	**8**	8	8	**8**
M	0	0	0	8	8	**8**	8	8	**8**
N	0	0	0	8	8	**8**	8	8	**8**
Total	**P_0= 0**	**C_0 = 0**	**CP_0 = 0**	**P_1 = 80**	**C_1 = 80**	**CP_1 = 80**	**P_2 = 8**	**C_2 = 80**	**CP_2 = 80**

3.2.4 Calculation and interpretation

3.2.4.1 Calculation of the specificity percentage (SP)

The percentage specificities (SP) of the reference method and of the alternative method, using the data after confirmation, based on the results of level L0 are the following (See **Table 24**).

Table 24 - Percentage specificity

Specificity for the reference method	$SP_{ref} = \left(1 - \left(\frac{P_0}{N\_}\right)\right) x\ 100\ \% =$	100.0 %
Specificity for the alternative method	$SP_{alt} = \left(1 - \left(\frac{CP_0}{N\_}\right)\right) x\ 100\ \% =$	100.0 %

N: number of all L0 tests
P_0 = total number of false-positive results obtained with the blank samples before confirmation
CP_0 = total number of false-positive results obtained with the blank samples

3.2.4.2 Calculation of the sensitivity (SE_{alt}), the sensitivity for the reference method (SE_{ref}), the relative trueness (RT) and the false positive ratio for the alternative method (FPR)

Fractional positive results were obtained for the low inoculation level (L1). This inoculation level was retained for calculation.

A summary of the results of the collaborators retained for interpretation, and obtained with the reference and the alternative methods for Level 1 is provided in **Table 25**.

Table 25 - Summary of the obtained results with the reference method and the alternative method for Level 1

Level	Response	Reference method positive (R+)	Reference method negative (R-)
1	**Alternative method positive (A+)**	Positive agreement (A+/R+) **PA = 77**	Positive deviation (R-/A+) **PD = 3**
	Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 0 (PPND=0)**	Negative agreement (A-/R-) **NA = 0 (PPNA=0)**

Based on the data summarized in **Table 25**, the values of sensitivity of the alternative and reference methods, as well as the relative trueness and false positive ratio for the alternative method taking account the confirmations, are the following (See **Table 26**).

Table 26 - Sensitivity, relative trueness and false positive ratio percentages

		Level 1
Sensitivity for the alternative method:	$SE_{alt} = \frac{(PA+PD)}{(PA+PD+ND)} \; x \; 100\% =$	100 %
Sensitivity for the reference method:	$SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)} \; x \; 100\% =$	96.3 %
Relative trueness	$RT = \frac{(PA+NA)}{N} \; x \; 100\% =$	96.3 %
False positive ratio for the alternative method	$FPR = \frac{FP}{NA} \; x \; 100\% =$	/

3.2.4.3 Interpretation of data

Three positive deviations were observed; they concern samples I2, J2 and J10.

For an **unpaired study design**, the difference between (ND – PD) is calculated for the level(s) where fractional recovery is obtained (so L_1 and possibly L_2). The observed value found for (ND – PD) shall not be higher than the AL. The AL is defined as $[(ND - PD)_{max}]$ and calculated per level where fractional recovery is obtained as described below using the following three parameters:

$$(p+)_{\text{ref}} = \frac{P_x}{N_x}$$

where

P_x = number of samples with a positive result obtained with the reference method at level x (L_1 or L_2) for all the collaborators

N_x = number of samples tested at level x (L_1 or L_2) with the reference method by all the collaborators

$$(p+)_{\text{alt}} = \frac{CP_x}{N_x}$$

where

CP_x = number of samples with a confirmed positive result obtained with the alternative method at level x (L_1 or L_2) for all the collaborators;

N_x = number of samples tested at level x (L_1 or L_2) with the alternative method by all the collaborators.

$$(\text{ND-PD})_{\max} = \sqrt{3N_x \times \left((p+)_{\text{ref}} + (p+)_{\text{alt}} - 2\left((p+)_{\text{ref}} \times (p+)_{\text{alt}}\right)\right)}$$

where

N_x = number of samples tested for level x (L_1 or L_2) with the reference method by all the collaborators.

The AL is not met when the observed value is higher than the AL. When the AL is not met, investigations should be made (e.g. root cause analysis) in order to provide an explanation of the observed results. Based on the AL and the additional information,

it is decided whether the alternative method is regarded as not fit for purpose. The reasons for acceptance of the alternative method when the AL is not met shall be stated in the study report.

In this study, fractional recovery was observed at Level 1 and Level 2. The calculations are the following, according to the EN ISO 16140-2:2016 (See **Table 27**).

Table 27 - Calculations

	Level 1
N_X	80
$(p+)_{ref}$	0.7
$(p+)_{alt}$	0.7
AL = (ND - PD) max	9.85
ND - PD	-3
Conclusion	ND-PD<AL

The ISO 16140-2 (2016) requirements are fulfilled as (ND - PD) met the AL.

The alternative and the reference methods are considered equivalent.

3.2.5 *Evaluation of the LOD 50%, LOD 95 % and RLOD between laboratories*

The RLOD was calculated using the EN ISO 16140-2:2016 Excel spreadsheet available at https://standards.iso.org/iso/16140/-5/ed-1/en/RLOD_inter-lab-study_16140-2_AnnexF_ver1_28-06-2017.xls. The results are used only for information (see **Table 38**).

Table 28 - LOD 50 %, LOD 95 % and RLOD

Method	LOD 50 %	LOD 95 %	RLOD
Reference	0.53	2.28	Comparison between both cannot be drawn as the result for the alternative method is not available
Alternative	Result not available as every inoculated sample gave a positive result		

3.3 General conclusion

The **method comparison study conclusions** are:

☒ The method comparison study scheme corresponds to an UNPAIRED STUDY design as the alternative and reference methods have different enrichment procedures.

☒ In the sensitivity study, 1 category was tested with two test portions (25 g and 375 g). The protocol of the alternative method shows 28 positive deviations (PD) and 19 negative deviations (ND) when combining both test portions. The ND - PD meet the acceptability limits (AL) whatever the test portion, and as well for the 2 tested portions. The number of PD is higher that the number of ND.

☒ Two APF were tested for the 25 g test portion (protocol ①, APF Classic for the initial validation study (2008) and APF Fast for the extension study (2020), similar results were obtained. Only the APF Fast was tested for protocol ② (375 g test portion) and satisfying results were obtained.

☒ The Relative Levels of Detection (RLOD) are all below the AL fixed at 2.5 for the unpaired data study for the two matrix/strain pairs tested.

☒ The inclusivity and exclusivity testing gave the expected results for the 50 target strains and the 36 non target strains.

☒ It is possible to store the enrichment broth for 72 h at 5 ± 3°C (375 g test portion)

☒ The alternative method allows a one-day screening of the negative samples.

☒ The alternative method fulfils all the EN ISO 16140-2:2016 and AFNOR technical rules (revision 6).

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

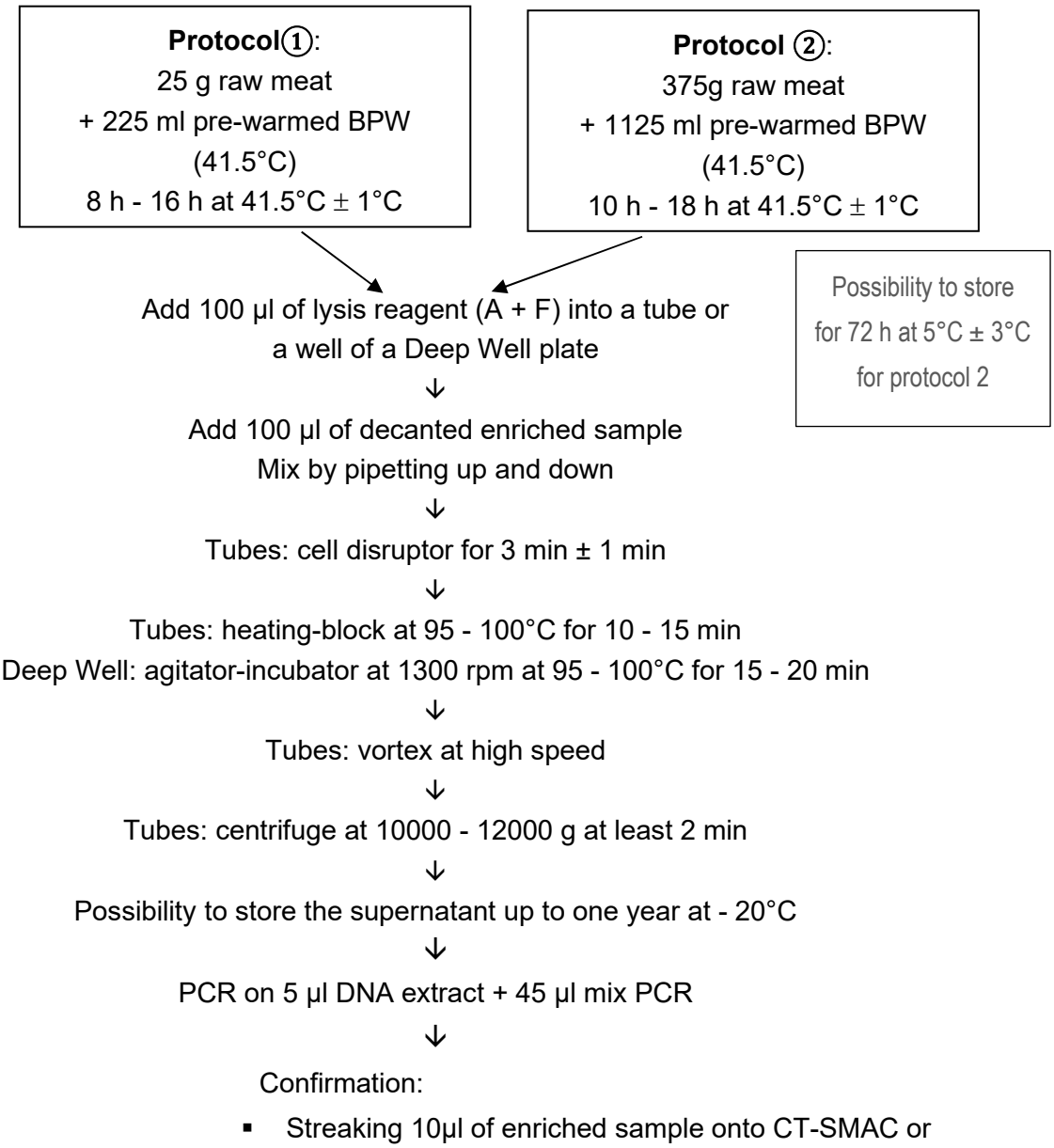
☒ The data and interpretations comply with the EN ISO 16140-2:2016 requirements. **The iQ-Check *E. coli* O157:H7 method is considered equivalent to the ISO standard.**

Quimper, 22 February 2023

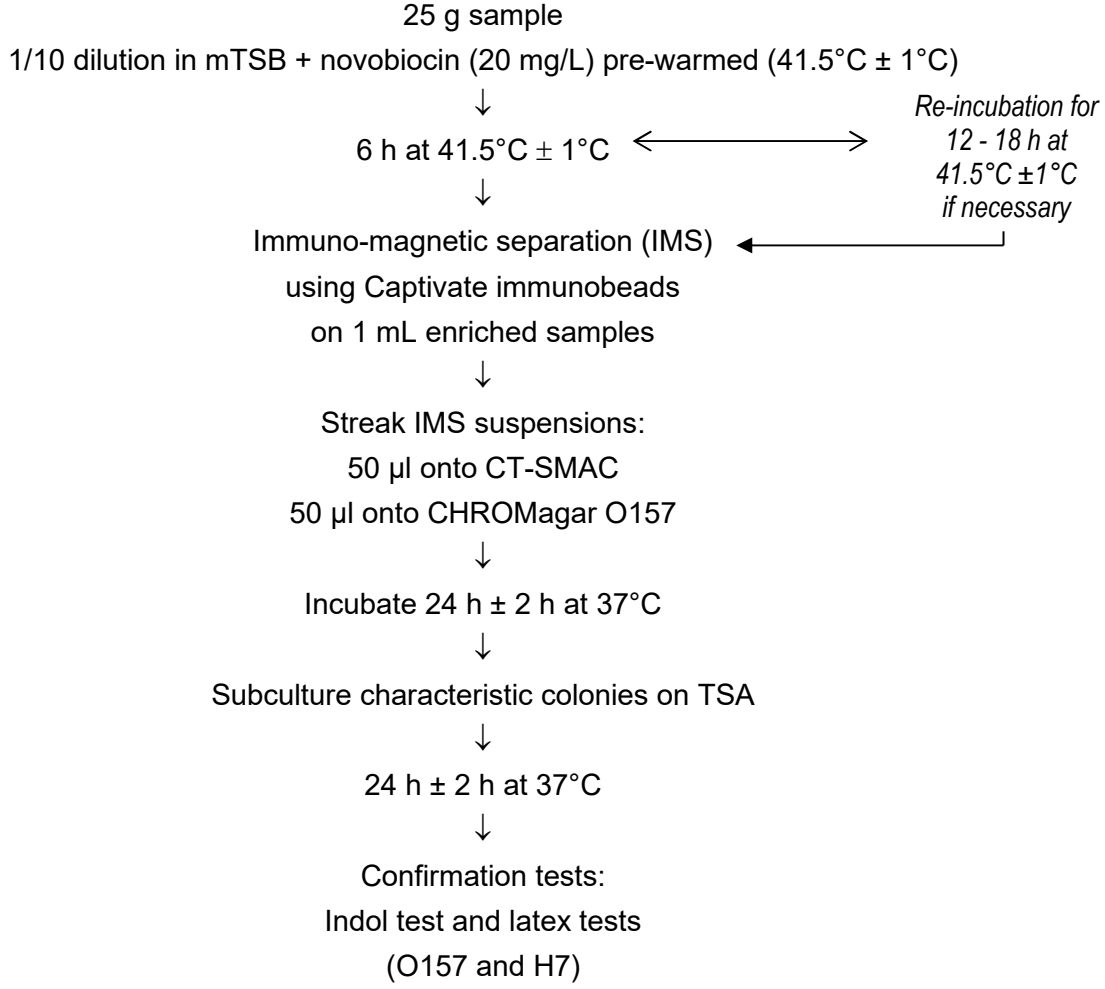
Maryse RANNOU
Project Manager
Validation of Alternative methods
Food Safety & Quality

I hereby attest to the validation of the verification of the conformity of the report (opinion and interpretation).

Appendix 1 – Flow diagram of the alternative method: iQ-Check *E. coli* O157:H7



Appendix 2 - Flow diagram of the reference method: ISO 16654 (2011): Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Escherichia coli* O157 - Amendment 1 (March 2017): annex B: result of inter-laboratory studies



