

**OXOID**  
**THERMO FISHER SCIENTIFIC**  
6 route de Paisy  
F-69571 DARDILLY

**Validation study according to the ISO 16140 standard:**  
**Synthesis**

**ISO 16140 validation  
of the BAX<sup>®</sup> Real-Time  
*E. coli* O157:H7 method**

This report gathers 40 pages, including 6 annexes.  
Only complete report copies shall be realised.

Expert laboratory competences are certified by COFRAC accreditation for all analysis marked with a symbol♦.

**Synthesis BAX<sup>®</sup> Real-Time *E. coli* O157:H7**  
**Version 1**  
September 6, 2010

**Cancel and replace the previous version,**  
*which must be returned to ADRIA Développement*  
*or destroyed internally.*

<b>1</b>	<b>INTRODUCTION</b>	<b>4</b>
1.1	Alternative method	4
1.2	Reference method	4
<b>2</b>	<b>COMPARISON METHOD STUDY</b>	<b>5</b>
2.1	Relative accuracy, relative specificity and relative sensitivity	5
2.2	Relative detection level	10
2.3	Inclusivity / exclusivity	12
<b>3</b>	<b>PRACTICABILITY</b>	<b>13</b>
<b>4</b>	<b>INTERLABORATORY STUDY ORGANISATION AND RESULTS</b>	<b>15</b>
4.1	Study organisation	15
4.2	Experimental parameters control	17
4.3	Results analysis	19
4.4	Results interpretation	21
4.5	Interpretation	23
<b>5</b>	<b>CONCLUSION</b>	<b>24</b>
<input type="checkbox"/>	<i>Annex 1 - BAX® Real-Time E. coli O157: protocol</i>	25
<input type="checkbox"/>	<i>Annex 2 - ISO 16654 : Horizontal method for the detection of Escherichia coli O157</i>	26
<input type="checkbox"/>	<i>Annex 3 - Artificial contamination of samples</i>	27
<input type="checkbox"/>	<i>Annex 4 - Relative accuracy : raw data</i>	30
<input type="checkbox"/>	<i>Annex 5 - Inclusivity and exclusivity : raw data</i>	37
<input type="checkbox"/>	<i>Annex 6 - Collaborator labs</i>	40

**A double line in the margin on the left indicates the report modifications.**

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### ***Before comment***

Elements which allow the certification of the analysis quality can be consulted by OXOID THERMO FISHER SCIENTIFIC.

The results are presented according to the ISO 16140 standard.

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- ✓ **Firm:** OXOID  
6 route de Paisy  
F-69571 DARDILLY
  
- ✓ **Expert laboratory :** ADRIA Développement  
ZA Creac'h Gwen  
F-29196 QUIMPER Cedex
  
- ✓ **Studied method:** **BAX<sup>®</sup> Real-Time *E. coli* O157:H7**
  
- ✓ **Validation standard:** ISO 16140 (October 2003): Food microbiology – Protocol for the validation of alternative methods
  
- ✓ **Reference method <sup>♦</sup>:** ISO 16654: Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Escherichia coli* O157
  
- ✓ **Products categories:** **Raw beef meat**  
**Raw vegetables**

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<sup>♦</sup> Analysis performed according to the COFRAC accreditation

# 1 INTRODUCTION

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The **validation study of BAX<sup>®</sup> Real-Time *E. coli* O157:H7 method** focuses on **raw beef and raw vegetables** analysis. The initial BAX<sup>®</sup> *E. coli* O157:H7 MP kit has been recently modified in order to

- increase the PCR performances by using **modified primers and new real time PCR chemistry based on internal probe;**
- **shorten enrichment steps (7 / 8 h – 24 h);**
- **shorten PCR cycles.**

## 1.1 Alternative method

The alternative method protocol (see annex 1) includes :

- enrichment in BAX<sup>®</sup> *E. coli* O157:H7 MP broth at  $42 \pm 1^\circ\text{C}$  during
  - 8 h to 24 h for the vegetable samples
  - 7 h to 24 h for the raw beef samples,
- DNA extraction step on 20  $\mu\text{l}$  / enrichment broth,
- Real Time PCR with internal probe.

The results are confirmed by streaking 50  $\mu\text{l}$  of enrichment broth onto CT-SMAC and confirming characteristic purified colonies with the Wellcolex<sup>™</sup> *E. coli* O157:H7 latex test. If the results are not confirmed using these simple tests, the Qualicon protocol is performed.

**Two incubation times were evaluated during the validation, i.e. 7 h / 8 h and 24 h.**

## 1.2 Reference method <sup>♦</sup>

The reference method is the NF EN ISO 16654 method: Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Escherichia coli* O157 (See Annex 2).

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<sup>♦</sup> Analysis performed according to the COFRAC accreditation

## 2 COMPARISON METHOD STUDY

### 2.1 Relative accuracy, relative specificity and relative sensitivity

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

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

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

#### 2.1.1 Number and nature of samples

Two categories were studied, with the following types:

- raw beef: fresh, frozen, flavoured or marinated,
- vegetable products: raw, ready-to-eat or ready-to-cook, frozen.

Breakdown per food category and food type is shown below:

Category	Type	Positives (number)	Negatives (number)	Total (number)
Raw beef	Fresh	17	13	30
	Frozen	11	13	24
	Flavoured or marinated	12	7	19
	<b>Total</b>	<b>40</b>	<b>33</b>	<b>73</b>
Vegetable products	Fresh	12	9	21
	Ready-to-eat or ready-to-cook	17	18	35
	Frozen	16	3	19
		<b>45</b>	<b>30</b>	<b>75</b>
<b>TOTAL</b>		<b>85</b>	<b>63</b>	<b>148</b>

148 samples were analysed, covering 85 positive and 63 negative samples.

### 2.1.2 Artificial contamination of samples

Artificial contaminations were carried out by spiking. For sample spiking, various injury protocols were used. Cellular stress/injury was verified by comparing the growth difference between non-selective and selective media, i.e. TSYEA and CT-SMAC agar. Artificial contaminations are presented in annex 3.

Note that four ground beef samples were naturally contaminated samples.

### 2.1.3 Confirmation protocols

The positive samples were confirmed by streaking (50 µl) enrichment broth onto CT-SMAC agar and confirming characteristic purified colonies with the Wellcolex *E. coli* O157:H7 latex test. When the results were not confirmed using these tests, the Qualicon protocol was used.

### 2.1.4 Test results

Raw data per category are presented annex 4.

**Table 1 – Paired results of the reference and alternative methods**

Short enrichment step (7h / 8h)		
Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) PA = 39	Positive deviation (R-/A+) PD = 26
Alternative method negative (A-)	Negative deviation (A-/R+) ND = 12	Negative agreement (A-/R-) NA = 71

24h enrichment step		
Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) PA = 46	Positive deviation (R-/A+) PD = 34
Alternative method negative (A-)	Negative deviation (A-/R+) ND = 5	Negative agreement (A-/R-) NA = 63

PPNC = 0

**Results per category of sample****Table 2 – Raw beef**

Short enrichment step (7h)		
Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) PA = 22	Positive deviation (R-/A+) PD = 11
Alternative method negative (A-)	Negative deviation (A-/R+) ND = 5	Negative agreement (A-/R-) NA = 35

24 h enrichment step		
Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) PA = 26	Positive deviation (R-/A+) PD = 13
Alternative method negative (A-)	Negative deviation (A-/R+) ND = 1	Negative agreement (A-/R-) NA = 33

**Table 3 – Vegetable products**

Short enrichment step (8h)		
Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) PA = 17	Positive deviation (R-/A+) PD = 15
Alternative method negative (A-)	Negative deviation (A-/R+) ND = 7	Negative agreement (A-/R-) NA = 36

24 h enrichment step		
Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) PA = 20	Positive deviation (R-/A+) PD = 21
Alternative method negative (A-)	Negative deviation (A-/R+) ND = 4	Negative agreement (A-/R-) NA = 30

**Table 4 – Calculation of relative accuracy (AC), relative sensitivity (SE) and relative specificity (SP)**

Short enrichment step										
Matrix	PA	NA	ND	PD	N	Relative accuracy AC (%) [100x(PA+NA)]/N]	N+ PA + ND	Relative sensitivity SE (%) [100xPA]/N+]	N- NA + PD	Relative specificity SP (%) [100xNA]/N-]
Raw beef products (7h enrichment step)	22	35	5	11	73	78.1	27	81.5	46	76.1
Vegetable products (8h enrichment step)	17	36	7	15	75	70.7	24	70.8	51	70.6
TOTAL	39	71	12	26	148	74.3	51	76.5	97	73.2

