ADRIA
FOOD EXPERTISE

cofrac
ESSAIS
(TESTING)

ACCREDITATION
N°1-0144
scope available
on www.COFRAC.fr

NF VALIDATION
Validation of alternative analytical methods
Application in food microbiology

Summary report
Validation study according to EN ISO 16140-2:2016

BAX® System Real-Time PCR Assay for *E. coli* O157:H7 method
(Certificate number: QUA 18/07 - 07/10)
for the detection of *E. coli* O157:H7
in raw beef meat, and fresh vegetables

Qualitative method

Expert Laboratory:	**ADRIA Développement** ZA Creac'h Gwen 29196 Quimper Cedex (France)
For:	**Hygiena- Qualicon Diagnostics** 2 Boulden Circle, Suite 6 New Castle, Delaware 19720 (USA)

This report consists of 62 pages, including 8 appendices.
Only copies including the totality of this report are authorized.
Competencies of the expert laboratory are certified by COFRAC accreditation for the analyses marked with the symbol◆.

Version 0
29 July 2022

Quality Assurance documents related to this study can be consulted upon request from **QUALICON DIAGNOSTICS LLC**.

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♦	NF EN ISO 16654 (2001): Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Escherichia coli* O157 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Escherichia coli O157 - Amendment 1 (2017): annex B : result of interlaboratory studies
Alternative method	**BAX® System Real-Time PCR Assay for *E. coli* O157:H7**
Scope	☒ **Raw beef meat** ☒ **Fresh vegetables**
Certification organism	AFNOR Certification (http://nf-validation.afnor.org/)

♦ Analyses performed according to the COFRAC accreditation

1 INTRODUCTION

The **BAX® System Real-Time PCR Assay for *E. coli* O157:H7 detection** was initially validated on 2nd of March 2010 (Certificate number QUA 18/07 - 07/10).

A summary of the different validation studies is listed below:

Date	Study
March 2011	Extension study for BAX® System software version 2.8 with the Q7 Instrument
March 2014	Renewal study
January 2018	Extension study for a software modification (from version 2.9 to version 3.6) as well as an update in order to be in agreement with ISO 16140-2:2016
May 2018	Renewal study
June 2022	Renewal study and extension study for moving from software version 3.6 to version 4.0 without any additional testing as the modification has no impact on the algorithm and are considered as minor

2 METHOD PROTOCOLS

2.1 Alternative method

2.1.1 Principle

The BAX® System is based on real-time PCR technology using internal Scorpions™ probes.

2.1.2 Protocol

The flow diagram of the alternative method is provided in **Appendices 1 and 2.**
Two enrichment protocols are proposed according to the food category (See Table 1).

Table 1 - Enrichment protocols

Category	Enrichment broth	Incubation and temperature time
Raw beef meat	Pre-warmed BAX® System MP Broth	7 h to 24 h at 42°C± 1°C
Fresh vegetables	Pre-warmed BAX® System MP Broth	8 h to 24 h at 42°C± 1°C

- *DNA extraction lysis*
  * Addition of 20 µl enrichment broth to 200 µl lysis buffer mixture (lysis buffer is prepared as follows: mix 150 µl protease to 12 ml lysis buffer)
  * 20 minutes at 37°C
  * 10 minutes at 95°C
  * Cool in a cooling block (2-8°C) for at least 5 minutes

- *Amplification*
  * Transfer 30 µl of the lysate in a PCR tube in a cooling block
  * Run the PCR in the automate

- *Detection*

  The fluorescence is measured directly by the BAX® System Q7 PCR instrument, which provides positive or negative result interpretation.

- *Confirmation of positive results* by streaking 50 µl of enrichment broth onto CT-SMAC and confirming characteristic colonies with a O157 and H7 latex test after purification step on a non-selective agar plate. If the results are not confirmed using this direct streaking protocol, the QUALICON's confirmation protocol is performed (See **Appendix 2 & Technical bulletin MTD-2001 "Confirmation Protocol for *E. coli* O157:H7"**).

2.1.3 Restriction

There is no restriction.

2.2 Reference methods♦

The reference method is EN ISO 16654 (2001) and its amendment the EN ISO 16654/A1 (2017) method: Microbiology of food and animal feeding stuffs -

♦ Analysis performed according to the COFRAC accreditation

Horizontal method for the detection of *Escherichia coli* O157 (See **Appendix 3**).

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 AND EXTENSION STUDIES: RESULTS

3.1 Method comparison study

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

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

3.1.1.1 Number and nature of the samples

Combining the different studies (initial and extension), 69 positive samples (for the short enrichment protocol), 76 positive samples (for 24 h enrichment protocol) and 65 negative samples (for 24 h enrichment protocol) were obtained for a total of 141 samples.

The repartition per category and type is given in Table 2.

Table 2 - Repartition per category and type

Category			Type		Protocol	Positive samples	Negative samples	Total
1	Raw beef meat	7h	a	Fresh	MP Broth 7h at 42°C	12	11	23
			b	Frozen	MP Broth 7h at 42°C	13	17	30
			c	Flavored or marinated	MP Broth 7h at 42°C	11	9	20
					Total	36	37	73
		24h	a	Fresh	MP Broth 24h at 42°C	12	11	23
			b	Frozen	MP Broth 24h at 42°C	14	16	30
			c	Flavored or marinated	MP Broth 24h at 42°C	12	8	20
					Total	38	35	73
2	Vegetable products	8h	a	Fresh	MP Broth 8h at 42°C	12	13	25
			b	RTE/RTC	MP Broth 8h at 42°C	9	12	21
			c	Frozen	MP Broth 8h at 42°C	12	10	22
					Total	33	35	68
		24h	a	Fresh	MP Broth 24h at 42°C	13	12	25
			b	RTE/RTC	MP Broth 24h at 42°C	11	10	21
			c	Frozen	MP Broth 24h at 42°C	14	8	22
					Total	38	30	68
All categories			**7 h or 8 h incubation time**			**69**	**72**	**141**
			24 h incubation time			**76**	**65**	**141**

3.1.1.2 Artificial contamination of the samples

The strains were stressed using various injury protocols. For the spiking protocol, the injury treatment and the efficiency were determined by enumeration on selective agar plate (CT-SMAC) and non-selective agar plate (TSYEA). The artificial contaminations are presented in **Appendix 4**.

76 samples were artificially contaminated. 66 samples for the short enrichment step and 72 samples for 24 h enrichment step gave a positive result. 3 samples for the short enrichment step and 4 samples for 24 h enrichment step were naturally contaminated.

The repartition of the positive naturally and artificially contaminated samples is provided in Table 3.

Table 3 - Repartition of the positive naturally and artificially contaminated samples

		Naturally contaminated	Artificially contaminated						Total
			Seeding protocol			Spiking protocol			
			≤3	3<x≤10	>10	≤5	5< x<10	>10	
7 or 8h protocol	Positive samples	3	19	0	0	31	11	5	69
	%	4,3	27,5	0,0	0,0	44,9	15,9	7,2	100
24h protocol	Positive samples	4	19	0	0	35	13	5	76
	%	5,3	25,0	0,0	0,0	46,1	17,1	6,6	100

Taking into account all the studies, 4.3 % (for the short enrichment step) and 5.3 % (for the 24 h enrichment step) of the samples were naturally contaminated.

3.1.1.3 Protocols applied during the validation study

- **Incubation time**

For the initial validation study and for the extension study, the shortest incubation times were tested (7h or 8h) as well as 24h incubation time.

- **Confirmations**

The confirmations were done by direct streaking of 50µl enrichment broth onto CT-SMAC.
The typical colonies were confirmed by O157 and H7 latex tests after purification step on a non-selective agar plate.
In case the direct streaking protocol failed to get confirmation, QUALICON's confirmation protocol was carried out (Technical bulletin MTD-2001 "Confirmation Protocol for *E. coli* O157:H7").

3.1.1.4 Test results

Raw data per category are given in **Appendix 5**. The results are given in Table 4.

Table 4 – Summary of results obtained with the reference and the alternative methods

Category			PA	NA*	PD	ND**	PPND	PPNA
1	Raw beef meat	7h	17	37	13	6	0	0
		24h	20	35	15	3	0	0
2	Vegetable products	8h	15	35	12	6	0	0
		24h	18	30	17	3	0	0
All categories		**7 h or 8 h incubation time**	**32**	**72**	**25**	**12**	**0**	**0**
		24 h incubation time	**38**	**65**	**32**	**6**	**0**	**0**

* 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			Type		Protocol	PA	NA*	PD	ND**	PPND	PPNA	SE_{alt} %	SE_{ref} %	RT %	FPR %
1	Raw beef meat	7h	a	Fresh	MP Broth 7h at 42°C	7	11	4	1	0	0	91,7	66,7	78,3	0,0
			b	Frozen	MP Broth 7h at 42°C	8	17	3	2	0	0	84,6	76,9	83,3	0,0
			c	Flavored or marinated	MP Broth 7h at 42°C	2	9	6	3	0	0	72,7	45,5	55,0	0,0
			Total			17	37	13	6	0	0	83,3	63,9	74,0	0,0
		24h	a	Fresh	MP Broth 24h at 42°C	8	11	4	0	0	0	100,0	66,7	82,6	0,0
			b	Frozen	MP Broth 24h at 42°C	9	16	4	1	0	0	92,9	71,4	83,3	0,0
			c	Flavored or marinated	MP Broth 24h at 42°C	3	8	7	2	0	0	83,3	41,7	55,0	0,0
			Total			20	35	15	3	0	0	92,1	60,5	75,3	0,0
2	Vegetable products	8h	a	Fresh	MP Broth 8h at 42°C	6	13	3	3	0	0	75,0	75,0	76,0	0,0
			b	RTE/RTC	MP Broth 8h at 42°C	2	12	7	0	0	0	100,0	22,2	66,7	0,0
			c	Frozen	MP Broth 8h at 42°C	7	10	2	3	0	0	75,0	83,3	77,3	0,0
			Total			15	35	12	6	0	0	81,8	63,6	73,5	0,0
		24h	a	Fresh	MP Broth 24h at 42°C	8	12	4	1	0	0	92,3	69,2	80,0	0,0
			b	RTE/RTC	MP Broth 24h at 42°C	2	10	9	0	0	0	100,0	18,2	57,1	0,0
			c	Frozen	MP Broth 24h at 42°C	8	8	4	2	0	0	85,7	71,4	72,7	0,0
			Total			18	30	17	3	0	0	92,1	55,3	70,6	0,0
All categories			**7h or 8 h incubation time**			**32**	**72**	**25**	**12**	**0**	**0**	**82,6**	**63,8**	**73,8**	**0,0**
			24 h incubation time			**38**	**65**	**32**	**6**	**0**	**0**	**92,1**	**57,9**	**73,0**	**0,0**

* PPNA not included ** PPND not included

A summary of the results is given in Table 6.

Table 6 - Summary of results

		Short enrichment step: 7h or 8 h	Enrichment step: 24 h
Sensitivity for the alternative method	$SE_{alt} = \frac{(PA+PD)}{(PA+ND+PD)} \times 100\%$	82.6 %	92.1 %
Sensitivity for the reference method	$SE_{ref} = \frac{(PA+ND)}{(PA+ND+PD)} \times 100\%$	63.8 %	57.9 %
Relative trueness	$RT = \frac{(PA+NA)}{N} \times 100\%$	73.8 %	73.0 %
False positive ratio for the alternative method* **FP = PPNA + PPND**	$FPR = \frac{(FP)}{NA} \times 100\%$	0.0 %	0.0 %

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

3.1.1.6 Analysis of discordant results

12 negative deviations (for the short enrichment step) and 6 negative deviations (for 24 h enrichment step) were observed. One sample (N° 720) was naturally contaminated and 11 samples (for the short enrichment step) and 5 samples (for 24 h enrichment step) were artificially contaminated.

For 4 samples (No 502, 506, 8669 and 8976), the confirmatory tests concluded to the presence of *Escherichia coli* O157:H7 in the enrichment broth. All were artificially contaminated. Note that these samples gave positive PCR tests after 24 h incubation time.

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

The presence of *E. coli* O157:H7 was not detected in the enrichment broth for samples in negative agreement, during the extension study.

Table 7 - Negative deviations

Sample N°	Product	Artificial contaminations (spiking protocol)		Global result		Reference method ISO 16654♦	BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - Short enrichment step				BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - 24h enrichment step				Category	Type
		Strain	Inoculation level/sample	7h or 8h	24h	Result	PCR result	Confirmatory tests	Final result	Agreement	PCR Result	Confirmatory tests	Final result	Agreement		
720	Frozen ground beef	/	/	+	+	+	-	-	-	**ND**	-	-	-	**ND**	1	b
744	**Chuck**	*E.coli* O157:H7 Ad565	0-2-3-2-4 (2,2)	+	+	+	-	-	-	**ND**	+	+	+	**PA**	1	a
498	**Spinach beet**	*E.coli* O157:H7 Ad556	5-3-3-3-4(3,6)	+	+	+	-	-	-	**ND**	-	-	-	**ND**	2	a
502	**Links**	*E.coli* O157:H7 Ad575	1-4-2-4-2(2,6)	+	+	+	-	+	-	**ND**	+	+	+	**PA**	2	a
506	**Lettuce**	*E.coli* O157:H7 Ad556	1-5-1-0-1(1,6)	+	+	+	-	+	-	**ND**	+	+	+	**PA**	2	a
523	**Vegetable based cake**	*E.coli* O157:H7 Ad575	2-5-4-4-2(3,4)	+	+	+	-	-	-	**ND**	+	+ (ims2)	+ (ims2)	**PA**	2	c
526	**Fried frozen mushrooms**	*E.coli* O157:H7 Ad575	2-5-4-4-2(3,4)	+	+	+	-	-	-	**ND**	-	-	-	**ND**	2	c
527	**Fried frozen vegetables**	*E.coli* O157:H7 Ad576	8-5-3-5-3(4,8)	+	+	+	-	-	-	**ND**	-	-	-	**ND**	2	c
8969	**Frozen ground beef**	*E.coli* O157:H7 Ad486	6-1-1-2-5 (3,0)	+	+	+	-/-/-	+	-	**ND**	+	+	+	**PA**	1	b
8975	**Rump steak with shallots**	*E.coli* O157:H7 Ad489	1-1-4-1-2 (1,8)	+	+	+	-	-	-	**ND**	-	-	-	**ND**	1	c
8976	**Parmesan carpaccio**	*E.coli* O157:H7 Ad489	1-1-4-1-2 (1,8)	+	+	+	-/-/-	+	-	**ND**	+	+	+	**PA**	1	c
8978	**Seasoned beef carpaccio**	*E.coli* O157:H7 Ad561	1-0-5-3-2 (2,2)	+	+	+	-	-	-	**ND**	-	-	-	**ND**	1	c