Appendix 3 – Artificial contamination of samples

Date of analysis	Sample N°	Product (french name)	Product	Artificial contaminations					Global result	Protocol	Type
				Strain	Origin	Injury applied	Injury evaluation	Inoculation level (CFU/sample)			
2008	**2679**	**Haché tradition pur bœuf**	**Ground beef meat**	*E. coli O157:H7 435*	Ground beef	48 h -18°c-72 h 4°c	2,7	4-4-7-4-4 (4,6)	+	1	a
2008	**2680**	**Haché tradition pur bœuf**	**Ground beef meat**	*E. coli O157:H7 Ad489*	Ground beef	48 h -18°C-72 h 4°C	1,4	2-0-3-1-4 (2)	+	1	a
2008	**2681**	**Haché tradition pur bœuf**	**Ground beef meat**	*E. coli O157:H7 Ad489*	Ground beef	48 h 4°C-24h -18°C-72 h 4°C	0,5	5-9-7-8-10 (7,8)	+	1	a
2008	**2682**	**Viande hachée**	**Ground beef meat**	*E. coli O157:H7* Ad485	Ground beef	48 h -18°C-72 h 4°C	1,2	2-0-0-1-1-0 (0,8)	+	1	a
2008	**2683**	**Viande hachée**	**Ground beef meat**	*E. coli O157:H7* Ad489	Ground beef	48 h 4°C-24h -18°C-72 h 4°C	0,5	5-9-7-8-10 (7,8)	+	1	a
2008	**2684**	**Viande hachée**	**Ground beef meat**	*E. coli O157:H7 435*	Ground beef	48 h -18°C-72 h 4°C	2,7	4-4-7-4-4 (4,6)	+	1	a
2008	**2687**	**Viande hachée 5%MG**	**Ground beef meat**	*E. coli O157:H7 Ad485*	Ground beef	48 h -18°C-72 h 4°C	1,2	2-0-0-1-1-0 (0,8)	+	1	a
2008	**2688**	**Steak haché 15%MG**	**Ground beef meat**	*E. coli O157:H7 Ad489*	Ground beef	48 h -18°C-72 h 4°C	1,4	2-0-3-1-4 (2)	+	1	a
2008	**2689**	**Steak haché 15%MG**	**Ground beef meat**	*E. coli O157:H7 435*	Ground beef	48 h -18°C-72 h 4°C	2,7	4-4-7-4-4 (4,6)	+	1	a
2008	**2693**	**Steak haché 15%MG**	**Ground beef meat**	*E. coli O157:H7 Ad485*	Ground beef	48 h 4°C-24h -18°C-72 h 4°C	0,4	8-6-3-9-7 (6,6)	+	1	a
2008	**2705**	**Viande hachée**	**Ground beef meat**	*E. coli O157:H7 A215-16*	Beef faeces	48 h -18°C-72 h 4°C	1,6	1-1-0-1-3 (1,2)	+	1	a
2008	**2706**	**Viande hachée**	**Ground beef meat**	*E. coli O157:H7 A215-16*	Beef faeces	48 h -18°C-72 h 4°C	1,6	1-1-0-1-3 (1,2)	+	1	a
2008	**2707**	**Viande hachée**	**Ground beef meat**	*E. coli O157:H7 B68*	Environment	48 h -18°C-72 h 4°C	1,1	2-12-9-8-4 (7)	+	1	a
2008	**2708**	**Viande hachée tradition 20%MG**	**Ground beef meat**	*E. coli O157:H7 A1814ID*	Ground beef	48 h -18°C-72 h 4°C	2,1	2-1-1-1-0 (1)	+	1	a
2008	**2709**	**Viande hachée tradition 20%MG**	**Ground beef meat**	*E. coli O157:H7 B68*	Environment	48 h -18°C-72 h 4°C	1,1	2-12-9-8-4 (7)	+	1	a
2008	**2710**	**Viande hachée tradition 20%MG**	**Ground beef meat**	*E. coli O157:H7 A215-16*	Beef faeces	48 h -18°C-72 h 4°C	1,6	1-1-0-1-3 (1,2)	+	1	a
2020	**1868**	**Filet mignon de porc**	**Chilled raw pork meat**	*E. coli* O157:H7 Ad1501	Meat product	Seeding 48 h à 3 ± 2°C	/	2-0-2-1-2 (1,4)	+	1	a
2020	**1869**	**Côte de veau**	**Chilled raw veal meat**	*E. coli* O157:H7 Ad1071	Meat product	Seeding 48 h à 3 ± 2°C	/	3-2-3-3-3 (2,8)	+	1	a
2020	**1870**	**Escalope de veau**	**Chilled raw veal meat**	*E. coli* O157:H7 Ad685	Environment	Seeding 48 h à 3 ± 2°C	/	3-2-4-2-3 (2,8)	+	1	a
2020	**1877**	**Viande de porc**	**Chilled raw pork meat**	*E. coli* O157:H7 Ad685	Environment	Seeding 48 h à 3 ± 2°C	/	3-2-4-2-3 (2,8)	-	1	a
2020	**2318**	**Collier d'agneau à mijoter**	**Chilled raw lamb meat**	*E. coli* O157:H7 Ad555	Environment	Seeding 48 h à 3 ± 2°C	/	3-2-2-2-5 (2,8)	+	1	a
2020	**2319**	**Gigot d'agneau à griller**	**Chilled raw lamb meat**	*E. coli* O157:H7 Ad557	Environment	Seeding 48 h à 3 ± 2°C	/	2-3-2-3-2 (2,4)	-	1	a
2020	**2963**	**Côte de porc**	**Chilled raw pork meat**	*E. coli* O157:H7 Ad925	Meat product	Seeding 48 h à 3 ± 2°C	/	1-0-4-5-3 (2,6)	+	1	a
2020	**2964**	**Côte échine de porc**	**Chilled raw pork meat**	*E. coli* O157:H7 Ad1174	Meat product	Seeding 48 h à 3 ± 2°C	/	1-2-0-5-1 (1,8)	+	1	a
2020	**2965**	**Côtes d'agneau**	**Chilled raw lamb meat**	*E. coli* O157:H7 Ad568	Environment	Seeding 48 h à 3 ± 2°C	/	2-4-1-6-2 (3,0)	+	1	a
2020	**2966**	**Tranche osso bucco veau**	**Chilled raw veal meat**	*E. coli* O157:H7 Ad924	Meat product	Seeding 48 h à 3 ± 2°C	/	0-1-2-3-1 (1,4)	+	1	a
2020	**2968**	**Gigot d'agneau**	**Chilled raw lamb meat**	*E. coli* O157:H7 Ad1174	Meat product	Seeding 48 h à 3 ± 2°C	/	1-2-0-5-1 (1,8)	+	1	a
2020	**2969**	**Pavé de veau**	**Chilled raw veal meat**	*E. coli* O157:H7 Ad568	Environment	Seeding 48 h à 3 ± 2°C	/	2-4-1-6-2 (3,0)	+	1	a
2020	**2970**	**Côte de veau**	**Chilled raw veal meat**	*E. coli* O157:H7 Ad1174	Meat product	Seeding 48 h à 3 ± 2°C	/	1-2-0-5-1 (1,8)	+	1	a
2020	**2971**	**Filet mignon porc**	**Chilled raw pork meat**	*E. coli* O157:H7 Ad924	Meat product	Seeding 48 h à 3 ± 2°C	/	0-1-2-3-1 (1,4)	+	1	a
2020	**2972**	**Selle d'agneau**	**Chilled raw lamb meat**	*E. coli* O157:H7 Ad925	Meat product	Seeding 48 h à 3 ± 2°C	/	1-0-4-5-3 (2,6)	+	1	a
2020	**2973**	**Rôti de porc**	**Chilled raw pork meat**	*E. coli* O157:H7 Ad568	Environment	Seeding 48 h à 3 ± 2°C	/	2-4-1-6-2 (3,0)	+	1	a
2020	**2974**	**Poitrine d'agneau**	**Chilled raw lamb meat**	*E. coli* O157:H7 Ad924	Meat product	Seeding 48 h à 3 ± 2°C	/	0-1-2-3-1 (1,4)	+	1	a
2008	**2694**	**Petit tendre haché surgelé**	**Frozen ground beef meat**	*E. coli O157:H7 Ad485*	Ground beef	48 h -18°C-72 h 4°C	1,2	2-0-0-1-1-0 (0,8)	+	1	b
2008	**2695**	**Petit tendre haché surgelé**	**Frozen ground beef meat**	*E. coli O157:H7 Ad489*	Ground beef	48 h -18°C-72 h 4°C	1,4	2-0-3-1-4 (2)	+	1	b
2008	**2696**	**Petit tendre haché surgelé**	**Frozen ground beef meat**	*E. coli O157:H7 Ad485*	Ground beef	48 h 4°C-24h -18°C-72 h 4°C	0,4	8-6-3-9-7 (6,6)	+	1	b
2008	**2697**	**Petit tendre haché surgelé**	**Frozen ground beef meat**	*E. coli O157:H7 435*	Ground beef	48 h 4°C-24h -18°C-72 h 4°C	1,1	5-4-5-1-5 (4)	+	1	b
2020	**1668**	**Souris d'agneau congelée**	**Frozen raw lamb meat**	*E. coli* O157:H7 Ad684	Meat product	Seeding 2 weeks at -20°C	/	5-3-4-2-6 (4,0)	+	1	b
2020	**1818**	**Filet mignon porc congelé**	**Frozen raw pork meat**	*E. coli* O157:H7 Ad684	Meat product	Seeding 2 weeks at -20°C	/	7-2-3-4-7 (4,6)	+	1	b
2020	**1819**	**Souris d'agneau congelée**	**Frozen raw lamb meat**	*E. coli* O157:H7 Ad684	Meat product	Seeding 2 weeks at -20°C	/	7-2-3-4-7 (4,6)	+	1	b
2020	**1820**	**Escalope de veau hâché congelée**	**Frozen ground veal meat**	*E. coli* O157:H7 Ad589	Meat product	Seeding 2 weeks at -20°C	/	2-0-2-0-0 (0,8)	-	1	b
2020	**1821**	**Filet mignon de porc congelé**	**Frozen raw pork meat**	*E. coli* O157:H7 Ad589	Meat product	Seeding 2 weeks at -20°C	/	2-0-2-0-0 (0,8)	+	1	b
2020	**1822**	**Côte d'agneau congelée**	**Frozen raw lamb meat**	*E. coli* O157:H7 Ad589	Meat product	Seeding 2 weeks at -20°C	/	2-0-2-0-0 (0,8)	+	1	b
2008	**2676**	**Boulettes au bœuf 15%MG**	**Seasoned raw beef meat**	*E. coli O157:H7* Ad1513	Ground beef	48 h -18°C-72 h 4°C	2,6	2-2-2-4-1 (2,2)	+	1	c
2008	**2677**	**Boulettes au bœuf 15%MG**	**Seasoned raw beef meat**	*E. coli O157:H7* Ad1513	Ground beef	48 h -18°C-72 h 4°C	2,6	2-2-2-4-1 (2,2)	+	1	c
2008	**2678**	**Boulettes au bœuf 15%MG**	**Seasoned raw beef meat**	*E. coli O157:H7 A1513*	Ground beef	48 h -18°C-72 h 4°C	2,6	2-2-2-4-1 (2,2)	+	1	c
2008	**2700**	**Boulettes au bœuf**	**Seasoned raw beef meat**	*E. coli O157:H7 435*	Ground beef	48 h 4°C-24h -18°C-72 h 4°C	1,1	5-4-5-1-5 (4)	+	1	c
2008	**2701**	**Boulettes au bœuf**	**Seasoned raw beef meat**	*E. coli O157:H7 A1814ID*	Ground beef	48 h -18°C-72 h 4°C	2,1	2-1-1-1-0 (1)	+	1	c
2008	**2702**	**Boulettes au bœuf**	**Seasoned raw beef meat**	*E. coli O157:H7 B68*	Environment	48 h -18°C-72 h 4°C	1,1	2-12-9-8-4 (7)	+	1	c

Date of analysis	Sample N°	Product (french name)	Product	Artificial contaminations					Global result	Protocol	Type
				Strain	Origin	Injury applied	Injury evaluation	Inoculation level (CFU/sample)			
2008	2703	Boulettes au bœuf	Seasoned raw beef meat	*E. coli O157:H7 A1814ID*	Ground beef	48 h -18°C-72 h 4°C	2,1	2-1-1-1-0 (1)	+	1	c
2008	2704	Boulettes au bœuf	Seasoned raw beef meat	*E. coli O157:H7 435*	Ground beef	48 h 4°C-24h -18°C-72 h 4°C	1,1	5-4-5-1-5 (4)	+	1	c
2020	1594	Lomo de porc mariné à l'andalouse	Marinated raw pork meat	*E. coli* O157:H7 Ad1248	Meat product	Seeding 48 h à 3 ± 2°C	/	2-2-1-2-0 (1,4)	+	1	c
2020	1595	Lomo de porc mariné au piment doux	Marinated raw pork meat	*E. coli* O157:H7 Ad1248	Meat product	Seeding 48 h à 3 ± 2°C	/	2-2-1-2-0 (1,4)	-	1	c
2020	1596	Brochette de porc à la provençale	Seasoned raw pork meat	*E. coli* O157:H7 Ad688	Environment	Seeding 48 h à 3 ± 2°C	/	5-1-1-5-3 (3,0)	-	1	c
2020	1871	Noisette d'agneau aux fines herbes	Seasoned raw lamb meat	*E. coli* O157:H7 Ad1501	Meat product	Seeding 48 h à 3 ± 2°C	/	2-0-2-1-2 (1,4)	+	1	c
2020	1872	Côte d'agneau aux fines herbes	Seasoned raw lamb meat	*E. coli* O157:H7 Ad1071	Meat product	Seeding 48 h à 3 ± 2°C	/	3-2-3-3-3 (2,8)	+	1	c
2020	1873	Brochette d'agneau à l'ail	Seasoned raw lamb meat	*E. coli* O157:H7 Ad1071	Meat product	Seeding 48 h à 3 ± 2°C	/	3-2-3-3-3 (2,8)	+	1	c
2020	1875	Grillade porc à la mexicaine	Seasoned raw pork meat	*E. coli* O157:H7 Ad1071	Meat product	Seeding 48 h à 3 ± 2°C	/	3-2-3-3-3 (2,8)	+	1	c
2020	1878	Pavé de veau mariné	Marinated raw veal meat	*E. coli* O157:H7 Ad685	Environment	Seeding 48 h à 3 ± 2°C	/	3-2-4-2-3 (2,8)	+	1	c
2020	1879	Gigot d'agneau mariné	Marinated raw lamb meat	*E. coli* O157:H7 Ad685	Environment	Seeding 48 h à 3 ± 2°C	/	3-2-4-2-3 (2,8)	+	1	c
2020	2688	Lomo de porc ail et persil	Seasoned raw pork meat	*E. coli* O157:H7 Ad976	Beef	Seeding 48 h à 3 ± 2°C	/	2-6-2-5-3 (3,6)	+	1	c
2020	2689	Pavé de rumsteak à l'échalote	Seasoned raw beef meat	*E. coli* O157:H7 Ad976	Beef	Seeding 48 h à 3 ± 2°C	/	2-6-2-5-3 (3,6)	+	1	c
2020	2691	Pavés de bœuf aux trois poivres	Seasoned raw beef meat	*E. coli* O157:H7 Ad562	Ground beef	Seeding 48 h à 3 ± 2°C	/	1-4-6-1-2 (2,8)	+	1	c
2020	2692	Côte de porc à la mexicaine	Seasoned raw pork meat	*E. coli* O157:H7 Ad562	Ground beef	Seeding 48 h à 3 ± 2°C	/	1-4-6-1-2 (2,8)	+	1	c
2020	2693	Côte de porc à l'américaine	Seasoned raw pork meat	*E. coli* O157:H7 Ad562	Ground beef	Seeding 48 h à 3 ± 2°C	/	1-4-6-1-2 (2,8)	+	1	c
2020	2694	Pavé d'agneau mariné ail et persil	Marinated raw lamb meat	*E. coli* O157:H7 Ad553	Environment	Seeding 48 h à 3 ± 2°C	/	4-2-1-2-0 (1,8)	+	1	c
2020	2695	Hâché de veau à la provençale	Seasoned ground veal meat	*E. coli* O157:H7 Ad553	Environment	Seeding 48 h à 3 ± 2°C	/	4-2-1-2-0 (1,8)	+	1	c
2020	2696	Tranche d'agneau à l'armoricaine	Seasoned raw lamb meat	*E. coli* O157:H7 Ad553	Environment	Seeding 48 h à 3 ± 2°C	/	4-2-1-2-0 (1,8)	+	1	c
2020	2697	Brochettes de veau marinés au curry	Marinated raw veal meat	*E. coli* O157:H7 Ad558	Environment	Seeding 48 h à 3 ± 2°C	/	3-4-2-2-4 (3,0)	+	1	c
2020	2698	Brochettes d'agneau ail et herbes	Seasoned raw lamb meat	*E. coli* O157:H7 Ad558	Environment	Seeding 48 h à 3 ± 2°C	/	3-4-2-2-4 (3,0)	+	1	c
2020	2699	Bœuf à la mexicaine	Seasoned raw beef meat	*E. coli* O157:H7 Ad558	Environment	Seeding 48 h à 3 ± 2°C	/	3-4-2-2-4 (3,0)	+	1	c
2020	1963	Côte de veau	Chilled raw veal meat	*E. coli* O157:H7 Ad570	Environment	Seeding 48 h à 3 ± 2°C	/	1-1-3-3-3 (2,2)	+	2	a
2020	1964	Escalope de veau	Chilled raw veal meat	*E. coli* O157:H7 Ad560	Meat product	Seeding 48 h à 3 ± 2°C	/	1-1-2-1-1 (1,2)	+	2	a
2020	1965	Rôti de veau	Chilled raw veal meat	*E. coli* O157:H7 Ad552	Environment	Seeding 48 h à 3 ± 2°C	/	1-2-5-5-5 (3,6)	+	2	a
2020	1966	Bœuf bourguignon	Chilled raw beef meat	*E. coli* O157:H7 Ad933	Meat product	Seeding 48 h à 3 ± 2°C	/	1-3-2-2-5 (2,6)	-	2	a
2020	1967	Basse côte de bœuf	Chilled raw beef meat	*E. coli* O157:H7 Ad570	Environment	Seeding 48 h à 3 ± 2°C	/	1-1-3-3-3 (2,2)	+	2	a
2020	1968	Basse côte de bœuf	Chilled raw beef meat	*E. coli* O157:H7 Ad560	Meat product	Seeding 48 h à 3 ± 2°C	/	1-1-2-1-1 (1,2)	+	2	a
2020	1969	Rouelle de porc	Chilled raw pork meat	*E. coli* O157:H7 Ad552	Environment	Seeding 48 h à 3 ± 2°C	/	1-2-5-5-5 (3,6)	+	2	a
2020	1970	Filet de porc	Chilled raw pork meat	*E. coli* O157:H7 Ad933	Meat product	Seeding 48 h à 3 ± 2°C	/	1-3-2-2-5 (2,6)	+	2	a
2020	1971	Sauté de porc	Chilled raw pork meat	*E. coli* O157:H7 Ad570	Environment	Seeding 48 h à 3 ± 2°C	/	1-1-3-3-3 (2,2)	+	2	a
2020	1972	Collier d'agneau	Chilled raw lamb meat	*E. coli* O157:H7 Ad560	Meat product	Seeding 48 h à 3 ± 2°C	/	1-1-2-1-1 (1,2)	+	2	a
2020	1973	Epaule d'agneau	Chilled raw lamb meat	*E. coli* O157:H7 Ad552	Environment	Seeding 48 h à 3 ± 2°C	/	1-2-5-5-5 (3,6)	+	2	a
2020	1974	Poitrine d'agneau	Chilled raw lamb meat	*E. coli* O157:H7 Ad933	Meat product	Seeding 48 h à 3 ± 2°C	/	1-3-2-2-5 (2,6)	+	2	a
2020	2031	Sauté de veau pour blanquette congelé	Frozen raw veal meat	*E. coli* O157:H7 Ad1501	Meat product	Seeding 2 weeks at -20°C	/	0-4-0-0-2 (1,2)	+	2	b
2020	2032	Escalope de veau congelée	Frozen raw veal meat	*E. coli* O157:H7 Ad1071	Meat product	Seeding 2 weeks at -20°C	/	0-4-1-1-0 (1,2)	-	2	b
2020	2033	Gigot d'agneau congelé	Frozen raw lamb meat	*E. coli* O157:H7 Ad686	Environment	Seeding 2 weeks at -20°C	/	0-5-6-1-3 (3,0)	+	2	b
2020	2034	Steak haché bœuf congelé	Frozen ground beef meat	*E. coli* O157:H7 Ad567	Environment	Seeding 2 weeks at -20°C	/	0-1-3-1-1 (1,2)	+	2	b
2020	2035	Fondant de charolais congelé	Frozen raw beef meat	*E. coli* O157:H7 Ad1501	Meat product	Seeding 2 weeks at -20°C	/	0-4-0-0-2 (1,2)	+	2	b
2020	2036	Viande hâchée de bœuf congelé	Frozen ground beef meat	*E. coli* O157:H7 Ad1071	Meat product	Seeding 2 weeks at -20°C	/	0-4-1-1-0 (1,2)	+	2	b
2020	2037	Filet mignon de porc congelé	Frozen raw pork meat	*E. coli* O157:H7 Ad567	Environment	Seeding 2 weeks at -20°C	/	0-1-3-1-1 (1,2)	+	2	b
2020	2038	Sauté de veau congelé	Frozen raw veal meat	*E. coli* O157:H7 Ad686	Environment	Seeding 2 weeks at -20°C	/	0-5-6-1-3 (3,0)	-	2	b
2020	2039	Escalope hâchée de veau congelée	Frozen ground veal meat	*E. coli* O157:H7 Ad567	Environment	Seeding 2 weeks at -20°C	/	0-1-3-1-1 (1,2)	-	2	b
2020	2040	Filet mignon de porc congelé	Frozen raw pork meat	*E. coli* O157:H7 Ad686	Environment	Seeding 2 weeks at -20°C	/	0-5-6-1-3 (3,0)	+	2	b
2020	2041	Echine côte de porc congelée	Frozen raw pork meat	*E. coli* O157:H7 Ad1071	Meat product	Seeding 2 weeks at -20°C	/	0-4-1-1-0 (1,2)	+	2	b
2020	2042	Sauté de porc congelé	Frozen raw pork meat	*E. coli* O157:H7 Ad1501	Meat product	Seeding 2 weeks at -20°C	/	0-4-0-0-2 (1,2)	+	2	b
2020	2676	Lomo de porc ail et persil	Seasoned raw pork meat	*E. coli* O157:H7 Ad976	Beef	Seeding 48 h à 3 ± 2°C	/	2-6-2-5-3 (3,6)	-	2	c
2020	2677	Pavé de rumsteak à l'échalote	Seasoned raw beef meat	*E. coli* O157:H7 Ad976	Beef	Seeding 48 h à 3 ± 2°C	/	2-6-2-5-3 (3,6)	+	2	c
2020	2678	Carpaccio bœuf au parmesan	Seasoned raw beef meat	*E. coli* O157:H7 Ad976	Beef	Seeding 48 h à 3 ± 2°C	/	2-6-2-5-3 (3,6)	+	2	c
2020	2679	Pavés de bœuf aux trois poivres	Seasoned raw beef meat	*E. coli* O157:H7 Ad562	Ground beef	Seeding 48 h à 3 ± 2°C	/	1-4-6-1-2 (2,8)	+	2	c
2020	2680	Côte de porc à la mexicaine	Seasoned raw pork meat	*E. coli* O157:H7 Ad562	Ground beef	Seeding 48 h à 3 ± 2°C	/	1-4-6-1-2 (2,8)	+	2	c