24 h enrichment step										
Matrix	PA	NA	ND	PD	N	Relative Accuracy AC (%) [100x(PA+NA)]/N]	N+ PA + ND	Relative sensitivity SE (%) [100xPA]/N+]	N- NA + PD	Relative specificity SP (%) [100xNA]/N-]
Raw beef products	26	33	1	13	73	80.8	27	96.3	46	71.7
Vegetable products	20	30	4	21	75	66.7	24	83.3	51	58.8
TOTAL	46	63	5	34	148	73.6	51	90.2	97	64.9

PA = positive agreement (R+/A+)

NA =negative agreement (R-/A-)

PD =positive deviation (R-/A+)

ND = negative deviation (A-/R+)

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

The alternative method percentage values are:

	Short enrichment step	24 h enrichment step
Relative accuracy	74.3	73.6
Relative specificity	73.2	64.9
Relative sensitivity	76.5	90.2

Taking into account the alternative method positive deviations, sensitivities of both tested methods are the following:

	Short enrichment step	24 h enrichment step
Alternative method	84.4	94.1
Reference method	66.2	60.0

**2.1.6 Analysis of discordant results:**

Breakdown of the discordant results is the following:

	ND	AC	NC	PD	AC	NC
<b>Short time enrichment step</b>						
Raw beef	5	4	1	11	11	0
Vegetable products	7	7		15	10	
<b>24 h enrichment step</b>						
Raw beef	1			13	12	1
Vegetable products	4	4		21	21	

AC: artificially contaminated sample

NC: naturally contaminated sample

Numerous positive deviations are observed whatever the enrichment incubation time, enhancing a McNemars test concluding that the compared methods are different.

	Short time enrichment step	24 h enrichment step
Y = ND + PD	Y = 12 + 26 = 38 Y > 22	Y = 5 + 34 = 39 Y > 22
McNemar test $\chi^2 = d^2/y$	D =  PD - ND  = 26 - 12 = 14 $\chi^2 = 14^2/38 = 5,15$ $\chi^2 > 3.841$	D =  PD - ND  = 34 - 5 = 29 $\chi^2 = 29^2/39 = 21,6$ $\chi^2 > 3.841$

**Differences are in favour of the alternative method which clearly shows higher performances than the reference method.**

## 2.2 Relative detection level

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

### 2.2.1 Matrices

The objective of this study is (i) to determine the minimal contamination level that can be detected in food matrices, (ii) to compare both method results.

Detection limits were defined by analysing different (matrix/strain) pairs. Four levels were tested, with six replicates per contamination level.

The following matrices were tested:

- ground beef inoculated with *Escherichia coli* O157:H7 Ad 663,
- frozen spinach, inoculated *Escherichia coli* O157:H7 Ad 557.

### 2.2.2 Contamination protocol

Contaminations and enumerations were realised according to the AFNOR technical rules (low levels inoculation protocol). Contamination levels are presented bellow:

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

Samples were analysed by both methods, and back ground microflora was enumerated.

### 2.2.3 Results

Detection levels are presented Table 5.

**Table 5 – Relative detection level results**  
(short time enrichment step and 24 h enrichment step)

Strain / matrix pairs	Relative detection level (CFU / 25 g) according to Spearman-Kärber test <sup>1</sup>	
	Reference method	Alternative method (7/8 h and 24 incubation time)
Ground beef / <i>Escherichia coli</i> O157:H7 Ad 663	0.224 [0.071; 0.71]	0.62 [0.384; 1.003]
Frozen spinach / <i>Escherichia coli</i> O157:H7 Ad 557	0.394 [0.166 ; 0.936]	0.32 [0.128 ; 0.797]

#### 2.2.4 Conclusion

The detection level varies from 0.1 to 0.9 CFU/25 g for the reference method and from 0.1 to 1.0 CFU/25 g for the alternative method whatever the incubation time (7 h / 8 h or 24 h).

The alternative and the standard methods show similar detection levels.

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

## 2.3 Inclusivity / exclusivity

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

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

### 2.3.1 Technical protocols

#### ✓ **Inclusivity**

50 target *E. coli* O157:H7 strains were cultivated in BHI broth and dilutions were done in order to inoculate the BAX<sup>®</sup> specific broth (MP broth) at 10 to 100 cells in 225 ml. The enrichment broth was then incubated for 7h at 42°C, and the alternative method protocol was realized.

#### ✓ **Exclusivity**

50 non-target *E. coli* O157:H7 strains were cultivated in BHI broth, and dilutions were done in order to inoculate the BPW at 10<sup>5</sup> cells in 225 ml. The enrichment broth was then incubated for 24h at 42°C, and the alternative method protocol was realised. Note that two *Escherichia coli* O157:H- strains were tested.

### 2.3.2 Results

Raw data are given in annex 5.

#### ✓ **Inclusivity**

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

#### ✓ **Exclusivity**

All the non target strains show negative PCR results.

### 2.3.3 Conclusion

**The alternative method shows satisfying exclusivity and inclusivity performances.**

### 3 PRACTICABILITY

The BAX<sup>®</sup> Real-Time *E. coli* O157:H7 method practicability performances were evaluated according to the AFNOR criteria relative to the preliminary study.

<p>✓ <b>Packaging</b></p> <p>✓ <b>Volume of reagents</b></p>	<p>For 96 tests, the following reagents are required:</p> <ul style="list-style-type: none"> <li>- 2 x 12 ml of lysis buffer</li> <li>- 400 µl of Protease enzyme</li> <li>- 12 x 8 PCR strips</li> <li>- 12 x 8 optical cap strips</li> </ul>
<p>✓ <b>Storage condition and kit</b></p>	<p>The storage temperature is 2-8°C, and is clearly mentioned on the packaging and reagents. The kit shelf-life is also specified.</p>
<p>✓ <b>Use after having open the kit</b></p>	<p>The reagents are stored at 2-8°C.</p>
<p>✓ <b>Required instruments</b></p>	<p>No specific room is required. The method is easy to use in labs for food microbiology analyses.</p>
<p>✓ <b>Reagents</b></p>	<p>The lysis buffer is prepared by mixing 150 µl of protease with 12 ml buffer. It can be then stored for 2 weeks at 2-8°C.</p>
<p>✓ <b>Training courses</b></p>	<p>2 days training are required for technicians that do not know the principles of PCR. Less than 1 days training is required for technicians familiar with PCR.</p>

✓ <b>Workflow study</b> (time required for analyses in minutes)	<b>Negative samples</b>				
	Steps	ISO 16654		BAX <i>E. coli</i> O157:H7	
		12 samples	32 samples	12 samples	32 samples
	Sampling	54	105	54	105
	IMS 6 h	48	85		
	IMS 24 h, if necessary	48	85		
	DNA extraction			3	9
	PCR			5	16
	Confirmatory tests	5	10		
	Total for negative samples analyses	155	285	62	130
	Total / negative samples	13	9	5	4
	<b>Presumptive positive or positive samples</b>				
	Steps	ISO 16654		BAX <i>E. coli</i> O157:H7	
		12 samples	32 samples	12 samples	32 samples
	Streak on CT-SMAC			5	10
	CT-SMAC reading			2	5
	Streak on Nutrient agar	10	25	10	25
	Indole test	24	72		
	Latex test	18	48	6	15
	Total for positive samples	207	430	85	185
Total / positive samples	17	13	7	6	
✓ <b>Time to results</b>	<b>Negative samples</b>				
	Steps	ISO 16654	BAX <i>E. coli</i> O157:H7		
			BAX 7 / 8 h	BAX 24 h	
	Sampling	D0	D0	D0	
	IMS 6 h	D0	/	/	
	IMS 24 h	D1	/	/	
	PCR	/	D0	D1	
	Final negative result	D1	D0	D1	
	<b>Presumptive positive or positive results</b>				
	Steps	ISO 16654	BAX <i>E. coli</i> O157:H7		
			BAX 7 / 8 h	BAX 24 h	
	Sampling	D0	D0	D0	
	IMS 6 h	D0	/	/	
	IMS 24 h	D1	/	/	
	PCR	/	D0	D1	
	Streak on CT SMAC	/	D0	D1	
Streak on nutritive agar	D1-D2	D1	D2		
Indol test	D2-D3	+	+		
Latex test	D3-D4	D2	D3		

✓ <b>Technician qualification</b>	Similar as the reference method
✓ <b>Common step with reference method</b>	No common step
✓ <b>Traceability</b>	Traceability requirements correspond to those that are usually realized in food microbiology labs
✓ <b>Maintenance</b>	/

The BAX<sup>®</sup> *E. coli* O157:H7 MP method clearly reduces time to results and handling time comparing to the reference method.