♦ Analyses performed according to the COFRAC accreditation

Table 8 - Positive deviations

Sample N°	Product	Artificial contaminations (spiking protocol)		Global result		Reference method ISO 16654♦	BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - Short enrichment step				BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - 24h enrichment step				Category	Type
		Strain	Inoculation level/sample	7h or 8h	24h	Result	PCR result	Confirmatory tests	Final result	Agreement	PCR Result	Confirmatory tests	Final result	Agreement		
373	**Ground beef (Bolognaise)**	*E.coli* O157:H7 Ad487	13-6-6-8-9(7,8)	-	+	-	-/-	+	-	**NA**	+	+	+	**PD**	1	c
378	**Ground beef (Bolognaise)**	*E.coli* O157:H7 Ad583	10-14-17-12-14(13,4)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	1	c
719	Frozen ground beef	/	/	-	+	-	-	-	-	**NA**	+	+	+	**PD**	1	b
745	**Rump steak**	*E.coli* O157:H7 Ad565	0-2-3-2-4 (2,2)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	1	a
747	**Sirloin**	*E.coli* O157:H7 Ad487	5-3-4-4-6 (4,4)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	1	a
855	**Marinated peace of beef with shallots**	*E.coli* O157:H7 Ad583	5-5-7-2-6(5,0)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	1	c
856	**Carpaccio**	*E.coli* O157:H7 Ad583	5-5-7-2-6(5,0)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	1	a
857	**Ground beef with onions**	*E.coli* O157:H7 Ad590	4-5-10-7-8(6,8)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	1	c
859	**Ground beef with onions**	*E.coli* O157:H7 Ad590	4-5-10-7-8(6,8)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	1	c
860	**Beef balls**	*E.coli* O157:H7 Ad590	4-5-10-7-8(6,8)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	1	a
500	**Parsley**	*E.coli* O157:H7 Ad558	3-6-5-4-3(4,2)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	2	a
504	**Endive**	*E.coli* O157:H7 Ad576	1-1-1-4-6(2,6)	-	+	-	-	+	-	**NA**	+	+	+	**PD**	2	a
508	**Fresh salad**	*E.coli* O157:H7 Ad556	5-3-3-3-4(3,6)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	2	b
510	**Fresh corn-salad**	*E.coli* O157:H7 Ad558	3-6-5-4-3(4,2)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	2	b
514	**Batavia**	*E.coli* O157:H7 Ad576	1-1-1-4-6(2,6)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	2	a
516	**White cabbage**	*E.coli* O157:H7 Ad556	1-5-1-0-1(1,6)	-	+	-	-	+	-	**NA**	+	+	+	**PD**	2	b
518	**Grated carrots**	*E.coli* O157:H7 Ad556	5-3-3-3-4(3,6)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	2	b
695	**Red cabbage (bagged pre-cut leafy vegetables)**	*E.coli* O157:H7 Ad575	1-2-11-2-8 (4,8)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	2	b
696	**Fresh mix vegetables (bagged pre-cut leafy vegetables)**	*E.coli* O157:H7 Ad575	1-2-11-2-8 (4,8)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	2	b
697	**White cabbage (bagged pre-cut leafy vegetables)**	*E.coli* O157:H7 Ad576	2-4-5-2-8 (4,0)	-	+	-	-	+	-	**NA**	+	+	+	**PD**	2	b
698	**Rocket (bagged pre-cut leafy vegetables)**	*E.coli* O157:H7 Ad576	2-4-5-2-8 (4,0)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	2	b
699	**Red cabbage (bagged pre-cut leafy vegetables)**	*E.coli* O157:H7 Ad576	2-4-5-2-8 (4,0)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	2	b
700	**Frozen vegetables for ratatouille**	*E.coli* O157:H7 Ad556	4-5-4-3-7 (4,6)	-	+	-	-	+	-	**NA**	+	+ (ims2)	+ (ims2)	**PD**	2	c
702	**Frozen sliced links**	*E.coli* O157:H7 Ad577	11-9-9-15-3 (9,4)	-	+	-	-	+	-	**NA**	+	+	+	**PD**	2	c
733	**Celery**	*E.coli* O157:H7 Ad556	4-8-8-4-6 (6,0)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	2	a
8968	**Frozen ground beef**	*E.coli* O157:H7 Ad486	6-1-1-2-5 (3,0)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	1	b
8971	**Frozen ground beef**	*E.coli* O157:H7 Ad488	3-5-1-1-1 (2,2)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	1	b
8973	**Frozen ground beef**	*E.coli* O157:H7 Ad488	3-5-1-1-1 (2,2)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	1	b
8974	**Seasoned beef Carpaccio**	*E.coli* O157:H7 Ad489	1-1-4-1-2 (1,8)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	1	c
8981	**Pistou Carpaccio**	*E.coli* O157:H7 Ad561	1-0-5-3-2 (2,2)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	1	c
8982	**Frozen spinach**	*E.coli* O157:H7 Ad685	1-1-3-5-2 (2,4)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	2	c
9021	**Frozen spinach**	*E.coli* O157:H7 Ad572	1-5-2-2-3 (2,6)	+	+	-	+	+	+	**PD**	+	+	+	**PD**	2	c

♦ Analyses performed according to the COFRAC accreditation

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

Table 9 - Analyses of discordant results

Category			Type		Protocol	N+	ND**	PPND	PD	Unpaired study (ND+PPND) -PD	Unpaired study AL
1	Raw beef meat	7h	a	Fresh	MP Broth 7h at 42°C	12	1	0	4	-3	
			b	Frozen	MP Broth 7h at 42°C	13	2	0	3	-1	
			c	Flavored or marinated	MP Broth 7h at 42°C	11	3	0	6	-3	
					Total	36	6	0	13	-7	3
		24h	a	Fresh	MP Broth 24h at 42°C	12	0	0	4	-4	
			b	Frozen	MP Broth 24h at 42°C	14	1	0	4	-3	
			c	Flavored or marinated	MP Broth 24h at 42°C	12	2	0	7	-5	
					Total	38	3	0	15	-12	3
2	Vegetable products	8h	a	Fresh	MP Broth 8h at 42°C	12	3	0	3	0	
			b	RTE/RTC	MP Broth 8h at 42°C	9	0	0	7	-7	
			c	Frozen	MP Broth 8h at 42°C	12	3	0	2	1	
					Total	33	6	0	12	-6	3
		24h	a	Fresh	MP Broth 24h at 42°C	13	1	0	4	-3	
			b	RTE/RTC	MP Broth 24h at 42°C	11	0	0	9	-9	
			c	Frozen	MP Broth 24h at 42°C	14	2	0	4	-2	
					Total	38	3	0	17	-14	3
All categories			**7 or 8h incubation time**			**69**	**12**	**0**	**25**	**-13**	**4**
			24h incubation time			**76**	**6**	**0**	**32**	**-26**	**4**

** PPND not included

The observed values for ((ND + PPND) - PD) meet the Acceptability Limit (AL) for each of the two individual categories and for all the combined categories whatever the incubation time applied (observed values < AL).

3.1.1.7 Confirmations

For all samples, typical colonies were observed by direct streaking onto CT-SMAC except for three samples. For one sample (n°748) in the short enrichment step protocol and two samples (n°523 and 700) in the 24h enrichment step protocol, the QUALICON's confirmation protocol (Technical bulletin MTD-2001 "Confirmation Protocol for *E. coli* O157:H7") was used and allowed to confirm the presence of *E. coli* O157:H7 in the enrichment broth.

3.1.1.8 PCR inhibitions

For all studies, 141 extracts/lysates were tested for each incubation time tested, the short enrichment step protocol and for the 24h enrichment step protocol. No inhibition was 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:

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

3.1.2.1 Experimental design

During the study carried out in 2010, two (matrix/strain) pairs were analyzed by the reference and the alternative methods. Contaminations and enumerations were done according to the AFNOR technical rules (protocol for low level inoculation). The contamination levels were:

- Level 1: 0 CFU/g or ml,
- Level 2: level necessary to obtain 0 to 50 % positive,
- Level 3: level necessary to obtain 50 to 75 % positive,
- Level 4: level necessary to obtain 100 % positive.

The matrices and the inoculated strains are given in Table 10.

Table 10 - Defined (matrix/strain) pairs for the RLOD determination

Study	Category	Matrix	Inoculated strain	Origin
2010	Raw beef meat	Ground beef	*Escherichia coli* O157:H7 Ad682	Meat product
2010	Vegetable	Spinach	*Escherichia coli* O157:H7 Ad683	Vegetables

3.1.2.2 Calculation and interpretation of the RLOD

The raw data are given in **Appendix 6**.

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 06.07.2015. The RLOD are given in Table 11.

Table 11 – RLOD

Name	RLOD	RLODL	RLODU	b=ln(RLOD)	sd(b)	z-Test statistic	p-value	AL
Ground beef / *E. coli* O157:H7 Ad682 Incubation time: 7h or 24h	1.450	0.487	4.316	0.371	0.546	0.681	0.496	2.5
Spinach / *E. coli* O157:H7 Ad683 Incubation time: 8h or 24h	0.599	0.248	1.447	-0.513	0.441	1.163	1.755	2.5
Combined for both incubation times	**0.842**	**0.444**	**1.596**	**-0.172**	**0.320**	**0.538**	**1.409**	**2.5**

Both software versions, 2.9 & 3.6, showed the same RLOD results.

The RLOD meet the Acceptability Limit for each matrix/strain pair.

The $LOD_{50\ \%}$ calculations according to Wilrich & Wilrich POD-LOD calculation program - version 10, 2021-05-04 test are given in Table 12.

Table 12 - LOD_{50} results

Category	(Strain / matrix) pair	Level of detection at 50% (CFU / test portion) according to Wilrich & Wilrich[1]	
		Reference method	Alternative method
1	Ground beef / *E. coli* O157:H7 Ad682 Incubation time: 7h or 24h	0,4 [0,2;0,7]	0,6 [0,3;1,1]
2	Spinach / *E. coli* O157:H7 Ad683 Incubation time: 8h or 24h	0,7 [0,3;1,2]	0,4 [0,2;0,7]
	Combined results	**0,5 [0,3 ;0,8]**	**0,4 [0,3 ;0,8]**

3.1.3 Inclusivity/Exclusivity

The inclusivity study involves pure target strains to be detected or enumerated by the alternative method.
The exclusivity study involves pure non-target strains, which can be potentially cross-reactive, but are not expected to be detected or enumerated by the alternative method.

3.1.3.1 Test protocols

❒ **Inclusivity**

50 target *Escherichia coli* O157:H7 strains were grown in BHI medium at 37°C. Dilutions were done in order to inoculate 10 to 100 cells/25 ml of BAX® System MP broth. The enrichment broth was inoculated for 7 h at 42°C. The alternative protocol was then performed. Confirmation tests were run by streaking the MP enrichment onto CT-SMAC plate. The colonies were confirmed by O157 and H7 latex agglutination tests.

❒ **Exclusivity**

36 non-target strains were grown in BHI medium at 37°C. Dilutions were carried out in order to inoculate 10^5 cells/25 ml of BPW. BPW was incubated for 24 h at 37°C. The alternative protocol PCR testing protocol was then performed.

3.1.3.2 Results

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

[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.

❒ Inclusivity

All the target strains gave positive PCR results and characteristic colonies on CT-SMAC agar.

❒ Exclusivity

All the non-target strains showed negative PCR results.

3.1.4 Practicability

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

Storage conditions, shelf-life and modalities of utilisation after first use	The storage temperature is 2 - 8°C. The shelf-life is given on the package. All the reagents shall be stored at the temperature mentioned on the package labels.			
Time to result	Steps	**Reference method**	**Alternative method**	
			Short enrichment step : 7 or 8h	**Enrichment step : 24h**
	Negative samples			
	Sampling, enrichment	Day 0	Day 0	
	Lysate	/	Day 0	Day 1
	PCR test	/	Day 0	Day 1
	IMS 6 h	Day 0	/	/
	IMS 24	Day 1	/	/
	Selective plate reading	Day 1 (IMS 6 h) Day 2 (IMS 24 h)	/	/
	Results	Day 2	/	/
	Presumptive positive or positive results			
	Subculture of typical colonies	Day 3	/	/
	Confirmation tests	Day 3 - Day 4	Day 1 to Day 2	Day 2 - Day 3
	Results	Day 4	Day 1 to Day 2	Day 2 - Day 3
Common step with the reference method	There is no common step			

The negative results are available either in less than a day (short enrichment step) or in a day and confirmed positive results in one to two days (short enrichment step) and two to three days (24h enrichment protocol).

3.1.5 *Method Comparison Study conclusion*

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

For the sensitivity study, two categories were tested: Raw beef meat and Vegetable products. The protocol of the alternative method shows 12 negative deviations (ND) for the short enrichment protocol, and 6 negative deviations for 24 h enrichment protocol. The ((ND + PPND) - PD) values are lower than the acceptability limits (AL) for each of the individual categories and for both categories whatever the incubation time.

The Relative Levels of Detections (RLOD) meet the Acceptability Limit for each matrix/strain pair.

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

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).

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 organization*

The inter-laboratory study was run in May 2010.

Samples were sent to 14 collaborators. Ground beef was inoculated with *Escherichia coli* O157:H7.
Two sets of 24 samples were prepared per laboratory, one for the BAX® System Real-Time PCR Assay for *E. coli* O157:H7 method and the other for the reference method (ISO 16654:2001). Each set of samples was divided in 3 levels of contamination, with 8 samples per level.

One sample for aerobic mesophilic flora enumeration by ISO 4833-1 method was sent.

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 25 CFU/25 g and 5 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 (See Table 13).

Table 13 - Strain stability

Day	Reference method (detection)			CFU/ 25 g (CT-SMAC)		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Day 0	+	+	+	18	27	20
Day 1	+	+	+	16	17	18
Day 2	+	+	+	9	8	0

A short decrease of the inoculated strain was observed during the storage at 5 ± 3°C, *i.e.* the inoculated bacterial population was divided by 2.

3.2.2.2 Contamination levels

The contamination levels and the sample codification were the following (see Table 14).

Table 14 - Contamination levels

Level	Samples	Theoretical target level (CFU/25 g)	True level (CFU/25 g sample)	Low limit CFU/ 25 g sample	High limit CFU/ 25 g sample
Level 0 (L0)	3 - 8 - 9 - 12 - 15 - 18 - 20 - 21	0	/	/	/
Low level (L1)	1 - 4 - 7 - 10 - 11 - 13 - 17 - 24	5	9.2	8.0	16.0
High level (L2)	2 - 5 - 6 - 14 - 16 - 19 - 22 - 23	25	25	43.0	57.9

3.2.2.3 Logistic conditions

Blind-coded samples were placed in isothermal boxes, which contained cooling blocks, and express-shipped to the different laboratories.

A temperature control flask containing a sensor was added to the package in order to register the temperature profile during the transport, the package delivery and storage until analyses.

Temperature conditions are given in Table 15.