Date of analysis	Sample N°	Product (french name)	Product	Artificial contaminations					Global result	Protocol	Type
				Strain	Origin	Injury applied	Injury evaluation	Inoculation level (CFU/sample)			
2020	**2681**	**Côte de porc à l'américaine**	**Seasoned raw pork meat**	*E. coli* O157:H7 Ad562	Ground beef	Seeding 48 h à 3 ± 2°C	/	1-4-6-1-2 (2,8)	**+**	2	c
2020	**2682**	**Pavé d'agneau mariné ail et persil**	**Marinated raw lamb meat**	*E. coli* O157:H7 Ad553	Environment	Seeding 48 h à 3 ± 2°C	/	4-2-1-2-0 (1,8)	**+**	2	c
2020	**2683**	**Hâché de veau à la provençale**	**Seasoned ground veal meat**	*E. coli* O157:H7 Ad553	Environment	Seeding 48 h à 3 ± 2°C	/	4-2-1-2-0 (1,8)	**+**	2	c
2020	**2684**	**Tranche d'agneau à l'armoricaine**	**Seasoned raw lamb meat**	*E. coli* O157:H7 Ad553	Environment	Seeding 48 h à 3 ± 2°C	/	4-2-1-2-0 (1,8)	**+**	2	c
2020	**2685**	**Brochettes de veau marinés au curry**	**Marinated raw veal meat**	*E. coli* O157:H7 Ad558	Environment	Seeding 48 h à 3 ± 2°C	/	3-4-2-2-4 (3,0)	**+**	2	c
2020	**2686**	**Brochettes d'agneau ail et herbes**	**Seasoned raw lamb meat**	*E. coli* O157:H7 Ad558	Environment	Seeding 48 h à 3 ± 2°C	/	3-4-2-2-4 (3,0)	**+**	2	c
2020	**2687**	**Bœuf à la mexicaine**	**Seasoned raw beef meat**	*E. coli* O157:H7 Ad558	Environment	Seeding 48 h à 3 ± 2°C	/	3-4-2-2-4 (3,0)	**+**	2	c

Appendix 4 – Sensitivity study: raw data

Bold typing: artificially inoculated samples

***E. coli* O157 detection results:**

m: minority level of target analyte
M: majority level of target analyte
p: pure culture level of target analyte
1/2: 50% level of target analyte
(x): number of colonies in the plate
-: no typical colonies but presence of background microflora
St: plate without any colony
d: doubtful result
NC: non-characteristic colony onto nutritive agar
a: auto-agglutinable strain
PA: positive agreement
NA: negative agreement
ND: negative deviation
PD: positive deviation
PPNA: positive presumptive negative agreement
PPND: positive presumptive negative deviation

Date of analysis	Sample N°	Product (French name)	Product	Reference method: ISO 16654♦					Alternative method: iQ-Check *E. coli* O157:H7 — BPW for 8 h at 41.5°C (25 g) -Protocol ①																											Type
				Typical colonies					PCR result 8 h (25 g) APF Classic (2008) APF Fast (2020)			Direct streaking									IMS												Final result with all confirmatory tests combined	Agreement 8 h	ISO 16654 tests after 18 h incubation time of the BPW	
				IMS 6H		IMS 24H						CT SMAC					RAPID'*E. coli* O157:H7					CT SMAC					RAPID'*E. coli* O157:H7								
													Latex					Latex					Latex					Latex								
				CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157	Result	Ct IC: HEX	Ct target: FAM	Result	Typical colonies	O157	H7	Final result	Agreement	Typical colonies	O157	H7	Final result	Agreement	Typical colonies	O157	H7	Final result	Concordance	Typical colonies	O157	H7	Final result	Agreement	Result			
2008	**2679**	**Haché tradition pur bœuf**	**Ground beef meat**	+m	+m	+m (2col)	+/-m (2col)	+	33,11	37,52	+	+m(1col)	+	+	+	PA	+/-m (2col)	a/a/a	/	-	ND	+1/2	+	+	+	PA	+m	+	+	+	PA	+	PA		a
2008	**2680**	**Haché tradition pur bœuf**	**Ground beef meat**	+m	+m	-	+m	+	33,01	38,02	+	+/-m(1-2col)	+	+	+	PA	+m	a/-/a	/	-	ND	+1/2	+	+	+	PA	+1/2	a/a/a	/	-	ND	+	PA		a
2008	**2681**	**Haché tradition pur bœuf**	**Ground beef meat**	+M	+m	+m	+m	+	33,54	31,98	+	+1/2	+	+	+	PA	+/-m (2col)	+	+	+	PA	+M	+	+	+	PA	+p	+	+	+	PA	+	PA		a
2008	**2682**	**Viande hachée**	**Ground beef meat**	-st	-st	-	-	-	33,45	31,67	+	+p	+	+	+	PD	+p	+	+	+	PD	+p	+	+	+	PD	+p	+	+	+	PD	+	PD		a
2008	**2683**	**Viande hachée**	**Ground beef meat**	-st	-st	-	-	-	33,73	30,43	+	+p	+	+	+	PD	+p	+	+	+	PD	+p	+	+	+	PD	+p	+	+	+	PD	+	PD		a
2008	**2684**	**Viande hachée**	**Ground beef meat**	+p	+M	+p	+1/2	+	34,06	35,91	+	+M	+	+	+	PA	+M	+	+	+	PA	+p	+	+	+	PA	+p	+	+	+	PA	+	PA		a
2008	**2687**	**Viande hachée 5%MG**	**Ground beef meat**	+p	+m	+m	-	+	33,72	-	-	-	/	/	-	ND	-	/	/	-	ND	+m	+	+	-	ND	-	/	/	-	ND	-	ND		a
2008	**2688**	**Steak haché 15%MG**	**Ground beef meat**	+M	+m	+M	+m	+	33,17	35,84	+	+1/2	+	+	+	PA	+1/2	a/a/a	/	-	ND	+p	+	+	+	PA	+M	+	+	+	PA	+	PA		a
2008	**2689**	**Steak haché 15%MG**	**Ground beef meat**	+p	+M	+M	+m	+	33,09	34,23	+	+M	+	+	+	PA	+/-1/2	+	+	+	PA	+p	+	+	+	PA	+p	+	+	+	PA	+	PA		a
2008	**2693**	**Steak haché 15%MG**	**Ground beef meat**	+p	+M	+m	+m (2col)	+	33,55	31,77	+	+m	+	+	+	PA	+/-(1col)	+	+	+	PA	+1/2	+	+	+	PA	+p	+	+	+	PA	+	PA		a
2008	**2705**	**Viande hachée**	**Ground beef meat**	+m (3col)	-	+m	-	+	34,04	-	-	+m(1-2col)	+	+	-	ND	-	/	/	-	ND	+1/2	+	+	-	ND	+1/2	+	+	-	ND	-	ND		a
2008	**2706**	**Viande hachée**	**Ground beef meat**	+p	+p	+p	+p	+	33,71	-	-	+p	+	+	-	ND	+p	+	+	-	ND	+p	+	+	-	ND	+p	+	+	-	ND	-	ND		a
2008	**2707**	**Viande hachée**	**Ground beef meat**	+p	+p	+p	+p	+	33,70	30,40	+	+p	+	+	+	PA	+p	+	+	+	PA	+p	+	+	+	PA	+p	+	+	+	PA	+	PA		a
2008	**2708**	**Viande hachée tradition 20%MG**	**Ground beef meat**	+p	+p	+p	+p	+	33,86	36,19	+	+p	+	+	+	PA	+p	+	+	+	PA	+p	+	+	+	PA	+p	+	+	+	PA	+	PA		a
2008	**2709**	**Viande hachée tradition 20%MG**	**Ground beef meat**	+M	+m	+m (3col)	+/-m (2col)	+	34,62	34,76	+	+m	+	+	+	PA	+/-m	-/a/+	+	+	PA	+p	+	+	+	PA	+M	+	+	+	PA	+	PA		a
2008	**2710**	**Viande hachée tradition 20%MG**	**Ground beef meat**	+M	+m	+m	+/-m (2col)	+	34,13	39,56	+	+m (4-5col)	+	+	+	PA	+/-m	-/a/a	/	-	ND	+M	+	+	+	PA	+1/2	+	+	+	PA	+	PA		a
2008	3111	Tartare de bœuf	Raw beef meat	-	-	-	-	-	31,67	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		a
2008	3113	Steak haché façon bouchère	Ground beef meat	+	-	-	-	-	31,83	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		a
2008	3116	Bifteck haché pur bœuf 5%MG	Ground beef meat	-	-	-	-	-	30,63	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		a
2008	3117	Steak haché pur bœuf	Ground beef meat	-	-	-	-	-	31,12	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		a
2008	3121	Steak haché pur bœuf	Ground beef meat	-	-	-	-	-	32,03	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		a
2008	3122	Steak haché frais pur bœuf	Ground beef meat	-	-	-	-	-	31,99	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		a
2008	3123	Rumsteak à griller	Raw beef meat	-	-	-	-	-	23,69	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		a
2008	3124	Haché façon boucherie	Ground beef meat	-	+	-	+/-	-	31,55	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		a
2008	3125	Tartare façon bouchère	Raw beef meat	-	-	-	-	-	30,95	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		a
2008	3127	Onglet à bifteck	Raw beef meat	+	+	+	+	+	31,49	37,68	+	+m	+	+	+	PA	-	/	/	-	ND	+M	+	+	+	PA	+/-m	+	+	+	PA	+	PA		a
2008	3128	Steak haché	Ground beef meat	-	-	-	-	-	32,07	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		a
2008	3130	Bavette	Raw beef meat	-	-	-	-	-	32,28	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		a

♦ Analyses performed according to the COFRAC accreditation

Date of analysis	Sample N°	Product (French name)	Product	Reference method: ISO 16654♦					Alternative method: iQ-Check *E. coli* O157:H7 — BPW for 8 h at 41.5°C (25 g) -Protocol ①																											Type
				Typical colonies					PCR result 8 h (25 g) APF Classic (2008) APF Fast (2020)			Direct streaking										IMS												Final result with all confirmatory tests combined	Agreement 8 h	ISO 16654 tests after 18 h incubation time of the BPW	
				IMS 6H		IMS 24H						CT SMAC					RAPID'*E. coli* O157:H7					CT SMAC					RAPID'*E. coli* O157:H7								
													Latex					Latex					Latex					Latex								
				CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157	Result	Ct IC: HEX	Ct target: FAM	Result	Typical colonies	O157	H7	Final result	Agreement	Typical colonies	O157	H7	Final result	Agreement	Typical colonies	O157	H7	Final result	Concordance	Typical colonies	O157	H7	Final result	Agreement	Result			
2008	3131	Steak haché façon bouchère	Ground beef meat	-	-	-	-	-	31,75	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		a
2008	3133	Rond de gite à biftek	Raw beef meat	-	-	-	-	-	31,45	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		a
2008	3135	Basse côte à griller	Raw beef meat	-	-	-	-	-	32,48	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		a
2008	3138	Aiguillette baronne à biftek	Raw beef meat	-	-	-	-	-	31,65	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		a
2008	3140	Steak haché façon bouchère	Ground beef meat	-	-	-	-	-	31,46	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		a
2008	3215	Steak haché pur bœuf frais	Ground beef meat	-	-	-	-	-	34,71	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		a
2020	**1868**	**Filet mignon de porc**	**Chilled raw pork meat**	+p	+p			+	33,23	-	-	st			-	ND	-			-	ND	st			-	ND	-			-	ND	-	ND	-	a
2020	**1869**	**Côte de veau**	**Chilled raw veal meat**	st	+d	+p	+Md	+	33,65	-	-	st			-	ND	st			-	ND	st			-	ND	st			-	ND	-	ND	-	a
2020	**1870**	**Escalope de veau**	**Chilled raw veal meat**	st	-	-	-	-	32,43	27,39	+	+p	+	+	+	PD	+p	+	+	+	PD	+p	+	+	+	PD	+p	+	+	+	PD	+	PD		a
2020	**1877**	**Viande de porc**	**Chilled raw pork meat**	-	-	-	-	-	33,66/ 33,33/ 33,47	38,59/-/-	+/-/- /+/-/ -/-/- /-/-	-			-	PPNA	-			-	PPNA	-			-	PPNA	-			-	PP NA	-	PPNA	-	a
2020	**2318**	**Collier d'agneau à mijoter**	**Chilled raw lamb meat**	st	+d	+p	+d	+	33,68	-	-	-			-	ND	-			-	ND	-			-	ND	-			-	ND	-	ND	-	a
2020	**2319**	**Gigot d'agneau à griller**	**Chilled raw lamb meat**	st	+1/2d (latex-)	st	+d (latex -)	-	33,38	-	-	-			-	NA	-			-	NA	st			-	NA	-			-	NA	-	NA	-	a
2020	2371	Sauté de veau	Chilled raw veal meat	st	-	-	-	-	33,92	-	-	-			-	NA	-			-	NA	st			-	NA	-			-	NA	-	NA	-	a
2020	2372	Sauté d'agneau congelé	Frozen chilled raw lamb meat	st	-	-	-	-	32,73	-	-	-			-	NA	-			-	NA	-			-	NA	-			-	NA	-	NA	-	b
2020	**2963**	**Côte de porc**	**Chilled raw pork meat**	st	-	st	-	-	33,52	24,70	+	+p	+	+	+	PD	+p	+	+	+	PD	+p	+	+	+	PD	+p	+	+	+	PD	+	PD		a
2020	**2964**	**Côte échine de porc**	**Chilled raw pork meat**	-	-	-	-	-	33,06	35,82	+	-			-	NA	-			-	NA	+d	+	+	+	PD	-			-	NA	+	PD		a
2020	**2965**	**Côtes d'agneau**	**Chilled raw lamb meat**	+p	+1/2			+	33,11	25,17	+	+1/2	+	+	+	PA	+1/2	+	+	+	PA	+M	+	+	+	PA	+M	+	+	+	PA	+	PA		a
2020	**2966**	**Tranche osso bucco veau**	**Chilled raw veal meat**	+p	+M			+	33,24	33,25	+	+M	+	+	+	PA	+m	+	+	+	PA	+M	+	+	+	PA	+M	+	+	+	PA	+	PA		a
2020	**2968**	**Gigot d'agneau**	**Chilled raw lamb meat**	+p	+M			+	33,33	-	-	+m	-(x5)	/	-	ND	-			-	ND	+m	-(x5)	/	-	ND	-			-	ND	-	ND	-	a
2020	**2969**	**Pavé de veau**	**Chilled raw veal meat**	+p	+M			+	33,27	-	-	-			-	ND	-			-	ND	-			-	ND	-			-	ND	-	ND	-	a
2020	**2970**	**Côte de veau**	**Chilled raw veal meat**	+p	+M			+	32,91	25,19	+	+M	+	+	+	PA	+M	+	+	+	PA	+M	+	+	+	PA	+M	+	+	+	PA	+	PA		a
2020	**2971**	**Filet mignon porc**	**Chilled raw pork meat**	+p	+M			+	32,62	27,25	+	+m	+	+	+	PA	+m	+	+	+	PA	+1/2	+	+	+	PA	+M	+	+	+	PA	+	PA		a
2020	**2972**	**Selle d'agneau**	**Chilled raw lamb meat**	+p	+1/2			+	32,71	28,67	+	+1/2	+	+	+	PA	-			-	ND	+M	+	+	+	PA	+M	+	+	+	PA	+	PA		a
2020	**2973**	**Rôti de porc**	**Chilled raw pork meat**	st	+(1)(indol -)	st	-	-	33,39	33,66	+	+M	+	+	+	PD	+1/2	+	+	+	PD	+M	+	+	+	PD	+M	+	+	+	PD	+	PD		a
2020	**2974**	**Poitrine d'agneau**	**Chilled raw lamb meat**	st	+1/2 (indol -)	-	+1/2d (indol -)	-	32,68	30,76	+	+m	+	+	+	PD	-			-	NA	+p	+	+	+	PD	+M	+	+	+	PD	+	PD		a
2020	2372	Sauté d'agneau congelé	Frozen chilled raw lamb meat	st	-	-	-	-	32,73	-	-	-			-	NA	-			-	NA	-			-	NA	-			-	NA	-	NA	-	b
2008	**2694**	**Petit tendre haché surgelé**	**Frozen ground beef meat**	+p	+p	+p	+1/2	+	34,58	-	-	+m	+	+	-	ND	+m (3col)	+	+	-	ND	+p	+	+	-	ND	+p	+	+	-	ND	-	ND		b
2008	**2695**	**Petit tendre haché surgelé**	**Frozen ground beef meat**	+p	+p	+p	+1/2	+	33,74	27,55	+	+p	+	+	+	PA	+1/2	+	+	+	PA	+p	+	+	+	PA	+p	+	+	+	PA	+	PA		b