## 4 INTERLABORATORY STUDY ORGANISATION AND RESULTS

### 4.1 Study organisation

#### Collaborators number

Samples were sent to 14 laboratories (See annex 6) but only 12 laboratories were able to perform the analyses. Lab I never received its parcel due to problems with Customs and Lab H could not analyse the samples because of illness of the technician, who was trained with the PCR System.

#### Matrix and strain used

The study was carried on a meat matrix (ground beef).

#### Samples

Samples were inoculated and sent on Monday 31<sup>st</sup> May 2010. Analysis was performed on Wednesday 2nd June 2010, as described below:

- 24 codified samples (25 g) (red labelled) for *Escherichia coli* O157:H7 for analysis by BAX<sup>®</sup> Real-Time *E. coli* O157:H7 method.
- 24 codified samples (25 g) (blue labelled) for analysis by the reference method ISO 16654 (2001).

- 1 ground beef sample (labelled “Sample for Total Count enumeration”) for aerobic mesophilic flora enumeration by ISO 4833 method,
- 1 water flask labelled “Temperature Control” with a temperature probe, which must follow the samples during analysis.

#### *Inoculation*

The targeted inoculation levels were:

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

Each laboratory received 48 samples of 25 g, i.e. 8 samples per inoculation level and method. Furthermore, one non-inoculated sample was added to the package for total viable count by the ISO 4833 method.

#### *Labelling and shipping*

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

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

Samples were shipped in 24 h to 48 h to the laboratories. Sample temperature should be lower or equal to 8.4°C during transport and between 0°C – 8.4°C at arrival.

#### *Analysis*

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

#### *Expedition conditions*

The collaborative study instructions were sent on May 6<sup>th</sup>, 2010.

## 4.2 Experimental parameters control

### 4.2.1 Contamination level before inoculation, levels obtained after samples artificial contamination

#### Before inoculation

In order to detect *E. coli* O157:H7, the ISO 16654 method was performed on five ground beef samples (25 g) before the samples were inoculated. All of the results were negative.

#### Samples stability

Preliminary assay was performed by inoculating the matrix at 25 CFU/25 g and 5 CFU/25 g. Enumeration was performed on 5 g of ground beef for the high contamination level and a study was performed for the low contamination level. The results are the following:

Day	Reference method (research)			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 4°C, i.e. the inoculated bacterial population is divided by 2.

#### Contamination levels

The contamination levels and the confidence intervals are:

Level	Samples	Theoretical target level (b/25 g)	True level (b/25 g sample)	Low limit / 25 g sample	High limit / 25 g sample
Level 0	3 – 8 – 9 – 12 – 15 – 18 – 20 – 21	0	/	/	/
Low level	1 – 4 – 7 – 10 – 11 – 13 – 17 – 24	5	9.2	8.0	16.0
High level	2 – 5 – 6 – 14 – 16 – 19 – 22 – 23	25	49.7	43.0	57.9

#### 4.2.2 Logistic conditions

Temperature conditions are given below:

##### Samples temperatures at receipt

Laboratories	Temperature measured by the sensor (°C)	Temperature measured at receipt (°C)	Receipt delay	Day of analysis
A	2.0	5.8	02/06/10 – 09h10	J2
B	3.0	5.0	01/06/10 – 09h30	J2
C	3.0	4.1	01/06/10 – 10h00	J2
D	2.5	5.1	01/06/10 – 14h00	J2
E	2.5	5.0	01/06/10 – 10h00	J2
F	3.5	7.3	01/06/10 – 11h15	J2
G	3.0	6.8	01/06/10 – 12h30	J2
H	This lab did not performed the analysis and did not return the probe.			
I	Parcel not dispatched due to Customs delay			
J	3.0	3.4	01/06/10 – 10h40	J2
K	2.5	2.3	01/06/10 – 08h40	J2
L	3.5	5.9	01/06/10 – 08h00	J2
M	2.0	3.3	01/06/10 – 09h30	J2
N	3.0	6.0	01/06/10 – 09h15	J2

The logistic temperatures vary from 2°C to 3.5°C.

#### 4.2.3 Conclusion

No problem was encountered during the transport or at receipt.

## 4.3 Results analysis

### 4.3.1 Aerobic mesophilic flora enumeration

Depending on the lab results, the enumeration levels varied from  $3.2 \times 10^4$  to  $6.2 \times 10^5$ CFU/g.

### 4.3.2 Expert lab results

One negative result was observed by the reference method for one low inoculated sample.

### 4.3.3 Incubation conditions and analyses

#### Alternative method enrichment incubation

In order to follow the incubation for the alternative method, the collaborating laboratories were asked to incubate the temperature probe along with the alternative enrichments.

The incubation time is given in the table below:

Laboratory	Beginning incubation hour	End incubation hour	Incubation time
A	13h50	21h50	08h00
B	07h06	13h51	06h45
C	08h30	15h45	07h15
D	09h23	15h53	06h30
E	07h54	14h54	07h00
F	09h40	16h25	06h45
G	08h56	16h00	07h00
J	08h15	15h15	07h00
K	09h13	16h13	07h00
L	07h00	14h00	07h00
M	09h15	16h15	07h00
N	08h15	15h00	06h45
ADRIA	10h00	17h00	07h00

The probe of Lab C was not kept in the incubator until the end of the reference enrichment and the curve indicates 24 h incubation time. According to the Lab C's traceability records, the alternative method enrichment was stopped at 15h45, which corresponds to an incubation time of 7h15.

Lab L forgot to put the probe in the incubator with the enrichments but the incubator temperature record was forwarded to ADRIA.

All of the Labs followed the instructions and incubated the enrichment for 7 hours, except Lab A which incubated the MP broth for 8 hours.

❑ Laboratories retained for the interpretation

The results of Lab A were not taken into account: as the MP enrichment broth was incubated for 8 hours, 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 I did not receive its parcel.

Note that the temperature probe was stored with the samples during this 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 *E. coli* O157:H7 behaviour. The Sym'Previus software<sup>2</sup> was used.

According to these simulations, it was decided that the results of Lab F were useable.

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<sup>2</sup> [www.symprevius.org](http://www.symprevius.org)

## 4.4 Results interpretation

### 4.4.1 Both methods specificity and sensitivity

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

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

with: N- = total number of all L0 essays

FP = number of false positive

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

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

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

TP = number of true positive

Results are reported in the following table:

Level	Reference method		Alternative method	
	SP/SE %	LCL%	SP/SE %	LCL%
L0	SP = 97.5	93	SP = 100	98
L1	SE = 91.3	84	SE = 100	98
L2	SE = 97.5	93	SE = 100	98
L1+L2	SE = 94.4	89	SE = 100	98

### 4.4.2 Relative accuracy (AC)

Results for all levels are given below:

**Table 6 - Paired results of the alternative and reference methods**

Alternative method	Reference method		Total
	+	-	
+	PA = 151	PD = 9	160
-	ND = 2	NA = 78	80
<b>Total</b>	N+ = 153	N- = 87	N = 240

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

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

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

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

**Table 7**

Level	AC %	LCL %
L0	97.5	93
L1	91.3	84
L2	97.5	93
L1 + L2	94.4	89
<b>Total</b>	<b>95.4</b>	<b>89</b>

#### 4.4.3 Discordant results

##### Negative deviations

Two negative deviations were observed for Lab F (samples F18 and F20). For sample 20, only one characteristic colony was observed on CT-SMAC and was confirmed as *E. coli* O157:H7. This was probably due to sample cross contamination.

##### Positive deviation

Nine positive deviations were observed, 7 for the low contamination level and 2 for the high contamination level.

##### Statistic interpretation

$$Y = PD + ND = 9 + 2 = 11$$

$$6 \leq y \leq 22 \quad m = ND = 2$$

$$M = 1 \quad m > M$$

There is no difference between the 2 methods at  $\alpha$  0.05.

## 4.5 Interpretation

### 4.5.1 *Relative accuracy, specificity and sensibility comparison values*

The values obtained for the two parts of the validation study (comparative and inter-laboratory studies) are reported in table 8.

