

NF VALIDATION
Validation of alternative analytical methods
Application in food microbiology

Summary report

Validation study according to the ISO 16140-2

BAX[®] System Real-Time PCR Assay
***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 vegetable products**

Qualitative method

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This report consists of 61 pages, including 8 appendices.
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The results in this report relate only to the item(s) submitted for testing.
Competencies of the laboratory are certified by COFRAC accreditation for the analyses marked with the symbol♦.

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LIST OF ABBREVIATIONS

Method & protocol

CFU	Colony Forming Units
ILS	Interlaboratory Study
MCS	Method Comparison Study
RLOD	Relative Level of Detection
RT	Relative Trueness
RTC	Ready to cook
RTE	Ready to eat
RTRH	Ready to reheat
SE	Relative Sensitivity
SP	Relative Specificity
IC	Internal Control

Interpretation

AL	Acceptability Limit
alt	Alternative method
$\bar{D}$	Average difference
FN	False Negative results
FNR	False Negative Ratio
FP	False Positive results
FPR	False Positive Ratio
LOD	Limit of Detection
NA	Negative agreement
NA _{FN (alt)}	Negative Agreement due to false negative alternative-method results
ND	Negative Deviation
ND _{FN (alt)}	Negative Deviation due to false negative alternative-method results
PA	Positive Agreement
PA _{FP (alt)}	Positive Agreement due to false positive alternative-method results
PD	Positive deviation
PD _{FP (alt)}	Positive Deviation due to false positive alternative-method results
ref	Reference method
TNA	Total Negative Agreement
TND	Total Negative Deviation

Raw data

-	No typical colonies but presence of background microflora
-A	No typical colonies but presence of high level of background microflora
-B	No typical colonies but presence of medium level of background microflora
-C	No typical colonies but presence of low level of background microflora
(x)	Number of colonies in the plate
1/2	50% level of target analyte
d	Doubtful result
i	Inhibition
ne	New DNA extraction
NC	Non-characteristic colony
NI	No identification
ni	Not isolated colony
No amp	Non characteristic PCR curve
m	Minority level of target analyte
M	Majority level of target analyte
P	Pure culture level of target analyte
st	Plate without any colony
w	Weak reaction
Bold typing	Artificially inoculated samples

Quality Assurance documents related to this study can be consulted upon request from **HYGIENA**.

The technical protocol and the result interpretation were carried out according to the ISO 16140-2:2016, ISO 16140-2/A1:2024 and the AFNOR technical rules (Revision 13).

Validation protocols	▪ ISO 16140-1:2016 - Microbiology of the food chain - Method validation — <i>Part 1: Vocabulary</i> ▪ ISO 16140-2:2016 & ISO 16140-2/A1:2024 - Microbiology of the food chain - Method validation — <i>Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method</i> ▪ AFNOR technical rules (Revision 13)
Reference method[♦]	ISO 16654:2001 - Microbiology of food and animal feeding stuffs - Horizontal method for the detection of <i>Escherichia coli</i> O157 - Amendment 1:2017: annex B: result of inter-laboratory studies. Amendment 2:2023 - Inclusion of performance testing of all culture media and reagents
Alternative method	BAX[®] System Real-Time PCR Assay <i>E. coli</i> O157:H7
Scope	> Raw beef meat > Vegetable products
Certification organisation	AFNOR Certification (http://nf-validation.afnor.org/)

♦ Analyses performed according to the COFRAC accreditation (Accreditation Testing n°1-0144, scope available on www.cofrac.fr)

1 INTRODUCTION

The **BAX® System Real-Time PCR Assay *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	Reference method	Validation standard	Conducted by
2011	Extension study for BAX® System software version 2.8 with the Q7 Instrument	ISO 16654:2001	ISO 16140:2003	ADRIA
2014	Renewal study	ISO 16654:2001	ISO 16140:2003	ADRIA
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	ISO 16654:2001 & ISO 16654/A1:2017	ISO 16140:2003 & ISO 16140-2:2016	ADRIA
2018	Renewal study	ISO 16654:2001 & ISO 16654/A1:2017	ISO 16140:2003 & ISO 16140-2:2016	ADRIA
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 is considered as minor	ISO 16654:2001 & ISO 16654/A1:2017	ISO 16140:2003 & ISO 16140-2:2016	ADRIA
2023	Upgrade of the BAX® software from version 4.0 to 4.22, used with the BAX® Instrument Q7 system.	/	/	Internal data by HYGIENA
2024	Upgrade of the BAX® software from version 4.22 to 5.0, used with the BAX® Instrument Q7 system.	/	/	Internal data by HYGIENA
2025	Upgrade of the BAX® software from version 5.0 to 5.1, used with the BAX® Instrument Q7 system.	/	/	Internal data by HYGIENA
2026	Renewal study with an update of the interpretation according to ISO 16140-2/A1:2024	ISO 16654:2001 & ISO 16654/A1:2017	ISO 16140-2:2016 & ISO 16140-2/A1:2024	ADRIA

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 to 24 h at 42°C± 1°C
Vegetable products	Pre-warmed BAX [®] System MP Broth	8 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 to 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 alternative method confirmation procedure is performed (See **Appendix 2**).

2.1.3 Restriction

There is no restriction.

2.2 Reference method♦

The reference method is ISO 16654:2001 - Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Escherichia coli* O157 - Amendment 1:2017: annex B: result of inter-laboratory studies & Amendment 2:2023 - Inclusion of performance testing of all culture media and reagents (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, an alternative method confirmation procedure can be performed (**Appendix 2**).

For the initial validation, the confirmations were performed only after positive PCR results or in case of discordant results between the PCR and the reference method. Therefore, for the sample generated during this study, it is not possible to estimate the presence of NA_{FN(alt)}.

For the extension performed in 2017, all samples were confirmed including the samples in negative agreement, allowing the evaluation of false negative among this group.

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 alternative methods (after confirmation) of all samples for each category

Category			PA	PD	TND	TNA	Total
1	Raw beef meat	7 h	17	13	6	37	73
		24 h	20	15	3	35	73
2	Vegetable products	8 h	15	12	6	35	68
		24 h	18	17	2	30	67
TOTAL		7 h or 8 h incubation time	32	25	12	72	141
		24 h incubation time	38	32	6	65	141

Unpaired evaluation: $TND = ND + ND_{FN(alt)} + PA_{FP(alt)}$ $TNA = NA + NA_{FN(alt)} + PD_{FP(alt)}$

3.1.1.5 Calculation of relative trueness (RT), sensitivity (SE), false positive ratio (FPR) and false negative ratio (FNR) for the alternative method

The calculations are presented in Table 5.

Table 5 – Calculation of relative trueness (RT), sensitivity (SE), false positive ratio (FPR) and false negative ratio (FNR) for the alternative method

Category			Type		Protocol	PA	PA _{FP(alt)}	NA	NA _{FN(alt)}	PD	ND	ND _{FN(alt)}	PD _{FP(alt)}	TND	TNA	SE _{alt} %	SE _{ref} %	RT %	FPR %	FNR%
1	Raw beef meat	7h	a	Fresh	MP Broth 7h at 42°C	7	0	11	0	4	0	1	0	1	11	91.7	66.7	78.3	0.0	8.3
			b	Frozen	MP Broth 7h at 42°C	8	0	16	1	3	1	1	0	2	17	84.6	76.9	83.3	0.0	15.4
			c	Flavored or marinated	MP Broth 7h at 42°C	2	0	8	1	6	2	1	0	3	9	72.7	45.5	55.0	0.0	18.2
			Total			17	0	35	2	13	3	3	0	6	37	83.3	63.9	74.0	0.0	13.9
		24h	a	Fresh	MP Broth 24h at 42°C	8	0	11	0	4	0	0	0	0	11	100.0	66.7	82.6	0.0	0.0
			b	Frozen	MP Broth 24h at 42°C	9	0	16	0	4	1	0	0	1	16	92.9	71.4	83.3	0.0	0.0
			c	Flavored or marinated	MP Broth 24h at 42°C	3	0	8	0	7	2	0	0	2	8	83.3	41.7	55.0	0.0	0.0
			Total			20	0	35	0	15	3	0	0	3	35	92.1	60.5	75.3	0.0	0.0
2	Vegetable products	8h	a	Fresh	MP Broth 8h at 42°C	6	0	12	1	3	1	2	0	3	13	75.0	75.0	76.0	0.0	25.0
			b	RTE/RTC	MP Broth 8h at 42°C	2	0	10	2	7	0	0	0	12	100.0	22.2	66.7	0.0	22.2	
			c	Frozen	MP Broth 8h at 42°C	7	0	8	2	2	3	0	0	3	10	75.0	83.3	77.3	0.0	16.7
			Total			15	0	30	5	12	4	2	0	6	35	81.8	63.6	73.5	0.0	21.2
		24h	a	Fresh	MP Broth 24h at 42°C	8	0	12	0	4	1	0	0	1	12	92.3	69.2	80.0	0.0	0.0
			b	RTE/RTC	MP Broth 24h at 42°C	2	0	10	0	9	0	0	0	10	100.0	18.2	57.1	0.0	0.0	
			c	Frozen	MP Broth 24h at 42°C	8	0	8	0	4	2	0	0	2	8	85.7	71.4	72.7	0.0	0.0
			Total			18	0	30	0	17	3	0	0	3	30	92.1	55.3	70.6	0.0	0.0
Total 7 or 8h incubation time						32	0	65	7	25	7	5	0	12	72	82.6	63.8	73.8	0.0	17.4
Total 24h incubation time						38	0	65	0	32	6	0	0	6	65	92.1	57.9	73.0	0.0	0.0