Date of analysis	Sample N°	Product (French name)	Product	Reference method: ISO 16654♦					Alternative method: iQ-Check *E. coli* O157:H7 — BPW for 8 h at 41.5°C (25 g) -Protocol ①																										Type
				Typical colonies					PCR result 8 h (25 g) APF Classic (2008) APF Fast (2020)			Direct streaking										IMS												Final result with all confirmatory tests combined	Agreement 8 h	ISO 16654 tests after 18 h incubation time of the BPW	
				IMS 6H		IMS 24H						CT SMAC					RAPID'*E. coli* O157:H7					CT SMAC					RAPID'*E. coli* O157:H7								
													Latex					Latex					Latex					Latex								
				CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157	Result	Ct IC: HEX	Ct target: FAM	Result	Typical colonies	O157	H7	Final result	Agreement	Typical colonies	O157	H7	Final result	Agreement	Typical colonies	O157	H7	Final result	Concordance	Typical colonies	O157	H7	Final result	Agreement	Result			
2008	**2696**	**Petit tendre haché surgelé**	**Frozen ground beef meat**	+p	+p	+p	+1/2	+	34,04	26,31	+	+p	+	+	+	**PA**	+p	+	+	+	**PA**	+p	+	+	+	**PA**	+p	+	+	+	**PA**	+	**PA**		b
2008	**2697**	**Petit tendre haché surgelé**	**Frozen ground beef meat**	+p	+p	+p	+1/2	+	33,03	29,69	+	+p	+	+	+	**PA**	+M	+	+	+	**PA**	+p	+	+	+	**PA**	+p	+	+	+	**PA**	+	**PA**		b
2008	3139	Steak surgelé	Frozen ground beef meat	-	-	-	-	-	31,63	-	-	/	/	/	-	**NA**	/	/	/	-	**NA**	/	/	/	-	**NA**	/	/	/	-	**NA**	-	**NA**		b
2020	**1668**	**Souris d'agneau congelée**	**Frozen raw lamb meat**	+p	+p			+	34,16	-	-	st			-	**ND**	st			-	**ND**	st			-	**ND**	st			-	**ND**	-	**ND**	**-**	b
2020	**1818**	**Filet mignon porc congelé**	**Frozen raw pork meat**	+M	+M			+	35,28	21,57	+	+p	+	+	+	**PA**	+p	+	+	+	**PA**	+p	+	+	+	**PA**	+p	+	+	+	**PA**	+	**PA**		b
2020	**1819**	**Souris d'agneau congelée**	**Frozen raw lamb meat**	+p	+p			+	35,94	20,47	+	+M	+	+	+	**PA**	+p	+	+	+	**PA**	+M	+	+	+	**PA**	+p	+	+	+	**PA**	+	**PA**		b
2020	**1820**	**Escalope de veau hâché congelée**	**Frozen ground veal meat**	st	-	st	-	-	38,64	-	-	-			-	**NA**	-			-	**NA**	st			-	**NA**	-			-	**NA**	-	**NA**	**-**	b
2020	**1821**	**Filet mignon de porc congelé**	**Frozen raw pork meat**	+p	+p			+	33,32	24,21	+	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+	**PA**		b
2020	**1822**	**Côte d'agneau congelée**	**Frozen raw lamb meat**	+p	+p			+	34,38	-	-	st			-	**ND**	st			-	**ND**	st			-	**ND**	st			-	**ND**	-	**ND**	**-**	b
2020	2373	Carré d'agneau congelé	Frozen raw lamb meat	st	st	-	-	-	34,22	-	-	st			-	**NA**	st			-	**NA**	st			-	**NA**	st			-	**NA**	-	**NA**	**-**	b
2020	2374	Souris d'agneau congelée	Frozen raw lamb meat	st	st	st	st	-	34,61	-	-	st			-	**NA**	-			-	**NA**	st			-	**NA**	st			-	**NA**	-	**NA**	**-**	b
2020	2375	Escalope de veau congelée	Frozen raw veal meat	st	st	st	-	-	33,82	-	-	-			-	**NA**	st			-	**NA**	st			-	**NA**	st			-	**NA**	-	**NA**	**-**	b
2020	2376	Sauté de veau congelé	Frozen raw veal meat	st	st	st	st	-	33,49	-	-	-			-	**NA**	-			-	**NA**	st			-	**NA**	-			-	**NA**	-	**NA**	**-**	b
2020	2377	Escalope de veau hâchée congelée	Frozen ground veal meat	st	st	st	-	-	33,55	-	-	st			-	**NA**	-			-	**NA**	st			-	**NA**	-			-	**NA**	-	**NA**	**-**	b
2020	2378	Filet mignon de porc congelé	Frozen raw pork meat	st	-	st	-	-	33,12	-	-	st			-	**NA**	-			-	**NA**	st			-	**NA**	-			-	**NA**	-	**NA**	**-**	b
2020	2379	Filet mignon de porc congelé	Frozen raw pork meat	st	-	st	-	-	33,32	-	-	-			-	**NA**	-			-	**NA**	st			-	**NA**	-			-	**NA**	-	**NA**	**-**	b
2020	2380	Côte de porc congelée	Frozen raw pork meat	st	-	st	-	-	33,11	-	-	-			-	**NA**	-			-	**NA**	st			-	**NA**	-			-	**NA**	-	**NA**	**-**	b
2008	**2676**	**Boulettes au bœuf 15%MG**	**Seasoned raw beef meat**	-st	-st	-	-	-	34,30	33,76	+	+p	+	+	+	**PD**	+M	+	+	+	**PD**	+p	+	+	+	**PD**	+p	+	+	+	**PD**	+	**PD**		c
2008	**2677**	**Boulettes au bœuf 15%MG**	**Seasoned raw beef meat**	-st	-st	-	-	-	33,47	34,12	+	+M	+	+	+	**PD**	+M	+	+	+	**PD**	+p	+	+	+	**PD**	+p	+	+	+	**PD**	+	**PD**		c
2008	**2678**	**Boulettes au bœuf 15%MG**	**Seasoned raw beef meat**	-st	-st	+p	+p	+	34,81	34,73	+	+m	+	+	+	**PA**	+/- m(2col)	+	+	+	**PA**	+p	+	+	+	**PA**	+p	+	+	+	**PA**	+	**PA**		c
2008	**2700**	**Boulettes au bœuf**	**Seasoned raw beef meat**	+p	+p	+p	+M	+	35,82	32,66	+	+p	+	+	+	**PA**	+p	+	+	+	**PA**	+p	+	+	+	**PA**	+p	+	+	+	**PA**	+	**PA**		c
2008	**2701**	**Boulettes au bœuf**	**Seasoned raw beef meat**	+p	+p	+p	+1/2	+	38,05	32,72	+	+p	+	+	+	**PA**	+M	+	+	+	**PA**	+p	+	+	+	**PA**	+p	+	+	+	**PA**	+	**PA**		c
2008	**2702**	**Boulettes au bœuf**	**Seasoned raw beef meat**	-st	-st	+p	-	+	34,53	37,82	+	+p	+	+	+	**PA**	+M	+	+	+	**PA**	+p	+	+	+	**PA**	+p	+	+	+	**PA**	+	**PA**		c
2008	**2703**	**Boulettes au bœuf**	**Seasoned raw beef meat**	+m	+m (1col)	+m (1col)	+/-(1col)	+	34,48	39,98	+	-	/	/	-	**ND**	-	/	/	-	**ND**	+1/2	+	+	+	**PA**	+m	+	+	+	**PA**	+	**PA**		c
2008	**2704**	**Boulettes au bœuf**	**Seasoned raw beef meat**	+p	+M	+p	+m	+	33,86	34,37	+	+1/2	+	+	+	**PA**	+/-m (1col)	+	+	+	**PA**	+p	+	+	+	**PA**	+M	+	+	+	**PA**	+	**PA**		c
2008	3110	Boulettes à base de bœuf	Seasoned raw beef meat	-	-	-	-	-	32,34	-	-	/	/	/	-	**NA**	/	/	/	-	**NA**	/	/	/	-	**NA**	/	/	/	-	**NA**	-	**NA**		c
2008	3112	Steak haché tomate	Seasoned ground beef meat	-	-	-	-	-	32,07	-	-	/	/	/	-	**NA**	/	/	/	-	**NA**	/	/	/	-	**NA**	/	/	/	-	**NA**	-	**NA**		c

Date of analysis	Sample N°	Product (French name)	Product	Reference method: ISO 16654♦					Alternative method: iQ-Check *E. coli* O157:H7 — BPW for 8 h at 41.5°C (25 g) -Protocol ①																												Type
				Typical colonies					PCR result 8 h (25 g) APF Classic (2008) APF Fast (2020)			Direct streaking										IMS												Final result with all confirmatory tests combined	Agreement 8 h	ISO 16654 tests after 18 h incubation time of the BPW	
				IMS 6H		IMS 24H						CT SMAC					RAPID'*E. coli* O157:H7					CT SMAC					RAPID'*E. coli* O157:H7								
													Latex					Latex					Latex					Latex								
				CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157	Result	Ct IC: HEX	Ct target: FAM	Result	Typical colonies	O157	H7	Final result	Agreement	Typical colonies	O157	H7	Final result	Agreement	Typical colonies	O157	H7	Final result	Concordance	Typical colonies	O157	H7	Final result	Agreement	Result			
2008	3114	Carpaccio parmesan	Seasoned raw beef meat	-	-	-	-	-	31,41	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		c
2008	3115	Boulettes à base de bœuf	Seasoned raw beef meat	-	-	-	-	-	31,64	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		c
2008	3118	Boulettes au boeuf	Seasoned raw beef meat	-	-	-	-	-	31,53	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		c
2008	3119	Haché bolognaise	Seasoned ground beef meat	-	-	-	-	-	31,49	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		c
2008	3120	Hamburgers viande hachée de bœuf	Seasoned ground beef meat	-	-	-	-	-	32,09	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		c
2008	3126	Boulettes de viande	Seasoned raw beef meat	-	-	-	-	-	31,45	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		c
2008	3129	Boulettes au boeuf	Seasoned raw beef meat	-	-	-	-	-	32,08	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		c
2008	3132	Steak haché assaisonné aux oignons	Seasoned ground beef meat	-	-	-	-	-	31,79	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		c
2008	3134	Boulettes kefta	Seasoned raw beef meat	-	-	-	-	-	31,97	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		c
2008	3136	Mini boulettes au bœuf	Seasoned raw beef meat	-	-	-	-	-	31,76	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		c
2008	3137	Carpaccio pur bœuf	Seasoned raw beef meat	-	-	-	-	-	32,01	-	-	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	/	/	/	-	NA	-	NA		c
2020	**1594**	**Lomo de porc marinés à l'andalouse**	**Marinated raw pork meat**	-	+M			+	33,52	-	-	-			-	ND	+1/2d	5x-		-	ND	-			-	ND	+md	5x-		-	ND	-	ND	-	c
2020	**1595**	**Lomo de porc mariné au piment doux**	**Marinated raw pork meat**	st	-	-	-	-	33,53	-	-	-			-	NA	-			-	NA	-			-	NA	+md	5x-		-	NA	-	NA	-	c
2020	**1596**	**Brochette de porc à la provençale**	**Seasoned raw pork meat**	st	-	st	-	-	33,19	-	-	st			-	NA	st			-	NA	st			-	NA	st			-	NA	-	NA	-	c
2020	**1871**	**Noisette d'agneau aux fines herbes**	**Seasoned raw lamb meat**	st	-	st	-	-	32,45	25,65	+	+p	+	+	+	PD	+p	+	+	+	PD	+p	+	+	+	PD	+p	+	+	+	PD	+	PD		c
2020	**1872**	**Côte d'agneau aux fines herbes**	**Seasoned raw lamb meat**	st	-	st	-	-	32,40	27,96	+	+1/2	+	+	+	PD	+M	+	+	+	PD	+md	+	+	+	PD	+Md	+	+	+	PD	+	PD		c
2020	**1873**	**Brochette d'agneau à l'ail**	**Seasoned raw lamb meat**	+d	+1/2			+	33,05	26,29	+	+d	+	+	+	PA	+d	+	+	+	PA	+d	+	+	+	PA	+d	+	+	+	PA	+	PA		c
2020	**1875**	**Grillade porc à la mexicaine**	**Seasoned raw pork meat**	-	+M			+	33,66	26,39	+	+M	+	+	+	PA	+M	+	+	+	PA	+M	+	+	+	PA	+M	+	+	+	PA	+	PA		c
2020	**1878**	**Pavé de veau mariné**	**Marinated raw veal meat**	+1/2	+m			+	32,94	32,94	+	+m	+	+	+	PA	-			-	ND	+1/2	+	+	+	PA	-			-	ND	+	PA	+ (REC et CT-SMAC)	c
2020	**1879**	**Gigot d'agneau mariné**	**Marinated raw lamb meat**	+p	+M			+	33,31	-	-	-			-	ND	-			-	ND	-			-	ND	-			-	ND	-	ND	-	c
2020	2381	Brochette de veau assaisonnée	Seasoned raw veal meat	st	-	-	-	-	33,02	-	-	-			-	NA	-			-	NA	-			-	NA	-			-	NA	-	NA	-	c
2020	**2688**	**Lomo de porc ail et persil**	**Seasoned raw pork meat**	+M	+1/2			+	32,76	29,80	+	-			-	ND	-			-	ND	+M	+	+	+	PA	+M	+	+	+	PA	+	PA		c
2020	**2689**	**Pavé de rumsteak à l'échalote**	**Seasoned raw beef meat**	-	+Md	-	+M	+	33,53	-	-	-			-	ND	-			-	ND	st			-	ND	-			-	ND	-	ND	-	c
2020	**2691**	**Pavés de bœuf aux trois poivres**	**Seasoned raw beef meat**	+M	+M			+	35,13	22,21	+	+p	+	+	+	PA	+M	+	+	+	PA	+M	+	+	+	PA	+M	+	+	+	PA	+	PA		c
2020	**2692**	**Côte de porc à la mexicaine**	**Seasoned raw pork meat**	st	-	st	-	-	33,83	28,90	+	+M	+	+	+	PD	+M	+	+	+	PD	+M	+	+	+	PD	+M	+	+	+	PD	+	PD		c

Date of analysis	Sample N°	Product (French name)	Product	Reference method: ISO 16654♦					Alternative method: iQ-Check *E. coli* O157:H7 — BPW for 8 h at 41.5°C (25 g) -Protocol ①																												Type
				Typical colonies					PCR result 8 h (25 g) APF Classic (2008) APF Fast (2020)			Direct streaking										IMS												Final result with all confirmatory tests combined	Agreement 8 h	ISO 16654 tests after 18 h incubation time of the BPW	
				IMS 6H		IMS 24H						CT SMAC					RAPID'*E. coli* O157:H7					CT SMAC					RAPID'*E. coli* O157:H7								
													Latex					Latex					Latex					Latex								
				CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157	Result	Ct IC: HEX	Ct target: FAM	Result	Typical colonies	O157	H7	Final result	Agreement	Typical colonies	O157	H7	Final result	Agreement	Typical colonies	O157	H7	Final result	Concordance	Typical colonies	O157	H7	Final result	Agreement	Result			
2020	**2693**	**Côte de porc à l'américaine**	**Seasoned raw pork meat**	+M	+1/2			+	33,10	32,31	+	+1/2d	+	+	+	**PA**	-			-	**ND**	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+	**PA**		c
2020	**2694**	**Pavé d'agneau mariné ail et persil**	**Marinated raw lamb meat**	+M	+1/2			+	34,52	31,69	+	-			-	**ND**	-			-	**ND**	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+	**PA**		c
2020	**2695**	**Haché de veau à la provençale**	**Seasoned ground veal meat**	+M	+M			+	32,78	27,44	+	+M	+	+	+	**PA**	+md	+	+	+	**PA**	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+	**PA**		c
2020	**2696**	**Tranche d'agneau à l'armoricaine**	**Seasoned raw lamb meat**	+M	+M			+	33,81	31,13	+	+m	+	+	+	**PA**	-			-	**ND**	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+	**PA**		c
2020	**2697**	**Brochettes de veau marinés au curry**	**Marinated raw veal meat**	+M	+M			+	35,19	22,35	+	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+	**PA**		c
2020	**2698**	**Brochettes d'agneau ail et herbes**	**Seasoned raw lamb meat**	+M	+M			+	34,70	24,60	+	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+	**PA**		c
2020	**2699**	**Bœuf à la mexicaine**	**Seasoned raw beef meat**	st	-	st	-	-	32,73	28,34	+	+M	+	+	+	**PD**	+1/2	+	+	+	**PD**	+M	+	+	+	**PD**	+M	+	+	+	**PD**	+	**PD**		c

Date of analysis	Sample N°	Product (French name)	Product	Reference method: ISO 16654♦					Alternative method: iQ-Check *E. coli* O157:H7 — BPW for 10 h at 41.5°C (375 g)-Protocol ②																										Type
				Typical colonies					PCR result 10 h (375 g) APF Fast			Direct streaking										IMS										Final result with all confirmatory tests combined	Agreement 10 h	ISO 16654 tests after 18 h incubation time of the BPW	
				IMS 6H		IMS 24H						CT SMAC					RAPID'*E. coli* O157:H7					CT SMAC					RAPID'*E. coli* O157:H7								
													Latex					Latex					Latex					Latex								
				CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157	Result	Ct IC: HEX	Ct target: FAM	Result	Typical colonies	O157	H7	Final result	Agreement	Typical colonies	O157	H7	Final result	Agreement	Typical colonies	O157	H7	Final result	Concordance	Typical colonies	O157	H7	Final result	Agreement	Result			
2020	**1963**	**Côte de veau**	**Chilled raw veal meat**	st	-	-	-	-	32,90	29,75	+	+M	+	+	+	**PD**	+(2)d	+	+	+	**PD**	+p	+	+	+	**PD**	+p	+	+	+	**PD**	+	**PD**		a
2020	**1964**	**Escalope de veau**	**Chilled raw veal meat**	-	+1/2	+M	+M	+	33,70	33,24	+	+M	+	+	+	**PA**	-			-	**ND**	+M	+	+	+	**PA**	+1/2	+	+	+	**PA**	+	**PA**		a
2020	**1965**	**Rôti de veau**	**Chilled raw veal meat**	st	+md	+M	+M	+	36,30	31,17	+	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+	**PA**		a
2020	**1966**	**Bœuf bourguignon**	**Chilled raw beef meat**	st	+md (latex -)	-	+md (latex -)	-	33,89	-	-	-			-	**NA**	-			-	**NA**	-			-	**NA**	-			-	**NA**	-	**NA**	**-**	a
2020	**1967**	**Basse côte de bœuf**	**Chilled raw beef meat**	st	+md (latex -)	st	+(3)d (latex -)	-	33,52	33,65	+	+(1)d	+	+	+	**PD**	-			-	**NA**	+1/2	+	+	+	**PD**	-			-	**NA**	+	**PD**		a
2020	**1968**	**Basse côte de bœuf**	**Chilled raw beef meat**	st	-	+md	+md	+	33,63	35,60	+	-			-	**ND**	-			-	**ND**	-			-	**ND**	-			-	**ND**	+	**PA**	**+ (only CT-SMAC)**	a
2020	**1969**	**Rouelle de porc**	**Chilled raw pork meat**	st	-	-	-	-	33,92	35,52	+	+md	-(5x)	/	-	**NA**	-			-	**NA**	+M	+	+	+	**PD**	+M	+	+	+	**PD**	+	**PD**		a
2020	**1970**	**Filet de porc**	**Chilled raw pork meat**	-	+m	+1/2	+m	+	33,12	-	-	-			-	**ND**	-			-	**ND**	-			-	**ND**	-			-	**ND**	-	**ND**	**-**	a
2020	**1971**	**Sauté de porc**	**Chilled raw pork meat**	-	-	-	-	-	32,96	32,12	+	+md	+	+	+	**PD**	-			-	**NA**	+M	+	+	+	**PD**	+M	+	+	+	**PD**	+	**PD**		a
2020	**1972**	**Collier d'agneau**	**Chilled raw lamb meat**	-	+md	+M	+m	+	33,05	-	-	-			-	**ND**	-			-	**ND**	-			-	**ND**	-			-	**ND**	-	**ND**	**-**	a
2020	**1973**	**Epaule d'agneau**	**Chilled raw lamb meat**	-	+1/2	+M	+1/2	+	33,06	32,54	+	+(1)d	+	+	+	**PA**	-			-	**ND**	+M	+	+	+	**PA**	-			-	**ND**	+	**PA**		a
2020	**1974**	**Poitrine d'agneau**	**Chilled raw lamb meat**	st	+1/2d	+M	+md	+	33,07	35,77	+	+(1)d	+	+	+	**PA**	-			-	**ND**	+m	+	+	+	**PA**	-			-	**ND**	+	**PA**		a
2020	2841	Sauté de porc	Chilled raw pork meat	st	-	st	-	-	33,29	-	-	-			-	**NA**	-			-	**NA**	st			-	**NA**	-			-	**NA**	-	**NA**	**-**	a
2020	2842	Filet mignon de porc	Chilled raw pork meat	st	st	st	-	-	33,60	-	-	st			-	**NA**	-			-	**NA**	st			-	**NA**	-			-	**NA**	-	**NA**	**-**	a
2020	2843	Bavette d'aloyau	Chilled raw beef meat	st	-	-	-	-	33,46	-	-	st			-	**NA**	-			-	**NA**	st			-	**NA**	-			-	**NA**	-	**NA**	**-**	a
2020	2844	Viande bovine pour fondue	Chilled raw beef meat	st	st	st	-	-	35,60	-	-	st			-	**NA**	-			-	**NA**	st			-	**NA**	-			-	**NA**	-	**NA**	**-**	a
2020	2846	Escalope de veau	Chilled raw veal meat	st	-	st	-	-	33,93	-	-	st			-	**NA**	-			-	**NA**	-			-	**NA**	-			-	**NA**	-	**NA**	**-**	a
2020	2847	Sauté de veau	Chilled raw veal meat	st	-	-	-	-	33,28	-	-	-			-	**NA**	-			-	**NA**	-			-	**NA**	-			-	**NA**	-	**NA**	**-**	a
2020	2848	Gigot d'agneau	Chilled raw lamb meat	-	st	-	-	-	33,06	-	-	-			-	**NA**	-			-	**NA**	-			-	**NA**	-			-	**NA**	-	**NA**	**-**	a
2020	2849	Sauté d'agneau	Chilled raw lamb meat	st	st	st	-	-	32,97	-	-	-			-	**NA**	-			-	**NA**	-			-	**NA**	-			-	**NA**	-	**NA**	**-**	a
2020	**2031**	**Sauté de veau pour blanquette congelé**	**Frozen raw veal meat**	st	st	-	-	-	35,05	29,19	+	+1/2	+	+	+	**PD**	+d	+	+	+	**PD**	+p	+	+	+	**PD**	+p	+	+	+	**PD**	+	**PD**		b
2020	**2032**	**Escalope de veau congelée**	**Frozen raw veal meat**	st	st	st	-	-	34,11	-	-	+d	a(x5)	a(x5)	-	**NA**	-			-	**NA**	-			-	**NA**	st			-	**NA**	-	**NA**	**-**	b
2020	**2033**	**Gigot d'agneau congelé**	**Frozen raw lamb meat**	st	st	st	st	-	34,79	24,11	+	+d	+	+	+	**PD**	-			-	**NA**	+p	+	+	+	**PD**	+p	+	+	+	**PD**	+	**PD**		b
2020	**2034**	**Steak haché bœuf congelé**	**Frozen ground beef meat**	st	st	st	-	-	33,17	29,24	+	+M	+	+	+	**PD**	+M	+	+	+	**PD**	+p	+	+	+	**PD**	+p	+	+	+	**PD**	+	**PD**		b
2020	**2035**	**Fondant de charolais congelé**	**Frozen raw beef meat**	st	st	st	-	-	33,75	34,25	+	+M	+	+	+	**PD**	+M	+	+	+	**PD**	+p	+	+	+	**PD**	+p	+	+	+	**PD**	+	**PD**		b
2020	**2036**	**Viande hâchée de bœuf congelé**	**Frozen ground beef meat**	st	+ (3)	+M	+M	+	33,41	30,90	+	-			-	**ND**	-			-	**ND**	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+	**PA**		b