**Tableau 8 - Alternative method values calculated during the comparative and inter-laboratory studies**

	Interlaboratory study	Methods comparative study
Relative accuracy (AC)	95.4	74.3
Sensibility (SE)	100.0	76.5
Specificity (SP)	100.0	73.2

### 4.5.2 *Accordance (DA)*

Accordance values for both methods are:

Level	Reference method (DA)	Alternative method (DA)
L0	96.3	100
L1	87.2	100
L2	95.6	100

### 4.5.3 *Concordance*

Both methods concordance values are:

Level	Reference method	Alternative method
L0	95.0	100
L1	83.7	100
L2	95.1	100

#### 4.5.4 Odds Ratio (COR)

The odds ratio value is determined according to:

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

Both method odds ratio values are:

Level	Reference method (COR)	Alternative method (COR)
L0	1.01	1.00
L1	1.04	1.00
L2	1.01	1.00

## 5 CONCLUSION

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The **methods comparative study conclusions** are:

- The BAX<sup>®</sup> Real-Time *E. coli* O157:H7 method shows satisfying relative accuracy, relative specificity and relative sensitivity results.
- The BAX<sup>®</sup> Real-Time *E. coli* O157:H7 and standard method have similar limit detection levels.
- The BAX<sup>®</sup> Real-Time *E. coli* O157:H7 shows satisfying exclusivity and inclusivity performances.
- The BAX<sup>®</sup> *E. coli* O157:H7 MP method has significant time and handling advantages.

The **interlaboratory study conclusions** are:

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

## Annex 1 - BAX<sup>®</sup> Real-Time *E. coli* O157: protocol

### Protocol

#### Raw beef and raw vegetables

25 g + 225 ml (dilution 1/10) BAX<sup>®</sup> 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



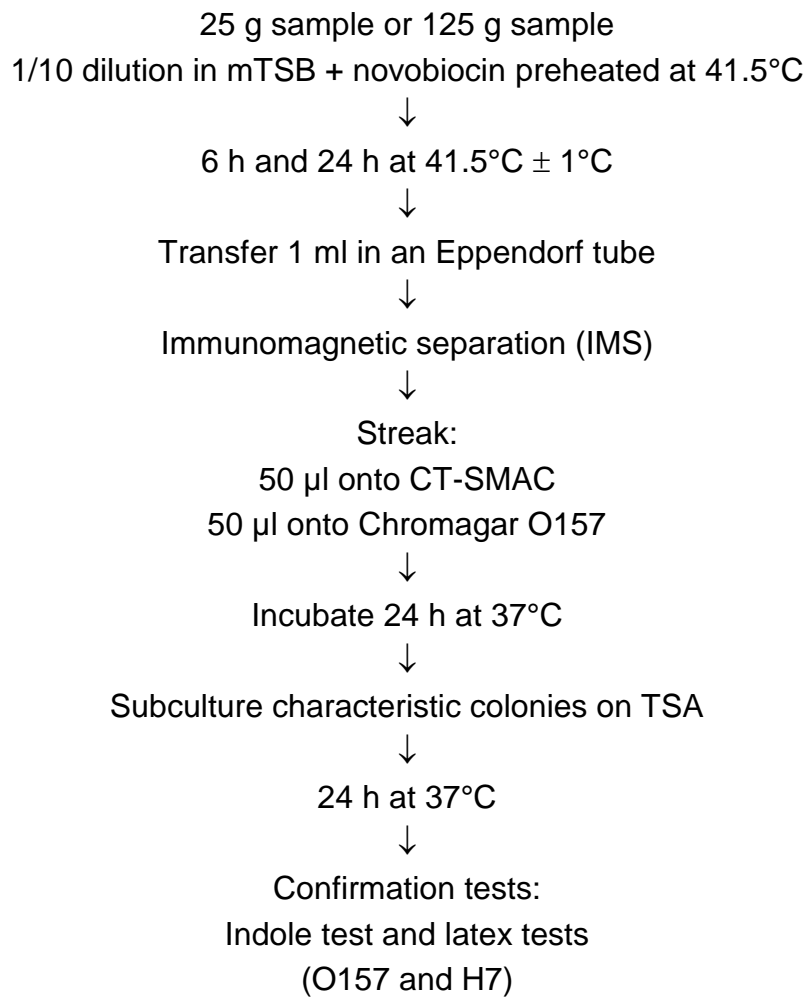
30 µl DNA extract / PCR tube



PCR



Confirmation by streaking 50 µl on  
CT -SMAC agar and confirm presumptive positive colonies with the Wellcolex *E. coli*  
O157:H7 latex test or use the Qualicon protocol

**Annex 2 – ISO 16654 : Horizontal method for the detection  
of *Escherichia coli* O157**

## Annex 3 - Artificial contamination of samples

Sample N°	Product	Artificial contaminations (spiking protocol)					Global result
		Strain	Origin	Injury protocol	Stress measurement	Inoculation level/25g	
365	Frozen ground beef preparation	E.coli O157:H7 Ad487	Ground beef	-20°C	>2,43	9-8-7-19-7(9,4)	+
366	Frozen ground beef preparation (80%)	E.coli O157:H7 Ad487	Ground beef	-20°C	>2,43	9-8-7-19-7(9,4)	+
367	Frozen ground beef	E.coli O157:H7 Ad487	Ground beef	-20°C	>2,43	9-8-7-19-7(9,4)	+
368	Frozen ground beef with onions	E.coli O157:H7 Ad564	Ground beef	-20°C	>2,82	11-15-20-16-11(14,6)	+
369	Frozen ground beef	E.coli O157:H7 Ad564	Ground beef	-20°C	>2,82	11-15-20-16-11(14,6)	+
370	Frozen groundbeef (meatballs)	E.coli O157:H7 Ad564	Ground beef	-20°C	>2,82	11-15-20-16-11(14,6)	+
371	Groundbeef (meatballs)	E.coli O157:H7 Ad565	Ground beef	-20°C	1,64	19-20-8-16-20(16,6)	+
372	Fresh ground beef	E.coli O157:H7 Ad487	Ground beef	10 days at 4°C	1,43	13-6-6-8-9(7,8)	+
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)	+
374	Fresh ground beef	E.coli O157:H7 Ad487	Ground beef	10 days at 4°C	1,43	13-6-6-8-9(7,8)	+
375	Fresh ground beef	E.coli O157:H7 Ad562	Ground beef	10 days at 4°C	1,73	10-11-8-12-7(9,6)	+
376	Fresh ground beef	E.coli O157:H7 Ad562	Ground beef	10 days at 4°C	1,73	10-11-8-12-7(9,6)	+
377	Fresh ground beef	E.coli O157:H7 Ad562	Ground beef	10 days at 4°C	1,73	10-11-8-12-7(9,6)	+
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)	+
379	Fresh ground beef	E.coli O157:H7 Ad583	Ground beef	10 days at 4°C	1,61	10-14-17-12-14(13,4)	+
380	Fresh ground beef (5%fat)	E.coli O157:H7 Ad583	Ground beef	10 days at 4°C	1,61	10-14-17-12-14(13,4)	+
381	Fresh ground beef (15%fat)	E.coli O157:H7 Ad590	Ground beef	10 days at 4°C	1,29	9-12-11-7-10(9,8)	+
382	Beef carpaccio	E.coli O157:H7 Ad590	Ground beef	10 days at 4°C	1,29	9-12-11-7-10(9,8)	+
383	Frozen ground beef	E.coli O157:H7 Ad565	Ground beef	-20°C	1,64	19-20-8-16-20(16,6)	+
384	Frozen beef preparation (51%)	E.coli O157:H7 Ad565	Ground beef	-20°C	1,64	19-20-8-16-20(16,6)	+
385	Frozen ground beef	E.coli O157:H7 Ad565	Ground beef	-20°C	1,64	19-20-8-16-20(16,6)	+
386	Frozen ground beef	E.coli O157:H7 Ad590	Ground beef	-20°C	2,46	8-8-10-4-5(7,0)	+
387	Frozen groundbeef (meatballs)	E.coli O157:H7 Ad590	Ground beef	-20°C	2,46	8-8-10-4-5(7,0)	+
388	Frozen groundbeef (51%) (meatballs)	E.coli O157:H7 Ad590	Ground beef	-20°C	2,46	8-8-10-4-5(7,0)	+
498	Spinach beet	E.coli O157:H7 Ad556	Ground beef	16 days at 4°C	1,70	5-3-3-3-4(3,6)	+
500	Parsley	E.coli O157:H7 Ad558	WWTP	16 days at 4°C	2,13	3-6-5-4-3(4,2)	+
502	Links	E.coli O157:H7 Ad575	Bovine feces	16 days at 4°C	0,93	1-4-2-4-2(2,6)	+
504	Endive	E.coli O157:H7 Ad576	Bovine feces	16 days at 4°C	1,70	1-1-1-4-6(2,6)	+
506	Lettuce	E.coli O157:H7 Ad556	WWTP	16 days at 4°C	1,87	1-5-1-0-1(1,6)	+