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	$SE_{alt} = \frac{(PA + PD)}{(PA + TND + PD)} \times 100 \%$	82.6 %	92.1 %
Sensitivity for the reference method	$SE_{ref} = \frac{(PA + TND)}{(PA + TND + PD)} \times 100 \%$	63.8 %	57.9 %
Relative trueness	$RT = \frac{(PA + TNA)}{N} \times 100 \%$	73.8 %	73.0 %
False positive ratio for the alternative method (unpaired evaluation)	$FPR = \frac{PA_{FP(alt)} + PD_{FP(alt)}}{TNA} \times 100 \%$	0.0 %	0.0 %
False negative ratio for the alternative method (unpaired evaluation)	$FNR = \frac{NA_{FN(alt)} + ND_{FN(alt)}}{PA + TND + PD} \times 100 \%$	17.4 %	0.0 %

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.

For 8 samples, the confirmatory tests concluded with the presence of *Escherichia coli* O157:H7 in the enrichment broth. All were artificially contaminated. Note that all 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 detected in the enrichment broth for 7 samples in negative agreement. Note that a majority of these samples were found during the extension study as none of the samples in negative agreement were confirmed during the initial validation. All those samples are described in Table 8.

Table 7 - Negative deviations

Sample N°	Product	Artificial contaminations (spiking protocol)		Global result		Reference method ISO 16654♦	BAX® System Real-Time PCR Assay for <i>E. coli</i> O157: H7 method - Short enrichment step				BAX® System Real-Time PCR Assay for <i>E. coli</i> 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		
744	Chuck	<i>E. coli</i> O157:H7 Ad565	0-2-3-2-4 (2.2)	+	+	+	-	+	-	ND _{FN(alt)}	+	+	+	PA	1	a
720	Frozen ground beef	/	/	+	+	+	-	-	-	ND	-	-	-	ND	1	b
8969	Frozen ground beef	<i>E. coli</i> O157:H7 Ad486	6-1-1-2-5 (3.0)	+	+	+	-/-/-	+	-	ND _{FN(alt)}	+	+	+	PA	1	b
8975	Rump steak with shallots	<i>E. coli</i> O157:H7 Ad489	1-1-4-1-2 (1.8)	+	+	+	-	-	-	ND	-	-	-	ND	1	c
8976	Parmesan carpaccio	<i>E. coli</i> O157:H7 Ad489	1-1-4-1-2 (1.8)	+	+	+	-/-/-	+	-	ND _{FN(alt)}	+	+	+	PA	1	c
8978	Seasoned beef carpaccio	<i>E. coli</i> O157:H7 Ad561	1-0-5-3-2 (2.2)	+	+	+	-	-	-	ND	-	-	-	ND	1	c
498	Spinach beet	<i>E. coli</i> O157:H7 Ad556	5-3-3-3-4 (3.6)	+	+	+	-	-	-	ND	-	-	-	ND	2	a
502	Links	<i>E. coli</i> O157:H7 Ad575	1-4-2-4-2 (2.6)	+	+	+	-	+	-	ND _{FN(alt)}	+	+	+	PA	2	a
506	Lettuce	<i>E. coli</i> O157:H7 Ad556	1-5-1-0-1 (1.6)	+	+	+	-	+	-	ND _{FN(alt)}	+	+	+	PA	2	a
523	Vegetable based cake	<i>E. coli</i> O157:H7 Ad575	2-5-4-4-2 (3.4)	+	+	+	-	-	-	ND	+	+ (ims2)	+ (ims2)	PA	2	c
526	Fried frozen mushrooms	<i>E. coli</i> O157:H7 Ad575	2-5-4-4-2 (3.4)	+	+	+	-	-	-	ND	-	-	-	ND	2	c
527	Fried frozen vegetables	<i>E. coli</i> O157:H7 Ad576	8-5-3-5-3 (4.8)	+	+	+	-	-	-	ND	-	-	-	ND	2	c