♦ Analyses performed according to the COFRAC accreditation

Date of analysis	Sample N°	Product (French name)	Product	Reference method: ISO 16654♦					Alternative method: iQ-Check *E. coli* O157:H7 — BPW for 10 h at 41.5°C (375 g)-Protocol ②																												Type
				Typical colonies					PCR result 10 h (375 g) APF Fast			Direct streaking										IMS												Final result with all confirmatory tests combined	Agreement 10 h	ISO 16654 tests after 18 h incubation time of the BPW	
				IMS 6H		IMS 24H						CT SMAC					RAPID'*E. coli* O157:H7					CT SMAC					RAPID'*E. coli* O157:H7								
													Latex					Latex					Latex					Latex								
				CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157	Result	Ct IC: HEX	Ct target: FAM	Result	Typical colonies	O157	H7	Final result	Agreement	Typical colonies	O157	H7	Final result	Agreement	Typical colonies	O157	H7	Final result	Concordance	Typical colonies	O157	H7	Final result	Agreement	Result			
2020	**2037**	**Filet mignon de porc congelé**	**Frozen raw pork meat**	st	st	st	-	-	33,45	31,07	+	+M	+	+	+	**PD**	+M	+	+	+	**PD**	+p	+	+	+	**PD**	+p	+	+	+	**PD**	+	**PD**		b
2020	**2038**	**Sauté de veau congelé**	**Frozen raw veal meat**	st	-	-	-	-	34,05	-	-	-			-	**NA**	-			-	**NA**	-			-	**NA**	-			-	**NA**	-	**NA**	**-**	b
2020	**2039**	**Escalope hachée de veau congelée**	**Frozen ground veal meat**	st	st	st	-	-	33,33/ 33,05/ 33,82	-/36,30/ 39,02	- /+/+ /+/+ /- /+/+ /+/+	+md	+	+	-	**NA**	-			-	**NA**	+M	+	+	-	**NA**	+M	+	+	-	**NA**	-	**NA**		b
2020	**2040**	**Filet mignon de porc congelé**	**Frozen raw pork meat**	+(1)	st	+M	+m	+	33,68	32,72	+	+p	+	+	+	**PA**	+p	+	+	+	**PA**	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+	**PA**		b
2020	**2041**	**Echine côte de porc congelée**	**Frozen raw pork meat**	st	-	-	-	-	33,21	36,66	+	-			-	**NA**	-			-	**NA**	+m	+	+	+	**PD**	+M	+	+	+	**PD**	+	**PD**		b
2020	**2042**	**Sauté de porc congelé**	**Frozen raw pork meat**	st	st	st	-	-	32,94	26,62	+	+p	+	+	+	**PD**	+p	+	+	+	**PD**	+p	+	+	+	**PD**	+p	+	+	+	**PD**	+	**PD**		b
2020	2845	Boeuf haché surgelé	Frozen ground beef meat	st	st	-	-	-	33,08	-	-	-			-	**NA**	-			-	**NA**	st			-	**NA**	-			-	**NA**	-	**NA**	**-**	b
2020	2850	Effeuillé de charolais surgelé	Frozen raw beef meat	st	st	st	-	-	33,22	-	-	st			-	**NA**	-			-	**NA**	st			-	**NA**	-			-	**NA**	-	**NA**	**-**	b
2020	2851	Sauté de veau surgelé	Frozen raw veal meat	st	st	st	-	-	33,31	-	-	st			-	**NA**	-			-	**NA**	-			-	**NA**	-			-	**NA**	-	**NA**	**-**	b
2020	2852	Tranche de collier d'agneau surgelé	Frozen raw lamb meat	st	st	-	-	-	33,99	-	-	-			-	**NA**	-			-	**NA**	-			-	**NA**	-			-	**NA**	-	**NA**	**-**	b
2020	2947	Collier d'agneau surgelé	Frozen raw lamb meat	st	st	st	-	-	33,80	-	-	-			-	**NA**	-			-	**NA**	-			-	**NA**	-			-	**NA**	-	**NA**	**-**	b
2020	2948	Viande hâchée bœuf surgelée	Frozen ground beef meat	st	st	st	-	-	33,47	-	-	st			-	**NA**	-			-	**NA**	st			-	**NA**	-			-	**NA**	-	**NA**	**-**	b
2020	2949	Sauté de porc surgelé	Frozen raw pork meat	st	st	st	-	-	33,37	-	-	-			-	**NA**	-			-	**NA**	-			-	**NA**	-			-	**NA**	-	**NA**	**-**	b
2020	2950	Bavette d'aloyau surgelée	Frozen raw beef meat	st	st	-	-	-	33,98	-	-	-			-	**NA**	-			-	**NA**	-			-	**NA**	-			-	**NA**	-	**NA**	**-**	b
2020	**2676**	**Lomo de porc ail et persil**	**Seasoned raw pork meat**	st	st	-	+md (latex -)	-	33,99	-	-	-			-	**NA**	-			-	**NA**	-			-	**NA**	-			-	**NA**	-	**NA**		c
2020	**2677**	**Pavé de rumsteak à l'échalote**	**Seasoned raw beef meat**	st	st	-	+1/2	+	33,77	39,29	+	-			-	**ND**	-			-	**ND**	-			-	**ND**	-			-	**ND**	+	PA	+ (only REC)	c
2020	**2678**	**Carpaccio bœuf au parmesan**	**Seasoned raw beef meat**	st	st	-	+m	+	33,80/ 33,74/ 33,02	-/37,59/-	-/+/-	st			-	**ND**	-			-	**ND**	-			-	**ND**	-			-	**ND**	-	**ND**	+	c
2020	**2679**	**Pavés de bœuf aux trois poivres**	**Seasoned raw beef meat**	st	st	st	-	-	33,85	33,52	+	+M	+	+	+	**PD**	+1/2	+	+	+	**PD**	st			-	**NA**	-			-	**NA**	+	**PD**	+	c
2020	**2680**	**Côte de porc à la mexicaine**	**Seasoned raw pork meat**	st	-	st	-	-	35,27	38,32	+	+m	+	+	+	**PD**	-			-	**NA**	+M	+	+	+	**PD**	-			-	**NA**	+	**PD**		c
2020	**2681**	**Côte de porc à l'américaine**	**Seasoned raw pork meat**	st	-	+M	+M	+	32,99/ 33,30/ 33,05	-/-/37,08	-/-/+	-			-	**ND**	-			-	**ND**	+md	+	+	-	**ND**	-			-	**ND**	-	**ND**		c
2020	**2682**	**Pavé d'agneau mariné ail et persil**	**Marinated raw lamb meat**	st	-	+m	+m	+	34,96	38,60	+	-			-	**ND**	-			-	**ND**	+1/2	+	+	+	**PA**	+d	+	+	+	**PA**	+	**PA**		c
2020	**2683**	**Haché de veau à la provençale**	**Seasoned ground veal meat**	st	-	-	+1/2	+	33,40	32,14	+	+d	+	+	+	**PA**	-			-	**ND**	+m	+	+	+	**PA**	-			-	**ND**	+	**PA**		c
2020	**2684**	**Tranche d'agneau à l'armoricaine**	**Seasoned raw lamb meat**	st	+(1)	-	+m	+	33,75	32,98	+	-			-	**ND**	-			-	**ND**	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+	**PA**		c
2020	**2685**	**Brochettes de veau marinés au curry**	**Marinated raw veal meat**	st	-	-	+(1)d (latex -)	-	33,53	38,20	+	-			-	**NA**	-			-	**NA**	+d	-(x5)	/	-	**NA**	-			-	**NA**	+	**PD**	+	c

Date of analysis	Sample N°	Product (French name)	Product	Reference method: ISO 16654♦					Alternative method: iQ-Check *E. coli* O157:H7 — BPW for 10 h at 41.5°C (375 g)-Protocol ②																													Type
				Typical colonies					PCR result 10 h (375 g) APF Fast			Direct streaking										IMS													Final result with all confirmatory tests combined	Agreement 10 h	ISO 16654 tests after 18 h incubation time of the BPW	
				IMS 6H		IMS 24H						CT SMAC					RAPID'*E. coli* O157:H7					CT SMAC					RAPID'*E. coli* O157:H7								
				CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157	Result	Ct IC: HEX	Ct target: FAM	Result	Typical colonies	Latex O157	Latex H7	Final result	Agreement	Typical colonies	Latex O157	Latex H7	Final result	Agreement	Typical colonies	Latex O157	Latex H7	Final result	Concordance	Typical colonies	Latex O157	Latex H7	Final result	Agreement	Result			
2020	**2686**	**Brochettes d'agneau ail et herbes**	**Seasoned raw lamb meat**	st	-	st	-	-	34,17	36,37	+	+m	+	+	+	**PD**	+m	+	+	+	**PD**	+M	+	+	+	**PD**	+m	+	+	+	**PD**	+	**PD**		c
2020	**2687**	**Bœuf à la mexicaine**	**Seasoned raw beef meat**	st	+1/2d	+1/2	+1/2	+	33,66/ 34,13/ 33,09	-/41,08/-	-/+/-	+m	+	+	-	**ND**	+m	- (x5)	/	-	**ND**	+M	+	+	-	**ND**	+M	+	+	-	**ND**	-	**ND**		c
2020	2951	Farce de veau	Seasoned raw veal meat	st	-	-	-	-	33,23	-	-	-			-	**NA**	-			-	**NA**	-			-	**NA**	-			-	**NA**	-	**NA**	-	c
2020	2952	Grillade de porc à la provençale	Seasoned raw pork meat	st	-	-	-	-	33,13	-	-	-			-	**NA**	-			-	**NA**	-			-	**NA**	-			-	**NA**	-	**NA**	-	c
2020	2953	Grillade de porc marinée	Marinated raw pork meat	st	st	st	-	-	32,82	-	-	-			-	**NA**	-			-	**NA**	-			-	**NA**	-			-	**NA**	-	**NA**	-	c
2020	2954	Paleron épicé	Seasoned raw beef meat	st	-	-	-	-	33,75	-	-	-			-	**NA**	-			-	**NA**	st			-	**NA**	-			-	**NA**	-	**NA**	-	c
2020	2955	Pavé d'agneau à la tomate	Seasoned raw lamb meat	st	-	-	-	-	35,37	-	-	-			-	**NA**	-			-	**NA**	-			-	**NA**	-			-	**NA**	-	**NA**	-	c
2020	2956	Brochette d'agneau à la provençale	Seasoned raw lamb meat	st	-	-	-	-	33,20	-	-	-			-	**NA**	-			-	**NA**	st			-	**NA**	-			-	**NA**	-	**NA**	-	c
2020	2957	Brochette de veau mariné kebab	Marinated raw veal meat	st	-	-	-	-	33,66	-	-	-			-	**NA**	-			-	**NA**	-			-	**NA**	-			-	**NA**	-	**NA**	-	c
2020	2958	Brochette de bœuf mariné	Marinated raw veal meat	st	-	-	-	-	33,10	-	-	-			-	**NA**	-			-	**NA**	st			-	**NA**	-			-	**NA**	-	**NA**	-	c
2020	2959	Côte de porc assaisonnée	Seasoned raw pork meat	st	st	-	-	-	33,51	-	-	st			-	**NA**	st			-	**NA**	st			-	**NA**	-			-	**NA**	-	**NA**	-	c

Date of analysis	Sample N°	Product (french name)	Product	Reference method: ISO 16654♦	Alternative method: iQ-Check *E. coli* O157:H7 — BPW for 10 h at 41.5°C + 72 h at 5°C ± 3°C - Protocol ②																						All confir-matory tests combined	Agree-ment 72 h	Type
					PCR result 72 h APF Fast			Direct streaking										IMS												
								CT SMAC					RAPID'*E. coli* O157:H7					CT SMAC					RAPID'*E. coli* O157:H7							
									Latex					Latex					Latex					Latex							
					Ct IC: HEX	Ct target: FAM	Result	Typical colonies	O157	H7	Final result	Agree-ment	Typical colonies	O157	H7	Final result	Agreement	Typical colonies	O157	H7	Final result	Agree-ment	Typical colonies	O157	H7	Final result	Agree-ment	Result		
2020	**1963**	**Côte de veau**	**Chilled raw veal meat**	-	33,28	30,52	+	+p	+	+	+	**PD**	+p	+	+	**+**	**PD**	+p	+	+	**+**	**PD**	+p	+	+	**+**	**PD**	**+**	**PD**	a
2020	**1964**	**Escalope de veau**	**Chilled raw veal meat**	+	33,66	34,21	+	+1/2	+	+	+	**PA**	-			**-**	**ND**	+p	+	+	**+**	**PA**	+p	+	+	**+**	**PA**	**+**	**PA**	a
2020	**1965**	**Rôti de veau**	**Chilled raw veal meat**	+	33,09	28,61	+	+p	+	+	+	**PA**	+M	+	+	**+**	**PA**	+p	+	+	**+**	**PA**	+p	+	+	**+**	**PA**	**+**	**PA**	a
2020	**1967**	**Basse côte de bœuf**	**Chilled raw beef meat**	-	33,45	32,46	+	-			-	**NA**	-			**-**	**NA**	+M	+	+	**+**	**PD**	-			**-**	**NA**	**+**	**PD**	a
2020	**1968**	**Basse côte de bœuf**	**Chilled raw beef meat**	+	33,10	37,33	+	-			-	**ND**	-			**-**	**ND**	+m	+	+	**+**	**PA**	-			**-**	**ND**	**+**	**PA**	a
2020	**1969**	**Rouelle de porc**	**Chilled raw pork meat**	-	33,44	33,98	+	+1/2	+	+	+	**PD**	+m	-(x5)	/	**-**	**NA**	+M	+	+	**+**	**PD**	+M	+	+	**+**	**PD**	**+**	**PD**	a
2020	**1970**	**Filet de porc**	**Chilled raw pork meat**	+	33,22	-	-	-			-	**ND**	-			**-**	**ND**	-			**-**	**ND**	-			**-**	**ND**	**-**	**ND**	a
2020	**1971**	**Sauté de porc**	**Chilled raw pork meat**	-	33,03	31,40	+	+m	+	+	+	**PD**	+m	+	+	**+**	**PD**	+M	+	+	**+**	**PD**	+M	+	+	**+**	**PD**	**+**	**PD**	a
2020	**1972**	**Collier d'agneau**	**Chilled raw lamb meat**	+	34,13	-	-	-			-	**ND**	-			**-**	**ND**	-			**-**	**ND**	-			**-**	**ND**	**-**	**ND**	a
2020	**1973**	**Epaule d'agneau**	**Chilled raw lamb meat**	+	33,44	33,21	+	-			-	**ND**	-			**-**	**ND**	+M	+	+	**+**	**PA**	+md	- /a(x4)	/	**-**	**ND**	**+**	**PA**	a
2020	**1974**	**Poitrine d'agneau**	**Chilled raw lamb meat**	+	33,29	35,09	+	-			-	**ND**	-			**-**	**ND**	+1/2	+	+	**+**	**PA**	-			**-**	**ND**	**+**	**PA**	a
2020	**2031**	**Sauté de veau pour blanquette congelé**	**Frozen raw veal meat**	-	35,97	30,32	+	+m	+	+	+	**PD**	+m	+	+	**+**	**PD**	+M	+	+	**+**	**PD**	+M	+	+	**+**	**PD**	**+**	**PD**	b
2020	**2033**	**Gigot d'agneau congelé**	**Frozen raw lamb meat**	-	33,02	23,19	+	+d	+	+	+	**PD**	+d	+	+	**+**	**PD**	+M	+	+	**+**	**PD**	+M	+	+	**+**	**PD**	**+**	**PD**	b
2020	**2034**	**Steak haché bœuf congelé**	**Frozen ground beef meat**	-	33,52	32,74	+	+p	+	+	+	**PD**	+m	+	+	**+**	**PD**	+M	+	+	**+**	**PD**	+m	+	+	**+**	**PD**	**+**	**PD**	b
2020	**2035**	**Fondant de charolais congelé**	**Frozen raw beef meat**	-	33,34	38,52	+	-			-	**NA**	-			**-**	**NA**	-			**-**	**NA**	-			**-**	**NA**	**-**	**NA**	b
2020	**2036**	**Viande hachée de bœuf congelé**	**Frozen ground beef meat**	+	33,10	36,90	+	+d	+	+	+	**PA**	+md	+	+	**+**	**PA**	+M	+	+	**+**	**PA**	+M	+	+	**+**	**PA**	**+**	**PA**	b
2020	**2037**	**Filet mignon de porc congelé**	**Frozen raw pork meat**	-	32,87	32,21	+	+p	+	+	+	**PD**	+md	- (x5)	/	**-**	**NA**	+M	+	+	**+**	**PD**	+M	+	+	**+**	**PD**	**+**	**PD**	b
2020	**2039**	**Escalope hachée de veau congelée**	**Frozen ground veal meat**	-	33,65	39,06	+	+m	+	+	+	**PD**	-			**-**	**NA**	+M	+	+	**+**	**PD**	+M	+	+	**+**	**PD**	**+**	**PD**	b
2020	**2040**	**Filet mignon de porc congelé**	**Frozen raw pork meat**	+	32,92	31,71	+	+p	+	+	+	**PA**	+p	+	+	**+**	**PA**	+M	+	+	**+**	**PA**	+M	+	+	**+**	**PA**	**+**	**PA**	b