Sample N°	Product	Artificial contaminations (spiking protocol)					Global result
		Strain	Origin	Injury protocol	Stress measurement	Inoculation level/25g	
508	Fresh salad	E.coli O157:H7 Ad556	WWTP	16 days at 4°C	1,70	5-3-3-3-4(3,6)	+
510	Fresh corn-salad	E.coli O157:H7 Ad558	WWTP	16 days at 4°C	2,13	3-6-5-4-3(4,2)	+
512	Lettuce	E.coli O157:H7 Ad575	Bovine feces	16 jours à 4°C	0,93	1-4-2-4-2(2,6)	+
514	Batavia	E.coli O157:H7 Ad576	Bovine feces	16 days at 4°C	1,70	1-1-1-4-6(2,6)	+
516	White cubbage	E.coli O157:H7 Ad556	WWTP	16 days at 4°C	1,87	1-5-1-0-1(1,6)	+
518	Grated carrots	E.coli O157:H7 Ad556	WWTP	16 days at 4°C	1,70	5-3-3-3-4(3,6)	+
520	Soya sprouts	E.coli O157:H7 Ad558	WWTP	16 days at 4°C	2,13	3-6-5-4-3(4,2)	-
522	Frozen mixed vegetables	E.coli O157:H7 Ad556	WWTP	-20°C	2,39	2-3-1-3-2(1,8)	+
523	Vegetable based cake	E.coli O157:H7 Ad575	Bovine feces	-20°C	>2,66	2-5-4-4-2(3,4)	+
524	Fried frozen vegetables	E.coli O157:H7 Ad576	Bovine feces	-20°C	>2,30	8-5-3-5-3(4,8)	+
525	Mixed vegetables	E.coli O157:H7 Ad556	WWTP	-20°C	2,39	2-3-1-3-2(1,8)	+
526	Fried frozen mushrooms	E.coli O157:H7 Ad575	Bovine feces	-20°C	>2,66	2-5-4-4-2(3,4)	+
527	Fried frozen vegetables	E.coli O157:H7 Ad576	Bovine feces	-20°C	>2,30	8-5-3-5-3(4,8)	+
693	Spinach (bagged pre-cut leafy vegetables)	E.coli O157:H7 Ad575	Bovine feces	26 days at 4°C	0,76	1-2-11-2-8 (4,8)	+
694	Fresh vegetable mix (cabbage,carrots,salad.) (bagged pre-cut leafy vegetables)	E.coli O157:H7 Ad575	Bovine feces	26 days at 4°C	0,76	1-2-11-2-8 (4,8)	+
695	Red cabbage (bagged pre-cut leafy vegetables)	E.coli O157:H7 Ad575	Bovine feces	26 days at 4°C	0,76	1-2-11-2-8 (4,8)	+
696	Fresh mix vegetables (bagged pre-cut leafy vegetables)	E.coli O157:H7 Ad575	Bovine feces	26 days at 4°C	0,76	1-2-11-2-8 (4,8)	+
697	White cabbage (bagged pre-cut leafy vegetables)	E.coli O157:H7 Ad576	Bovine feces	26 days at 4°C	1,38	2-4-5-2-8 (4,0)	+
698	Rocket (bagged pre-cut leafy vegetables)	E.coli O157:H7 Ad576	Bovine feces	26 days at 4°C	1,38	2-4-5-2-8 (4,0)	+
699	Red cabbage (bagged pre-cut leafy vegetables)	E.coli O157:H7 Ad576	Bovine feces	26 days at 4°C	1,38	2-4-5-2-8 (4,0)	+
700	Frozen vegetables for ratatouille	E.coli O157:H7 Ad556	WWTP	-20°C	>1,81	4-5-4-3-7 (4,6)	+
701	Frozen vegetables mix	E.coli O157:H7 Ad556	WWTP	-20°C	>1,81	4-5-4-3-7 (4,6)	-
702	Frozen sliced links	E.coli O157:H7 Ad577	Bovine feces	-20°C	1,91	11-9-9-15-3 (9,4)	+
703	Cauliflower	E.coli O157:H7 Ad577	Bovine feces	-20°C	1,91	11-9-9-15-3 (9,4)	+
704	Frozen mushrooms	E.coli O157:H7 Ad558	WWTP	-20°C	1,68	9-17-16-14-21 (15,4)	+
732	Green cabbage	E.coli O157:H7 Ad556	WWTP	27 days at 4°C	1,82	4-8-8-4-6 (6,0)	+
733	Celery	E.coli O157:H7 Ad556	WWTP	27 days at 4°C	1,82	4-8-8-4-6 (6,0)	+
734	Sprouts	E.coli O157:H7 Ad556	WWTP	27 days at 4°C	1,82	4-8-8-4-6 (6,0)	+
735	Courgette	E.coli O157:H7 Ad556	WWTP	27 days at 4°C	1,82	4-8-8-4-6 (6,0)	+
736	Artichoke	E.coli O157:H7 Ad577	Bovine feces	27 days at 4°C	1,82	12-9-5-7-8 (8,2)	+

Sample N°	Product	Artificial contaminations (spiking protocol)					Global result
		Strain	Origin	Injury protocol	Stress measurement	Inoculation level/25g	
737	Broccoli (bagged pre-cut leafy vegetables)	E.coli O157:H7 Ad577	Bovine feces	27 days at 4°C	1,82	12-9-5-7-8 (8,2)	+
738	Green beans (bagged pre-cut leafy vegetables)	E.coli O157:H7 Ad577	Bovine feces	27 days at 4°C	1,82	12-9-5-7-8 (8,2)	+
739	Vegetable mix (bagged pre-cut leafy vegetables)	E.coli O157:H7 Ad577	Bovine feces	27 days at 4°C	1,82	12-9-5-7-8 (8,2)	+
740	Green asparagus	E.coli O157:H7 Ad556	WWTP	-20°C	>0,90	7-4-2-5-4 (4,4)	+
741	Peas	E.coli O157:H7 Ad577	Bovine feces	-20°C	>0,90	4-5-4-3-4 (4,0)	+
742	Beans	E.coli O157:H7 Ad558	WWTP	-20°C	3,35	10-10-6-7-7 (8,0)	+
743	Vegetable mix (bagged pre-cut soup vegetables)	E.coli O157:H7 Ad558	WWTP	-20°C	3,35	10-10-6-7-7 (8,0)	+
744	Chuck	E.coli O157:H7 Ad565	Ground beef	28 days at 4°C	1,14	0-2-3-2-4 (2,2)	+
745	Rump steak	E.coli O157:H7 Ad565	Ground beef	28 days at 4°C	1,14	0-2-3-2-4 (2,2)	+
746	Tournedos	E.coli O157:H7 Ad565	Ground beef	28 days at 4°C	1,14	0-2-3-2-4 (2,2)	+
747	Sirloin	E.coli O157:H7 Ad487	Ground beef	28 days at 4°C	0,48	5-3-4-4-6 (4,4)	+
748	Shin	E.coli O157:H7 Ad487	Ground beef	28 days at 4°C	0,48	5-3-4-4-6 (4,4)	+
854	Marinated piece of beef	E.coli O157:H7 Ad583	Ground beef	33 days at 4°C	1,34	5-5-7-2-6(5,0)	+
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)	+
856	Carpaccio	E.coli O157:H7 Ad583	Ground beef	33 days at 4°C	1,34	5-5-7-2-6(5,0)	+
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)	+
858	Ground beef	E.coli O157:H7 Ad590	Ground beef	33 days at 4°C	1,50	4-5-10-7-8(6,8)	+
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)	+
860	Beef balls	E.coli O157:H7 Ad590	Ground beef	33 days at 4°C	1,50	4-5-10-7-8(6,8)	+
861	Courgette	E.coli O157:H7 Ad576	Bovine feces	33 days at 4°C	0,88	14-18-11-6-9(11,6)	+
862	Turnip	E.coli O157:H7 Ad576	Bovine feces	33 days at 4°C	0,88	14-18-11-6-9(11,6)	+
863	Swedish turnip	E.coli O157:H7 Ad558	WWTP	33 days at 4°C	1,62	8-7-6-6-7(6,8)	+
864	Artichoke	E.coli O157:H7 Ad558	WWTP	33 days at 4°C	1,62	8-7-6-6-7(6,8)	+
865	Vegetables for ratatouille	E.coli O157:H7 Ad558	WWTP	33 days at 4°C	1,62	8-7-6-6-7(6,8)	+