Table 8 - Positive deviations

Sample N°	Product	Artificial contaminations (spiking protocol)		Global result		Reference method	BAX® System Real-Time PCR Assay for <i>E. coli</i> O157: H7 method - Short enrichment step				BAX® System Real-Time PCR Assay for <i>E. coli</i> O157: H7 method - 24h enrichment step				Category	Type
						ISO 16654♦	PCR result	Confirmatory tests	Final result	Agreement	PCR Result	Confirmatory tests	Final result	Agreement		
		Result														
373	Ground beef (Bolognaise)	<i>E. coli</i> O157:H7 Ad487	13-6-6-8-9 (7.8)	-	+	-	-/-	+	-	NA _{FN(alt)}	+	+	+	PD	1	c
378	Ground beef (Bolognaise)	<i>E. coli</i> O157:H7 Ad583	10-14-17-12-14 (13.4)	+	+	-	+	+	+	PD	+	+	+	PD	1	c
719	Frozen ground beef	/	/	-	+	-	-	/ (+24h)	-	NA _{FN(alt)}	+	+	+	PD	1	b
745	Rump steak	<i>E. coli</i> O157:H7 Ad565	0-2-3-2-4 (2.2)	+	+	-	+	+	+	PD	+	+	+	PD	1	a
747	Sirloin	<i>E. coli</i> O157:H7 Ad487	5-3-4-4-6 (4.4)	+	+	-	+	+	+	PD	+	+	+	PD	1	a
855	Marinated peace of beef with shallots	<i>E. coli</i> O157:H7 Ad583	5-5-7-2-6(5.0)	+	+	-	+	+	+	PD	+	+	+	PD	1	c
856	Carpaccio	<i>E. coli</i> O157:H7 Ad583	5-5-7-2-6(5.0)	+	+	-	+	+	+	PD	+	+	+	PD	1	a
857	Ground beef with onions	<i>E. coli</i> O157:H7 Ad590	4-5-10-7-8(6.8)	+	+	-	+	+	+	PD	+	+	+	PD	1	c
859	Ground beef with onions	<i>E. coli</i> O157:H7 Ad590	4-5-10-7-8(6.8)	+	+	-	+	+	+	PD	+	+	+	PD	1	c
860	Beef balls	<i>E. coli</i> O157:H7 Ad590	4-5-10-7-8(6.8)	+	+	-	+	+	+	PD	+	+	+	PD	1	a
500	Parsley	<i>E. coli</i> O157:H7 Ad558	3-6-5-4-3(4.2)	+	+	-	+	+	+	PD	+	+	+	PD	2	a
504	Endive	<i>E. coli</i> O157:H7 Ad576	1-1-1-4-6(2.6)	-	+	-	-	+	-	NA _{FN(alt)}	+	+	+	PD	2	a
508	Fresh salad	<i>E. coli</i> O157:H7 Ad556	5-3-3-3-4(3.6)	+	+	-	+	+	+	PD	+	+	+	PD	2	b
510	Fresh corn-salad	<i>E. coli</i> O157:H7 Ad558	3-6-5-4-3(4.2)	+	+	-	+	+	+	PD	+	+	+	PD	2	b
514	Batavia	<i>E. coli</i> O157:H7 Ad576	1-1-1-4-6(2.6)	+	+	-	+	+	+	PD	+	+	+	PD	2	a
516	White cabbage	<i>E. coli</i> O157:H7 Ad556	1-5-1-0-1(1.6)	-	+	-	-	+	-	NA _{FN(alt)}	+	+	+	PD	2	b
518	Grated carrots	<i>E. coli</i> O157:H7 Ad556	5-3-3-3-4(3.6)	+	+	-	+	+	+	PD	+	+	+	PD	2	b
695	Red cabbage (bagged pre-cut leafy vegetables)	<i>E. coli</i> O157:H7 Ad575	1-2-11-2-8 (4.8)	+	+	-	+	+	+	PD	+	+	+	PD	2	b
696	Fresh mix vegetables (bagged pre-cut leafy vegetables)	<i>E. coli</i> O157:H7 Ad575	1-2-11-2-8 (4.8)	+	+	-	+	+	+	PD	+	+	+	PD	2	b
697	White cabbage (bagged pre-cut leafy vegetables)	<i>E. coli</i> O157:H7 Ad576	2-4-5-2-8 (4.0)	-	+	-	-	+	-	NA _{FN(alt)}	+	+	+	PD	2	b
698	Rocket (bagged pre-cut leafy vegetables)	<i>E. coli</i> O157:H7 Ad576	2-4-5-2-8 (4.0)	+	+	-	+	+	+	PD	+	+	+	PD	2	b
699	Red cabbage (bagged pre-cut leafy vegetables)	<i>E. coli</i> O157:H7 Ad576	2-4-5-2-8 (4.0)	+	+	-	+	+	+	PD	+	+	+	PD	2	b
700	Frozen vegetables for ratatouille	<i>E. coli</i> O157:H7 Ad556	4-5-4-3-7 (4.6)	-	+	-	-	+	-	NA _{FN(alt)}	+	+	+	PD	2	c
702	Frozen sliced links	<i>E. coli</i> O157:H7 Ad577	11-9-9-15-3 (9.4)	-	+	-	-	+	-	NA _{FN(alt)}	+	+	+	PD	2	c
733	Celery	<i>E. coli</i> O157:H7 Ad556	4-8-8-4-6 (6.0)	+	+	-	+	+	+	PD	+	+	+	PD	2	a
8968	Frozen ground beef	<i>E. coli</i> O157:H7 Ad486	6-1-1-2-5 (3.0)	+	+	-	+	+	+	PD	+	+	+	PD	1	b
8971	Frozen ground beef	<i>E. coli</i> O157:H7 Ad488	3-5-1-1-1 (2.2)	+	+	-	+	+	+	PD	+	+	+	PD	1	b
8973	Frozen ground beef	<i>E. coli</i> O157:H7 Ad488	3-5-1-1-1 (2.2)	+	+	-	+	+	+	PD	+	+	+	PD	1	b
8974	Seasoned beef Carpaccio	<i>E. coli</i> O157:H7 Ad489	1-1-4-1-2 (1.8)	+	+	-	+	+	+	PD	+	+	+	PD	1	c
8981	Pistou Carpaccio	<i>E. coli</i> O157:H7 Ad561	1-0-5-3-2 (2.2)	+	+	-	+	+	+	PD	+	+	+	PD	1	c
8982	Frozen spinach	<i>E. coli</i> O157:H7 Ad685	1-1-3-5-2 (2.4)	+	+	-	+	+	+	PD	+	+	+	PD	2	c
9021	Frozen spinach	<i>E. coli</i> O157:H7 Ad572	1-5-2-2-3 (2.6)	+	+	-	+	+	+	PD	+	+	+	PD	2	c

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

Table 9 - Analyses of discordant results

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

The observed values for (TND - 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 alternative method confirmation procedure 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. Contamination 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	<i>Escherichia coli</i> O157:H7 Ad682	Meat product
2010	Vegetable products	Spinach	<i>Escherichia coli</i> 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 version 4 (2024-01-10). The RLOD are given in Table 11.

Table 11 – Presentation of RLOD before and after confirmation of the alternative method results

Category	Matrix / strain	AL	RLOD [95% confidence limit]
1	Ground beef / <i>E. coli</i> O157:H7 Ad682 Incubation time: 7h or 24h	2.5	2.0 [0.7; 5.7]
2	Spinach / <i>E. coli</i> O157:H7 Ad683 Incubation time: 8h or 24h		0.6 [0.3; 1.5]
Combined for both incubation times			1.0 [0.5; 2.0]

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

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

The LOD₅₀ calculations were done using the Excel spreadsheet available at <http://standards.iso.org/iso/16140> POD-LOD calculation program - version 12, 2024-03-05. The tests are given in Table 12.

Table 12 - LOD₅₀ results

Category	(Strain / matrix) pair	Level of detection at 50% (CFU / test portion)		Level of detection at 95% (CFU / test portion)	
		Reference method	Alternative method	Reference method	Alternative method
1	Ground beef / <i>E. coli</i> O157:H7 Ad682 Incubation time: 7h or 24h	0.4 [0.2; 0.7]	0.6 [0.3; 1.1]	1.6 [0.9; 3.0]	2.6 [1.4; 4.9]
2	Spinach / <i>E. coli</i> O157:H7 Ad683 Incubation time: 8h or 24h	0.7 [0.3; 1.2]	0.4 [0.2; 0.7]	2.8 [1.5; 5.3]	1.7 [0.9; 3.1]
Combined results		0.5 [0.3; 0.8]	0.5 [0.3; 0.8]	2.2 [1.4; 3.4]	2.1 [1.4; 3.3]

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/225 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/225 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**.

> **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).

3.2 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.

The ILS was performed in May 2010. Data were initially generated following the ISO 16140:2003. The alternative method was tested in comparison to the ISO 16654:2001.

The results were re-interpreted according to the ISO 16140-2:2016/Amd1:2024 standard during the renewal in 2026.

3.2.1 Study organization

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 $5 \pm 3^\circ\text{C}$. *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 in the inoculated strain was observed during the storage at $5 \pm 3^\circ\text{C}$, *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 delays		
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'Previus¹ 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.

¹ Sym'Previus: www.symprevius.org

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 $3.2 \cdot 10^4$ to $6.2 \cdot 10^5$ CFU/g.

> *Escherichia coli O157:H7 detection*

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

**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	P₀ = 4	P₁ = 81	P₂ = 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	P₀ = 0	CP₀ = 0	P₁ = 88	CP₁ = 88	P₂ = 88	CP₂ = 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 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	P₀ = 2	P₁ = 73	P₂ = 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	P₀ = 0	CP₀ = 0	P₁ = 80	CP₁ = 80	P₂ = 80	CP₂ = 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	$SP_{ref} = \left(1 - \left(\frac{P_0}{N_-} \right) \right) \times 100 \% =$	97.5 %
Specificity for the alternative method	$SP_{alt} = \left(1 - \left(\frac{CP_0}{N_-} \right) \right) \times 100 \% =$	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 of the alternative method (SE_{alt}), the sensitivity for the reference method (SE_{ref}), the relative trueness (RT) and the false positive ratio for the alternative method (FPR)

In this study, partial positive results were obtained for the low (91.5%) and the high inoculation levels (97.5%) but above the fractional positive range described in the ISO 16140-1 (25 – 75%). Nevertheless, L1 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 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-)	Total Negative deviation (A-/R+) TND = 0	Total Negative agreement (A-/R-) TNA = 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
Sensitivity for the alternative method	$SE_{alt} = \frac{(PA + PD)}{(PA + TND + PD)} \times 100 \%$	100.0 %
Sensitivity for the reference method	$SE_{ref} = \frac{(PA + TND)}{(PA + TND + PD)} \times 100 \%$	91.3 %
Relative trueness	$RT = \frac{(PA + TNA)}{N} \times 100 \%$	91.3 %
False positive ratio for the alternative method (unpaired evaluation)	$FPR = \frac{PA_{FP(alt)} + PD_{FP(alt)}}{TNA} \times 100 \%$	0.0 %
False negative ratio for the alternative method (unpaired evaluation)	$FNR = \frac{NA_{FN(alt)} + ND_{FN(alt)}}{PA + TND + PD} \times 100 \%$	0.0 %

3.2.4.3 Interpretation of data

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

$$(p+)_{ref} = \frac{P_{x(ref)}}{N_{x(ref)}}$$

where

$P_{x(ref)}$ = is number of samples with a positive result obtained with the reference method at level x (L_1 or L_2) for all laboratories

$N_{x(ref)}$ = is number of samples tested at level x (L_1 or L_2) with the reference method by all laboratories

$$(p+)_{alt} = \frac{CP_{x(alt)}}{N_{x(alt)}}$$

where

$CP_{x(alt)}$ = is number of samples with a confirmed positive result obtained with the alternative method at level x (L_1 or L_2) for all laboratories

$N_{x(alt)}$ = is number of samples tested at level x (L_1 or L_2) with the alternative method by all laboratories

$$(TND - PD)_{max} = \sqrt{3N_{x(ref)} \times ((p+)_{ref} + (p+)_{alt} - 2((p+)_{ref} \times (p+)_{alt}))}$$

where

$N_{x(ref)}$ = is number of samples tested for level x (L_1 or L_2) with the reference method by all laboratories.