Table 15 - Sample temperatures at receipt

Collaborators	Temperature measured by the probe (°C)	Temperature measured at receipt (°C)	Analysis date
A	5.8	2.0	Day 2
B	5.0	3.0	Day 2
C	4.1	3.0	Day 2
D	5.1	2.5	Day 2
E	5.0	2.5	Day 2
F	7.3	3.5	Day 2
G	6.8	3.0	Day 2
H	These Labs did not perform the analysis and did not return the probe parcel		
I	Not dispatched due to custom delivery		
J	*3.4*	3.0	Day 2
K	2.3	2.5	Day 2
L	5.9	3.5	Day 2
M	3.3	2.0	Day 2
N	6.0	3.0	Day 2

Samples were shipped within 24 h to the collaborators except Lab I which did not receive its parcel.

Note that the temperature probe was stored with the samples during the inter-laboratory study. Lab F stored the samples before analysis between 8.7 and 9.3°C. According to the AFNOR technical rules, the delivery and storage temperature conditions cannot exceed 8.4°C. Simulations were performed in order to compare the temperature storage impact on the *Escherichia coli* O157:H7 behavior. The Sym'Previus[2] software was used. According to the simulations, it was decided that the results of Lab F were useable.

3.2.3 Results analysis

The raw data are given in **Appendix 8**.

3.2.3.1 Expert laboratory results

The results obtained by the expert laboratory are given in Table 16.

Table 16 – Results obtained by the expert Lab.

Level	Reference method	Alternative method
L0	0/8	0/8
L1	7/8	8/8
L2	8/8	8/8

3.2.3.2 Results observed by the collaborative laboratories

- ***Aerobic mesophilic flora enumeration***

According to the Lab results, the enumeration levels varied from $3.2\ 10^4$ to $6.2\ 10^5$ CFU/g.

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

11 collaborators participated to the study. The results obtained are provided in Table 17 (reference method) and Table 18 (alternative method).

[2] Sym'Previus: www.symprevius.org

Table 17 - Positive results by the reference method (ALL the collaborators)

Collaborator	Contamination level		
	L0	L1	L2
A	2	8	8
B	0	7	7
D	0	6	8
E	0	8	8
F	2	8	8
G	0	8	8
J	0	8	8
K	0	8	8
L	0	8	8
M	0	7	8
N	0	5	7
TOTAL	$P_0 = 4$	$P_1 = 81$	$P_2 = 86$

Table 18 - Positive results (before and after confirmation) by the alternative method (ALL the collaborators)

Collaborators	Contamination level					
	L0		*L1*		*L2*	
	Before confirmation	*After confirmation*	*Before confirmation*	*After confirmation*	*Before confirmation*	*After confirmation*
A	0	0	8	8	8	8
B	0	0	8	8	8	8
D	0	0	8	8	8	8
E	0	0	8	8	8	8
F	0	0	8	8	8	8
G	0	0	8	8	8	8
J	0	0	8	8	8	8
K	0	0	8	8	8	8
L	0	0	8	8	8	8
M	0	0	8	8	8	8
N	0	0	8	8	8	8
TOTAL	$P_0 = 0$	$CP_0 = 0$	$P_1 = 88$	$CP_1 = 88$	$P_2 = 88$	$CP_2 = 88$

The results of Lab A were not taken into account as the MP enrichment broth was incubated for 8 h and the Lab performed the IMS step of the reference method after storage of the enrichment broth for 4 days (the materials were not available to do the IMS earlier).

Lab C did not carry out the reference method.

Lab H could not perform the analysis.

Lab F obtained two positive results with the reference method for unspiked samples Level 0).

Despite the fact that according to the AFNOR technical rules, it is possible to include the results from a collaborator with maximum one cross contamination at Level 0, the results from Lab F were kept for interpretation. This lab obtained two positive results for unspiked samples using the reference method. This was accepted by the AFNOR Technical committee when presenting the initial validation study.

3.2.3.3 Results of the collaborators retained for interpretation

The results from 4 collaborators were excluded:

- Lab A: delay for the IMS step,
- Lab C: did not carry out the reference method,
- Lab H: did not carry out the analysis,
- Lab I: did not receive the package.

The results obtained with the 10 labs kept for interpretation are presented in Table 19 (reference method) and Table 20 (alternative method).

Table 19 - Positive results by the reference method (Excluding Lab A)

Collaborators	Contamination level		
	L0	L1	L2
B	0	7	7
D	0	6	8
E	0	8	8
F	2	8	8
G	0	8	8
J	0	8	8
K	0	8	8
L	0	8	8
M	0	7	8
N	0	5	7
TOTAL	$P_0 = 2$	$P_1 = 73$	$P_2 = 78$

Table 20 - Positive results (before and after confirmation) by the alternative method (Excluding Lab A)

Collaborators	Contamination level					
	L0		*L1*		*L2*	
	Before confirmation	*After confirmation*	*Before confirmation*	*After confirmation*	*Before confirmation*	*After confirmation*
B	0	0	8	8	8	8
D	0	0	8	8	8	8
E	0	0	8	8	8	8
F	0	0	8	8	8	8
G	0	0	8	8	8	8
J	0	0	8	8	8	8
K	0	0	8	8	8	8
L	0	0	8	8	8	8
M	0	0	8	8	8	8
N	0	0	8	8	8	8
TOTAL	$P_0 = 0$	$CP_0 = 0$	$P_1 = 80$	$CP_1 = 80$	$P_2 = 80$	$CP_2 = 80$

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 21).

Table 21 - Percentage specificity

Specificity for the reference method	$SP_{ref} = \left(1 - \left(\frac{P_0}{N\_}\right)\right) x\ 100\ \% =$	97.5 %
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 both inoculation levels, L1 and L2. Both inoculation levels were 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 and Level 2 is provided in Table 22.

Table 22 - Summary of the obtained results with the reference method and the alternative method for Level 1 and Level 2

	Response	Reference method positive (R+)	Reference method negative (R-)
Level 1	Alternative method positive (A+)	Positive agreement (A+/R+) **PA = 73**	Positive deviation (R-/A+) **PD = 7**
	Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 0**	Negative agreement (A-/R-) **NA = 0**
Level 2	Alternative method positive (A+)	Positive agreement (A+/R+) **PA = 78**	Positive deviation (R-/A+) **PD = 2**
	Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 0**	Negative agreement (A-/R-) **NA = 0**

Based on the data summarized in Table 22, 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 into account the confirmations, are the following (See Table 23).

Table 23 - Sensitivity, relative trueness and false positive ratio percentages

		Level 1	Level 2
Sensitivity for the alternative method:	$SE_{alt} = \frac{(PA+PD)}{(PA+PD+ND)} \; x \; 100\% =$	100.0 %	100.0%
Sensitivity for the reference method:	$SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)} \; x \; 100\% =$	91.3 %	97,5%
Relative trueness	$RT = \frac{(PA+NA)}{N} \; x \; 100\% =$	91.3 %	97,5%
False positive ratio for the alternative method	$FPR = \frac{FP}{NA} \; x \; 100\% =$	0	0

3.2.4.3 Interpretation of data

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.

In this study, fractional recovery was observed at Level 1 and Level 2. The calculations are the following, according to EN ISO 16140-2:2016 (See Table 23).

Table 24 - Calculations

	Level 1	Level 2
N_X	80	80
$(p+)_{ref}$	0.9	1.0
$(p+)_{alt}$	0.0	0.0
AL = (ND - PD) max	14.80	15.30
ND - PD	- 7	-2
Conclusion	ND - PD < AL	ND - PD < AL

The ISO 16140-2 (2016) requirements are fulfilled as (ND - PD) is lower than the AL for both inoculation levels.

3.2.4.4 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 but as 2 positive samples were obtained at Level 0 with the reference method for Lab F, the calculation is not possible.

3.2.5 Inter-laboratory Study conclusion

The data and interpretations comply with the EN ISO 16140-2:2016 requirements. **The BAX® System Real-Time PCR Assay *E. coli* O157:H7 method is considered equivalent to the ISO standard.**

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, two categories were tested: Raw beef meat and Vegetable products. The protocol of the alternative method shows 12 negative deviations (ND) and 25 positive deviations (PD) for the short enrichment step, 6 negative deviations and 32 positive deviations for 24 h enrichment step. The ((ND + PPND) - PD) calculated values meet the acceptability limits (AL) for each of the individual categories and for both categories tested.

The Relative Levels of Detection (RLOD) meet the AL fixed at 2.5 for the unpaired data study whatever the matrix/strain pairs.

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

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 BAX® System Real-Time PCR Assay *E. coli* O157:H7 method is considered equivalent to the ISO standard.**

Quimper, 29 July 2022

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 BAX® System Real-Time PCR Assay for *E. coli* O157:H7

Protocol for Raw beef meat and vegetables

25 g + 225 ml (dilution 1/10) BAX® System MP broth
preheated at 42°C

↓

7 / 8 - 24 h at 42°C ± 1°C

↓

20 µl enrichment broth
+ 200 µl lysis buffer

↓

Lysis: 20 min at 37°C
10 min at 95°C
Cooling: 5 min

↓

30 µl DNA extract / PCR tube

↓

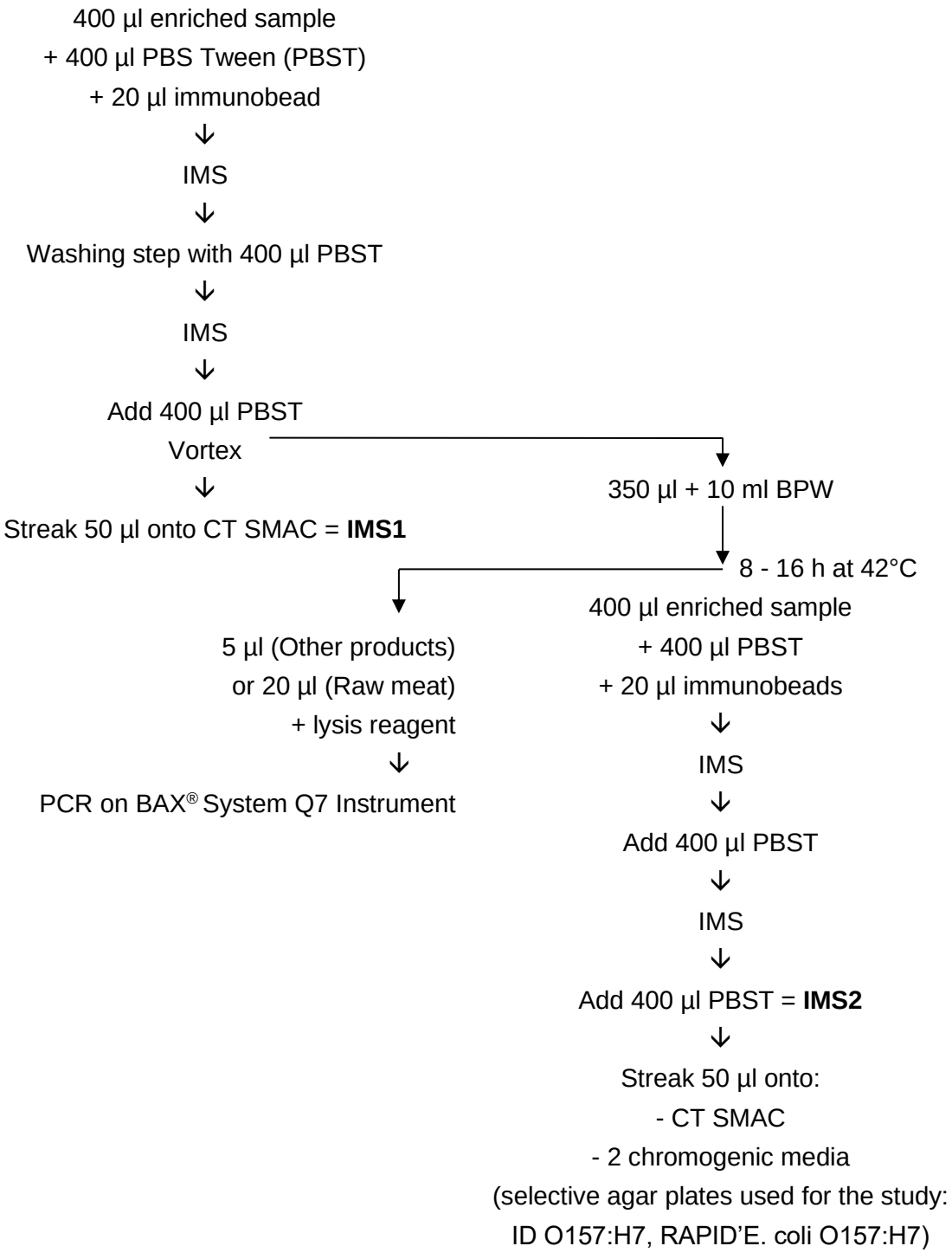
PCR

↓

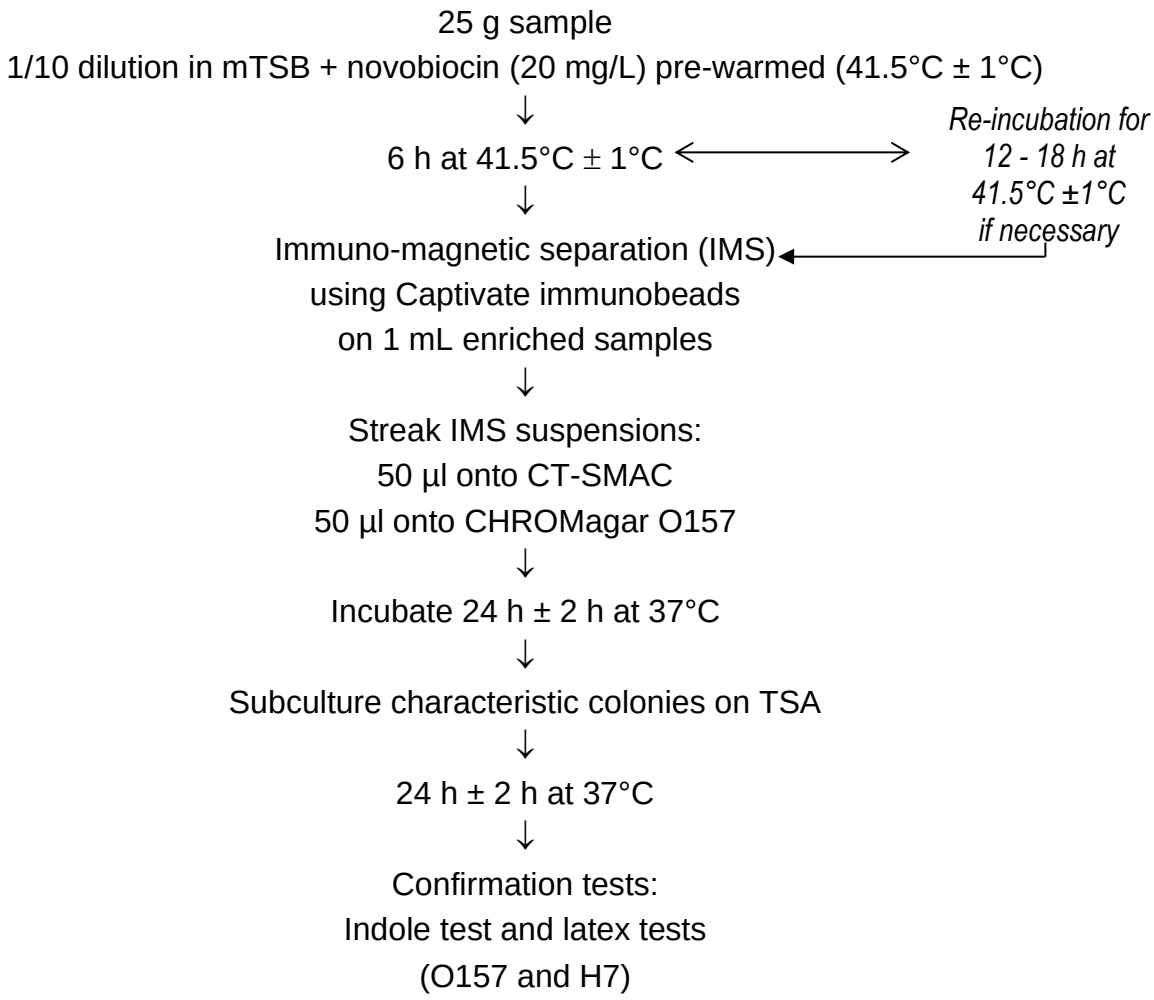
Confirmation by streaking 50 µl onto CT -SMAC agar
and confirm presumptive positive colonies with
O157and H7 latex tests
or use the QUALICON's confirmation protocol