## Annex 4 – Relative accuracy : raw data

+/-: doubtful colonies

NC : non characteristic colonies on TSA

Ims2: Qualicon confirmation protocol performed

VEGETABLE PRODUCTS																		
N°	Product	Reference method ISO 16654♦					BAX E.coli O157: H7-Real Time method – Short enrichment step (8 h)						BAX E.coli O157: H7-Real Time method – 24h enrichment step					
		Characteristic colonies				ISO 16654 result	PCR	Confirmatory tests			PCR		PCR-24H	Confirmatory tests			PCR	
		IMS 6H		IMS 24H				CT SMAC Characteristic colonies	O157 latex	H7 Latex	Final result	Agreement		CT SMAC Characteristic colonies	O157 latex	H7 Latex	Final result	Agreement
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157													
498	Spinach beet	+	+	/	/	+	-	/	/	/	-	ND	-	/	/	/	-	ND
499	Spinach beet	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
500	Parsley	-	-	-	-	-	+	+	+	+	+	PD	+	+	+	+	+	PD
501	Parsley	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
502	Links	+	+	/	/	+	-	/	/	/	-	ND	+	+	+	+	+	=
503	Links	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
504	Endive	-	-	-	-	-	-	/	/	/	-	=	+	+	+	+	+	PD
505	Endive	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
506	Lettuce	-	-	+	+	+	-	/	/	/	-	ND	+	+	+	+	+	=
507	Lettuce	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
508	Fresh salad	-	-	-	-	-	+	+	+	+	+	PD	+	+	+	+	+	PD
509	Fresh salad	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
510	Fresh corn-salad	-	-	-	-	-	+	+	+	+	+	PD	+	+	+	+	+	PD
511	Fresh corn-salad	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
512	Lettuce	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	=
513	Lettuce	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
514	Batavia	-	-	-	-	-	+	+	+	+	+	PD	+	+	+	+	+	PD
515	Batavia	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
516	White cabbage	-	-	-	-	-	-	/	/	/	-	=	+	+	+	+	+	PD
517	White cabbage	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
518	Grated carrots	-	-	-	-	-	+	+	+	+	+	PD	+	+	+	+	+	PD
519	Grated carrots	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
520	Soya sprouts	+(NC)	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=

♦ Analysis performed according to the COFRAC accreditation

+/-: doubtful colonies

NC : non characteristic colonies on TSA

Ims2: Qualicon confirmation protocol performed

VEGETABLE PRODUCTS																		
N°	Product	Reference method ISO 16654♦					BAX E.coli O157: H7-Real Time method – Short enrichment step (8 h)						BAX E.coli O157: H7-Real Time method – 24h enrichment step					
		Characteristic colonies				ISO 16654 result	PCR	Confirmatory tests			PCR		PCR-24H	Confirmatory tests			PCR	
		IMS 6H		IMS 24H				CT SMAC Characteristic colonies	O157 latex	H7 Latex	Final result	Agreement		CT SMAC Characteristic colonies	O157 latex	H7 Latex	Final result	Agreement
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157													
521	Soya sprouts	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
522	Frozen mixed vegetables	-	-	+	+	+	+	+	+	+	+	=	+	+	+	+	+	=
523	Vegetable based cake	-	-	+	-	+	-	/	/	/	-	ND	+	+(ims2)	+	+	+	+(ims2)
524	Fried frozen vegetables	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	=
525	Mixed vegetables	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	=
526	Fried frozen mushrooms	+(1col)	-	+	-	+	-	/	/	/	-	ND	-	/	/	/	-	ND
527	Fried frozen vegetables	-	+(NC)	+	+(NC)	+	-	/	/	/	-	ND	-	/	/	/	-	ND
683	Frozen spinach	-	-	-	+	-	-	/	/	/	-	=	-	/	/	/	-	=
684	Frozen fried vegetables	-	+(2)	-	+	-	-	/	/	/	-	=	-	/	/	/	-	=
685	Frozen fried vegetables	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
686	Frozen mixed vegetables	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
687	Frozen mixed vegetables	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
688	Aubergine	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
689	Courgette	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
690	Carrots	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
691	Sprouts	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
692	Broccoli	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
693	Spinach (bagged pre-cut leafy vegetables)	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	=
694	Fresh vegetable mix (cabbage,carrots,salad.) (bagged pre-cut leafy vegetables)	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	=
695	Red cabbage (bagged pre-cut leafy vegetables)	-	-	-	-	-	+	+/-	+	+	+	PD	+	+	+	+	+	PD
696	Fresh mix vegetables (bagged pre-cut leafy vegetables)	-	-	-	+	-	+	+	+	+	+	PD	+	+	+	+	+	PD
697	White cabbage (bagged pre-cut leafy vegetables)	-	-	-	-	-	-	/	/	/	-	=	+	+	+	+	+	PD
698	Rocket (bagged pre-cut leafy vegetables)	-	+/-	-	+	-	+	+	+	+	+	PD	+	+	+	+	+	PD
699	Red cabbage (bagged pre-cut leafy vegetables)	-	-	-	-	-	+	+	+	+	+	PD	+	+	+	+	+	PD

+/-: doubtful colonies

NC : non characteristic colonies on TSA

Ims2: Qualicon confirmation protocol performed

VEGETABLE PRODUCTS																			
N°	Product	Reference method ISO 16654♦					BAX E.coli O157: H7-Real Time method – Short enrichment step (8 h)						BAX E.coli O157: H7-Real Time method – 24h enrichment step						
		Characteristic colonies				ISO 16654 result	PCR	Confirmatory tests			PCR		PCR- 24H	Confirmatory tests			PCR		
		IMS 6H		IMS 24H				CT SMAC Characteristic colonies	O157 latex	H7 Latex	Final result	Agreement		CT SMAC Characteristic colonies	O157 latex	H7 Latex	Final result	Agreement	
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157														
700	Frozen vegetables for ratatouille	-	-	-	-	-	-	/	/	/	-	=	+	+/- (2)	+	+	+	+ (ims2)	PD
701	Frozen vegetables mix	-	-	+	-	-	-	/	-	/	-	=	/	/	/	/	/	-	=
702	Frozen sliced links	-	-	-	-	-	-	/	/	/	-	=	+	+	+	+	+	+	PD
703	Cauliflower	+	+	/	/	+	-	/	/	/	-	ND	+	+	+	+	+	+	=
704	Frozen mushrooms	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	+	=
715	White cabbage (bagged pre-cut leafy vegetables)	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	/	-	=
716	Fresh vegetable mix (cabbage,carots,salad.) (bagged pre-cut leafy vegetables)	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	/	-	=
717	Spinach(bagged pre-cut leafy vegetables)	-	-	-	+	-	-	/	/	/	-	=	-	/	/	/	/	-	=
718	Red cabbage (bagged pre-cut leafy vegetables)	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	/	-	=
732	Green cabbage	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	+	=
733	Celery	+(1)	+(2)	+	+	-	+	+	+	+	+	PD	+	+	+	+	+	+	PD
734	Sprouts	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	+	=
735	Courgette	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	+	=
736	Artichoke	-	-	-	-	-	+	+	+	+	+	PD	+	+	+	+	+	+	PD
737	Broccoli (bagged pre-cut leafy vegetables)	-	-	-	-	-	+	+	+	+	+	PD	+	+	+	+	+	+	PD
738	Green beans (bagged pre-cut leafy vegetables)	-	-	-	-	-	-	/	/	/	-	=	+	+/-	+	+	+	+	PD
739	Vegetable mix (bagged pre-cut leafy vegetables)	-	-	-	-	-	+	+	+	+	+	PD	+	+	+	+	+	+	PD
740	Green asparagus	+(1)	-	+	+	+	+	+	+	+	+	=	+	+	+	+	+	+	=
741	Peas	-	-	+	+	+	+	+	+	+	+	=	+	+	+	+	+	+	=
742	Beans	+(3)	+(1)	+	+	+	+	+	+	+	+	=	+	+	+	+	+	+	=
743	Vegetable mix (bagged pre-cut soup vegetables)	+	+	/	/	+	+	+	+	+	+	=	+	+/-	+	+	+	+	=