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 24).

Table 24 - Calculations

	Level 1
N_x	80
$(p+)_{ref}$	0.9
$(p+)_{alt}$	0.0
$AL = (TND - PD)_{max}$	14.80
TND - PD	- 7
Conclusion	TND - PD < AL

The ISO 16140-2:2016 and ISO 16140-2/A1:2024 requirements are fulfilled as (TND - PD) is lower than the AL for both inoculation levels.

3.2.4.4 Evaluation of the $LOD_{50\%}$ and RLOD between laboratories

The $LOD_{50\%}$ was calculated using the ISO 16140-2 Excel spreadsheet available at https://standards.iso.org/iso/16140/-2/ed-1/en/amd/1/PODL0D-interlab_ver2.xlsm.

The RLOD is defined as the ratio of the LODs of the alternative method and the reference method: **RLOD = LOD_{alt}/LOD_{ref}** .

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 ISO 16140-2 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. Extended incubation beyond 7 hours improved detection of *E. coli* O157:H7 for the alternative method however, in comparison to the reference method, performance at 7 hours was acceptable. The TND - 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 ISO 16140-2 and AFNOR technical rules (Revision 13).

The inter-laboratory study conclusions are:

The data and interpretations comply with the ISO 16140-2:2016, ISO 16140-2/A1:2024 requirements and AFNOR technical rules (Revision 13).

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

Quimper, 17 July 2026

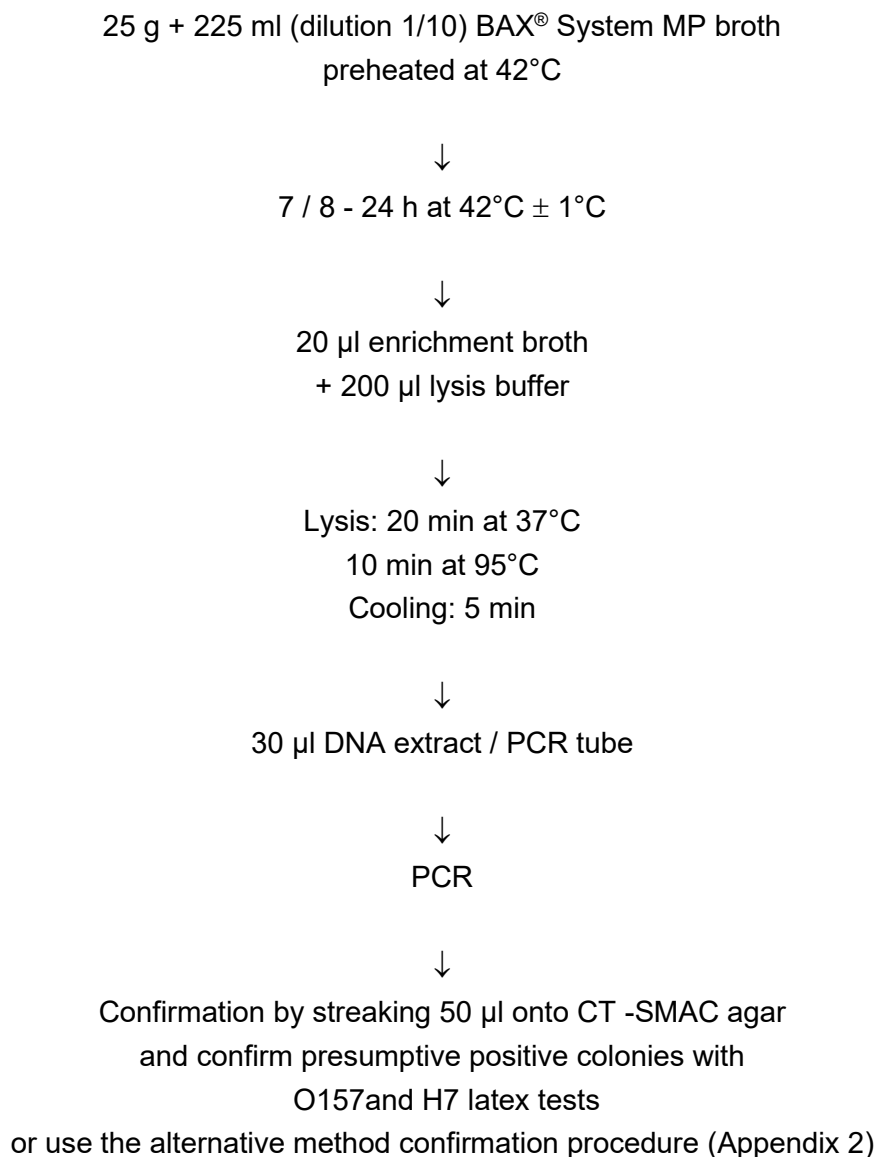
Astrid CARIOU
Manager
Method performance in food microbiology



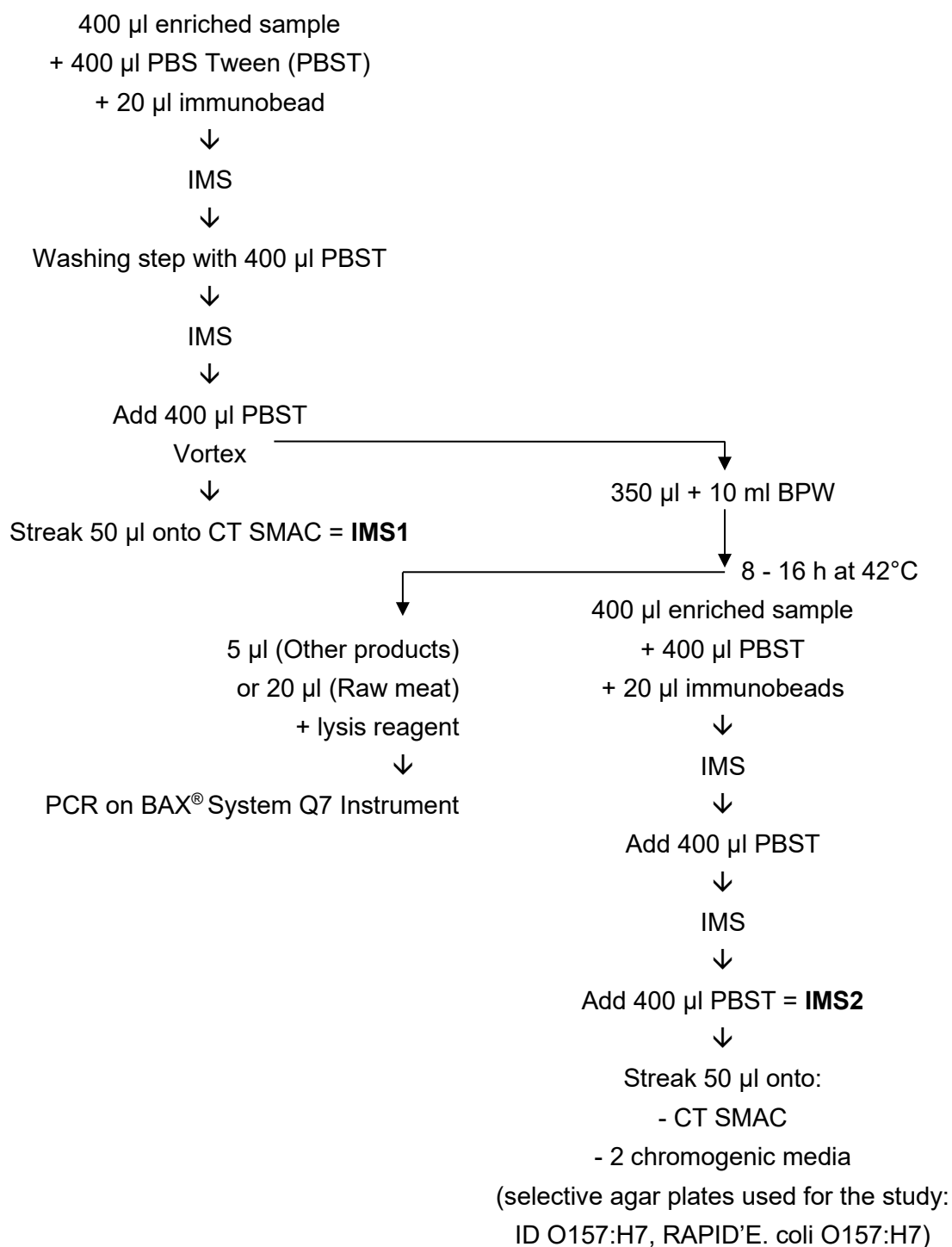
I hereby attest to the validation of the verification of the conformity of the report (opinion and interpretation).