Appendix 2 – QUALICON's confirmation protocol

Technical bulletin MTD-2001 "Confirmation Protocol for E. coli O157:H7"



Appendix 3 – Flow diagram of the reference method: ISO 16654: Horizontal method for the detection of *Escherichia coli* O157



Appendix 4 – Artificial contamination of the samples

Study	Sample N°	Product	Artificial contaminations (spiking protocol)					Global result		Category	Type
			Strain	Origin	Injury protocol	Stress measurement	Inoculation level/25g	7h or 8h	24h		
2010	**370**	**Frozen ground beef (meatballs)**	*E.coli* O157:H7 Ad564	Ground beef	-20°C	>2,82	11-15-20-16-11(14,6)	+	+	1	b
2010	**371**	**Ground beef (meatballs)**	*E.coli* O157:H7 Ad565	Ground beef	-20°C	1,64	19-20-8-16-20(16,6)	+	+	1	a
2010	**373**	**Ground beef (Bolognaise)**	*E.coli* O157:H7 Ad487	Ground beef	10 days at 4°C	1,43	13-6-6-8-9(7,8)	-	+	1	c
2010	**378**	**Ground beef (Bolognaise)**	*E.coli* O157:H7 Ad583	Ground beef	10 days at 4°C	1,61	10-14-17-12-14(13,4)	+	+	1	c
2010	**386**	**Frozen ground beef**	*E.coli* O157:H7 Ad590	Ground beef	-20°C	2,46	8-8-10-4-5(7,0)	+	+	1	b
2010	**387**	**Frozen ground beef (meatballs)**	*E.coli* O157:H7 Ad590	Ground beef	-20°C	2,46	8-8-10-4-5(7,0)	+	+	1	b
2010	**388**	**Frozen ground beef (51%) (meatballs)**	*E.coli* O157:H7 Ad590	Ground beef	-20°C	2,46	8-8-10-4-5(7,0)	+	+	1	b
2010	**744**	**Chuck**	*E.coli* O157:H7 Ad565	Ground beef	28 days at 4°C	1,14	0-2-3-2-4 (2,2)	+	+	1	a
2010	**745**	**Rumsteak**	*E.coli* O157:H7 Ad565	Ground beef	28 days at 4°C	1,14	0-2-3-2-4 (2,2)	+	+	1	a
2010	**746**	**Beef meat**	*E.coli* O157:H7 Ad565	Ground beef	28 days at 4°C	1,14	0-2-3-2-4 (2,2)	+	+	1	a
2010	**747**	**Sirloin**	*E.coli* O157:H7 Ad487	Ground beef	28 days at 4°C	0,48	5-3-4-4-6 (4,4)	+	+	1	a
2010	**748**	**Shin**	*E.coli* O157:H7 Ad487	Ground beef	28 days at 4°C	0,48	5-3-4-4-6 (4,4)	+	+	1	a
2010	**854**	**Marinated peace of beef**	*E.coli* O157:H7 Ad583	Ground beef	33 days at 4°C	1,34	5-5-7-2-6(5,0)	+	+	1	c
2010	**855**	**Marinated peace of beef with shallots**	*E.coli* O157:H7 Ad583	Ground beef	33 days at 4°C	1,34	5-5-7-2-6(5,0)	+	+	1	c
2010	**856**	**Carpaccio**	*E.coli* O157:H7 Ad583	Ground beef	33 days at 4°C	1,34	5-5-7-2-6(5,0)	+	+	1	a
2010	**857**	**Ground beef with onions**	*E.coli* O157:H7 Ad590	Ground beef	33 days at 4°C	1,50	4-5-10-7-8(6,8)	+	+	1	c
2010	**858**	**Ground beef**	*E.coli* O157:H7 Ad590	Ground beef	33 days at 4°C	1,50	4-5-10-7-8(6,8)	+	+	1	a
2010	**859**	**Ground beef with onions**	*E.coli* O157:H7 Ad590	Ground beef	33 days at 4°C	1,50	4-5-10-7-8(6,8)	+	+	1	c
2010	**860**	**Beef balls**	*E.coli* O157:H7 Ad590	Ground beef	33 days at 4°C	1,50	4-5-10-7-8(6,8)	+	+	1	a
2010	**498**	**Spinach beet**	*E.coli* O157:H7 Ad556	Ground beef	16 days at 4°C	1,70	5-3-3-3-4(3,6)	+	+	2	a

Study	Sample N°	Product	Artificial contaminations (spiking protocol)					Global result		Category	Type
			Strain	Origin	Injury protocol	Stress measurement	Inoculation level/25g	7h or 8h	24h		
2010	**500**	**Parsley**	*E.coli* O157:H7 Ad558	WWTP	16 days at 4°C	2,13	3-6-5-4-3(4,2)	+	+	2	a
2010	**502**	**Links**	*E.coli* O157:H7 Ad575	Bovine feces	16 days at 4°C	0,93	1-4-2-4-2(2,6)	+	+	2	a
2010	**504**	**Endive**	*E.coli* O157:H7 Ad576	Bovine feces	16 days at 4°C	1,70	1-1-1-4-6(2,6)	-	+	2	a
2010	**506**	**Lettuce**	*E.coli* O157:H7 Ad556	WWTP	16 days at 4°C	1,87	1-5-1-0-1(1,6)	+	+	2	a
2010	**508**	**Fresh salad**	*E.coli* O157:H7 Ad556	WWTP	16 days at 4°C	1,70	5-3-3-3-4(3,6)	+	+	2	b
2010	**510**	**Fresh corn-salad**	*E.coli* O157:H7 Ad558	WWTP	16 days at 4°C	2,13	3-6-5-4-3(4,2)	+	+	2	b
2010	**512**	**Lettuce**	*E.coli* O157:H7 Ad575	Bovine feces	16 days à 4°C	0,93	1-4-2-4-2(2,6)	+	+	2	a
2010	**514**	**Batavia**	*E.coli* O157:H7 Ad576	Bovine feces	16 days at 4°C	1,70	1-1-1-4-6(2,6)	+	+	2	a
2010	**516**	**White cabbage**	*E.coli* O157:H7 Ad556	WWTP	16 days at 4°C	1,87	1-5-1-0-1(1,6)	-	+	2	b
2010	**518**	**Grated carrots**	*E.coli* O157:H7 Ad556	WWTP	16 days at 4°C	1,70	5-3-3-3-4(3,6)	+	+	2	b
2010	**520**	**Soya sprouts**	*E.coli* O157:H7 Ad558	WWTP	16 days at 4°C	2,13	3-6-5-4-3(4,2)	-	-	2	b
2010	**522**	**Frozen mixed vegetables**	*E.coli* O157:H7 Ad556	WWTP	-20°C	2,39	2-3-1-3-2(1,8)	+	+	2	c
2010	**523**	**Vegetable based cake**	*E.coli* O157:H7 Ad575	Bovine feces	-20°C	>2,66	2-5-4-4-2(3,4)	+	+	2	c
2010	**524**	**Fried frozen vegetables**	*E.coli* O157:H7 Ad576	Bovine feces	-20°C	>2,30	8-5-3-5-3(4,8)	+	+	2	c
2010	**525**	**Mixed vegetables**	*E.coli* O157:H7 Ad556	WWTP	-20°C	2,39	2-3-1-3-2(1,8)	+	+	2	c
2010	**526**	**Fried frozen mushrooms**	*E.coli* O157:H7 Ad575	Bovine feces	-20°C	>2,66	2-5-4-4-2(3,4)	+	+	2	c
2010	**527**	**Fried frozen vegetables**	*E.coli* O157:H7 Ad576	Bovine feces	-20°C	>2,30	8-5-3-5-3(4,8)	+	+	2	c
2010	**693**	**Spinach (bagged pre-cut leafly vegetables)**	*E.coli* O157:H7 Ad575	Bovine feces	26 days at 4°C	0,76	1-2-11-2-8 (4,8)	+	+	2	b

Study	Sample N°	Product	Artificial contaminations (spiking protocol)					Global result		Category	Type
			Strain	Origin	Injury protocol	Stress measurement	Inoculation level/25g	7h or 8h	24h		
2010	694	Fresh vegetable mix (cabbage, carrots, salad...) (bagged pre-cut leafly vegetables)	*E.coli* O157:H7 Ad575	Bovine feces	26 days at 4°C	0,76	1-2-11-2-8 (4,8)	+	+	2	b
2010	695	Red cabbage (bagged pre-cut leafly vegetables)	*E.coli* O157:H7 Ad575	Bovine feces	26 days at 4°C	0,76	1-2-11-2-8 (4,8)	+	+	2	b
2010	696	Fresh mix vegetables (bagged pre-cut leafly vegetables)	*E.coli* O157:H7 Ad575	Bovine feces	26 days at 4°C	0,76	1-2-11-2-8 (4,8)	+	+	2	b
2010	697	White cabbage (bagged pre-cut leafly vegetables)	*E.coli* O157:H7 Ad576	Bovine feces	26 days at 4°C	1,38	2-4-5-2-8 (4,0)	-	+	2	b
2010	698	Rocket (bagged pre-cut leafly vegetables)	*E.coli* O157:H7 Ad576	Bovine feces	26 days at 4°C	1,38	2-4-5-2-8 (4,0)	+	+	2	b
2010	699	Red cabbage (bagged pre-cut leafly vegetables)	*E.coli* O157:H7 Ad576	Bovine feces	26 days at 4°C	1,38	2-4-5-2-8 (4,0)	+	+	2	b
2010	700	Frozen vegetables for ratatouille	*E.coli* O157:H7 Ad556	WWTP	-20°C	>1,81	4-5-4-3-7 (4,6)	-	+	2	c
2010	701	Frozen vegetables mix	*E.coli* O157:H7 Ad556	WWTP	-20°C	>1,81	4-5-4-3-7 (4,6)	-	-	2	c
2010	702	Frozen sliced links	*E.coli* O157:H7 Ad577	Bovine feces	-20°C	1,91	11-9-9-15-3 (9,4)	-	+	2	c
2010	732	Green cabbage	*E.coli* O157:H7 Ad556	WWTP	27 days at 4°C	1,82	4-8-8-4-6 (6,0)	+	+	2	a
2010	733	Celery	*E.coli* O157:H7 Ad556	WWTP	27 days at 4°C	1,82	4-8-8-4-6 (6,0)	+	+	2	a
2010	734	Sprouts	*E.coli* O157:H7 Ad556	WWTP	27 days at 4°C	1,82	4-8-8-4-6 (6,0)	+	+	2	a
2010	735	Zucchini	*E.coli* O157:H7 Ad556	WWTP	27 days at 4°C	1,82	4-8-8-4-6 (6,0)	+	+	2	a
2010	740	Green asparagus	*E.coli* O157:H7 Ad556	WWTP	-20°C	>0,90	7-4-2-5-4 (4,4)	+	+	2	c
2010	741	Peas	*E.coli* O157:H7 Ad577	Bovine feces	-20°C	>0,90	4-5-4-3-4 (4,0)	+	+	2	c
2010	861	Zucchini	*E.coli* O157:H7 Ad576	Bovine feces	33 days at 4°C	0,88	14-18-11-6-9(11,6)	+	+	2	a

Study	Sample N°	Product	Artificial contaminations (spiking protocol)					Global result		Category	Type
			Strain	Origin	Injury protocol	Stress measurement	Inoculation level/25g	7h or 8h	24h		
2010	862	Turnip	*E.coli* O157:H7 Ad576	Bovine feces	33 days at 4°C	0,88	14-18-11-6-9(11,6)	+	+	2	a
2017	8965	Ground beef	*E.coli* O157:H7 Ad485	Beef	Seeding 48h 2-8°C	/	1-3-1-2-2 (1,8)	+	+	1	a
2017	8966	Bifteck	*E.coli* O157:H7 Ad485	Beef	Seeding 48h 2-8°C	/	1-3-1-2-2 (1,8)	+	+	1	a
2017	8967	Beef steak	*E.coli* O157:H7 Ad485	Beef	Seeding 48h 2-8°C	/	1-3-1-2-2 (1,8)	+	+	1	a
2017	8968	Frozen ground beef	*E.coli* O157:H7 Ad486	Beef	Seeding 48h 2-8°C	/	6-1-1-2-5 (3,0)	+	+	1	b
2017	8969	Frozen ground beef	*E.coli* O157:H7 Ad486	Beef	Seeding 48h 2-8°C	/	6-1-1-2-5 (3,0)	+	+	1	b
2017	8970	Frozen ground beef	*E.coli* O157:H7 Ad486	Beef	Seeding 48h 2-8°C	/	6-1-1-2-5 (3,0)	+	+	1	b
2017	8971	Frozen ground beef	*E.coli* O157:H7 Ad488	Beef	Seeding 48h 2-8°C	/	3-5-1-1-1 (2,2)	+	+	1	b
2017	8972	Frozen ground beef	*E.coli* O157:H7 Ad488	Beef	Seeding 48h 2-8°C	/	3-5-1-1-1 (2,2)	+	+	1	b
2017	8973	Frozen ground beef	*E.coli* O157:H7 Ad488	Beef	Seeding 48h 2-8°C	/	3-5-1-1-1 (2,2)	+	+	1	b
2017	8974	Seasoned beef Carpaccio	*E.coli* O157:H7 Ad489	Beef	Seeding 48h 2-8°C	/	1-1-4-1-2 (1,8)	+	+	1	c
2017	8975	Rump steak with shallots	*E.coli* O157:H7 Ad489	Beef	Seeding 48h 2-8°C	/	1-1-4-1-2 (1,8)	+	+	1	c
2017	8976	Parmesan Carpaccio	*E.coli* O157:H7 Ad489	Beef	Seeding 48h 2-8°C	/	1-1-4-1-2 (1,8)	+	+	1	c
2017	8977	Pistou Carpaccio	*E.coli* O157:H7 Ad489	Beef	Seeding 48h 2-8°C	/	1-1-4-1-2 (1,8)	-	-	1	c
2017	8978	Seasoned beef Carpaccio	*E.coli* O157:H7 Ad561	Beef	Seeding 48h 2-8°C	/	1-0-5-3-2 (2,2)	+	+	1	c
2017	8979	Rump steak with shallots	*E.coli* O157:H7 Ad561	Beef	Seeding 48h 2-8°C	/	1-0-5-3-2 (2,2)	-	-	1	c
2017	8980	Parmesan Carpaccio	*E.coli* O157:H7 Ad561	Beef	Seeding 48h 2-8°C	/	1-0-5-3-2 (2,2)	+	+	1	c
2017	8981	Pistou Carpaccio	*E.coli* O157:H7 Ad561	Beef	Seeding 48h 2-8°C	/	1-0-5-3-2 (2,2)	+	+	1	c
2017	8982	Frozen spinach	*E.coli* O157:H7 Ad685	Feces	Seeding 48h 2-8°C	/	1-1-3-5-2 (2,4)	+	+	2	c
2017	8983	Frozen carrots	*E.coli* O157:H7 Ad685	Feces	Seeding 48h 2-8°C	/	1-1-3-5-2 (2,4)	+	+	2	c
2017	9021	Frozen spinach	*E.coli* O157:H7 Ad572	Feces	Seeding 48h 2-8°C	/	1-5-2-2-3 (2,6)	+	+	2	c
2017	9022	Frozen carrots	*E.coli* O157:H7 Ad572	Feces	Seeding 48h 2-8°C	/	1-5-2-2-3 (2,6)	+	+	2	c