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Date of analysis	Sample N°	Product (french name)	Product	Reference method: ISO 16654♦	Alternative method: iQ-Check *E. coli* O157:H7 — BPW for 10 h at 41.5°C + 72 h at 5°C ± 3°C - Protocol ②																										Type
					PCR result 72 h APF Fast			Direct streaking														IMS									All confirmatory tests combined	Agreement 72 h	
								CT SMAC					RAPID'*E. coli* O157:H7					CT SMAC					RAPID'*E. coli* O157:H7							
									Latex					Latex					Latex					Latex							
					Ct IC: HEX	Ct target: FAM	Result	Typical colonies	O157	H7	Final result	Agreement	Typical colonies	O157	H7	Final result	Agreement	Typical colonies	O157	H7	Final result	Agreement	Typical colonies	O157	H7	Final result	Agreement	Result		
2020	**2041**	**Echine côte de porc congelée**	**Frozen raw pork meat**	-	33,45	35,59	+	-			-	**NA**	-			-	**NA**	+M	+	+	+	**PD**	+M	+	+	+	**PD**	+	**PD**	b
2020	**2042**	**Sauté de porc congelé**	**Frozen raw pork meat**	-	33,24	30,32	+	+1/2	+	+	+	**PD**	+1/2	+	+	+	**PD**	+p	+	+	+	**PD**	+1/2	+	+	+	**PD**	+	**PD**	b
2020	**2676**	**Lomo de porc ail et persil**	**Seasoned raw pork meat**	-	34,07/ 32,99/ 32,86	34,75/ 37,37/ 37,95	+/+/+	+(x5)	-		-	**PPNA**	+(x5)	-		-	**PPNA**	+(x5)	-		-	**PPNA**	+(x5)	+	+	+	**PD**	+	**PD**	c
2020	**2677**	**Pavé de rumsteak à l'échalote**	**Seasoned raw beef meat**	+	33,93	37,11	+	-			-	**ND**	-			-	**ND**	-			-	**ND**	+1/2	+	+	+	**PA**	+	**PA**	c
2020	**2678**	**Carpaccio bœuf au parmesan**	**Seasoned raw beef meat**	+	33,74/ 33,15/ 33,01	-/40,19/ 39,61	-/+/+/ +/+/+/+/-	-			-	**ND**	+m	+	+	-	**ND**	+M	+	+	-	**ND**	+M	+	+	-	**ND**	-	**ND**	c
2020	**2679**	**Pavés de bœuf aux trois poivres**	**Seasoned raw beef meat**	-	34,19	36,20	+	+(4)	+	+	+	**PD**	+(2)	+	+	+	**PD**	st			-	**NA**	st			-	**NA**	+	**PD**	c
2020	**2680**	**Côte de porc à la mexicaine**	**Seasoned raw pork meat**	-	34,46	35,84	+	+m	+	+	+	**PD**	-			-	**NA**	+M	+	+	+	**PD**	+m	+	+	+	**PD**	+	**PD**	c
2020	**2681**	**Côte de porc à l'américaine**	**Seasoned raw pork meat**	+	34,07	38,31	+	+(1)d	+	+	+	**PA**	-			-	**ND**	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+	**PA**	c
2020	**2682**	**Pavé d'agneau mariné ail et persil**	**Marinated raw lamb meat**	+	34,70	33,48	+	+(2)d	-(x2)	/	-	**ND**	-			-	**ND**	+1/2	+	+	+	**PA**	+m	+	+	+	**PA**	+	**PA**	c
2020	**2683**	**Haché de veau à la provençale**	**Seasoned ground veal meat**	+	34,04	33,05	+	+md	-(x5)	/	-	**ND**	+(1)d	+	+	+	**PA**	+1/2	+	+	+	**PA**	-			-	**ND**	+	**PA**	c
2020	**2684**	**Tranche d'agneau à l'armoricaine**	**Seasoned raw lamb meat**	+	33,67	33,42	+	-			-	**ND**	-			-	**ND**	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+	**PA**	c
2020	**2685**	**Brochettes de veau marinés au curry**	**Marinated raw veal meat**	-	33,76	40,65	+/-/+/-/+/+/+/+	+(x5)	-		-	**PPNA**	+(x5)	-		-	**PPNA**	+(x5)	-		-	**PPNA**	+(x5)	+	+	+	**PD**	+	**PD**	c
2020	**2686**	**Brochettes d'agneau ail et herbes**	**Seasoned raw lamb meat**	-	35,06	38,64	+	+1/2	+	+	+	**PD**	+(2)	+	+	+	**PD**	+M	+	+	+	**PD**	+M	+	+	+	**PD**	+	**PD**	c
2020	**2687**	**Bœuf à la mexicaine**	**Seasoned raw beef meat**	+	33,87	38,28	+	+1/2	+	+	+	**PA**	+m	+	+	+	**PA**	+M	+	+	+	**PA**	+M	+	+	+	**PA**	+	**PA**	c

Appendix 5 – Relative level of detection study: raw data

Matrix: Veal meat 25 g test portion-Protocol ①

Strain: *E. coli* O157:H7 Ad485
Aerobic mesophilic flora: 9,6.10⁷ CFU/g

Sample N°	Inoculation level (CFU/sample)	Reference method: ISO 16654♦						Alternative method: iQ-Check *E. coli* O157:H7																				
								8 h at 41.5°C																				
								PCR APF Fast			Confirmation																	
											Direct streaking									IMS								
											CT SMAC				RAPID'*E. coli* O157:H7					CT SMAC				RAPID'*E. coli* O157:H7				
		IMS 6H		IMS 24H		Result	Positive/Total	Ct IC: HEX	Ct target: FAM	Result	Typical colonies	Latex		Final result	Typical colonies	Latex		Final result	Positive/Total	Typical colonies	Latex		Final result	Typical colonies	Latex		Final result	Positive/Total
		CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157							O157	H7			O157	H7				O157	H7			O157	H7		
1698	/	st	-	-	-	-	0/5	34,15	-	-	st			-	-			-		st			-	-			-	0/5
1699		st	-	st	-	-		33,91	-	-	st			-	-			-		st			-	-			-	
1700		st	-	st	-	-		33,18	-	-	st			-	-			-		st			-	-			-	
1701		st	-	st	-	-		33,35	-	-	st			-	-			-		st			-	-			-	
1702		st	-	st	-	-		33,22	-	-	-			-	-			-		st			-	-			-	
1703	1,1	+m	+m	/	/	+	14/20	33,02	29,33	+	+m	+	+	+	+m	+	+	+	15/20	+p	+	+	+	+p	+	+	+	15/20
1704		+p	+m	/	/	+		33,24	-	-	-			-	+d/-			-		st			-	-			-	
1705		+p	+M	/	/	+		32,42	28,44	+	+M	+	+	+	+1/2	+	+	+		+p	+	+	+	+p	+	+	+	
1706		+p	+M	/	/	+		33,53	32,13	+	+1/2	+	+	+	-			-		+p	+	+	+	+p	+	+	+	
1707		st	-	st	-	-		33,74	-	-	-			-	-			-		st			-	-			-	
1708		+p	+1/2	/	/	+		32,86	25,26	+	+M	+	+	+	+M	+	+	+		+p	+	+	+	+p	+	+	+	
1709		+p	+1/2	/	/	+		32,58	26,78	+	+M	+	+	+	+m	+	+	+		+p	+	+	+	+p	+	+	+	
1710		+p	+m	/	/	+		33,01	26,53	+	+m	+	+	+	+m	+	+	+		+p	+	+	+	+p	+	+	+	
1711		+p	+M	/	/	+		33,59	26,42	+	+M	+	+	+	+m	+	+	+		+p	+	+	+	+p	+	+	+	
1712		+p	+p	/	/	+		34,05	-	-	-			-	-			-		st			-	-			-	
1713		st	-	st	-	-		33,25	26,58	+	+M	+	+	+	+m	+	+	+		+p	+	+	+	+p	+	+	+	
1714		+p	+1/2	/	/	+		32,85	27,67	+	+M	+	+	+	+m	+	+	+		+p	+	+	+	+p	+	+	+	
1715		st	-	st	-	-		33,72	-	-	-			-	-			-		st			-	-			-	
1716		-	-	+M	+d	+		32,57	29,36	+	+M	+	+	+	+1/2	+	+	+		+p	+	+	+	+p	+	+	+	
1717		+p	+1/2	/	/	+		33,00	25,48	+	+M	+	+	+	+1/2	+	+	+		+p	+	+	+	+p	+	+	+	
1718		+p	+1/2	/	/	+		33,36	24,89	+	+M	+	+	+	+M	+	+	+		+p	+	+	+	+p	+	+	+	
1719		st	-	st	-	-		32,66	27,36	+	+M	+	+	+	+m	+	+	+		+p	+	+	+	+p	+	+	+	
1720		st	-	st	-	-		32,76	29,05	+	+M	+	+	+	+m	+	+	+		+p	+	+	+	+M	+	+	+	
1721		+p	+M			+		33,36	-	-	-			-	-			-		st			-	-			-	
1722		st	-	st	-	-		33,23	26,10	+	+M	+	+	+	+M	+	+	+		+p	+	+	+	+p	+	+	+	
1723	5,6	+p	+1/2	/	/	+	5/5	32,65	26,59	+	+M	+	+	+	+M	+	+	+	5/5	+p	+	+	+	+M	+	+	+	5/5
1724		+p	+M	/	/	+		33,34	24,49	+	+M	+	+	+	+1/2	+	+	+		+p	+	+	+	+p	+	+	+	
1725		+p	+M	/	/	+		36,91	22,50	+	+p	+	+	+	+M	+	+	+		+p	+	+	+	+p	+	+	+	
1726		+p	+M	/	/	+		32,64	24,79	+	+p	+	+	+	+1/2	+	+	+		+p	+	+	+	+M	+	+	+	
1727		+p	+M	/	/	+		34,18	23,79	+	+p	+	+	+	+M	+	+	+		+p	+	+	+	+p	+	+	+	

♦ Analyses performed according to the COFRAC accreditation

Matrix: Ground beef 375 g test portion- Protocol ②

Strain: *E.coli* O157:H7 Ad2222
Aerobic mesophilic flora : 1,5.10³CFU/g

Sample N°	Inoculation level (CFU/sample)	Reference method: ISO 16654 ♦				Result	Positive/Total	Alternative method : iQ-Check *E. coli* O157:H7 10 h at 41.5°C																		Positive/Total
		IMS 6H		IMS 24H				PCR APF Fast			Confirmation															
											Direct streaking							IMS								
											CT SMAC				RAPID'*E. coli* O157:H7				CT SMAC				RAPID'*E. coli* O157:H7				
											Typical colonies	Latex		Final result	Typical colonies	Latex		Final result	Typical colonies	Latex		Final result	Typical colonies	Latex		Final result	
		CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157			Ct IC: HEX	Ct target: FAM	Result		O157	H7			O157	H7			O157	H7			O157	H7	
2445	/	st	st	st	-	-	0/5	33,51	-	-	st			-	-			-	st			-	-			-	0/5
2446		st	st	st	-	-		33,57	-	-	st			-	-			-	st			-	-			-	
2447		st	st	st	-	-		33,69	-	-	st			-	-			-	st			-	-			-	
2448		st	st	st	-	-		33,35	-	-	st			-	-			-	st			-	-			-	
2449		st	st	st	-	-		33,64	-	-	st			-	-			-	st			-	-			-	
2536	1,0	st	st	st	-	-	7/20	34,40	-	-	st			-	-			-	st			-	st			-	13/20
2537		st	st	st	-	-		33,50	-	-	st			-	-			-	st			-	-			-	
2538		st	st	st	-	-		33,09	34,33	+	+p	+	+	+	+M	+	+	+	+p	+	+	+	+M	+	+	+	
2539		st	st	st	-	-		32,75	29,97	+	+p	+	+	+	+M	+	+	+	+p	+	+	+	+M	+	+	+	
2540		st	st	st	-	-		33,36	-	-	st			-	-			-	st			-	-			-	
2541		st	st	st	-	-		33,05	33,24	+	+p	+	+	+	+M	+	+	+	+p	+	+	+	+M	+	+	+	
2542		st	st	+M	+p	+		33,32	-	-	st			-	-			-	st			-	-			-	
2543		st	+(3)	/	/	+		33,45	36,27	+	+M	+	+	+	+1/2	+	+	+	+p	+	+	+	+1/2	+	+	+	
2544		st	+(1)	/	/	+		33,24	33,28	+	+p	+	+	+	+M	+	+	+	+p	+	+	+	+M	+	+	+	
2545		st	st	st	-	-		33,18	-	-	-			-	-			-	st			-	-			-	
2546		st	st	+M	+p	+		33,08	36,66	+	+p	+	+	+	+M	+	+	+	+p	+	+	+	+1/2	+	+	+	
2547		st	st	-	-	-		33,34	36,67	+	+p	+	+	+	+M	+	+	+	+p	+	+	+	+1/2	+	+	+	
2548		st	st	st	-	-		33,25	36,48	+	+p	+	+	+	+1/2	+	+	+	+p	+	+	+	+M	+	+	+	
2549		st	st	+p	+M	+		33,02	35,85	+	+M	+	+	+	+m	+	+	+	+p	+	+	+	+M	+	+	+	
2550		st	st	st	-	-		33,21	-	-	st			-	-			-	st			-	st			-	
2551		st	st	+M	+M	+		32,89	32,40	+	+p	+	+	+	+M	+	+	+	+p	+	+	+	+M	+	+	+	
2552		st	st	st	-	-		33,35	37,70	+	+p	+	+	+	+m	+	+	+	+p	+	+	+	+M	+	+	+	
2553		st	st	st	-	-		33,25	35,99	+	+p	+	+	+	+M	+	+	+	+p	+	+	+	+p	+	+	+	
2554		st	st	st	-	-		33,28	-	-	st			-	-			-	st			-	st			-	
2555		st	+p	/	/	+		33,09	38,39	+	+p	+	+	+	+1/2	+	+	+	+p	+	+	+	+M	+	+	+	
2450	6,8	+p	+p	/	/	+	5/5	32,86	23,72	+	+p	+	+	+	+M	+	+	+	+p	+	+	+	+M	+	+	+	5/5
2451		+p	+p	/	/	+		32,63	28,39	+	+p	+	+	+	+M	+	+	+	+p	+	+	+	+M	+	+	+	
2452		+(2)	+(1)	/	/	+		33,26	34,51	+	+p	+	+	+	+M	+	+	+	+p	+	+	+	+M	+	+	+	
2453		+p	+p	/	/	+		34,06	30,31	+	+p	+	+	+	+M	+	+	+	+p	+	+	+	+M	+	+	+	
2454		+p	+p	/	/	+		33,17	23,95	+	+p	+	+	+	+M	+	+	+	+p	+	+	+	+M	+	+	+	

♦ Analyses performed according to the COFRAC accreditation

Appendix 6 - Inclusivity / exclusivity: raw data (initial validation study, 2008)

INCLUSIVITY								
N°	Genus/ species	Serotype	Strain N°	Origin	Inoculation level (CFU/225mL)	PCR APF Classic		
						IC: HEX	Target: FAM	Result
1.	*Escherichia coli*	O157:H7	B177	Slaughterhouse	51	30,96	27,62	+
2.	*Escherichia coli*	O157:H7	BV2	Slaughterhouse	96	31,05	28,28	+
3.	*Escherichia coli*	O157:H7	BR3	Slaughterhouse	73	30,73	28,47	+
4.	*Escherichia coli*	O157:H7	BD4	Slaughterhouse	95	30,39	27,7	+
5.	*Escherichia coli*	O157:H7	ENV177	STEP	85	30,71	28,53	+
6.	*Escherichia coli*	O157:H7	ET8	STEP	132	30,46	27,99	+
7.	*Escherichia coli*	O157:H7	EK9	STEP	117	30,53	26,51	+
8.	*Escherichia coli*	O157:H7	435	Ground beef	73	31,18	28,82	+
9.	*Escherichia coli*	O157:H7	670T	Ground beef	48	30,66	28,25	+
10.	*Escherichia coli*	O157:H7	730T	Ground beef	71	30,7	27,02	+
11.	*Escherichia coli*	O157:H7	226T	Ground beef	105	30,14	27,61	+
12.	*Escherichia coli*	O157:H7	42197-1	Ground beef	98	30,61	27,18	+
13.	*Escherichia coli*	O157:H7	A3612	Ground beef	39	30,47	26,52	+
14.	*Escherichia coli*	O157:H7	A4513	Ground beef	74	30,89	26,65	+
15.	*Escherichia coli*	O157:H7	A1075	Ground beef	114	30,55	26,31	+
16.	*Escherichia coli*	O157:H7	B68	Slaughterhouse	79	30,33	25,33	+
17.	*Escherichia coli*	O157:H7	AT40	Slaughterhouse	61	30,29	25,11	+
18.	*Escherichia coli*	O157:H7	AV36	Slaughterhouse	96	30,61	25,11	+
19.	*Escherichia coli*	O157:H7	AR15	Slaughterhouse	83	30,32	25,12	+
20.	*Escherichia coli*	O157:H7	LS3	Faeces	108	30,55	25,61	+
21.	*Escherichia coli*	O157:H7	AMVT6	Faeces	81	30,02	24,57	+
22.	*Escherichia coli*	O157:H7	ATKP8	Faeces	105	30,24	25,01	+
23.	*Escherichia coli*	O157:H7	AZRS15	Faeces	111	30,16	25,22	+
24.	*Escherichia coli*	O157:H7	R33-9	Bovine faeces	79	29,82	25,07	+