**Appendix 1 – Flow diagram of the alternative method
BAX® System Real-Time PCR Assay *E. coli* O157:H7**

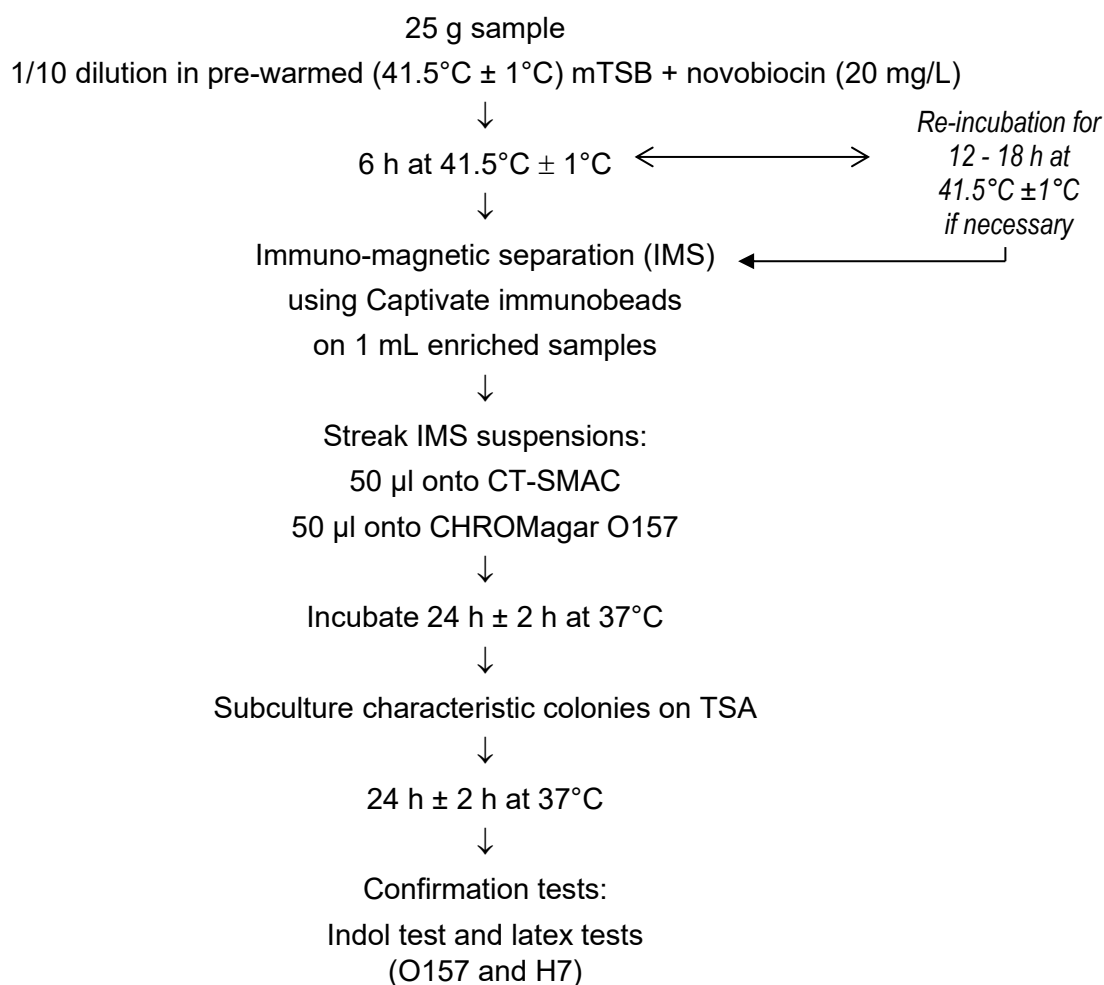
Protocol for Raw beef meat and vegetable products



Appendix 2 – Alternative method confirmation procedure



Appendix 3 – Flow diagram of the reference method: ISO 16654:2001 - Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Escherichia coli* O157 - Amendment 1:2017: annex B: result of inter-laboratory studies. Amendment 2:2023 - Inclusion of performance testing of all culture media and reagents