Appendix 5 – Sensitivity study: raw data

Bold typing: artificially inoculated samples

***E. coli* O157: H7 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
-:	no typical colonies but presence of background microflora
st:	plate without any colony
i:	PCR inhibition
PA:	positive agreement
NA:	negative agreement
ND:	negative deviation
PD:	positive deviation
PPNA:	positive presumptive negative agreement
PPND :	positive presumptive negative deviation
NC:	non-characteristic colony on nutrient agar
d:	doubtful colony
ni:	not isolated colony
col :	colony
ims :	immuno separation (QUALICON's confirmation protocol)

RAW BEEF MEAT																				
Sample N°	Product	Reference method ISO 16654♦					BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - Short enrichment step						BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - 24h enrichment step						Category	Type
		Characteristic colonies				Result	PCR result	Confirmatory tests			Final result	Agreement	PCR Result	Confirmatory tests			Final result	Agreement		
		IMS 6H		IMS 24H				CT SMAC Characteristic colonies	O157 latex	H7 Latex				CT SMAC Characteristic colonies	O157 latex	H7 Latex				
		CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157															
371	**Ground beef (meatballs)**	+	+	/	/	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	1	a
664	Marinated steak	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	a
665	Fresh ground beef	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	a
666	Beef Carpaccio	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	a
667	Beef meatballs	-	-	+/-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	a
668	Ground beef	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	a
669	Beef meat	+	-	+/-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	a
671	Steak	+/-	-	+	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	a
672	Rump steak	+	-	+ (3)	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	a
706	Beef meat pieces	-	-	+	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	a
707	Beef pieces (faux filet)	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	a
708	Beef piece (tournedos)	-	-	+	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	a
744	**Chuck**	+	+	/	/	+	-	+	- (5)	/	-	**ND**	+	d	+	+	+	**PA**	1	a
745	**Rump steak**	-	-	+	+	-	+	+	+	+	+	**PD**	+	d	+	+	+	**PD**	1	a
746	**Tournedos**	-	-	+	+	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	1	a
747	**Sirloin**	+ (1)	-	+	+	-	+	+	+	+	+	**PD**	+	+	+	+	+	**PD**	1	a
748	**Shin**	+	+	/	/	+	+	+	+	+	+ (ims2)	**PA**	+	+	+	+	+	**PA**	1	a
856	**Carpaccio**	-	-	-	-	-	+	+	+	+	+	**PD**	+	+	+	+	+	**PD**	1	a
858	**Ground beef**	-	-	+	+/-	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	1	a
860	**Beef balls**	-	-	-	-	-	+	+	+	+	+	**PD**	+	+	+	+	+	**PD**	1	a

♦ Analyses performed according to the COFRAC accreditation

RAW BEEF MEAT																				
Sample N°	Product	Reference method ISO 16654♦					BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - Short enrichment step						BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - 24h enrichment step						Category	Type
		Characteristic colonies				Result	PCR result	Confirmatory tests			Final result	Agreement	PCR Result	Confirmatory tests			Final result	Agreement		
		IMS 6H		IMS 24H				CT SMAC Characteristic colonies	O157 latex	H7 Latex				CT SMAC Characteristic colonies	O157 latex	H7 Latex				
		CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157															
8965	**Ground beef**	+p	+p	/	/	+	+	+M	+	+	+	**PA**	+	+1/2	+	+	+	**PA**	1	a
8966	**Beef steak**	+p	+p	/	/	+	+	+p	+	+	+	**PA**	+	+p	+	+	+	**PA**	1	a
8967	**Beef steak**	+p	+p	/	/	+	+	+p	+	+	+	**PA**	+	+p	+	+	+	**PA**	1	a
370	**Frozen ground beef (meatballs)**	+	+	/	/	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	1	b
386	**Frozen ground beef**	+	+	/	/	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	1	b
387	**Frozen ground beef (meatballs)**	+(1col)	+(1col)	+	+	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	1	b
388	**Frozen ground beef (51%) (meatballs)**	+	+	/	/	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	1	b
673	Frozen ground beef preparation (80%)	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	b
674	Frozen ground beef (meatballs) (51%)	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	b
675	Frozen ground beef preparation (51%)	-	-	-	+ (1)	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	b
676	Frozen ground beef	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	b
678	Frozen ground beef	-	-	+/-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	b
679	Frozen ground beef	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	b
681	Frozen ground beef	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	b
682	Frozen ground beef (balls)	-	-	+	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	b
709	Frozen ground beef for Bolognaise	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	b
710	Frozen ground beef	-	-	-	+/-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	b
712	Frozen ground beef (51%)	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	b
713	Frozen ground beef with onions	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	b

RAW BEEF MEAT																				
Sample N°	Product	Reference method ISO 16654♦					BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - Short enrichment step						BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - 24h enrichment step						Category	Type
		Characteristic colonies				Result	PCR result	Confirmatory tests			Final result	Agreement	PCR Result	Confirmatory tests			Final result	Agreement		
		IMS 6H		IMS 24H				CT SMAC Characteristic colonies	O157 latex	H7 Latex				CT SMAC Characteristic colonies	O157 latex	H7 Latex				
		CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157															
714	Frozen ground beef	-	-	-	-	-	-	/	/	/	-	NA	-	/	/	/	-	NA	1	b
719	Frozen ground beef	-	-	-	-	-	-	/	/	/	-	NA	+	+	+	+	+	PD	1	b
720	Frozen ground beef	-	+/-	+	+	+	-	-	/	/	-	ND	-	-	/	/	-	ND	1	b
721	Frozen ground beef	-	-	-	-	-	-	/	/	/	-	NA	-	/	/	/	-	NA	1	b
722	Frozen ground beef	+	+	+	+	+	+	+	+	+	+	PA	+	+	+	+	+	PA	1	b
723	Frozen ground beef	+	+	+	+	+	+	+	+	+	+	PA	+	+	+	+	+	PA	1	b
724	Frozen ground beef	-	-	-	+/- (1)	-	-	/	/	/	-	NA	-	/	/	/	-	NA	1	b
725	Frozen ground beef	-	-	+/-	+/-	-	-	/	/	/	-	NA	-	/	/	/	-	NA	1	b
8968	**Frozen ground beef**	-	-	-	-	-	+	+1/2	+	+	+	PD	+	+M	+	+	+	PD	1	b
8969	**Frozen ground beef**	+p	+p	/	/	+	-/-/-	+d	+	+	-	ND	+	+m	+	+	+	PA	1	b
8970	**Frozen ground beef**	+p	+p	/	/	+	+	+M	+	+	+	PA	+	+1/2	+	+	+	PA	1	b
8971	**Frozen ground beef**	-	-	-	-	-	+	+m	+	+	+	PD	+	+m	+	+	+	PD	1	b
8972	**Frozen ground beef**	+M	+M	/	/	+	+	+m	+	+	+	PA	+	+m	+	+	+	PA	1	b
8973	**Frozen ground beef**	-	-	-	-	-	+	+M	+	+	+	PD	+	+m	+	+	+	PD	1	b
373	**Ground beef (Bolognaise)**	-	-	-	-	-	-/-	/	/	/	-	NA	+	+	+	+	+	PD	1	c
378	**Ground beef (Bolognaise)**	-	-	-	-	-	+	+	+	+	+	PD	+	+	+	+	+	PD	1	c
663	Ground beef preparation (Provençal)	-	-	+	-	-	-	/	/	/	-	NA	-	/	/	/	-	NA	1	c
670	Ground beef (Bolognaise)	-	-	-	-	-	-	/	/	/	-	NA	-	/	/	/	-	NA	1	c
677	Ground beef with onions	+/-	-	-	-	-	-	/	/	/	-	NA	-	/	/	/	-	NA	1	c
680	Frozen ground beef (balls) (51%)	-	-	-	-	-	-	/	/	/	-	NA	-	/	/	/	-	NA	1	c

RAW BEEF MEAT																				
Sample N°	Product	Reference method ISO 16654♦					BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - Short enrichment step						BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - 24h enrichment step						Category	Type
		Characteristic colonies				Result	PCR result	Confirmatory tests			Final result	Agreement	PCR Result	Confirmatory tests			Final result	Agreement		
		IMS 6H		IMS 24H				CT SMAC Characteristic colonies	O157 latex	H7 Latex				CT SMAC Characteristic colonies	O157 latex	H7 Latex				
		CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157															
705	Marinated peace of beef	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	c
711	Beef meat balls (51%)	-	-	-	+	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	1	c
854	**Marinated peace of beef**	-	-	-	+	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	1	c
855	**Marinated peace of beef with shallots**	-	-	-	+/-	-	+	+	+	+	+	**PD**	+	+	+	+	+	**PD**	1	c
857	**Ground beef with onions**	-	-	-	+/-	-	+	+	+	+	+	**PD**	+	+	+	+	+	**PD**	1	c
859	**Ground beef with onions**	-	-	-	-	-	+	+	+	+	+	**PD**	+	+	+	+	+	**PD**	1	c
8974	**Seasoned beef Carpaccio**	-	-	-	-	-	+	+p	+	+	+	**PD**	+	+p	+	+	+	**PD**	1	c
8975	**Rump steak with shallots**	+M	+M	/	/	+	-	+d (NC)	/	/	-	**ND**	-	+d (NC)	/	/	-	**ND**	1	c
8976	**Parmesan Carpaccio**	+p	+p	/	/	+	-/-/-	+d	+	+	-	**ND**	+	+M	+	+	+	**PA**	1	c
8977	**Pistou Carpaccio**	st	st	st	-	-	-	st	/	/	-	**NA**	-	st	/	/	-	**NA**	1	c
8978	**Seasoned beef Carpaccio**	+p	+p	/	/	+	-	-	/	/	-	**ND**	-	-	/	/	-	**ND**	1	c
8979	**Rump steak with shallots**	-	-	-	-	-	-	-	/	/	-	**NA**	-	-	/	/	-	**NA**	1	c
8980	**Parmesan Carpaccio**	+(9)	+(9)	+p	+1/2	+	+	+p	+	+	+	**PA**	+	+p	+	+	+	**PA**	1	c
8981	**Pistou Carpaccio**	st	-	-	-	-	+	+p	+	+	+	**PD**	+	+p	+	+	+	**PD**	1	c

VEGETABLES PRODUCTS																					
Sample N°	Product	Reference method ISO 16654♦					BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - Short enrichment step						BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - 24h enrichment step						Category	Type
		Characteristic colonies				Result	PCR result	Confirmatory tests			Final result	Agreement	PCR Result	Confirmatory tests			Final result	Agreement		
		IMS 6H		IMS 24H				CT SMAC Characteristic colonies	O157 latex	H7 Latex				CT SMAC Characteristic colonies	O157 latex	H7 Latex				
		CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157															
498	**Spinach beet**	+	+	/	/	+	-	-	/	/	-	**ND**	-	-	/	/	-	**ND**	2	a
499	Spinach beet	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	a
500	**Parsley**	-	-	-	-	-	+	+	+	+	+	**PD**	+	+	+	+	+	**PD**	2	a
501	Parsley	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	a
502	**Links**	+	+	/	/	+	-	/	/	/	-	**ND**	+	+	+	+	+	**PA**	2	a
503	Links	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	a
504	**Endive**	-	-	-	-	-	-	/	/	/	-	**NA**	+	+	+	+	+	**PD**	2	a
505	Endive	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	a
506	**Lettuce**	-	-	+	+	+	-	/	/	/	-	**ND**	+	+	+	+	+	**PA**	2	a
507	Lettuce	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	a
512	**Lettuce**	+	+	/	/	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	2	a
513	Lettuce	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	a
514	**Batavia**	-	-	-	-	-	+	+	+	+	+	**PD**	+	+	+	+	+	**PD**	2	a
515	Batavia	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	a
688	Eggplant	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	a
689	Zucchini	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	a
690	Carrots	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	a
691	Sprouts	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	a
692	Broccoli	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	a
732	**Green cabbage**	+	+	/	/	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	2	a

♦ Analyses performed according to the COFRAC accreditation

VEGETABLES PRODUCTS																				
Sample N°	Product	Reference method ISO 16654♦					BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - Short enrichment step						BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - 24h enrichment step						Category	Type
		Characteristic colonies				Result	PCR result	Confirmatory tests			Final result	Agreement	PCR Result	Confirmatory tests			Final result	Agreement		
		IMS 6H		IMS 24H				CT SMAC Characteristic colonies	O157 latex	H7 Latex				CT SMAC Characteristic colonies	O157 latex	H7 Latex				
		CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157															
733	**Celery**	+ (1)	+ (2)	+	+	-	+	+	+	+	+	**PD**	+	+	+	+	+	**PD**	2	a
734	**Sprouts**	+	+	/	/	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	2	a
735	**Zucchini**	+	+	/	/	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	2	a
861	**Zucchini**	+	+	/	/	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	2	a
862	**Turnip**	+	+	/	/	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	2	a
508	**Fresh salad**	-	-	-	-	-	+	+	+	+	+	**PD**	+	+	+	+	+	**PD**	2	b
509	Fresh salad	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	b
510	**Fresh corn-salad**	-	-	-	-	-	+	+	+	+	+	**PD**	+	+	+	+	+	**PD**	2	b
511	Fresh corn-salad	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	b
516	**White cabbage**	-	-	-	-	-	-	/	/	/	-	**NA**	+	+	+	+	+	**PD**	2	b
517	White cabbage	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	b
518	**Grated carrots**	-	-	-	-	-	+	+	+	+	+	**PD**	+	+	+	+	+	**PD**	2	b
519	Grated carrots	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	b
520	**Soya sprouts**	+(NC)	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	b
521	Soya sprouts	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	b
693	**Spinach (bagged pre-cut leafy vegetables)**	+	+	/	/	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	2	b
694	**Fresh vegetable mix (cabbage, carrots, salad..) (Bagged pre-cut leafy vegetables)**	+	+	/	/	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	2	b
695	**Red cabbage (bagged pre-cut leafy vegetables)**	-	-	-	-	-	+	d	+	+	+	**PD**	+	+	+	+	+	**PD**	2	b