+/-: doubtful colonies

NC : non characteristic colonies on TSA

Ims2: Qualicon confirmation protocol performed

VEGETABLE PRODUCTS																			
N°	Product	Reference method ISO 16654♦					BAX E.coli O157: H7-Real Time method – Short enrichment step (8 h)						BAX E.coli O157: H7-Real Time method – 24h enrichment step						
		Characteristic colonies				ISO 16654 result	PCR	Confirmatory tests			PCR		PCR- 24H	Confirmatory tests			PCR		
		IMS 6H		IMS 24H				CT SMAC Characteristic colonies	O157 latex	H7 Latex	Final result	Agreement		CT SMAC Characteristic colonies	O157 latex	H7 Latex	Final result	Agreement	
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157														
861	<b>Courgette</b>	+	+	/	/	+	+	+	+	+	+	+	=	+	+	+	+	=	
862	<b>Turnip</b>	+	+	/	/	+	+	+	+	+	+	+	=	+	+	+	+	=	
863	<b>Swedish turnip</b>	-	-	-	-	-	+	+	+	+	+	+	PD	+	+	+	+	PD	
864	<b>Artichoke</b>	+	+	/	/	+	+	+	+	+	-	/	/	/	/	/	/	-	ND
865	<b>Vegetables for ratatouille</b>	-	-	-	-	-	+	+	+	+	+	+	PD	+	+	+	+	+	PD
924	Vegetables julienne	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	/	-	=
925	Ratatouille	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	/	-	=

RAW BEEF MEAT																		
N°	Product	Reference method ISO 16654♦					BAX E.coli O157: H7-Real Time method – Short enrichment step (7 h)						BAX E.coli O157: H7-Real Time method – 24h enrichment step					
		Characteristic colonies				ISO 16654 result	PCR	Confirmatory tests			PCR		PCR-24H	Confirmatory tests			PCR	
		IMS 6H		IMS 24H				CT SMAC Characteristic colonies	O157 latex	H7 Latex	Final result	Agreement		CT SMAC Characteristic colonies	O157 latex	H7 Latex	Final result	Agreement
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157													
365	Frozen ground beef preparation	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	=
366	Frozen ground beef preparation (80%)	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	=
367	Frozen ground beef	+	+	/	/	+	-	/	/	/	-	ND	+	+	+	+	+	=
368	Frozen ground beef with onions	+	+	/	/	+	+	+	+	+	+	=	+	+/-	+	+	+	=
369	Frozen ground beef	+	+	/	/	+	-	/	/	/	-	ND	+	+	+	+	+	=
370	Frozen ground beef (meatballs)	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	=
371	Ground beef (meatballs)	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	=
372	Fresh ground beef	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	=
373	Ground beef (Bolognaise)	-	-	-	-	-	-	/	/	/	-	=	+	+	+	+	+	PD
374	Fresh ground beef	+	+	/	/	+	-	/	/	/	-	ND	+	+	+	+	+	=
375	Fresh ground beef	-	-	-	-	-	+	+	+	+	+	PD	+	+	+	+	+	PD
376	Fresh ground beef	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	=
377	Fresh ground beef	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	=
378	Ground beef (Bolognaise)	-	-	-	-	-	+	+	+	+	+	PD	+	+	+	+	+	PD
379	Fresh ground beef	+/-	+/-	+/-	+/-	+	+	+	+	+	+	=	+	+	+	+	+	=
380	Fresh ground beef (5%fat)	-	-	-	-	-	+	+	+	+	+	PD	+	+	+	+	+	PD
381	Fresh ground beef (15%fat)	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	=
382	Beef carpaccio	-	-	-	-	-	+	+	+	+	+	PD	+	+	+	+	+	PD
383	Frozen ground beef	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	=
384	Frozen beef preparation (51%)	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	=
385	Frozen ground beef	+	+	/	/	+	+	+	+	+	+	=	+	+/-	+	+	+	=
386	Frozen ground beef	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	=
387	Frozen ground beef (meatballs)	+(1col)	+(1col)	+	+	+	+	+	+	+	+	=	+	+	+	+	+	=
388	Frozen ground beef (51% (meatballs)	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	=

♦ Analysis performed according to the COFRAC accreditation

RAW BEEF MEAT																		
N°	Product	Reference method ISO 16654♦					BAX E.coli O157: H7-Real Time method – Short enrichment step (7 h)						BAX E.coli O157: H7-Real Time method – 24h enrichment step					
		Characteristic colonies				ISO 16654 result	PCR	Confirmatory tests			PCR		PCR-24H	Confirmatory tests			PCR	
		IMS 6H		IMS 24H				CT SMAC Characteristic colonies	O157 latex	H7 Latex	Final result	Agreement		CT SMAC Characteristic colonies	O157 latex	H7 Latex	Final result	Agreement
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157													
663	Ground beef preparation (Provençal)	-	-	+	-	-	/	/	/	-	=	-	/	/	/	-	=	
664	Marinated steak	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=	
665	Fresh ground beef	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=	
666	Beef carpaccio	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=	
667	Beef meatballs	-	-	+/-	-	-	/	/	/	-	=	-	/	/	/	-	=	
668	Ground beef	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=	
669	Beef meat	+	-	+/-	-	-	/	/	/	-	=	-	/	/	/	-	=	
670	Ground beef (Bolognaise)	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=	
671	Steak	+/-	-	+	-	-	/	/	/	-	=	-	/	/	/	-	=	
672	Rump steak	+	-	+ (3)	-	-	/	/	/	-	=	-	/	/	/	-	=	
673	Frozen ground beef preparation (80%)	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=	
674	Frozen ground beef (meatballs) (51%)	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=	
675	Frozen ground beef preparation (51%)	-	-	-	+ (1)	-	/	/	/	-	=	-	/	/	/	-	=	
676	Frozen ground beef	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=	
677	Ground beef with onions	+/-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=	
678	Frozen ground beef	-	-	+/-	-	-	/	/	/	-	=	-	/	/	/	-	=	
679	Frozen ground beef	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=	
680	Frozen ground beef (balls) (51%)	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=	
681	Frozen ground beef	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=	
682	Frozen ground beef (balls)	-	-	+	-	-	/	/	/	-	=	-	/	/	/	-	=	
705	Marinated piece of beef	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=	
706	Beef meat pieces	-	-	+	-	-	/	/	/	-	=	-	/	/	/	-	=	
707	Beef pieces (faux filet)	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=	
708	Beef piece (tournedos)	-	-	+	-	-	/	/	/	-	=	-	/	/	/	-	=	
709	Frozen ground beef for Bolognaise	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=	
710	Frozen ground beef	-	-	-	+/-	-	/	/	/	-	=	-	/	/	/	-	=	
711	Beef meat balls (51%)	-	-	-	+	-	/	/	/	-	=	-	/	/	/	-	=	
712	Frozen ground beef (51%)	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=	

RAW BEEF MEAT																		
N°	Product	Reference method ISO 16654♦					BAX E.coli O157: H7-Real Time method – Short enrichment step (7 h)						BAX E.coli O157: H7-Real Time method – 24h enrichment step					
		Characteristic colonies				ISO 16654 result	PCR	Confirmatory tests			PCR		PCR- 24H	Confirmatory tests			PCR	
		IMS 6H		IMS 24H				CT SMAC Characteristic olonies	O157 latex	H7 Latex	Final result	Agreement		CT SMAC Characteristic colonies	O157 latex	H7 Latex	Final result	Agreement
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157													
713	Frozen ground beef with onions	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
714	Frozen ground beef	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
719	Frozen ground beef	-	-	-	-	-	-	/	/	/	-	=	+	+	+	+	+	PD
720	Frozen ground beef	-	+/-	+	+	+	-	/	/	/	-	ND	-	/	/	/	-	ND
721	Frozen ground beef	-	-	-	-	-	-	/	/	/	-	=	-	/	/	/	-	=
722	Frozen ground beef	+	+	+	+	+	+	+	+	+	+	=	+	+	+	+	+	=
723	Frozen ground beef	+	+	+	+	+	+	+	+	+	+	=	+	+	+	+	+	=
724	Frozen ground beef	-	-	-	+/- (1)	-	-	/	/	/	-	=	-	-	/	/	-	=
725	Frozen ground beef	-	-	+/-	+/-	-	-	/	/	/	-	=	-	/	/	/	-	=
744	Chuck	+	+	/	/	+	-	/	/	/	-	ND	+	+/-	+	+	+	=
745	Rump steak	-	-	+	+	-	+	+	+	+	+	PD	+	+/-	+	+	+	PD
746	Tournedos	-	-	+	+	+	+	+	+	+	+	=	+	+	+	+	+	=
747	Sirloin	+ (1)	-	+	+	-	+	+	+	+	+	PD	+	+	+	+	+	PD
748	Shin	+	+	/	/	+	+	+	+	+	+	=	+	+	+	+	+	=
854	Marinated piece of beef	-	-	-	+	+	+	+	+	+	+	=	+	+	+	+	+	=
855	Marinated peace of beef with shallots	-	-	-	+/-	-	+	+	+	+	+	PD	+	+	+	+	+	PD
856	Carpaccio	-	-	-	-	-	+	+	+	+	+	PD	+	+	+	+	+	PD
857	Ground beef with onions	-	-	-	+/-	-	+	+	+	+	+	PD	+	+	+	+	+	PD
858	Ground beef	-	-	+	+/-	+	+	+	+	+	+	=	+	+	+	+	+	=
859	Ground beef with onions	-	-	-	-	-	+	+	+	+	+	PD	+	+	+	+	+	PD
860	Beef balls	-	-	-	-	-	+	+	+	+	+	PD	+	+	+	+	+	PD