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)	<i>E. coli</i> O157:H7 Ad564	Ground beef	-20°C	>2.82	11-15-20-16-11(14.6)	+	+	1	b
2010	371	Ground beef (meatballs)	<i>E. coli</i> O157:H7 Ad565	Ground beef	-20°C	1.64	19-20-8-16-20(16.6)	+	+	1	a
2010	373	Ground beef (Bolognaise)	<i>E. coli</i> 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)	<i>E. coli</i> 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	<i>E. coli</i> O157:H7 Ad590	Ground beef	-20°C	2.46	8-8-10-4-5(7.0)	+	+	1	b
2010	387	Frozen ground beef (meatballs)	<i>E. coli</i> 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)	<i>E. coli</i> O157:H7 Ad590	Ground beef	-20°C	2.46	8-8-10-4-5(7.0)	+	+	1	b
2010	744	Chuck	<i>E. coli</i> O157:H7 Ad565	Ground beef	28 days at 4°C	1.14	0-2-3-2-4 (2.2)	+	+	1	a
2010	745	Rumsteak	<i>E. coli</i> 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	<i>E. coli</i> O157:H7 Ad565	Ground beef	28 days at 4°C	1.14	0-2-3-2-4 (2.2)	+	+	1	a
2010	747	Sirloin	<i>E. coli</i> O157:H7 Ad487	Ground beef	28 days at 4°C	0.48	5-3-4-4-6 (4.4)	+	+	1	a
2010	748	Shin	<i>E. coli</i> O157:H7 Ad487	Ground beef	28 days at 4°C	0.48	5-3-4-4-6 (4.4)	+	+	1	a
2010	854	Marinated piece of beef	<i>E. coli</i> 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	<i>E. coli</i> O157:H7 Ad583	Ground beef	33 days at 4°C	1.34	5-5-7-2-6(5.0)	+	+	1	c
2010	856	Carpaccio	<i>E. coli</i> 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	<i>E. coli</i> 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	<i>E. coli</i> 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	<i>E. coli</i> 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	<i>E. coli</i> 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	<i>E. coli</i> 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	<i>E. coli</i> O157:H7 Ad558	WWTP	16 days at 4°C	2.13	3-6-5-4-3(4.2)	+	+	2	a
2010	502	Links	<i>E. coli</i> O157:H7 Ad575	Bovine feces	16 days at 4°C	0.93	1-4-2-4-2(2.6)	+	+	2	a
2010	504	Endive	<i>E. coli</i> O157:H7 Ad576	Bovine feces	16 days at 4°C	1.70	1-1-1-4-6(2.6)	-	+	2	a
2010	506	Lettuce	<i>E. coli</i> O157:H7 Ad556	WWTP	16 days at 4°C	1.87	1-5-1-0-1(1.6)	+	+	2	a
2010	508	Fresh salad	<i>E. coli</i> 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	<i>E. coli</i> O157:H7 Ad558	WWTP	16 days at 4°C	2.13	3-6-5-4-3(4.2)	+	+	2	b
2010	512	Lettuce	<i>E. coli</i> O157:H7 Ad575	Bovine feces	16 days at 4°C	0.93	1-4-2-4-2(2.6)	+	+	2	a
2010	514	Batavia	<i>E. coli</i> 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	<i>E. coli</i> O157:H7 Ad556	WWTP	16 days at 4°C	1.87	1-5-1-0-1(1.6)	-	+	2	b
2010	518	Grated carrots	<i>E. coli</i> O157:H7 Ad556	WWTP	16 days at 4°C	1.70	5-3-3-3-4(3.6)	+	+	2	b
2010	520	Soya sprouts	<i>E. coli</i> 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	<i>E. coli</i> O157:H7 Ad556	WWTP	-20°C	2.39	2-3-1-3-2(1.8)	+	+	2	c
2010	523	Vegetable based cake	<i>E. coli</i> O157:H7 Ad575	Bovine feces	-20°C	>2.66	2-5-4-4-2(3.4)	+	+	2	c
2010	524	Fried frozen vegetables	<i>E. coli</i> O157:H7 Ad576	Bovine feces	-20°C	>2.30	8-5-3-5-3(4.8)	+	+	2	c
2010	525	Mixed vegetables	<i>E. coli</i> O157:H7 Ad556	WWTP	-20°C	2.39	2-3-1-3-2(1.8)	+	+	2	c
2010	526	Fried frozen mushrooms	<i>E. coli</i> O157:H7 Ad575	Bovine feces	-20°C	>2.66	2-5-4-4-2(3.4)	+	+	2	c
2010	527	Fried frozen vegetables	<i>E. coli</i> O157:H7 Ad576	Bovine feces	-20°C	>2.30	8-5-3-5-3(4.8)	+	+	2	c
2010	693	Spinach (bagged pre-cut leafy vegetables)	<i>E. coli</i> O157:H7 Ad575	Bovine feces	26 days at 4°C	0.76	1-2-11-2-8 (4.8)	+	+	2	b
2010	694	Fresh vegetable mix (cabbage, carrots, salad...) (bagged pre-cut leafy vegetables)	<i>E. coli</i> 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 leafy vegetables)	<i>E. coli</i> 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 leafy vegetables)	<i>E. coli</i> 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	697	White cabbage (bagged pre-cut leafy vegetables)	<i>E. coli</i> 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 leafy vegetables)	<i>E. coli</i> 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 leafy vegetables)	<i>E. coli</i> 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	<i>E. coli</i> O157:H7 Ad556	WWTP	-20°C	>1.81	4-5-4-3-7 (4.6)	-	+	2	c
2010	701	Frozen vegetables mix	<i>E. coli</i> O157:H7 Ad556	WWTP	-20°C	>1.81	4-5-4-3-7 (4.6)	-	-	2	c
2010	702	Frozen sliced links	<i>E. coli</i> O157:H7 Ad577	Bovine feces	-20°C	1.91	11-9-9-15-3 (9.4)	-	+	2	c
2010	732	Green cabbage	<i>E. coli</i> O157:H7 Ad556	WWTP	27 days at 4°C	1.82	4-8-8-4-6 (6.0)	+	+	2	a
2010	733	Celery	<i>E. coli</i> O157:H7 Ad556	WWTP	27 days at 4°C	1.82	4-8-8-4-6 (6.0)	+	+	2	a
2010	734	Sprouts	<i>E. coli</i> O157:H7 Ad556	WWTP	27 days at 4°C	1.82	4-8-8-4-6 (6.0)	+	+	2	a
2010	735	Zucchini	<i>E. coli</i> O157:H7 Ad556	WWTP	27 days at 4°C	1.82	4-8-8-4-6 (6.0)	+	+	2	a
2010	740	Green asparagus	<i>E. coli</i> O157:H7 Ad556	WWTP	-20°C	>0.90	7-4-2-5-4 (4.4)	+	+	2	c
2010	741	Peas	<i>E. coli</i> O157:H7 Ad577	Bovine feces	-20°C	>0.90	4-5-4-3-4 (4.0)	+	+	2	c
2010	861	Zucchini	<i>E. coli</i> O157:H7 Ad576	Bovine feces	33 days at 4°C	0.88	14-18-11-6-9(11.6)	+	+	2	a
2010	862	Turnip	<i>E. coli</i> 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	<i>E. coli</i> O157:H7 Ad485	Beef	Seeding 48h 2-8°C	/	1-3-1-2-2 (1.8)	+	+	1	a
2017	8966	Bifteck	<i>E. coli</i> O157:H7 Ad485	Beef	Seeding 48h 2-8°C	/	1-3-1-2-2 (1.8)	+	+	1	a
2017	8967	Beef steak	<i>E. coli</i> O157:H7 Ad485	Beef	Seeding 48h 2-8°C	/	1-3-1-2-2 (1.8)	+	+	1	a
2017	8968	Frozen ground beef	<i>E. coli</i> O157:H7 Ad486	Beef	Seeding 48h 2-8°C	/	6-1-1-2-5 (3.0)	+	+	1	b
2017	8969	Frozen ground beef	<i>E. coli</i> O157:H7 Ad486	Beef	Seeding 48h 2-8°C	/	6-1-1-2-5 (3.0)	+	+	1	b
2017	8970	Frozen ground beef	<i>E. coli</i> O157:H7 Ad486	Beef	Seeding 48h 2-8°C	/	6-1-1-2-5 (3.0)	+	+	1	b
2017	8971	Frozen ground beef	<i>E. coli</i> O157:H7 Ad488	Beef	Seeding 48h 2-8°C	/	3-5-1-1-1 (2.2)	+	+	1	b
2017	8972	Frozen ground beef	<i>E. coli</i> O157:H7 Ad488	Beef	Seeding 48h 2-8°C	/	3-5-1-1-1 (2.2)	+	+	1	b
2017	8973	Frozen ground beef	<i>E. coli</i> O157:H7 Ad488	Beef	Seeding 48h 2-8°C	/	3-5-1-1-1 (2.2)	+	+	1	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		
2017	8974	Seasoned beef Carpaccio	<i>E. coli</i> O157:H7 Ad489	Beef	Seeding 48h 2-8°C	/	1-1-4-1-2 (1.8)	+	+	1	c
2017	8975	Rump steak with shallots	<i>E. coli</i> O157:H7 Ad489	Beef	Seeding 48h 2-8°C	/	1-1-4-1-2 (1.8)	+	+	1	c
2017	8976	Parmesan Carpaccio	<i>E. coli</i> O157:H7 Ad489	Beef	Seeding 48h 2-8°C	/	1-1-4-1-2 (1.8)	+	+	1	c
2017	8977	Pistou Carpaccio	<i>E. coli</i> O157:H7 Ad489	Beef	Seeding 48h 2-8°C	/	1-1-4-1-2 (1.8)	-	-	1	c
2017	8978	Seasoned beef Carpaccio	<i>E. coli</i> O157:H7 Ad561	Beef	Seeding 48h 2-8°C	/	1-0-5-3-2 (2.2)	+	+	1	c
2017	8979	Rump steak with shallots	<i>E. coli</i> O157:H7 Ad561	Beef	Seeding 48h 2-8°C	/	1-0-5-3-2 (2.2)	-	-	1	c
2017	8980	Parmesan Carpaccio	<i>E. coli</i> O157:H7 Ad561	Beef	Seeding 48h 2-8°C	/	1-0-5-3-2 (2.2)	+	+	1	c
2017	8981	Pistou Carpaccio	<i>E. coli</i> O157:H7 Ad561	Beef	Seeding 48h 2-8°C	/	1-0-5-3-2 (2.2)	+	+	1	c
2017	8982	Frozen spinach	<i>E. coli</i> O157:H7 Ad685	Feces	Seeding 48h 2-8°C	/	1-1-3-5-2 (2.4)	+	+	2	c
2017	8983	Frozen carrots	<i>E. coli</i> O157:H7 Ad685	Feces	Seeding 48h 2-8°C	/	1-1-3-5-2 (2.4)	+	+	2	c
2017	9021	Frozen spinach	<i>E. coli</i> O157:H7 Ad572	Feces	Seeding 48h 2-8°C	/	1-5-2-2-3 (2.6)	+	+	2	c
2017	9022	Frozen carrots	<i>E. coli</i> O157:H7 Ad572	Feces	Seeding 48h 2-8°C	/	1-5-2-2-3 (2.6)	+	+	2	c

Appendix 5 – Sensitivity study: raw data

RAW BEEF MEAT																				
Sample N°	Product	Reference method ISO 16654♦				BAX® System Real-Time PCR Assay <i>E. coli</i> O157: H7 method - Short enrichment step						BAX® System Real-Time PCR Assay <i>E. coli</i> 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 _{FN(alt)}	+	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
8965	Ground beef	+p	+p	/	/	+	+	+M	+	+	+	PA	+	+1/2	+	+	+	PA	1	a