VEGETABLES PRODUCTS																					
Sample N°	Product	Reference method ISO 16654♦					BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - Short enrichment step						BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - 24h enrichment step						Category	Type
		Characteristic colonies				Result	PCR result	Confirmatory tests			Final result	Agreement	PCR Result	Confirmatory tests			Final result	Agreement		
		IMS 6H		IMS 24H				CT SMAC Characteristic colonies	O157 latex	H7 Latex				CT SMAC Characteristic colonies	O157 latex	H7 Latex				
		CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157															
696	**Fresh mix vegetables (bagged pre-cut leafy vegetables)**	-	-	-	+	-	+	+	+	+	+	**PD**	+	+	+	+	+	**PD**	2	b
697	**White cabbage (bagged pre-cut leafy vegetables)**	-	-	-	-	-	-	/	/	/	-	**NA**	+	+	+	+	+	**PD**	2	b
698	**Rocket (bagged pre-cut leafy vegetables)**	-	+/-	-	+	-	+	+	+	+	+	**PD**	+	+	+	+	+	**PD**	2	b
699	**Red cabbage (bagged pre-cut leafy vegetables)**	-	-	-	-	-	+	+	+	+	+	**PD**	+	+	+	+	+	**PD**	2	b
715	White cabbage (bagged pre-cut leafy vegetables)	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	b
716	Fresh vegetable mix (cabbage, carrots, salad...) (bagged pre-cut leafy vegetables)	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	b
717	Spinach (bagged pre-cut leafy vegetables)	-	-	-	+	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	b
718	Red cabbage (bagged pre-cut leafy vegetables)	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	b
522	**Frozen mixed vegetables**	-	-	+	+	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	2	c
523	**Vegetable based cake**	-	-	+	-	+	-	-	/	/	-	**ND**	+	+ (ims2)	+	+	+ (ims 2)	**PA**	2	c
524	**Fried frozen vegetables**	+	+	/	/	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	2	c
525	**Mixed vegetables**	+	+	/	/	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	2	c
526	**Fried frozen mushrooms**	+(1col)	-	+	-	+	-	-	/	/	-	**ND**	-	-	/	/	-	**ND**	2	c
527	**Fried frozen vegetables**	-	+(NC)	+	+(NC)	+	-	/	/	/	-	**ND**	-	/	/	/	-	**ND**	2	c
683	Frozen spinach	-	-	-	+	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	c
684	Frozen fried vegetables	-	+ (2)	-	+	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	c

VEGETABLES PRODUCTS																				
Sample N°	Product	Reference method ISO 16654♦					BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - Short enrichment step						BAX® System Real-Time PCR Assay for *E. coli* O157: H7 method - 24h enrichment step						Category	Type
		Characteristic colonies				Result	PCR result	Confirmatory tests			Final result	Agreement	PCR Result	Confirmatory tests			Final result	Agreement		
		IMS 6H		IMS 24H				CT SMAC Characteristic colonies	O157 latex	H7 Latex				CT SMAC Characteristic colonies	O157 latex	H7 Latex				
		CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157															
685	Frozen fried vegetables	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	c
686	Frozen mixed vegetables	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	c
687	Frozen mixed vegetables	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	c
700	**Frozen vegetables for ratatouille**	-	-	-	-	-	-	/	/	/	-	**NA**	+	+/- (2)	+	+	+ (ims 2)	**PD**	2	c
701	**Frozen vegetables mix**	-	-	+	-	-	-	+ (1)	-	/	-	**NA**	-	/	/	/	-	**NA**	2	c
702	**Frozen sliced links**	-	-	-	-	-	-	/	/	/	-	**NA**	+	+	+	+	+	**PD**	2	c
740	**Green asparagus**	+ (1)	-	+	+	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	2	c
741	**Peas**	-	-	+	+	+	+	+	+	+	+	**PA**	+	+	+	+	+	**PA**	2	c
924	Vegetables julienne	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	c
925	Ratatouille	-	-	-	-	-	-	/	/	/	-	**NA**	-	/	/	/	-	**NA**	2	c
8982	**Frozen spinach**	st	-	-	-	-	+	+M	+	+	+	**PD**	+	+1/2	+	+	+	**PD**	2	c
8983	**Frozen carrots**	+p	+p	/	/	+	+	+p	+	+	+	**PA**	+	+p	+	+	+	**PA**	2	c
9021	**Frozen spinach**	st	st	-	-	-	+	+M	+	+	+	**PD**	+	+p	+	+	+	**PD**	2	c
9022	**Frozen carrots**	+p	+p	/	/	+	+	+p	+	+	+	**PA**	+	+p	+	+	+	**PA**	2	c

Appendix 6 - Relative level of detection: raw data

Spinach
***E. coli* O157 :H7 Ad683** Total viable count:14000 ufc/g

Sample N°	Level	Inoculation level (cfu/25g)	Reference method ISO 16654♦						BAX® System *E.coli* O157: H7 Real-Time method - 8h enrichment step						BAX® System *E.coli* O157: H7 Real-Time method - 24h enrichment step					
			Characteristic colonies				Result	Positive results/ Total	PCR-8H	Confirmatory tests			PCR 8H	Positive Results/ Total	PCR	Confirmatory tests			PCR 24H	Positive Results/ Total
			IMS 6H		IMS 24H					CT SMAC-Characteristic colonies	Latex O157	Latex H7	Final result			CT SMAC-Characteristic colonies	Latex O157	Latex H7	Final result	
			CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157														
560	0	0	-	-	-	-	-	0/6	-	/	/	/	-	0 /6	-	/	/	/	-	0 /6
561			-	-	-	-	-		-	/	/	/	-		-	/	/	/	-	
562			-	-	-	-	-		-	/	/	/	-		-	/	/	/	-	
563			-	-	-	-	-		-	/	/	/	-		-	/	/	/	-	
564			-	-	-	-	-		-	/	/	/	-		-	/	/	/	-	
565			-	-	-	-	-		-	/	/	/	-		-	/	/	/	-	
566	1	0,2	-	-	-	-	-	1/6	+	+	+	+	+	1 /6	+	+	+	+	+	1 /6
567			-	-	-	-	-		-	-	/	/	-		-	/	/	/	-	
568			-	-	-	-	-		-	-	/	/	-		-	/	/	/	-	
569			-	-	-	-	-		-	-	/	/	-		-	/	/	/	-	
570			-	-	-	-	-		-	-	/	/	-		-	/	/	/	-	
571			+	+	/	/	+		-	-	/	/	-		-	/	/	/	-	
572	2	0,4	-	-	-	-	-	1/6	-	-	/	/	-	4 /6	-	/	/	/	-	4 /6
573			+	+	/	/	+		+	+	+	+	+		+	+	+	+	+	
574			-	-	-	-	-		+	+	+	+	+		+	+ (ims1)	+	+	+ (ims1)	
575			-	-	-	-	-		+	+	+	+	+		+	+ (ims1)	+	+	+ (ims1)	
576			-	-	-	-	-		+	+	+	+	+		+	+	+	+	+	
577			-	-	-	-	-		-	-	/	/	-		-	/	/	/	-	
578	3	0,8	-	-	-	-	-	5/6	+	+	+	+	+	4 /6	+	+	+	+	+	4 /6
579			+	+	/	/	+		-	-	/	/	-		-	/	/	/	-	
580			+	+	/	/	+		-	-	/	/	-		-	/	/	/	-	
581			+	+	/	/	+		+	+	+	+	+		+	+ (ims1)	+	+	+ (ims1)	
582			+	+	/	/	+		+	+	+	+	+		+	+	+	+	+	
583			+	+	/	/	+		+	+	+	+	+		+	+	+	+	+	
584	4	2,1	-	-	-	-	-	5/6	+	+	+	+	+	6 /6	+	+	+	+	+	6 /6
585			+	+	/	/	+		+	+	+	+	+		+	+	+	+	+	
586			+	+	/	/	+		+	+	+	+	+		+	+	+	+	+	
587			+	+	/	/	+		+	+	+	+	+		+	+	+	+	+	
588			+	+	/	/	+		+	+	+	+	+		+	+	+	+	+	
589			+	+	/	/	+		+	+	+	+	+		+	+(1col)	+	+	+	

♦ Analyses performed according to the COFRAC accreditation

Fresh ground beef Total viable count: 5000 cfu/g

E. coli O157:H7 Ad682

Sample N°	Level	Inoculation level (cfu/25g)	Reference method ISO 16654 ♦						BAX® System *E.coli* O157: H7 Real-Time method - 7h enrichment step						BAX® System *E.coli* O157: H7 Real-Time method - 8h enrichment step						BAX® System *E.coli* O157: H7 Real-Time method - 24h enrichment step					
			characteristics colonies				ISO 16654 result	Positive Results/Total	PCR-7H	Confirmatory tests			PCR 7H	Positive Results/Total	PCR-8H	Confirmatory tests			PCR 8H	Positive Results/Total	PCR	Confirmatory tests			PCR	Positive Results/Total
			IMS 6H		IMS 24H					CT SMAC-Characteristic colonies	Latex O157	Latex H7	Final result			CT SMAC-Characteristic colonies	Latex O157	Latex H7	final result			CT SMAC-Characteristic colonies	Latex O157	Latex H7	Final result	
			CT SMAC	CHROMagar O157	CT SMAC	CHROMagar O157																				
442	0	0	-	-	-	-	-	0/6	-	/	/	/	-	0/6	-	/	/	/	-	0/6	-	/	/	/	-	0/6
443			-	-	-	-	-		-	/	/	/	-		-	/	/	/	-		-	/	/	/	-	
444			-	-	-	-	-		-	/	/	/	-		-	/	/	/	-		-	/	/	/	-	
445			-	-	-	-	-		-	/	/	/	-		-	/	/	/	-		-	/	/	/	-	
446			-	-	-	-	-		-	/	/	/	-		-	/	/	/	-		-	/	/	/	-	
447			-	-	-	-	-		-	/	/	/	-		-	/	/	/	-		-	/	/	/	-	
448	1	0,2	+	+	/	/	+	2/6	-	-	/	/	-	0/6	-	-	/	/	-	0/6	-	-	/	/	-	0/6
449			-	-	-	-	-		-	-	/	/	-		-	-	/	/	-		-	-	/	/	-	
450			+	+	/	/	+		-	NC	/	/	-		-	NC	/	/	-		-	-	/	/	-	
451			-	-	-	+/-	-		-	-	/	/	-		-	-	/	/	-		-	-	/	/	-	
452			-	-	-	-	-		-	-	/	/	-		-	-	/	/	-		-	-	/	/	-	
453			-	-	-	-	-		-	-	/	/	-		-	-	/	/	-		-	-	/	/	-	
454	2	0,4	-	-	-	-	-	4/6	-	-	/	/	-	2/6	-	-	/	/	-	2/6	-	-	/	/	-	2/6
455			+	+	/	/	+		-	-	/	/	-		-	-	/	/	-		-	-	/	/	-	
456			+	+	/	/	+		+	+	+	+	+		+	+	+	+	+		+	+	+	+	+	
457			-	-	-	+/-	-		-	-	/	/	-		-	-	/	/	-		-	+	+	+	-	
458			-	-	+	+	+		+	+	+	+	+		+	+	+	+	+		+	+	+	+	+	
459			+	+	/	/	+		-	-	/	/	-		-	-	/	/	-		-	-	/	/	-	
460	3	0,9	+	+	/	/	+	4/6	-	-	/	/	-	4/6	-	-	/	/	-	4/6	-	-	/	/	-	4/6
461			+	+	/	/	+		+	+	+	+	+		+	+	+	+	+		+	+	+	+	+	
462			-	-	-	-	-		+	+	+	+	+		+	+	+	+	+		+	+	+	+	+	
463			-	-	-	-	-		+	+	+	+	+		+	+	+	+	+		+	+	+	+	+	
464			+	+	/	/	+		+	+	+	+	+		+	+	+	+	+		+	+	+	+	+	
465			+	+	/	/	+		-	-	/	/	-		-	-	/	/	-		-	-	/	/	-	
590	4	2,2	+	+	/	/	+	6/6	+	+	+	+	+	6/6	+	+	+	+	+	6/6	+	+	+	+	+	6/6
591			+	+	/	/	+		+	+	+	+	+		+	+	+	+	+		+	+	+	+	+	
592			+	+	/	/	+		+	+	+	+	+		+	+	+	+	+		+	+	+	+	+	
593			+	+	/	/	+		+	+	+	+	+		+	+	+	+	+		+	+	+	+	+	
594			+	+	/	/	+		+	+	+	+	+		+	+	+	+	+		+	+	+	+	+	
595			+	+	/	/	+		+	+	+	+	+		+	+	+	+	+		+	+	+	+	+	