INCLUSIVITY								
N°	Genus/ species	Serotype	Strain N°	Origin	Inoculation level (CFU/225mL)	PCR APF Classic		
						IC: HEX	Target: FAM	Result
25.	*Escherichia coli*	O157:H7	AZ15-6	Bovine faeces	87	30,38	26,27	+
26.	*Escherichia coli*	O157:H7	AQ29-4	Bovine faeces	150	31,02	25,02	+
27.	*Escherichia coli*	O157:H7	AA18-3	Bovine faeces	84	30,45	25,51	+
28.	*Escherichia coli*	O157:H7	LS56	Faeces	155	30,63	24,47	+
29.	*Escherichia coli*	O157:H7	A425TK	Faeces	116	30,11	24,92	+
30.	*Escherichia coli*	O157:H7	A206RP	Faeces	87	30,49	24,4	+
31.	*Escherichia coli*	O157:H7	A778EF	Faeces	169	38,33	21,66	+
32.	*Escherichia coli*	O157:H7	MK41242	Ground beef	163	37,6	22,36	+
33.	*Escherichia coli*	O157:H7	AMK2608	Ground beef	101	37,44	21,88	+
34.	*Escherichia coli*	O157:H7	AMK1506	Ground beef	148	36,95	21,7	+
35.	*Escherichia coli*	O157:H7	AMK1311	Ground beef	149	36,65	21,87	+
36.	*Escherichia coli*	O157:H7	37006ID	Ground beef	183	38,35	21,36	+
37.	*Escherichia coli*	O157:H7	A1518ID	Ground beef	110	35,25	23,09	+
38.	*Escherichia coli*	O157:H7	A1512ID	Ground beef	66	37,83	23,6	+
39.	*Escherichia coli*	O157:H7	A1814ID	Ground beef	57	39,63	23,36	+
40.	*Escherichia coli*	O157:H7	A1989ID	Ground beef	88	37,13	22,94	+
41.	*Escherichia coli*	O157:H7	EF190	Faeces	76	36,55	23,29	+
42.	*Escherichia coli*	O157:H7	Ad686	Bovine slaughterhouse	5	N/A	19,42	+
43.	*Escherichia coli*	O157:H7	CIP103571 (ATCC 35150)	Clinic	91	36,67	22,59	+
44.	*Escherichia coli*	O157:H7	ATCC 43888	/	102	36,38	23,52	+
45.	*Escherichia coli*	O157:H7	Ad485	Ground beef	66	37,09	23,98	+
46.	*Escherichia coli*	O157:H7	Ad486	Ground beef	73	36,08	25,67	+
47.	*Escherichia coli*	O157:H7	Ad487	Ground beef	65	37,08	23,33	+
48.	*Escherichia coli*	O157:H7	Ad488	Ground beef	81	39,34	22,19	+
49.	*Escherichia coli*	O157:H7	Ad489	Ground beef	71	36,84	23,22	+
50.	*Escherichia coli*	O157:H7	ATCC 700728	/	73	36,25	24,02	+

EXCLUSIVITY								
N°	Genus/ species	Serotype	Strain N°	Origin	Inoculation level (CFU/mL)	PCR APF Classic		
						IC: HEX	Target: FAM	Result
1	*Escherichia coli*	O92:H33	JM221	Human (Mexico)	$3,7.10^5$	33,21	N/A	-
2.	*Escherichia coli*	O3:H2	38765	Human (Chili)	$5,0.10^5$	33,84	N/A	-
3.	*Escherichia coli*	O78:H11	H10407	ATCC 35401	$2,5.10^5$	33,45	N/A	-
4.	*Escherichia coli*	O6:H6	EDL1493	Unknown	$3,5.10^5$	33,36	N/A	-
5.	*Escherichia coli*	O6:H10	ECOR10	Human (Sweden)	$4,2.10^5$	33,12	N/A	-
6.	*Escherichia coli*	O111:H21	DEC6a	Human (USA)	$3,2.10^5$	33,34	N/A	-
7.	*Escherichia coli*	O86:H43	ECOR23	Animal (elephant)	$3,5.10^5$	33,54	N/A	-
8.	*Escherichia coli*	O26:H11	DEC9a	Human (USA)	$4,7.10^5$	33,09	N/A	-
9.	*Escherichia coli*	O111:H8	DEC8b	Human (USA)	$4,3.10^5$	33,92	N/A	-
10.	*Escherichia coli*	O128:H2	DEC11a	Human (USA)	$5,6.10^5$	33,59	N/A	-
11.	*Escherichia coli*	O111:H2	DEC12a	Human (UK)	$4,7.10^5$	33,33	N/A	-
12.	*Escherichia coli*	O128:H7	DEC13a	Human (USA)	$3,6.10^5$	33,34	N/A	-
13.	*Escherichia coli*	O78:H12	TX-1	ATCC 43896	$4,2.10^5$	32,67	N/A	-
14.	*Escherichia coli*	O104:H21	ECOR26	Human (USA)	$5,7.10^5$	33,12	N/A	-
15.	*Escherichia coli*	O157:H43	DEC7a	Animal (pork USA)	$4,5.10^5$	33,02	N/A	-
16.	*Escherichia coli*	O55:H7	DEC5d	Human (Sri Lanka)	$4,6.10^5$	33,54	N/A	-
17.	*Escherichia coli*	O44:H18	42	Human (Peru)	$5,6.10^5$	33,02	N/A	-
18.	*Escherichia coli*	O127:H6	E2348/69	Human (UK)	$5,5.10^5$	33,25	N/A	-
19.	*Escherichia coli*	O55:H6	DEC1a	Human (USA)	$4,5.10^5$	33,43	N/A	-
20.	*Escherichia coli*	O18:K1:H7	RS218	Human	$4,8.10^5$	33,46	N/A	-
21.	*Salmonella*	Landau	Ad499	Unknown	$4,3.10^5$	33,12	N/A	-
22.	*Salmonella*	Sternchanze	Ad500	Unknown	$2,6.10^5$	34,03	N/A	-
23.	*Salmonella*	Urbana	Ad501	Unknown	$3,5.10^5$	33,54	N/A	-
24.	*Salmonella*	Wayne	Ad502	Unknown	$3,6.10^5$	33,3	N/A	-
25.	*Hafnia alvei*		88	Pastry	$4,6.10^5$	33,83	N/A	-
26.	*Hafnia alvei*		167	Sausage	$4,5.10^5$	34,59	N/A	-

EXCLUSIVITY								
N°	Genus/ species	Serotype	Strain N°	Origin	Inoculation level (CFU/mL)	PCR APF Classic		
						IC: HEX	Target: FAM	Result
27.	*Citrobacter freundii*		25	Frozen spinach	$3{,}7.10^5$	33,47	N/A	-
28.	*Citrobacter freundii*		104	Ground beef	$6{,}2.10^5$	34,61	N/A	-
29.	*Escherichia vulneris*		127	Raw milk	$3{,}7.10^5$	33,43	N/A	-
30.	*Escherichia vulneris*		134	Pork liver	$5{,}5.10^4$	33,98	N/A	-
31	*Escherichia coli*	O157	Ad525	Bovine feces	$4{,}2.10^5$	33,7	N/A	-
32	*Escherichia coli*	O157	Ad527	Clinic	$5{,}3.10^5$	33,52	N/A	-
33	*Escherichia coli*	O157	Ad524	Dairy industry environment	$4{,}5.10^5$	32,94	N/A	-
34	*Escherichia coli*	O157	Ad526	Bovine feces	$7{,}4.10^5$	34,01	N/A	-
35	*Escherichia coli*	O157:H7-	O1.12.903	Unknown	70	33,42 (8 h)	N/A	-
						35,1 (24 h)	N/A	-
36	*Escherichia coli*	O157:H7-	O1.12.905	Unknown	56	32,72 (8 h)	N/A	-
						35,09 (24 h)	N/A	-

Appendix 7 – Inter-laboratory study: results obtained by the collaborative laboratories and the expert laboratory

Laboratory: **A**
Aerobic mesophilic flora: 2600/g

N° Sample	Reference method: ISO 16654				Alternative method: iQ-Check *E. coli* O157:H7						Agreement
	IMS 24 H		Confirmation	Final result	HEX	FAM	PCR result	Confirmation		Final result	
	CT SMAC	CHROMagar O157						CT SMAC	LatexO157:H7		
A3	-	-	/	-	32,7	N/A	-	/	/	-	**NA**
A4	-	-	/	-	33,3	N/A	-	/	/	-	**NA**
A8	-	-	/	-	33,1	N/A	-	/	/	-	**NA**
A11	-	-	/	-	32,2	N/A	-	/	/	-	**NA**
A14	-	-	/	-	33,1	N/A	-	/	/	-	**NA**
A16	-	-	/	-	32,7	N/A	-	/	/	-	**NA**
A21	-	-	/	-	32,8	N/A	-	/	/	-	**NA**
A22	-	-	/	-	32,8	N/A	-	/	/	-	**NA**
A2	+	+	+	+	40,4	24,5	+	+	+	+	**PA**
A7	+	+	+	+	35,4	26,5	+	+	+	+	**PA**
A10	+	+	+	+	44,1	22,8	+	+	+	+	**PA**
A12	+	+	+	+	N/A	23,2	+	+	+	+	**PA**
A17	+	+	+	+	N/A	23,1	+	+	+	+	**PA**
A18	+	+	+	+	47,5	21,6	+	+	+	+	**PA**
A20	+	+	+	+	41,8	23,5	+	+	+	+	**PA**
A24	+	+	+	+	N/A	21,4	+	+	+	+	**PA**
A1	+	+	+	+	42,2	24,3	+	+	+	+	**PA**
A5	+	+	+	+	36,6	25,7	+	+	+	+	**PA**
A6	+	+	+	+	33,6	26,2	+	+	+	+	**PA**
A9	+	+	+	+	36,7	26,3	+	+	+	+	**PA**
A13	+	+	+	+	43,3	23,6	+	+	+	+	**PA**
A15	+	+	+	+	44,2	23,3	+	+	+	+	**PA**
A19	+	+	+	+	35,7	26,9	+	+	+	+	**PA**
A23	+	+	+	+	N/A	21,2	+	+	+	+	**PA**

Laboratory: **B**
Aerobic mesophilic flora: 9500/g

N° Sample	Reference method: ISO 16654				Alternative method: iQ-Check *E. coli* O157:H7						Agreement
	IMS 24 H		Confirmation	Final result	HEX	FAM	PCR result	Confirmation		Final result	
	CT SMAC	CHROMagar O157						CT SMAC	LatexO157:H7		
B3	-	-	/	-	31,86	34,78	+	-	/	-	**NA**
B4	-	-	/	-	32,06	35,86	+	-	/	-	**NA**
B8	+	-	+(H7-)	+	33,18	37,52	+	+	+	+	**PA**
B11	-	-	/	-	31,87	N/A	-	/	/	-	**NA**
B14	-	-	/	-	32,26	30,84	+	-	/	-	**NA**
B16	-	+	-	-	32,96	38,29	+	-	/	-	**NA**
B21	-	-	/	-	31,62	N/A	-	-	/	-	**NA**
B22	-	-	/	-	33,39	31,39	+	-	/	-	**NA**
B2	+	+	+	+	34,48	34,51	+	+	+	+	**PA**
B7	+	+	+	+	37,95	23,16	+	+	+	+	**PA**
B10	-	+	+	+	33,7	26,03	+	+	+	+	**PA**
B12	+	+	+(H7-)	+	32,6	25,64	+	+	+(H7-)	+	**PA**
B17	+	+	+	+	32,99	26,96	+	+	+	+	**PA**
B18	+	+	+(H7-)	+	32,84	27,71	+	+	+	+	**PA**
B20	-	+	+	+	33,51	26,27	+	+	+	+	**PA**
B24	+	+	+	+	33,93	24,79	+	+	+	+	**PA**
B1	+	+	+	+	34,06	25,09	+	+	+	+	**PA**
B5	+	+	+	+	32,06	24,75	+	+	+	+	**PA**
B6	+	+	+	+	33,62	24,5	+	+	+	+	**PA**
B9	+	+	+	+	31,96	26,11	+	+	+(H7-)	+	**PA**
B13	+	+	+	+	33,43	23,45	+	+	+	+	**PA**
B15	-	+	+	+	33,97	24,81	+	+	+(H7-)	+	**PA**
B19	+	+	+	+	33,05	26,56	+	+	+	+	**PA**
B23	+	+	+(H7-)	+	33,81	25,21	+	+	+	+	**PA**

Laboratory: **C**
Aerobic mesophilic flora: 7100/g

N° Sample	Reference method: ISO 16654				Alternative method: iQ-Check *E. coli* O157:H7						Agreement
	IMS 24 H		Confirmation	Final result	HEX	FAM	PCR result	Confirmation		Final result	
	CT SMAC	CHROMagar O157						CT SMAC	LatexO157:H7		
C3	-	-	/	-	29,97	N/A	-	/	/	-	**NA**
C4	-	-	/	-	31,53	N/A	-	/	/	-	**NA**
C8	-	-	/	-	30,22	N/A	-	/	/	-	**NA**
C11	-	-	/	-	30,19	N/A	-	/	/	-	**NA**
C14	-	-	/	-	30,58	N/A	-	/	/	-	**NA**
C16	-	-	/	-	31,17	N/A	-	/	/	-	**NA**
C21	-	-	/	-	30,33	N/A	-	/	/	-	**NA**
C22	-	-	/	-	29,33	N/A	-	/	/	-	**NA**
C2	+	+	+	+	31,46	30,78	+	+	+	+	**PA**
C7	+	+	+	+	30,58	30,32	+	+	+	+	**PA**
C10	+	+	+	+	32,32	27,27	+	+	+	+	**PA**
C12	+	+	+	+	31,14	30,45	+	+	+	+	**PA**
C17	+	+	+	+	28,57	30,13	+	+	+	+	**PA**
C18	+	+	+	+	31,1	29,39	+	+	+	+	**PA**
C20	+	+	+	+	30,21	29,12	+	+	+	+	**PA**
C24	+	+	+	+	31,42	29,82	+	+	+	+	**PA**
C1	+	+	+	+	31,13	30,4	+	+	+	+	**PA**
C5	+	+	+	+	30,24	29,19	+	+	+	+	**PA**
C6	+	+	+	+	30,78	29,92	+	+	+	+	**PA**
C9	+	+	+	+	29,66	29,19	+	+	+	+	**PA**
C13	+	+	+	+	31,11	29,51	+	+	+	+	**PA**
C15	+	+	+	+	31,83	29,74	+	+	+	+	**PA**
C19	+	+	+	+	35,55	23,72	+	+	+	+	**PA**
C23	+	+	+	+	30,75	28,86	+	+	+	+	**PA**

Laboratory: **D**
Aerobic mesophilic flora: 770/g

N° Sample	Reference method: ISO 16654				Alternative method: iQ-Check *E. coli* O157:H7						Agreement
	IMS 24 H		Confirmation	Final result	HEX	FAM	PCR result	Confirmation		Final result	
	CT SMAC	CHROMagar O157						CT SMAC	LatexO157:H7		
D3	-	-	/	-	39,69	N/A	-	/	/	-	**NA**
D4	-	-	/	-	38,98	N/A	-	/	/	-	**NA**
D8	-	-	/	-	39,27	N/A	-	/	/	-	**NA**
D11	-	-	/	-	38,54	N/A	-	/	/	-	**NA**
D14	-	-	/	-	39,26	N/A	-	/	/	-	**NA**
D16	-	-	/	-	39,14	N/A	-	/	/	-	**NA**
D21	-	-	/	-	37,32	N/A	-	/	/	-	**NA**
D22	-	-	/	-	39,36	N/A	-	/	/	-	**NA**
D2	+	+	+	+	N/A	28,77	+	+ims	+	+	**PA**
D7	+	+	+	+	N/A	27,88	+	+	+	+	**PA**
D10	+	+	+	+	N/A	26,45	+	+ims	+	+	**PA**
D12	+	+	+	+	N/A	26,46	+	+ims	+	+	**PA**
D17	+	+	+	+	N/A	27,43	+	+ims	+	+	**PA**
D18	+	+	+	+	N/A	25,74	+	+ims	+	+	**PA**
D20	+	+	+	+	N/A	26,71	+	+ims	+	+	**PA**
D24	+	+	+	+	N/A	25,55	+	+	+	+	**PA**
D1	+	+	+	+	N/A	24,64	+	+	+	+	**PA**
D5	+	+	+	+	N/A	25,73	+	+	+	+	**PA**
D6	+	+	+	+	N/A	24,56	+	+	+	+	**PA**
D9	+	+	+	+	N/A	24,56	+	+	+	+	**PA**
D13	+	+	+	+	N/A	26,58	+	+	+	+	**PA**
D15	+	+	+	+	N/A	26,5	+	+	+	+	**PA**
D19	+	+	+	+	N/A	25,46	+	+	+	+	**PA**
D23	+	+	+	+	N/A	24,04	+	+	+	+	**PA**

Laboratory: **E**
Aerobic mesophilic flora: 2700/g

N° Sample	Reference method: ISO 16654				Alternative method: iQ-Check *E. coli* O157:H7						Agreement
	IMS 24 H		Confirmation	Final result	HEX	FAM	PCR result	Confirmation		Final result	
	CT SMAC	CHROMagar O157						CT SMAC	LatexO157:H7		
E3	+	-	-	-	39,54	N/A	-	/	/	-	**NA**
E4	+	-	-	-	32,31	N/A	-	/	/	-	**NA**
E8	-	-	/	-	33,94	N/A	-	/	/	-	**NA**
E11	+	-	-	-	33,67	N/A	-	/	/	-	**NA**
E14	-	-	/	-	34,37	N/A	-	/	/	-	**NA**
E16	+	-	-	-	35,16	N/A	-	/	/	-	**NA**
E21	+	-	-	-	36,65	N/A	-	/	/	-	**NA**
E22	+	-	-	-	33,7	N/A	-	/	/	-	**NA**
E2	+	+	+	+	32,2	27,82	+	+	+	+	**PA**
E7	+	+	+	+	36,1	27,97	+	+	+	+	**PA**
E10	+	+	+	+	33,38	26,14	+	+	+	+	**PA**
E12	+	+	+	+	34,06	28,29	+	+	+	+	**PA**
E17	+	+	+	+	33,57	27,32	+	+	+	+	**PA**
E18	+	+	+	+	33,69	27,75	+	+	+	+	**PA**
E20	+	+	+	+	34,56	27,91	+	+	+	+	**PA**
E24	+	+	+	+	37,82	24,6	+	+	+	+	**PA**
E1	+	+	+	+	32,85	29,41	+	+	+	+	**PA**
E5	+	+	+	+	33,09	25,69	+	+	+	+	**PA**
E6	+	+	+	+	32,77	27,23	+	+	+	+	**PA**
E9	+	+	+	+	35,21	26,86	+	+	+	+	**PA**
E13	+	+	+	+	31,97	27,39	+	+	+	+	**PA**
E15	+	+	+	+	35,5	24,94	+	+	+	+	**PA**
E19	+	+	+	+	34,16	26,94	+	+	+	+	**PA**
E23	+	+	+	+	33,35	27,38	+	+	+	+	**PA**