RAW BEEF MEAT																				
Sample N°	Product	Reference method ISO 16654♦					BAX® System Real-Time PCR Assay E. coli O157: H7 method - Short enrichment step						BAX® System Real-Time PCR Assay 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															
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
714	Frozen ground beef	-	-	-	-	-	-	/	/	/	-	NA	-	/	/	/	-	NA	1	b

RAW BEEF MEAT																				
Sample N°	Product	Reference method ISO 16654♦					BAX® System Real-Time PCR Assay E. coli O157: H7 method - Short enrichment step						BAX® System Real-Time PCR Assay 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															
719	Frozen ground beef	-	-	-	-	-	-	/	/	/	-	NA _{FN(alt)}	+	+	+	+	+	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 _{FN(alt)}	+	+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 _{FN(alt)}	+	+	+	+	+	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
705	Marinated piece of beef	-	-	-	-	-	-	/	/	/	-	NA	-	/	/	/	-	NA	1	c
711	Beef meat balls (51%)	-	-	-	+	-	-	/	/	/	-	NA	-	/	/	/	-	NA	1	c

RAW BEEF MEAT

Sample N°	Product	Reference method ISO 16654♦					BAX® System Real-Time PCR Assay <i>E. coli</i> O157: H7 method - Short enrichment step						BAX® System Real-Time PCR Assay <i>E. coli</i> 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															
854	Marinated piece 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 _{FN(alt)}	+	+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 <i>E. coli</i> O157: H7 method - Short enrichment step							BAX® System Real-Time PCR Assay <i>E. coli</i> 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 _{FN(alt)}	+	+	+	+	+	PA	2	a
503	Links	-	-	-	-	-	-	/	/	/	-	NA	-	/	/	/	-	NA	2	a
504	Endive	-	-	-	-	-	-	/	/	/	-	NA	+	+	+	+	+	PD	2	a
505	Endive	-	-	-	-	-	-	/	/	/	-	NA	-	/	/	/	-	NA	2	a
506	Lettuce	-	-	+	+	+	-	+	+	+	-	ND _{FN(alt)}	+	+	+	+	+	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
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

VEGETABLES PRODUCTS

Sample N°	Product	Reference method ISO 16654♦					BAX® System Real-Time PCR Assay <i>E. coli</i> O157: H7 method - Short enrichment step						BAX® System Real-Time PCR Assay <i>E. coli</i> 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															
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 _{FN(alt)}	+	+	+	+	+	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
696	Fresh mix vegetables (bagged pre-cut leafy vegetables)	-	-	-	+	-	+	+	+	+	+	PD	+	+	+	+	+	PD	2	b
697	White cabbage (bagged pre-cut leafy vegetables)	-	-	-	-	-	-	/	/	/	-	NA _{FN(alt)}	+	+	+	+	+	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

VEGETABLES PRODUCTS

Sample N°	Product	Reference method ISO 16654♦					BAX® System Real-Time PCR Assay E. coli O157: H7 method - Short enrichment step							BAX® System Real-Time PCR Assay 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																	
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)	+	+	+	(ims2)	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		
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 _{FN(alt)}	+	+/-(2)	+	+	+	+	(ims2)	PD	2	c
701	Frozen vegetables mix	-	-	+	-	-	-	+(1)	-	/	-	NA	-	/	/	/	-	NA	2	c		
702	Frozen sliced links	-	-	-	-	-	-	/	/	/	-	NA _{FN(alt)}	+	+	+	+	+	+	PD	2	c	

VEGETABLES PRODUCTS

Sample N°	Product	Reference method ISO 16654♦				BAX® System Real-Time PCR Assay <i>E. coli</i> O157: H7 method - Short enrichment step							BAX® System Real-Time PCR Assay <i>E. coli</i> 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															
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 CFU/g

Sample N°	Level	Inoculation level (cfu/25g)	Reference method ISO 16654♦						BAX System Real-Time PCR Assay <i>E. coli</i> O157:H7 method													
									8h enrichment step						24h enrichment step							
			Characteristic colonies						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			-	-	-	-	-		+	+	+	+	+		+	+	+	+				
575			-	-	-	-	-		+	+	+	+	+		+	+	+	+				
576			-	-	-	-	-		+	+	+	+	+		+	+	+	+				
577			-	-	-	-	-		-	-	/	/	-		-	/	/	/	-			
578	3	0.8	-	-	-	-	-	5/6	+	+	+	+	+	4 /6	+	+	+	+	+	4 /6		
579			+	+	/	/	+		-	-	/	/	-		-	/	/	/	-			
580			+	+	/	/	+		-	-	/	/	-		-	/	/	/	-			
581			+	+	/	/	+		+	+	+	+	+		+	+	+	+				
582			+	+	/	/	+		+	+	+	+	+		+	+	+	+				
583			+	+	/	/	+		+	+	+	+	+		+	+	+	+				
584	4	2.1	-	-	-	-	-	5/6	+	+	+	+	+	6 /6	+	+	+	+	+	6 /6		
585			+	+	/	/	+		+	+	+	+	+		+	+	+	+				
586			+	+	/	/	+		+	+	+	+	+		+	+	+	+				
587			+	+	/	/	+		+	+	+	+	+		+	+	+	+				
588			+	+	/	/	+		+	+	+	+	+		+	+	+	+				
589			+	+	/	/	+		+	+	+	+	+		+	+	+	+				

Fresh ground beef
E. coli O157:H7 Ad682

Total viable count: 5000 CFU/g

Sample N°	Level	Inoculation level (cfu/25g)	Reference method ISO 16654 ♦						BAX System Real-Time PCR Assay <i>E. coli</i> O157:H7 method																				
									7h enrichment step						8h enrichment step						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			+	+	/	/	+		+	+	+	+	+		+	+	+	+	+		+	+	+	+					

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 <i>E. coli</i> O157:H7	Confirmatory tests	
							CT-SMAC Characteristic colonies	O157 and H7 latex test
1.	<i>Escherichia coli</i>	O157:H7	B177	WWTP	21	+	+	+
2.	<i>Escherichia coli</i>	O157:H7	BV2	Slaughterhouse	15	+	+	+
3.	<i>Escherichia coli</i>	O157:H7	BR3	Slaughterhouse	47	+	+	+
4.	<i>Escherichia coli</i>	O157:H7	BD4	Slaughterhouse	53	+	+	+
5.	<i>Escherichia coli</i>	O157:H7	ENV177	WWTP	17	+	+	+
6.	<i>Escherichia coli</i>	O157:H7	ET8	WWTP	19	+	+	+
7.	<i>Escherichia coli</i>	O157:H7	EK9	WWTP	72	+	+	+
8.	<i>Escherichia coli</i>	O157:H7	435	Ground beef	14	+	+	+
9.	<i>Escherichia coli</i>	O157:H7	670T	Ground beef	57	+	+	+
10.	<i>Escherichia coli</i>	O157:H7	730T	Ground beef	16	+	+	+
11.	<i>Escherichia coli</i>	O157:H7	226T	Ground beef	82	+	+	+
12.	<i>Escherichia coli</i>	O157:H7	42197-1	Ground beef	57	+	+	+
13.	<i>Escherichia coli</i>	O157:H7	A3612	Ground beef	23	+	+	+
14.	<i>Escherichia coli</i>	O157:H7	A4513	Ground beef	32	+	+	+
15.	<i>Escherichia coli</i>	O157:H7	A1075	Ground beef	14	+	+	+
16.	<i>Escherichia coli</i>	O157:H7	B68	Slaughterhouse	32	+	+	+
17.	<i>Escherichia coli</i>	O157:H7	AT40	Slaughterhouse	47	+	+	+
18.	<i>Escherichia coli</i>	O157:H7	AV36	Slaughterhouse	51	+	+	+
19.	<i>Escherichia coli</i>	O157:H7	AR15	Slaughterhouse	36	+	+	+
20.	<i>Escherichia coli</i>	O157:H7	LS3	Feces	37	+	+	+
21.	<i>Escherichia coli</i>	O157:H7	AMVT6	Feces	21	+	+	+
22.	<i>Escherichia coli</i>	O157:H7	ATKP8	Feces	43	+	+	+
23.	<i>Escherichia coli</i>	O157:H7	AZRS15	Feces	51	+	+	+
24.	<i>Escherichia coli</i>	O157:H7	R33-9	Bovine faeces	17	+	+	+
25.	<i>Escherichia coli</i>	O157:H7	AZ15-6	Bovine faeces	21	+	+	+
26.	<i>Escherichia coli</i>	O157:H7	AQ29-4	Bovine faeces	42	+	+	+
27.	<i>Escherichia coli</i>	O157:H7	AA18-3	Bovine faeces	47	+	+	+
28.	<i>Escherichia coli</i>	O157:H7	LS56	Faeces	51	+	+	+
29.	<i>Escherichia coli</i>	O157:H7	A425TK	Faeces	32	+	+	+
30.	<i>Escherichia coli</i>	O157:H7	A206RP	Faeces	27	+	+	+
31.	<i>Escherichia coli</i>	O157:H7	A778EF	Faeces	17	+	+	+
32.	<i>Escherichia coli</i>	O157:H7	MK41242	Ground beef	10	+	+	+
33.	<i>Escherichia coli</i>	O157:H7	AMK2608	Ground beef	37	+	+	+
34.	<i>Escherichia coli</i>	O157:H7	AMK1506	Ground beef	42	+	+	+
35.	<i>Escherichia coli</i>	O157:H7	AMK1311	Ground beef	71	+	+	+
36.	<i>Escherichia coli</i>	O157:H7	37006ID	Ground beef	64	+	+	+
37.	<i>Escherichia coli</i>	O157:H7	A1518ID	Ground beef	39	+	+	+
38.	<i>Escherichia coli</i>	O157:H7	A1512ID	Ground beef	27	+	+	+
39.	<i>Escherichia coli</i>	O157:H7	A1814ID	Ground beef	23	+	+	+