♦ Analyses performed according to the COFRAC accreditation

Appendix 7 – Inclusivity and exclusivity: raw data

INCLUSIVITY								
No	**Species**	**Serotype**	**Strain identification**	**Origin**	Inoculation level cfu/225ml	PCR result BAX® System Real-Time PCR Assay for *E.coli* O157:H7	Confirmatory tests	
							CT-SMAC Characteristic colonies	O157 and H7 latex test
1.	*Escherichia coli*	O157:H7	B177	WWTP	21	+	+	+
2.	*Escherichia coli*	O157:H7	BV2	Slaughterhouse	15	+	+	+
3.	*Escherichia coli*	O157:H7	BR3	Slaughterhouse	47	+	+	+
4.	*Escherichia coli*	O157:H7	BD4	Slaughterhouse	53	+	+	+
5.	*Escherichia coli*	O157:H7	ENV177	WWTP	17	+	+	+
6.	*Escherichia coli*	O157:H7	ET8	WWTP	19	+	+	+
7.	*Escherichia coli*	O157:H7	EK9	WWTP	72	+	+	+
8.	*Escherichia coli*	O157:H7	435	Ground beef	14	+	+	+
9.	*Escherichia coli*	O157:H7	670T	Ground beef	57	+	+	+
10.	*Escherichia coli*	O157:H7	730T	Ground beef	16	+	+	+
11.	*Escherichia coli*	O157:H7	226T	Ground beef	82	+	+	+
12.	*Escherichia coli*	O157:H7	42197-1	Ground beef	57	+	+	+
13.	*Escherichia coli*	O157:H7	A3612	Ground beef	23	+	+	+
14.	*Escherichia coli*	O157:H7	A4513	Ground beef	32	+	+	+
15.	*Escherichia coli*	O157:H7	A1075	Ground beef	14	+	+	+
16.	*Escherichia coli*	O157:H7	B68	Slaughterhouse	32	+	+	+
17.	*Escherichia coli*	O157:H7	AT40	Slaughterhouse	47	+	+	+
18.	*Escherichia coli*	O157:H7	AV36	Slaughterhouse	51	+	+	+
19.	*Escherichia coli*	O157:H7	AR15	Slaughterhouse	36	+	+	+
20.	*Escherichia coli*	O157:H7	LS3	Feces	37	+	+	+
21.	*Escherichia coli*	O157:H7	AMVT6	Feces	21	+	+	+
22.	*Escherichia coli*	O157:H7	ATKP8	Feces	43	+	+	+
23.	*Escherichia coli*	O157:H7	AZRS15	Feces	51	+	+	+
24.	*Escherichia coli*	O157:H7	R33-9	Bovine faeces	17	+	+	+
25.	*Escherichia coli*	O157:H7	AZ15-6	Bovine faeces	21	+	+	+
26.	*Escherichia coli*	O157:H7	AQ29-4	Bovine faeces	42	+	+	+
27.	*Escherichia coli*	O157:H7	AA18-3	Bovine faeces	47	+	+	+
28.	*Escherichia coli*	O157:H7	LS56	Faeces	51	+	+	+
29.	*Escherichia coli*	O157:H7	A425TK	Faeces	32	+	+	+
30.	*Escherichia coli*	O157:H7	A206RP	Faeces	27	+	+	+
31.	*Escherichia coli*	O157:H7	A778EF	Faeces	17	+	+	+
32.	*Escherichia coli*	O157:H7	MK41242	Ground beef	10	+	+	+
33.	*Escherichia coli*	O157:H7	AMK2608	Ground beef	37	+	+	+
34.	*Escherichia coli*	O157:H7	AMK1506	Ground beef	42	+	+	+
35.	*Escherichia coli*	O157:H7	AMK1311	Ground beef	71	+	+	+
36.	*Escherichia coli*	O157:H7	37006ID	Ground beef	64	+	+	+
37.	*Escherichia coli*	O157:H7	A1518ID	Ground beef	39	+	+	+
38.	*Escherichia coli*	O157:H7	A1512ID	Ground beef	27	+	+	+

INCLUSIVITY								
No	**Species**	**Serotype**	**Strain identification**	**Origin**	Inoculation level cfu/225ml	PCR result BAX® System Real-Time PCR Assay for *E.coli* O157:H7	Confirmatory tests	
							CT-SMAC Characteristic colonies	O157 and H7 latex test
39.	*Escherichia coli*	O157:H7	A1814ID	Ground beef	23	+	+	+
40.	*Escherichia coli*	O157:H7	A1989ID	Ground beef	41	+	+	+
41.	*Escherichia coli*	O157:H7	EF190	Faeces	32	+	+	+
42.	*Escherichia coli*	O157:H7	Ad686	Slaughterhouse	48	+	+	+
43.	*Escherichia coli*	O157:H7	CIP103571 (ATCC 35150)	Clinical origin	39	+	+	+
44.	*Escherichia coli*	O157:H7	ATCC 43888	/	47	+	+	+
45.	*Escherichia coli*	O157:H7	Ad485	Ground beef	51	+	+	+
46.	*Escherichia coli*	O157:H7	Ad486	Ground beef	42	+	+	+
47.	*Escherichia coli*	O157:H7	Ad487	Ground beef	37	+	+	+
48.	*Escherichia coli*	O157:H7	Ad488	Ground beef	29	+	+	+
49.	*Escherichia coli*	O157:H7	Ad489	Ground beef	33	+	+	+
50.	*Escherichia coli*	O157:H7	ATCC 700728	/	41	+	+	+

EXCLUSIVITY								
No	Species	Serotype	Strain identification	Origin	Inoculation level cfu/225ml	PCR result BAX® System Real-Time PCR Assay for *E.coli* O157:H7	Confirmatory tests	
							CT-SMAC Characteristic colonies	O157 and H7 latex test
1.	*Escherichia coli*	O92:H33	JM221	Clinical origin (Mexico)	$5.7\ 10^5$	-	-	/
2.	*Escherichia coli*	O3:H2	38765	Clinical origin (Chili)	$5.0\ 10^5$	-	-	/
3.	*Escherichia coli*	O78:H11	H10407	ATCC 35401	$4.9\ 10^5$	-	-	/
4.	*Escherichia coli*	O6:H6	EDL1493	/	$3.6\ 10^5$	-	-	/
5.	*Escherichia coli*	O6:H10	ECOR10	Clinical origin (Sweden)	$7.6\ 10^5$	-	-	/
6.	*Escherichia coli*	O111:H21	DEC6a	Clinical origin (USA)	$2.5\ 10^5$	-	-	/
7.	*Escherichia coli*	O86:H43	ECOR23	Animal origin (elephant USA)	$3.7\ 10^5$	-	-	/
8.	*Escherichia coli*	O26:H11	DEC9a	Clinical origin (USA)	$3.9\ 10^5$	-	-	/
9.	*Escherichia coli*	O111:H8	DEC8b	Clinical origin (USA)	$4.9\ 10^5$	-	-	/
10.	*Escherichia coli*	O128:H2	DEC11a	Clinical origin (USA)	$8.0\ 10^5$	-	-	/
11.	*Escherichia coli*	O111:H2	DEC12a	Clinical origin (UK)	$5.7\ 10^5$	-	-	/
12.	*Escherichia coli*	O128:H7	DEC13a	Clinical origin (USA)	$3.9\ 10^5$	-	-	/
13.	*Escherichia coli*	O78:H12	TX-1	ATCC 43896	$4.1\ 10^5$	-	-	/
14.	*Escherichia coli*	O104:H21	ECOR26	Clinical origin (USA)	$7.0\ 10^5$	-	-	/
15.	*Escherichia coli*	O157:H43	DEC7a	Pork (USA)	$4.0\ 10^5$	-	-	/
16.	*Escherichia coli*	O55:H7	DEC5d	Clinical origin (Sri Lanka)	$4.2\ 10^5$	-	-	/
17.	*Escherichia coli*	O44:H18	42	Clinical origin (Peru)	$7.6\ 10^5$	-	-	/
18.	*Escherichia coli*	O127:H6	E2348/69	Clinical origin (UK)	$8.5\ 10^5$	-	+	O157-
19.	*Escherichia coli*	O55:H6	DEC1a	Clinical origin (USA)	$4.5\ 10^5$	-	+	O157-
20.	*Escherichia coli*	O18:K1:H7	RS218	Clinical origin	$4.7\ 10^5$	-	-	/
21.	*Salmonella*	Landau	Ad499	/	$2.9\ 10^5$	-	-	/
22.	*Salmonella*	Sternhauze	Ad500	/	$3.8\ 10^5$	-	-	/
23.	*Salmonella*	Urbana	Ad501	/	$3.7\ 10^5$	-	-	/
24.	*Salmonella*	Wayne	Ad502	/	$3.0\ 10^5$	-	-	/
25.	*Hafnia alvei*		88	Bakery	$4.2\ 10^5$	-	-	/
26.	*Hafnia alvei*		167	Sausage	$3.2\ 10^5$	-	-	/
27.	*Citrobacter freundii*		25	Frozen raw spinach	$3.9\ 10^5$	-	-	/
28.	*Citrobacter freundii*		104	Ground beef	$3.6\ 10^5$	-	-	/
29.	*Escherichia vulneris*		127	Raw milk	$5.5\ 10^5$	-	-	/
30.	*Pantoea spp.*		134	Pork	$8.5\ 10^4$	-	-	/
31.	*Escherichia coli*	O157	Ad524	Environment (dairy product)	$4.2\ 10^5$	-	-	/
32.	*Escherichia coli*	O157	Ad525	Faeces	$5.7\ 10^5$	-	-	/
33.	*Escherichia coli*	O157	Ad526	Faeces	$6.4\ 10^5$	-	-	/
34.	*Escherichia coli*	O157	Ad527	Clinical origin	$2.3\ 10^5$	-	-	/
35.	*Escherichia coli*	O157:H-	O1.12.903	/	$2.8\ 10^5$	-	-	/
36.	*Escherichia coli*	O157:H-	O1.12.905	/	$2.9\ 10^5$	-	-	/

Appendix 8 - Inter-laboratory study: results obtained by the collaborative laboratories and the expert laboratory

Laboratory: **A** IMS were done on 07/06/2010

Aerobic mesophilic flora 2,9.10^5/g

N°Sample	Reference method ISO 16654				Alternative method- BAX® System O157:H7 Real Time				Agreement
	IMS 24H		Confirmation result	Final result	PCR result	CT SMAC	Latex O157:H7	Final result	
	CT SMAC	O157:H7 ID							
A3	+	+	-	-	-	-	/	-	**NA**
A8	+	+	-	-	-	-	/	-	**NA**
A9	+	+	-	-	-	-	/	-	**NA**
A12	+	+	+	+	-	-	/	-	**ND**
A15	+	+	+	+	-	-	/	-	**ND**
A18	-	+	-	-	-	-	/	-	**NA**
A20	+	+	-	-	-	-	/	-	**NA**
A21	+	+	-	-	-	-	/	-	**NA**
A1	+	+	+	+	+	+	+	+	**PA**
A4	+	+	+	+	+	+	+	+	**PA**
A7	+	+	+	+	+	+	+	+	**PA**
A10	+	+	+	+	+	+	+	+	**PA**
A11	+	+	+	+	+	+	+	+	**PA**
A13	+	+	+	+	+	+	+	+	**PA**
A17	+	+	+	+	+	+	+	+	**PA**
A24	+	+	+	+	+	+	+	+	**PA**
A2	+	+	+	+	+	+	+	+	**PA**
A5	+	+	+	+	+	+	+	+	**PA**
A6	+	+	+	+	+	+	+	+	**PA**
A14	+	+	+	+	+	+	+	+	**PA**
A16	+	+	+	+	+	+	+	+	**PA**
A19	+	+	+	+	+	+	+	+	**PA**
A22	+	+	+	+	+	+	+	+	**PA**
A23	+	+	+	+	+	+	+	+	**PA**

Laboratory: **B**

Aerobic mesophilic flora $2{,}8.10^5$/g

Sample No	Reference method ISO 16654				Alternative method-BAX® System O157:H7 Real Time				Agreement
	IMS 24H		Confirmation result	Final result	PCR result	CT SMAC	Latex O157:H7	Final result	
	CT SMAC	O157:H7 ID							
B3	-	-	/	-	-	/	/	-	**NA**
B8	+/-	-	-	-	-	/	/	-	**NA**
B9	+	-	/	-	-	/	/	-	**NA**
B12	+/-	-	-	-	-	/	/	-	**NA**
B15	-	-	/	-	-	/	/	-	**NA**
B18	-	-	/	-	-	/	/	-	**NA**
B20	-	-	/	-	-	/	/	-	**NA**
B21	-	-	/	-	-	/	/	-	**NA**
B1	+	+	+	+	+	+	+	+	**PA**
B4	+	+	+	+	+	+	+	+	**PA**
B7	+	+	+	+	+	+	+	+	**PA**
B10	+	+	+	+	+	+	+	+	**PA**
B11	+	+	+	+	+	+	+	+	**PA**
B13	+	+	+	+	+	+	+	+	**PA**
B17	+	+	+	+	+	+	+	+	**PA**
B24	-	-	/	-	+	+	+	+	**PD**
B2	+	+	+	+	+	+	+	+	**PA**
B5	+	+	+	+	+	+	+	+	**PA**
B6	+	+	+	+	+	+	+	+	**PA**
B14	+	+	+	+	+	+	+	+	**PA**
B16	+	+	+	+	+	+	+	+	**PA**
B19	+	+	+	+	+	+	+	+	**PA**
B22	-	-	/	-	+	+	+	+	**PD**
B23	+	+	+	+	+	+	+	+	**PA**

+/-: doubtful colonies

Laboratory: **D**

Aerobic mesophilic flora 1,6.10^5/g

Sample No	Reference method ISO 16654				Alternative method-BAX® System O157:H7 Real Time				Agreement
	IMS 24H		Confirmation result	Final result	PCR result	CT SMAC	Latex O157:H7	Final result	
	CT SMAC	O157:H7 ID							
D3	-	+	-	-	-	/	-	-	**NA**
D8	-	+	-	-	-	/	-	-	**NA**
D9	-	+	-	-	-	/	-	-	**NA**
D12	+	+	-	-	-	/	-	-	**NA**
D15	+	+	-	-	-	/	-	-	**NA**
D18	+	+	-	-	-	/	-	-	**NA**
D20	+	+	-	-	-	/	-	-	**NA**
D21	+	+	-	-	-	/	-	-	**NA**
D1	+	+	+	+	+	+	+	+	**PA**
D4	+	+	+	+	+	+	+	+	**PA**
D7	+	+	+	+	+	+	+	+	**PA**
D10	+	+	O-	-	+	+	+	+	**PD**
D11	+	+	+	+	+	+	+	+	**PA**
D13	+	+	+	+	+	+	+	+	**PA**
D17	-	+	O-	-	+	+	+	+	**PD**
D24	+	+	+	+	+	+	+	+	**PA**
D2	+	+	+	+	+	+	+	+	**PA**
D5	+	+	+	+	+	+	+	+	**PA**
D6	+	+	+	+	+	+	+	+	**PA**
D14	+	+	+	+	+	+	+	+	**PA**
D16	+	+	+	+	+	+	+	+	**PA**
D19	+	+	+	+	+	+	+	+	**PA**
D22	+	+	+	+	+	+	+	+	**PA**
D23	+	+	+	+	+	+	+	+	**PA**

Laboratory: **E**

Aerobic mesophilic flora 4,5.10^5/g

Sample No	Reference method ISO 16654				Alternative method-BAX® System O157:H7 Real Time				Agreement
	IMS 24H		Confirmation result	Final result	PCR result	CT SMAC	Latex O157:H7	Final result	
	CT SMAC	O157:H7 ID							
E3	-	-	/	-	-	-	/	-	**NA**
E8	-	-	/	-	-	-	/	-	**NA**
E9	-	-	/	-	-	-	/	-	**NA**
E12	-	-	/	-	-	-	/	-	**NA**
E15	-	-	/	-	-	-	/	-	**NA**
E18	-	-	/	-	-	-	/	-	**NA**
E20	-	-	/	-	-	-	/	-	**NA**
E21	-	-	/	-	-	-	/	-	**NA**
E1	+	+	+	+	+	+	+	+	**PA**
E4	+	+	+	+	+	+	+	+	**PA**
E7	+	+	+	+	+	+	+	+	**PA**
E10	+	+	+	+	+	+	+	+	**PA**
E11	+	+	+	+	+	+	+	+	**PA**
E13	+	+	+	+	+	+	+	+	**PA**
E17	+	+	+	+	+	+	+	+	**PA**
E24	+	+	+	+	+	+	+	+	**PA**
E2	+	+	+	+	+	+	+	+	**PA**
E5	+	+	+	+	+	+	+	+	**PA**
E6	+	+	+	+	+	+	+	+	**PA**
E14	+	+	+	+	+	+	+	+	**PA**
E16	+	+	+	+	+	+	+	+	**PA**
E19	+	+	+	+	+	+	+	+	**PA**
E22	+	+	+	+	+	+	+	+	**PA**
E23	+	+	+	+	+	+	+	+	**PA**