Laboratory: **G**
Aerobic mesophilic flora: 8000/g

N° Sample	Reference method: ISO 16654				Alternative method: iQ-Check *E. coli* O157:H7						Agreement
	IMS 24 H		Confirmation	Final result	HEX	FAM	PCR result	Confirmation		Final result	
	CT SMAC	CHROMagar O157						CT SMAC	LatexO157:H7		
G3	-	-	/	-	36,12	N/A	-	/	/	-	**NA**
G4	-	-	/	-	35,27	N/A	-	/	/	-	**NA**
G8	-	-	/	-	35,38	N/A	-	/	/	-	**NA**
G11	-	-	/	-	36,28	N/A	-	/	/	-	**NA**
G14	-	-	/	-	34,88	N/A	-	/	/	-	**NA**
G16	-	-	/	-	35,04	N/A	-	/	/	-	**NA**
G21	-	-	/	-	36,16	N/A	-	/	/	-	**NA**
G22	-	-	/	-	35,54	N/A	-	/	/	-	**NA**
G2	+	+	+	+	N/A	23,9	+	+	+	+	**PA**
G7	+	+	+	+	34,76	31,62	+	+	+	+	**PA**
G10	+	+	+(H7-)	+	37,81	30,16	+	+	+	+	**PA**
G12	+	+	+	+	35,58	31,52	+	+	+	+	**PA**
G17	+	+	+	+	N/A	24,24	+	+	+	+	**PA**
G18	+	+	+	+	36,86	30,9	+	+	+	+	**PA**
G20	+	+	+	+	35,45	31,28	+	+	+	+	**PA**
G24	+	+	+	+	34,64	30,12	+	+	+	+	**PA**
G1	+	+	+	+	N/A	25,28	+	+	+	+	**PA**
G5	+	+	+	+	43,28	26,02	+	+	+	+	**PA**
G6	+	+	+	+	N/A	22,31	+	+	+	+	**PA**
G9	+	+	+	+	37,84	28,63	+	+	+	+	**PA**
G13	+	+	+	+	36,71	29,63	+	+	+	+	**PA**
G15	+	+	+(H7-)	+	35,42	30,25	+	+	+	+	**PA**
G19	+	+	+	+	36,32	29,26	+	+	+	+	**PA**
G23	+	+	+	+	37,05	29,23	+	+	+	+	**PA**

Laboratory: **H**
Aerobic mesophilic flora: 3000/g

N° Sample	Reference method: ISO 16654				Alternative method: iQ-Check *E. coli* O157:H7						Agreement
	IMS 24 H		Confirmation	Final result	HEX	FAM	PCR result	Confirmation		Final result	
	CT SMAC	CHROMagar O157						CT SMAC	LatexO157:H7		
H3	-	-	/	-	32,78	N/A	-	/	/	-	**NA**
H4	-	-	/	-	45,03	N/A	-	/	/	-	**NA**
H8	-	-	/	-	33,05	N/A	-	/	/	-	**NA**
H11	-	-	/	-	40,6	N/A	-	/	/	-	**NA**
H14	-	-	/	-	37,65	N/A	-	/	/	-	**NA**
H16	-	-	/	-	30,78	N/A	-	/	/	-	**NA**
H21	-	-	/	-	36,12	N/A	-	/	/	-	**NA**
H22	-	-	/	-	37,18	N/A	-	/	/	-	**NA**
H2	+	+	+	+	32,63	27,08	+	+	+	+	**PA**
H7	+	+	+	+	38,28	25,59	+	+	+	+	**PA**
H10	+	+	+	+	40,46	27,17	+	+	+	+	**PA**
H12	+	+	+	+	43,73	26,48	+	+	+	+	**PA**
H17	+	+	+	+	34,27	27,15	+	+	+	+	**PA**
H18	+	+	+	+	36,75	26,61	+	+	+	+	**PA**
H20	+	+	+	+	37,82	26,34	+	+	+	+	**PA**
H24	+	+	+	+	33,08	26,98	+	+	+	+	**PA**
H1	+	+	+	+	36,38	26,3	+	+	+	+	**PA**
H5	+	+	+	+	31,85	26,99	+	+	+	+	**PA**
H6	+	+	+	+	39,57	26,76	+	+	+	+	**PA**
H9	+	+	+	+	37,04	25,34	+	+	+	+	**PA**
H13	+	+	+	+	36,32	25,44	+	+	+	+	**PA**
H15	+	+	+	+	34,64	27,04	+	+	+	+	**PA**
H19	+	+	+	+	36,36	23,55	+	+	+	+	**PA**
H23	+	+	+	+	39,01	23,81	+	+	+	+	**PA**

Laboratory: **I**
Aerobic mesophilic flora: 3100/g

N° Sample	Reference method: ISO 16654				Alternative method: iQ-Check *E. coli* O157:H7						Agreement
	IMS 24 H		Confirmation	Final result	HEX	FAM	PCR result	Confirmation		Final result	
	CT SMAC	CHROMagar O157						CT SMAC	LatexO157:H7		
I3	-	-	/	-	33,4	N/A	-	/	/	-	**NA**
I4	-	-	/	-	34,1	N/A	-	/	/	-	**NA**
I8	-	-	/	-	32,4	N/A	-	/	/	-	**NA**
I11	-	-	/	-	35,5	N/A	-	/	/	-	**NA**
I14	-	-	/	-	32,8	N/A	-	/	/	-	**NA**
I16	-	-	/	-	33,8	N/A	-	/	/	-	**NA**
I21	-	-	/	-	35,4	N/A	-	/	/	-	**NA**
I22	-	-	/	-	35	N/A	-	/	/	-	**NA**
I2	-	-	/	-	38,7	25,5	+	+	+	+	**PD**
I7	+	+	+	+	35,2	26	+	+	+	+	**PA**
I10	+	+	+	+	32,5	26,8	+	+	+	+	**PA**
I12	+	+	+	+	37	26,2	+	+	+	+	**PA**
I17	+	+	+	+	36,8	25,9	+	+	+	+	**PA**
I18	+	+	+	+	32,9	30,7	+	+	+	+	**PA**
I20	+	+	+	+	33,4	27	+	+	+	+	**PA**
I24	+	+	+	+	38,3	34,7	+	+	+	+	**PA**
I1	+	+	+	+	36,1	24,2	+	+	+	+	**PA**
I5	+	+	+	+	38,3	23,2	+	+	+	+	**PA**
I6	+	+	+	+	38,2	24,3	+	+	+	+	**PA**
I9	+	+	+	+	42,9	23,3	+	+	+	+	**PA**
I13	+	+	+	+	35,4	23,8	+	+	+	+	**PA**
I15	+	+	+	+	42,6	23,4	+	+	+	+	**PA**
I19	+	+	+	+	37,3	24,4	+	+	+	+	**PA**
I23	+	+	+	+	43,2	23,3	+	+	+	+	**PA**

Laboratory: **J**
Aerobic mesophilic flora: 4700/g

N° Sample	Reference method: ISO 16654				Alternative method: iQ-Check *E. coli* O157:H7						Agreement
	IMS 24 H		Confirmation	Final result	HEX	FAM	PCR result	Confirmation		Final result	
	CT SMAC	CHROMagar O157						CT SMAC	LatexO157:H7		
J3	-	-	/	-	34,1	N/A	-	/	/	-	**NA**
J4	-	+	-	-	34,8	N/A	-	/	/	-	**NA**
J8	-	-	/	-	33,9	N/A	-	/	/	-	**NA**
J11	-	-	/	-	34,0	N/A	-	/	/	-	**NA**
J14	-	+	-	-	33,7	N/A	-	/	/	-	**NA**
J16	-	+	-	-	34,3	N/A	-	/	/	-	**NA**
J21	-	-	/	-	33,9	N/A	-	/	/	-	**NA**
J22	-	-	/	-	34,3	N/A	-	/	/	-	**NA**
J2	-	-	/	-	35,2	30,7	+	+	+	+	**PD**
J7	+	+	+	+	36,2	33,0	+	+	+	+	**PA**
J10	+	-	-	-	34,6	31,0	+	+	+	+	**PD**
J12	+	+	+	+	34,7	31,9	+	+	+	+	**PA**
J17	+	+	+	+	34,8	31,0	+	+	+	+	**PA**
J18	+	+	+	+	34,3	36,7	+	+	+	+	**PA**
J20	+	+	+	+	34,3	32,3	+	+	+	+	**PA**
J24	+	+	+	+	35,2	33,5	+	+	+	+	**PA**
J1	+	+	+	+	35,4	31,4	+	+	+	+	**PA**
J5	+	+	+	+	35,7	30,0	+	+	+	+	**PA**
J6	+	+	+	+	35,0	30,0	+	+	+	+	**PA**
J9	+	+	+	+	35,5	28,0	+	+	+	+	**PA**
J13	+	+	+	+	35,2	30,9	+	+	+	+	**PA**
J15	+	+	+	+	35,4	29,0	+	+	+	+	**PA**
J19	+	+	+	+	35,2	30,9	+	+	+	+	**PA**
J23	+	+	+	+	35,1	29,0	+	+	+	+	**PA**

Laboratory: **K**
Aerobic mesophilic flora: 6900/g

N° Sample	Reference method: ISO 16654				Alternative method: iQ-Check *E. coli* O157:H7						Agreement
	IMS 24 H		Confirmation	Final result	HEX	FAM	PCR result	Confirmation		Final result	
	CT SMAC	CHROMagar O157						CT SMAC	LatexO157:H7		
K3	-	-	/	-	+	-	-	/	/	-	**NA**
K4	-	-	/	-	+	-	-	/	/	-	**NA**
K8	-	-	/	-	+	-	-	/	/	-	**NA**
K11	+	+	+	+	+	+	+	+	+	+	**PA**
K14	+	+	+	+	+	+	+	+	+	+	**PA**
K16	+	+	+(H7-)	+	+	-	-	/	/	-	**ND**
K21	+	-	-	-	+	-	-	/	/	-	**NA**
K22	-	-	/	-	+	-	-	/	/	-	**NA**
K2	+	+	+	+	+	+	+	+	+	+	**PA**
K7	+	+	+	+	+	+	+	+	+	+	**PA**
K10	+	+	+	+	+	+	+	+	+	+	**PA**
K12	+	+	+	+	+	+	+	+	+	+	**PA**
K17	+	+	+	+	+	+	+	+	+	+	**PA**
K18	+	+	+	+	+	+	+	+	+	+	**PA**
K20	+	+	+	+	+	+	+	+	+	+	**PA**
K24	+	+	+	+	+	+	+	+	+	+	**PA**
K1	+	+	+	+	+	+	+	+	+	+	**PA**
K5	+	+	+	+	+	+	+	+	+	+	**PA**
K6	+	+	+	+	+	+	+	+	+	+	**PA**
K9	+	+	+	+	+	+	+	+	+	+	**PA**
K13	+	+	+	+	+	+	+	+	+	+	**PA**
K15	+	+	+	+	+	+	+	+	+	+	**PA**
K19	+	+	+	+	+	+	+	+	+	+	**PA**
K23	+	+	+	+	+	+	+	+	+	+	**PA**

Laboratory: **L**
Aerobic mesophilic flora: 2500/g

N° Sample	Reference method: ISO 16654				Alternative method: iQ-Check *E. coli* O157:H7						Agreement
	IMS 24 H		Confirmation	Final result	HEX	FAM	PCR result	Confirmation		Final result	
	CT SMAC	CHROMagar O157						CT SMAC	LatexO157:H7		
L3	+	+	+	+	33,07	N/A	-	/	/	-	**ND**
L4	+	-	+	+	33,55	N/A	-	/	/	-	**ND**
L8	+	-	-	-	34,01	N/A	-	/	/	-	**NA**
L11	-	+	-	-	32,75	N/A	-	/	/	-	**NA**
L14	-	-	/	-	33,6	N/A	-	/	/	-	**NA**
L16	-	+	+	+	32,54	N/A	-	/	/	-	**ND**
L21	+	-	-	-	33,8	N/A	-	/	/	-	**NA**
L22	+	-	+	+	33,24	N/A	-	/	/	-	**ND**
L2	+	+	+	+	33,6	31,28	+	+	+	+	**PA**
L7	+	+	+	+	33,6	34,17	+	+	+	+	**PA**
L10	+	+	+	+	33,41	32,47	+	+	+	+	**PA**
L12	+	+	+	+	32,9	30,97	+	+	+	+	**PA**
L17	+	+	+	+	33,35	32,51	+	+	+	+	**PA**
L18	+	+	+	+	32,65	31,48	+	+	+	+	**PA**
L20	+	+	+	+	34,3	29,11	+	+	+	+	**PA**
L24	+	+	+	+	33,41	28,7	+	+	+	+	**PA**
L1	+	+	+	+	34,37	29,13	+	+	+	+	**PA**
L5	+	+	+	+	34,43	30,25	+	+	+	+	**PA**
L6	+	+	+	+	34,93	30,4	+	+	+	+	**PA**
L9	+	+	+	+	34,0	30,6	+	+	+	+	**PA**
L13	+	+	+	+	33,67	29,24	+	+	+	+	**PA**
L15	+	+	+	+	34,44	30,66	+	+	+	+	**PA**
L19	+	+	+	+	34,74	29,21	+	+	+	+	**PA**
L23	+	+	+	+	41,31	25,74	+	+	+	+	**PA**

Laboratory: **M**
Aerobic mesophilic flora: 3200/g

N° Sample	Reference method: ISO 16654				Alternative method: iQ-Check *E. coli* O157:H7						Agreement
	IMS 24 H		Confirmation	Final result	HEX	FAM	PCR result	Confirmation		Final result	
	CT SMAC	CHROMagar O157						CT SMAC	LatexO157:H7		
M3	-	-	/	-	36,7	N/A	-	/	/	-	**NA**
M4	-	-	/	-	36	N/A	-	/	/	-	**NA**
M8	-	-	/	-	35,7	N/A	-	/	/	-	**NA**
M11	-	-	/	-	35,8	N/A	-	/	/	-	**NA**
M14	-	-	/	-	35,3	N/A	-	/	/	-	**NA**
M16	-	-	/	-	35,8	N/A	-	/	/	-	**NA**
M21	-	-	/	-	36	N/A	-	/	/	-	**NA**
M22	-	-	/	-	35,5	N/A	-	/	/	-	**NA**
M2	+	+	+	+	40,8	28,3	+	+	+	+	**PA**
M7	+	+	+	+	46,5	26,6	+	+	+	+	**PA**
M10	+	+	+	+	N/A	24,1	+	+	+	+	**PA**
M12	+	+	+	+	42,4	26,2	+	+	+	+	**PA**
M17	+	+	+	+	47,2	26,4	+	+	+	+	**PA**
M18	+	+	+	+	39,4	27,5	+	+	+	+	**PA**
M20	+	+	+	+	47,7	26	+	+	+	+	**PA**
M24	+	+	+	+	37,3	29,4	+	+	+	+	**PA**
M1	+	+	+	+	N/A	23,5	+	+	+	+	**PA**
M5	+	+	+	+	N/A	24	+	+	+	+	**PA**
M6	+	+	+	+	39,7	24,9	+	+	+	+	**PA**
M9	+	+	+	+	N/A	23,3	+	+	+	+	**PA**
M13	+	+	+	+	N/A	24,1	+	+	+	+	**PA**
M15	+	+	+	+	N/A	25,2	+	+	+	+	**PA**
M19	+	+	+	+	47,9	26,1	+	+	+	+	**PA**
M23	+	+	+	+	45,5	26,6	+	+	+	+	**PA**

Laboratory: **N**
Aerobic mesophilic flora: 3400/g

N° Sample	Reference method: ISO 16654				Alternative method: iQ-Check *E. coli* O157:H7						Agreement
	IMS 24 H		Confirmation	Final result	HEX	FAM	PCR result	Confirmation		Final result	
	CT SMAC	CHROMagar O157						CT SMAC	LatexO157:H7		
N3	-	-	/	-	34,33	N/A	-	/	/	-	**NA**
N4	-	-	/	-	32,69	N/A	-	/	/	-	**NA**
N8	-	-	/	-	34,11	N/A	-	/	/	-	**NA**
N11	-	-	/	-	31,48	N/A	-	/	/	-	**NA**
N14	-	-	/	-	34,79	N/A	-	/	/	-	**NA**
N16	-	-	/	-	34,88	N/A	-	/	/	-	**NA**
N21	-	-	/	-	33,53	N/A	-	/	/	-	**NA**
N22	-	-	/	-	33,73	N/A	-	/	/	-	**NA**
N2	+	+	+	+	35,05	30,17	+	+	+	+	**PA**
N7	+	+	+	+	31,37	31,76	+	+	+	+	**PA**
N10	+	+	+	+	32,35	31,46	+	+	+	+	**PA**
N12	+	+	+	+	34,28	33,83	+	+	+	+	**PA**
N17	+	+	+	+	33,69	31,52	+	+	+	+	**PA**
N18	+	+	+	+	34,63	33,18	+	+	+	+	**PA**
N20	+	+	+	+	34,67	30,59	+	+	+	+	**PA**
N24	+	+	+	+	34,74	33,71	+	+	+	+	**PA**
N1	+	+	+	+	35,6	30,5	+	+	+	+	**PA**
N5	+	+	+	+	41,29	27,02	+	+	+	+	**PA**
N6	+	+	+	+	34,85	30,28	+	+	+	+	**PA**
N9	+	+	+	+	32,12	29,7	+	+	+	+	**PA**
N13	+	+	+	+	34,86	30,53	+	+	+	+	**PA**
N15	+	+	+	+	35,14	30,84	+	+	+	+	**PA**
N19	+	+	+	+	34,49	29,48	+	+	+	+	**PA**
N23	+	+	+	+	34,47	29,63	+	+	+	+	**PA**

Laboratory: **ADRIA**
Aerobic mesophilic flora: 2900/g

N° Sample	Reference method: ISO 16654♦				Alternative method: iQ-Check *E. coli* O157:H7						Agreement
	IMS 24 H		Confirmation	Final result	HEX	FAM	PCR result	Confirmation		Final result	
	CT SMAC	CHROMagar O157						CT SMAC	LatexO157:H7		
O3	-	-	/	-	38,19	N/A	-	/	/	-	**NA**
O4	-	-	/	-	39,77	N/A	-	/	/	-	**NA**
O8	-	-	/	-	35,44	N/A	-	/	/	-	**NA**
O11	-	-	/	-	36,39	N/A	-	/	/	-	**NA**
O14	-	-	/	-	35,3	N/A	-	/	/	-	**NA**
O16	-	-	/	-	36,41	N/A	-	/	/	-	**NA**
O21	-	-	/	-	35,67	N/A	-	/	/	-	**NA**
O22	-	-	/	-	36,67	N/A	-	/	/	-	**NA**
O2	+	+	+	+	42,93	31,25	+	+	+	+	**PA**
O7	+	+	+	+	42,29	29,58	+	+	+	+	**PA**
O10	+	+	+	+	38,17	31,65	+	+	+	+	**PA**
O12	-	-	/	-	N/A	29,68	+	+	+	+	**PD**
O17	+	+	+	+	N/A	29,15	+	+	+	+	**PA**
O18	-	-	/	-	44,89	29,62	+	+	+	+	**PD**
O20	+	+	+	+	N/A	29,82	+	+	+	+	**PA**
O24	+	+	+	+	43,95	28,4	+	+	+	+	**PA**
O1	+	+	+	+	N/A	29,34	+	+	+	+	**PA**
O5	+	+	+	+	36,14	32,14	+	+	+	+	**PA**
O6	+	+	+	+	28,56	30,05	+	+	+	+	**PA**
O9	+	+	+	+	N/A	28,72	+	+	+	+	**PA**
O13	+	+	+	+	N/A	27,78	+	+	+	+	**PA**
O15	+	+	+	+	N/A	28,09	+	+	+	+	**PA**
O19	+	+	+	+	N/A	27,03	+	+	+	+	**PA**
O23	+	+	+	+	N/A	27,57	+	+	+	+	**PA**

♦ Analyses performed according to the COFRAC accreditation