## Annex 5 – Inclusivity and exclusivity : raw data

### ☐ Inclusivity

Target strains								
	Species	Serotype	Strain identification	Origin	Inoculation level cfu/225ml	PCR result BAX O157:H7 Real Time	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	+	+	+

Target strains								
	Species	Serotype	Strain identification	Origin	Inoculation level cfu/225ml	PCR result BAX O157:H7 Real Time	Confirmatory tests	
							CT-SMAC Characteristic colonies	O157 and H7 latex test
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	+	+	+
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**

Non target strains								
	Species	Serotype	Strain identification	Origin	Inoculation level cfu/225ml	PCR result BAX O157:H7 Real Time	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 <sup>5</sup>	-	-	/
2.	<i>Escherichia coli</i>	O3:H2	38765	Clinical origin (Chili)	5,0.10 <sup>5</sup>	-	-	/
3.	<i>Escherichia coli</i>	O78:H11	H10407	ATCC 35401	4,9.10 <sup>5</sup>	-	-	/
4.	<i>Escherichia coli</i>	O6:H6	EDL1493	/	3,6.10 <sup>5</sup>	-	-	/
5.	<i>Escherichia coli</i>	O6:H10	ECOR10	Clinical origin (Sweden)	7,6.10 <sup>5</sup>	-	-	/
6.	<i>Escherichia coli</i>	O111:H21	DEC6a	Clinical origin (USA)	2,5.10 <sup>5</sup>	-	-	/
7.	<i>Escherichia coli</i>	O86:H43	ECOR23	Animal origin (elephant USA)	3,7.10 <sup>5</sup>	-	-	/
8.	<i>Escherichia coli</i>	O26:H11	DEC9a	Clinical origin (USA)	3,9.10 <sup>5</sup>	-	-	/
9.	<i>Escherichia coli</i>	O111:H8	DEC8b	Clinical origin (USA)	4,9.10 <sup>5</sup>	-	-	/
10.	<i>Escherichia coli</i>	O128:H2	DEC11a	Clinical origin (USA)	8,0.10 <sup>5</sup>	-	-	/
11.	<i>Escherichia coli</i>	O111:H2	DEC12a	Clinical origin (UK)	5,7.10 <sup>5</sup>	-	-	/
12.	<i>Escherichia coli</i>	O128:H7	DEC13a	Clinical origin (USA)	3,9.10 <sup>5</sup>	-	-	/
13.	<i>Escherichia coli</i>	O78:H12	TX-1	ATCC 43896	4,1.10 <sup>5</sup>	-	-	/
14.	<i>Escherichia coli</i>	O104:H21	ECOR26	Clinical origin (USA)	7,0.10 <sup>5</sup>	-	-	/
15.	<i>Escherichia coli</i>	O157:H43	DEC7a	Pork (USA)	4,0.10 <sup>5</sup>	-	-	/
16.	<i>Escherichia coli</i>	O55:H7	DEC5d	Clinical origin (Sri Lanka)	4,2.10 <sup>5</sup>	-	-	/
17.	<i>Escherichia coli</i>	O44:H18	42	Clinical origin (Peru)	7,6.10 <sup>5</sup>	-	-	/
18.	<i>Escherichia coli</i>	O127:H6	E2348/69	Clinical origin (UK)	8,5.10 <sup>5</sup>	-	+	O157-
19.	<i>Escherichia coli</i>	O55:H6	DEC1a	Clinical origin (USA)	4,5.10 <sup>5</sup>	-	+	O157-
20.	<i>Escherichia coli</i>	O18:K1:H7	RS218	Clinical origin	4,7.10 <sup>5</sup>	-	-	/
21.	<i>Salmonella</i>	Landau	Ad499	/	2,9.10 <sup>5</sup>	-	-	/
22.	<i>Salmonella</i>	Sternhauze	Ad500	/	3,8.10 <sup>5</sup>	-	-	/
23.	<i>Salmonella</i>	Urbana	Ad501	/	3,7.10 <sup>5</sup>	-	-	/
24.	<i>Salmonella</i>	Wayne	Ad502	/	3,0.10 <sup>5</sup>	-	-	/
25.	<i>Hafnia alvei</i>		88	Bakery	4,2.10 <sup>5</sup>	-	-	/
26.	<i>Hafnia alvei</i>		167	Sausage	3,2.10 <sup>5</sup>	-	-	/
27.	<i>Citrobacter freundii</i>		25	Frozen raw spinach	3,9.10 <sup>5</sup>	-	-	/
28.	<i>Citrobacter freundii</i>		104	Ground beef	3,6.10 <sup>5</sup>	-	-	/
29.	<i>Escherichia vulneris</i>		127	Raw milk	5,5.10 <sup>5</sup>	-	-	/
30.	<i>Pantoea spp.</i>		134	Pork	8,5.10 <sup>4</sup>	-	-	/
31.	<i>Escherichia coli</i>	O157	Ad524	Environment (dairy product)	4,2.10 <sup>5</sup>	-	-	/
32.	<i>Escherichia coli</i>	O157	Ad525	Faeces	5,7.10 <sup>5</sup>	-	-	/
33.	<i>Escherichia coli</i>	O157	Ad526	Faeces	6,4.10 <sup>5</sup>	-	-	/
34.	<i>Escherichia coli</i>	O157	Ad527	Clinical origin	2,3.10 <sup>5</sup>	-	-	/
35.	<i>Escherichia coli</i>	O157:H-	O1.12.903	/	2,8.10 <sup>5</sup>	-	-	/
36.	<i>Escherichia coli</i>	O157:H-	O1.12.905	/	2,9.10 <sup>5</sup>	-	-	/

**Annex 6 – Collaborator labs**

<b>Laboratory</b>	<b>Address</b>	<b>Country</b>
Arla Foods amba	Korsvej mejeri, Højrupvej 116, 7000 Fredericia	Danemark/Denmark
Campden BRI	Microbiology Dept, Campden BRI, Chipping Campden, Gloucestershire, GL55 6LD	Royaume-Uni/UK
Delegacion de Sanidad y Consumo de Asturias c/	Julio Alvarez Mendo 3, Post code 33011, Asturias	Espagne/Spain
Ecole Nationale Vétérinaire de Lyon - Unité UMAP	Unité de Microbiologie alimentaire- 1, Avenue Bourgelet- 69280 Marcy l'Etoile	France
Eurofins Cervac Ouest	33 Rue Lavoisier, 35230 Noyal Châtillon sur Seiche	France
ILVO-Melle	Brusselsesteenweg 370, 9090 Melle	Belgique/Belgium
IRTA Edific B Granja Camps i Armet	Post code 17121, Monells, Girona, Spain	Espagne/Spain
Laboratoire Départemental Vétérinaire	Plate forme Alimentec - Rue Heri Deboissieux- 01000 Bourg en Bresse	France
Laboratoire Départemental Vétérinaire Touraine	LDV Touraine - ZA N°1 Papillon - Rue de l'aviation - 37120 Parçay-Meslay	France
Laboratoire Franck Duncombe	1, route Rosel- Saint Contest- 14053 Caen Cedex 04	France
Leatherhead Food Research	Randalls Road, Leatherhead, Surrey, KT22 7RY	Royaume-Uni/UK
RIVM	RIVM / Goederen ontvangststation, 1e Brandenburgerweg 78B, 3721 MK Bilthoven	Pays-Bas/The Netherlands
Section for food bacteriology and GMO, Norwegian Veterinary Institute	National Veterinary Institute, Ullevålsveien 68, Pb 750 Sentrum, N-0106 Oslo	Norvège/Norway
Seinäjoen Rintarivke- Ja YmpäristöLaboratorio	Vaasantie 1 C, 60100 Seinäjoki	Finlande/Finland