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

EXCLUSIVITY								
No	Species	Serotype	Strain identification	Origin	Inoculation level cfu/225ml	PCR result BAX® System Real-Time PCR Assay <i>E. coli</i> O157:H7	Confirmatory tests	
							CT-SMAC Characteristic colonies	O157 and H7 latex test
1.	<i>Escherichia coli</i>	O92:H33	JM221	Clinical origin (Mexico)	5.7 10 ⁵	-	-	/
2.	<i>Escherichia coli</i>	O3:H2	38765	Clinical origin (Chili)	5.0 10 ⁵	-	-	/
3.	<i>Escherichia coli</i>	O78:H11	H10407	ATCC 35401	4.9 10 ⁵	-	-	/
4.	<i>Escherichia coli</i>	O6:H6	EDL1493	/	3.6 10 ⁵	-	-	/
5.	<i>Escherichia coli</i>	O6:H10	ECOR10	Clinical origin (Sweden)	7.6 10 ⁵	-	-	/
6.	<i>Escherichia coli</i>	O111:H21	DEC6a	Clinical origin (USA)	2.5 10 ⁵	-	-	/
7.	<i>Escherichia coli</i>	O86:H43	ECOR23	Animal origin (elephant USA)	3.7 10 ⁵	-	-	/
8.	<i>Escherichia coli</i>	O26:H11	DEC9a	Clinical origin (USA)	3.9 10 ⁵	-	-	/
9.	<i>Escherichia coli</i>	O111:H8	DEC8b	Clinical origin (USA)	4.9 10 ⁵	-	-	/
10.	<i>Escherichia coli</i>	O128:H2	DEC11a	Clinical origin (USA)	8.0 10 ⁵	-	-	/
11.	<i>Escherichia coli</i>	O111:H2	DEC12a	Clinical origin (UK)	5.7 10 ⁵	-	-	/
12.	<i>Escherichia coli</i>	O128:H7	DEC13a	Clinical origin (USA)	3.9 10 ⁵	-	-	/
13.	<i>Escherichia coli</i>	O78:H12	TX-1	ATCC 43896	4.1 10 ⁵	-	-	/
14.	<i>Escherichia coli</i>	O104:H21	ECOR26	Clinical origin (USA)	7.0 10 ⁵	-	-	/
15.	<i>Escherichia coli</i>	O157:H43	DEC7a	Pork (USA)	4.0 10 ⁵	-	-	/
16.	<i>Escherichia coli</i>	O55:H7	DEC5d	Clinical origin (Sri Lanka)	4.2 10 ⁵	-	-	/
17.	<i>Escherichia coli</i>	O44:H18	42	Clinical origin (Peru)	7.6 10 ⁵	-	-	/
18.	<i>Escherichia coli</i>	O127:H6	E2348/69	Clinical origin (UK)	8.5 10 ⁵	-	+	O157-
19.	<i>Escherichia coli</i>	O55:H6	DEC1a	Clinical origin (USA)	4.5 10 ⁵	-	+	O157-
20.	<i>Escherichia coli</i>	O18:K1:H7	RS218	Clinical origin	4.7 10 ⁵	-	-	/
21.	<i>Salmonella</i>	Landau	Ad499	/	2.9 10 ⁵	-	-	/
22.	<i>Salmonella</i>	Sternhauze	Ad500	/	3.8 10 ⁵	-	-	/
23.	<i>Salmonella</i>	Urbana	Ad501	/	3.7 10 ⁵	-	-	/
24.	<i>Salmonella</i>	Wayne	Ad502	/	3.0 10 ⁵	-	-	/
25.	<i>Hafnia alvei</i>		88	Bakery	4.2 10 ⁵	-	-	/
26.	<i>Hafnia alvei</i>		167	Sausage	3.2 10 ⁵	-	-	/
27.	<i>Citrobacter freundii</i>		25	Frozen raw spinach	3.9 10 ⁵	-	-	/
28.	<i>Citrobacter freundii</i>		104	Ground beef	3.6 10 ⁵	-	-	/
29.	<i>Escherichia vulneris</i>		127	Raw milk	5.5 10 ⁵	-	-	/
30.	<i>Pantoea spp.</i>		134	Pork	8.5 10 ⁴	-	-	/
31.	<i>Escherichia coli</i>	O157	Ad524	Environment (dairy product)	4.2 10 ⁵	-	-	/
32.	<i>Escherichia coli</i>	O157	Ad525	Faeces	5.7 10 ⁵	-	-	/
33.	<i>Escherichia coli</i>	O157	Ad526	Faeces	6.4 10 ⁵	-	-	/
34.	<i>Escherichia coli</i>	O157	Ad527	Clinical origin	2.3 10 ⁵	-	-	/
35.	<i>Escherichia coli</i>	O157:H-	O1.12.903	/	2.8 10 ⁵	-	-	/
36.	<i>Escherichia coli</i>	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 2.9x10⁵/g

Sample No	Reference method ISO 16654				Alternative method BAX System RT PCR Assay <i>E. coli</i> O157:H7				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 2.8x10⁵/g

Sample No	Reference method ISO 16654				Alternative method BAX System RT PCR Assay <i>E. coli</i> O157:H7				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 1.6x10⁵/g

Sample No	Reference method ISO 16654				Alternative method BAX System RT PCR Assay <i>E. coli</i> O157:H7				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 4.5x10⁵/g

Sample No	Reference method ISO 16654				Alternative method BAX System RT PCR Assay <i>E. coli</i> O157:H7				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 3.0x10⁵/g

Sample No	Reference method ISO 16654				Alternative method BAX System RT PCR Assay <i>E. coli</i> O157:H7				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 1.7x10⁵/g

Sample No	Reference method ISO 16654				Alternative method BAX System RT PCR Assay <i>E. coli</i> O157:H7				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 2.5x10⁵/g

Sample No	Reference method ISO 16654				Alternative method BAX System RT PCR Assay <i>E. coli</i> O157:H7				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 3.2x10⁴/g

Sample No	Reference method ISO 16654				Alternative method BAX System RT PCR Assay <i>E. coli</i> O157:H7				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 6.2x10⁵/g

Sample No	Reference method ISO 16654				Alternative method BAX System RT PCR Assay <i>E. coli</i> O157:H7				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 2.8x10⁵/g

Sample No	Reference method ISO 16654				Alternative method BAX System RT PCR Assay <i>E. coli</i> O157:H7				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 8.6x10⁴/g

Sample No	Reference method ISO 16654				Alternative method BAX System RT PCR Assay <i>E. coli</i> O157:H7				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 1.5x10⁶/g

Sample No	Reference method ISO 16654♦				Alternative method BAX System RT PCR Assay <i>E. coli</i> O157:H7				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