Laboratory: **F** Samples storage between 8,7 and 9,3°c

Aerobic mesophilic flora 3,0.10^5/g

Sample No	Reference method ISO 16654				Alternative method-BAX® System O157:H7 Real Time				Agreement
	IMS 24H		Confirmation result	Final result	PCR result	CT SMAC	Latex O157:H7	Final result	
	CT SMAC	O157:H7 ID							
F3	+	+	-	-	-	/	/	-	**NA**
F8	+	+	-	-	-	/	/	-	**NA**
F9	+	+	-	-	-	/	/	-	**NA**
F12	+	+	-	-	-	/	/	-	**NA**
F15	-	-	/	-	-	/	/	-	**NA**
F18	+	+	+	+	-	/	/	-	**ND**
F20	+(1colony)	-	+	+	-	/	/	-	**ND**
F21	+	+	-	-	-	/	/	-	**NA**
F1	+	+	+	+	+	+	+	+	**PA**
F4	+	+	+	+	+	+	+	+	**PA**
F7	+	+	+	+	+	+	+	+	**PA**
F10	+	+	+	+	+	+	+	+	**PA**
F11	+	+	+	+	+	+	+	+	**PA**
F13	+	+	+	+	+	+	+	+	**PA**
F17	+	+	+	+	+	+	+	+	**PA**
F24	+	+	+	+	+	+	+	+	**PA**
F2	+	+	+	+	+	+	+	+	**PA**
F5	+	+	+	+	+	+	+	+	**PA**
F6	+	+	+	+	+	+	+	+	**PA**
F14	+	+	+	+	+	+	+	+	**PA**
F16	+	+	+	+	+	+	+	+	**PA**
F19	+	+	+	+	+	+	+	+	**PA**
F22	+	+	+	+	+	+	+	+	**PA**
F23	+	+	+	+	+	+	+	+	**PA**

Laboratory: **G**

Aerobic mesophilic flora 1,7.10^5/g

Sample No	Reference method ISO 16654				Alternative method-BAX® System O157:H7 Real Time				Agreement
	IMS 24H		Confirmation result	Final result	PCR result	CT SMAC	Latex O157:H7	Final result	
	CT SMAC	O157:H7 ID							
G3	-	-	/	-	-	/	/	-	**NA**
G8	-	+	-	-	-	/	/	-	**NA**
G9	-	+	-	-	-	/	/	-	**NA**
G12	-	-	/	-	-	/	/	-	**NA**
G15	+	+	-	-	-	/	/	-	**NA**
G18	+	-	-	-	-	/	/	-	**NA**
G20	-	-	/	-	-	/	/	-	**NA**
G21	-	-	/	-	-	/	/	-	**NA**
G1	+	+	+	+	+	+	+	+	**PA**
G4	+	+	+	+	+	+	+	+	**PA**
G7	+	+	+	+	+	+	+	+	**PA**
G10	+	+	+	+	+	+	+	+	**PA**
G11	+	+	+	+	+	+	+	+	**PA**
G13	+	+	+	+	+	+	+	+	**PA**
G17	+	+	+	+	+	+	+	+	**PA**
G24	+	+	+	+	+	+	+	+	**PA**
G2	+	+	+	+	+	+	+	+	**PA**
G5	+	+	+	+	+	+	+	+	**PA**
G6	+	+	+	+	+	+	+	+	**PA**
G14	+	+	+	+	+	+	+	+	**PA**
G16	+	+	+	+	+	+	+	+	**PA**
G19	+	+	+	+	+	+	+	+	**PA**
G22	+	+	+	+	+	+	+	+	**PA**
G23	+	+	+	+	+	+	+	+	**PA**

Laboratory: **J**

Aerobic mesophilic flora 2,5.10^5/g

Sample No	Reference method ISO 16654				Alternative method-BAX® System O157:H7 Real Time				Agreement
	IMS 24H		Confirmation result	Final result	PCR result	CT SMAC	Latex O157:H7	Final result	
	CT SMAC	O157:H7 ID							
J3	-	-	/	-	-	/	/	-	**NA**
J8	-	-	/	-	-	/	/	-	**NA**
J9	-	-	/	-	-	/	/	-	**NA**
J12	-	-	/	-	-	/	/	-	**NA**
J15	-	-	/	-	-	/	/	-	**NA**
J18	-	-	/	-	-	/	/	-	**NA**
J20	-	-	/	-	-	/	/	-	**NA**
J21	-	-	/	-	-	/	/	-	**NA**
J1	+	+	+	+	+	+	+	+	**PA**
J4	+	+	+	+	+	+	+	+	**PA**
J7	+	+	+	+	+	+	+	+	**PA**
J10	+	+	+	+	+	+	+	+	**PA**
J11	+	+	+	+	+	+	+	+	**PA**
J13	+	+	+	+	+	+	+	+	**PA**
J17	+	+	+	+	+	+	+	+	**PA**
J24	+	+	+	+	+	+	+	+	**PA**
J2	+	+	+	+	+	+	+	+	**PA**
J5	+	+	+	+	+	+	+	+	**PA**
J6	+	+	+	+	+	+	+	+	**PA**
J14	+	+	+	+	+	+	+	+	**PA**
J16	+	+	+	+	+	+	+	+	**PA**
J19	+	+	+	+	+	+	+	+	**PA**
J22	+	+	+	+	+	+	+	+	**PA**
J23	+	+	+	+	+	+	+	+	**PA**

Laboratory: **K**

Aerobic mesophilic flora 3,2.10⁴/g

Sample No	Reference method ISO 16654				Alternative method-BAX® System O157:H7 Real Time				Agreement
	IMS 24H		Confirmation result	Final result	PCR result	CT SMAC	Latex O157:H7	Final result	
	CT SMAC	O157:H7 ID							
K3	-	-	/	-	-	-	/	-	**NA**
K8	-	-	/	-	-	-	/	-	**NA**
K9	-	-	/	-	-	-	/	-	**NA**
K12	-	-	/	-	-	-	/	-	**NA**
K15	-	-	/	-	-	-	/	-	**NA**
K18	-	-	/	-	-	-	/	-	**NA**
K20	-	-	/	-	-	-	/	-	**NA**
K21	-	-	/	-	-	-	/	-	**NA**
K1	+	+	+	+	+	+	+	+	**PA**
K4	+	+	+	+	+	+	+	+	**PA**
K7	+	+	+	+	+	+	+	+	**PA**
K10	+	+	+	+	+	+	+	+	**PA**
K11	+	+	+	+	+	+	+	+	**PA**
K13	+	+	+	+	+	+	+	+	**PA**
K17	+	+	+	+	+	+	+	+	**PA**
K24	+	+	+	+	+	+	+	+	**PA**
K2	+	+	+	+	+	+	+	+	**PA**
K5	+	+	+	+	+	+	+	+	**PA**
K6	+	+	+	+	+	+	+	+	**PA**
K14	+	+	+	+	+	+	+	+	**PA**
K16	+	+	+	+	+	+	+	+	**PA**
K19	+	+	+	+	+	+	+	+	**PA**
K22	+	+	+	+	+	+	+	+	**PA**
K23	+	+	+	+	+	+	+	+	**PA**

Laboratory: **L**

Aerobic mesophilic flora 6,2;10^5/g

Sample No	Reference method ISO 16654				Alternative method-BAX® System O157:H7 Real Time				Agreement
	IMS 24H		Confirmation result	Final result	PCR result	CT SMAC	Latex O157:H7	Final result	
	CT SMAC	O157:H7 ID							
L3	-	-	/	-	-	/	/	-	**NA**
L8	-	-	/	-	-	/	/	-	**NA**
L9	-	-	/	-	-	/	/	-	**NA**
L12	-	-	/	-	-	/	/	-	**NA**
L15	-	-	/	-	-	/	/	-	**NA**
L18	-	-	/	-	-	/	/	-	**NA**
L20	-	-	/	-	-	/	/	-	**NA**
L21	-	-	/	-	-	/	/	-	**NA**
L1	+	+	+	+	+	+	+	+	**PA**
L4	+	+	+	+	+	+	+	+	**PA**
L7	+	+	+	+	+	+	+	+	**PA**
L10	+	+	+	+	+	+	+	+	**PA**
L11	+	+	+	+	+	+	+	+	**PA**
L13	+	+	+	+	+	+	+	+	**PA**
L17	+	+	+	+	+	+	+	+	**PA**
L24	+	+	+	+	+	+	+	+	**PA**
L2	+	+	+	+	+	+	+	+	**PA**
L4	+	+	+	+	+	+	+	+	**PA**
L6	+	+	+	+	+	+	+	+	**PA**
L14	+	+	+	+	+	+	+	+	**PA**
L16	+	+	+	+	+	+	+	+	**PA**
L19	+	+	+	+	+	+	+	+	**PA**
L22	+	+	+	+	+	+	+	+	**PA**
L23	+	+	+	+	+	+	+	+	**PA**

Laboratory: **M**

Aerobic mesophilic flora $2{,}8.10^5$/g

Sample No	Reference method ISO 16654				Alternative method-BAX® System O157:H7 Real Time				Agreement
	IMS 24H		Confirmation result	Final result	PCR result	CT SMAC	Latex O157:H7	Final result	
	CT SMAC	O157:H7 ID							
M3	-	-	/	-	-	-	/	-	**NA**
M8	+	-	-	-	-	+/-	-	-	**NA**
M9	-	-	/	-	-	+/-	-	-	**NA**
M12	-	-	/	-	-	-	/	-	**NA**
M15	-	-	/	-	-	-	/	-	**NA**
M18	-	-	/	-	-	-	/	-	**NA**
M20	-	-	/	-	-	-	/	-	**NA**
M21	-	-	/	-	-	-	/	-	**NA**
M1	+	+	+	+	+	+	+	+	**PA**
M4	+	+	+	+	+	+	+	+	**PA**
M7	+	+	+	+	+	+	+	+	**PA**
M10	+	+	+	+	+	+	+	+	**PA**
M11	+	+	+	+	+	+	+	+	**PA**
M13	-	-	/	-	+	+	+	+	**PD**
M17	+	+	+	+	+	+	+	+	**PA**
M24	+	+	+	+	+	+	+	+	**PA**
M2	+	+	+	+	+	+	+	+	**PA**
M5	+	+	+	+	+	+	+	+	**PA**
M6	+	+	+	+	+	+	+	+	**PA**
M14	+	+	+	+	+	+	+	+	**PA**
M16	+	+	+	+	+	+	+	+	**PA**
M19	+	+	+	+	+	+	+	+	**PA**
M22	+	+	+	+	+	+	+	+	**PA**
M23	+	+	+	+	+	+	+	+	**PA**

+/-: doubtful colonies

Laboratory: **N**

Aerobic mesophilic flora 8,6.10⁴/g

Sample No	Reference method ISO 16654				Alternative method-BAX® System O157:H7 Real Time				Agreement
	IMS 24H		Confirmation result	Final result	PCR result	CT SMAC	Latex O157:H7	Final result	
	CT SMAC	O157:H7 ID							
N3	-	-	/	-	-	/	/	-	**NA**
N8	+	-	-	-	-	/	/	-	**NA**
N9	-	-	/	-	-	/	/	-	**NA**
N12	+	+	-	-	-	/	/	-	**NA**
N15	-	-	/	-	-	/	/	-	**NA**
N18	+	-	-	-	-	/	/	-	**NA**
N20	-	-	/	-	-	/	/	-	**NA**
N21	-	-	/	-	-	/	/	-	**NA**
N1	-	+	-	-	+	+	+	+	**PD**
N4	+	-	-	-	+	+	+	+	**PD**
N7	+	+	+	+	+	+	+	+	**PA**
N10	+	+	+	+	+	+	+	+	**PA**
N11	+	+	-	-	+	+	+	+	**PD**
N13	+	+	+	+	+	+	+	+	**PA**
N17	+	+	+	+	+	+	+	+	**PA**
N24	+	+	+	+	+	+	+	+	**PA**
N2	+	+	+	+	+	+	+	+	**PA**
N5	+	-	-	-	+	+	+	+	**PD**
N6	+	+	+	+	+	+	+	+	**PA**
N14	+	+	+	+	+	+	+	+	**PA**
N16	+	+	+	+	+	+	+	+	**PA**
N19	+	+	+	+	+	+	+	+	**PA**
N22	+	+	+	+	+	+	+	+	**PA**
N23	+	+	+	+	+	+	+	+	**PA**

Laboratory: **ADRIA**

Aerobic mesophilic flora $1{,}5.10^6$/g

Sample No	Reference method ISO 16654♦				Alternative method-BAX® System O157:H7 Real Time				Agreement
	IMS 24H		Confirmation result	Final result	PCR result	CT SMAC	Latex O157:H7	Final result	
	CT SMAC	O157:H7 ID							
O3	-	-	/	-	-	/	/	-	**NA**
O8	+	-	-	-	-	/	/	-	**NA**
O9	-	-	/	-	-	/	/	-	**NA**
O12	+	-	-	-	-	/	/	-	**NA**
O15	+	-	-	-	-	/	/	-	**NA**
O18	-	-	/	-	-	/	/	-	**NA**
O20	-	-	/	-	-	/	/	-	**NA**
O21	-	-	/	-	-	/	/	-	**NA**
O1	+	-	+	+	+	+	+	+	**PA**
O4	+	+	+	+	+	+	+	+	**PA**
O7	+	+	+	+	+	+	+	+	**PA**
O10	+	-	-	-	+	+	+	+	**PD**
O11	+	+	+	+	+	+	+	+	**PA**
O13	+	+	+	+	+	+	+	+	**PA**
O17	+	+	+	+	+	+	+	+	**PA**
O24	+	+	+	+	+	+	+	+	**PA**
O2	+	+	+	+	+	+	+	+	**PA**
O5	+	+	+	+	+	+	+	+	**PA**
O6	+	-	+	+	+	+	+	+	**PA**
O14	+	+	+	+	+	+	+	+	**PA**
O16	+	+	+	+	+	+	+	+	**PA**
O19	+	+	+	+	+	+	+	+	**PA**
O22	+	+	+	+	+	+	+	+	**PA**
O23	+	+	+	+	+	+	+	+	**PA**

♦ Analyses performed according to the COFRAC accreditation