

3M

Boulevard de l'Oise

F-95029 CERGY PONTOISE CEDEX

NF VALIDATION

Validation of alternative analytical methods

Application in food microbiology

Summary report

**EN ISO 16140 validation study of the 3M™
Molecular Detection Assay *E. coli* O157 (including
H7) for the detection of *Escherichia coli* O157
(including H7) in raw beef meat, raw dairy
products, raw fruits & vegetables**

Qualitative method

This report includes 82 pages, with 11 appendixes.

Only copies including the totality of this document are authorised.

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

Version 0

July 19, 2016

ADRIA DEVELOPPEMENT

Creac'h Gwen - F. 29196 QUIMPER Cedex - Tél. (33) 02.98.10.18.18 - Fax (33) 02.98.10.18.08

E-mail : adria.developpement@adria.tm.fr - Site web : <http://www.adria.tm.fr>

ASSOCIATION LOI DE 1901 - N° SIRET 306 964 271 00036 - N° EXISTENCE 532900006329 - N°TVA FR4530696427100036

1	INTRODUCTION.....	4
1.1	Date of the initial validation study and renewal study.....	4
1.2	Reference method	4
1.3	Alternative method.....	4
2	INITIAL VALIDATION: RESULTS.....	6
2.1	Method comparison study.....	6
2.1.1	Relative accuracy, relative specificity and relative sensitivity	6
2.1.2	Relative detection level	16
2.1.3	Inclusivity / exclusivity	17
2.2	Practicability.....	18
2.3	Inter-laboratory study: organization and results	21
2.3.1	Study organization	21
2.3.2	Experimental parameters control	23
2.3.3	Results analysis	25
2.3.4	Results interpretation	27
2.3.5	Interpretation	29
2.4	Conclusion	31
	Appendix 1 – ISO 16654: Horizontal method for the detection of <i>Escherichia coli</i> O157	32
	Appendix 2 – Flow diagram of the alternative method	33
	Appendix 3 – Artificial contamination of samples	34
	Appendix 4 – Relative accuracy: raw data	38
	Appendix 5 – Calculation of relative accuracy (AC), relative sensitivity (SE) and relative specificity (SP) integrating LCL (lower confidence limit)	54
	Appendix 6 – Relative detection levels: raw data	55
	Appendix 7 – Inclusivity and exclusivity: raw data	61
	Appendix 8 – Results obtained by the expert laboratory	65
	Appendix 9 – Results obtained by each collaborator	66
	Appendix 10 – Specificity and sensitivity	81
	Appendix 11 – Paired results of the alternative and reference methods for each level	82

Before comment

Quality assurance documents related to this study can be consulted upon request from 3M.

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

✓ Company:	3M Boulevard de l'Oise F-95029 CERGY PONTOISE CEDEX
✓ Expert Laboratory:	ADRIA Développement ZA Creac'h Gwen F-29196 QUIMPER Cedex
✓ Studied method:	3M™ Molecular Detection Assay <i>E. coli</i> O157 (including H7)
✓ Validation standard:	EN ISO 16140 (October 2003) : Food microbiology – Protocol for the validation of alternative methods
✓ Reference method[♦]:	ISO 16654: Microbiology of food and animal feeding stuffs - Horizontal method for the detection of <i>Escherichia coli</i> O157
✓ Scope:	Raw beef meat, raw dairy products, raw fruits & vegetables
✓ Certification body:	AFNOR Certification

[♦] Analyses performed according to the COFRAC accreditation

1 INTRODUCTION

1.1 Date of the initial validation study and renewal study

The **3M™ Molecular Detection Assay *E. coli* O157 (including H7)** for the detection of *Escherichia coli* O157 (including H7) was validated in 2013 according to the EN ISO 16140 protocol (2003) and the AFNOR technical rules (Certificate number n° 3M 01/12 - 03/13). The method was renewed in June 2016.

1.2 Reference method ♦

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 **Appendix 1**).

1.3 Alternative method

□ **Principle**

The **3M™ Molecular Detection Assay** uses loop-mediated isothermal amplification to rapidly amplify nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification.

□ **Protocol** (see **Appendix 2**)

- Enrichment step in pre-warmed Buffered Peptone Water (BPW):
 - * 8 – 18 h at 41.5°C for raw beef meat (**the lower and the upper limits of the enrichment time were tested during the validation**)
 - * 18 – 24 h at 41.5°C for raw beef meats, raw fruits, vegetables and raw dairy products (the minimum incubation time was tested during the validation).
- Sample lysis
- DNA amplification

- **Confirmation:**

- * Option 1: Using the ISO 16654 standard starting from the buffered peptone water (BPW) enrichment.
 - * Option 2: Implementing a confirmation method consisting of the following: streak 50 µl of the BPW enrichment onto CT-SMAC agar plate. Incubate for 24 h ± 3 h at 37°C. Streak characteristic colonies onto nutrient agar and perform latex agglutination tested directly onto isolated colonies. If the 3M Molecular Detection Assay *E. coli* O157 (including H7) results are not confirmed, perform an immunomagnetic separation step and then streak 50 µl onto CT-SMA.
 - * Option 3: Using nucleic acid probes as described in the EN ISO 7218 standard, performed on isolate colonies (purified or not) from CT-SMAC (see option 1 or 2). The nucleic acid probes must be different from those used in the 3M Molecular Detection Assay *E. coli* O157 (including H7).
 - * Option 4: Using any other method certified NF VALIDATION, the principle of which must be different from 3M Molecular Detection Assay *E. coli* O157 (including H7). The complete protocol described for this second validated method must be used. All steps prior to the start of confirmation must be common to both methods.
- Enrichment and lysate storage for 72 h at 2 – 8°C was also evaluated on positive samples in order to offer sufficient practicability to the users. This was done on positive samples in the relative accuracy, specificity and selectivity study.

☐ **Reference of the kit insert since the last validation study**

The reference of the current kit insert is: 34-8711-2653-7. No modification has been done since the last validation study.

2 INITIAL VALIDATION: RESULTS

2.1 Method comparison study

2.1.1 Relative accuracy, relative specificity and relative sensitivity

The relative 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).

2.1.1.1 Number and nature of samples

185 samples were analysed. The distribution per tested category, type and pre-enrichment protocol is given in Table 1.

Table 1 - Distribution per tested category and type

Category	Type	Positive samples	Negative samples	Total
Raw beef meat	Raw meat	9	11	20
	Minced meat preparations (Carpaccio, ...)	11	11	22
	Ground beef	10	10	20
	Total	30	32	62
Dairy products	Raw milks	11	10	21
	Raw fermented or acidified milks	10	10	20
	Raw milk cheeses	11	10	21
	Total	32	30	62
Fruits and vegetables	Produces and leafy greens	16	15	31
	RTE foods (deli salads)	5	5	10
	Juices and fermented products	10	10	20
	Total	31	30	61
TOTAL		93	92	185

2.1.1.2 Artificial contamination of samples

Artificial contaminations were done by spiking. For sample spiking, strains were stressed using various injury protocols. The injury efficiency was evaluated by comparing enumeration results onto selective and non selective agars (respectively TSYEA and CT-SMAC agar). The artificial contaminations are presented in **Appendix 3**.

97 samples were artificially contaminated, using 22 different strains and 16 injury protocols; 91 gave a positive result. 78 samples were inoculated at a level lower or equal to 5 CFU/sample (Cf. figures 1 and 2). 13 samples were inoculated between 5.2 and 7.8 CFU. No more than 6 positive results were obtained with the same strain.

Figure 1 – Inoculation levels used for spiking

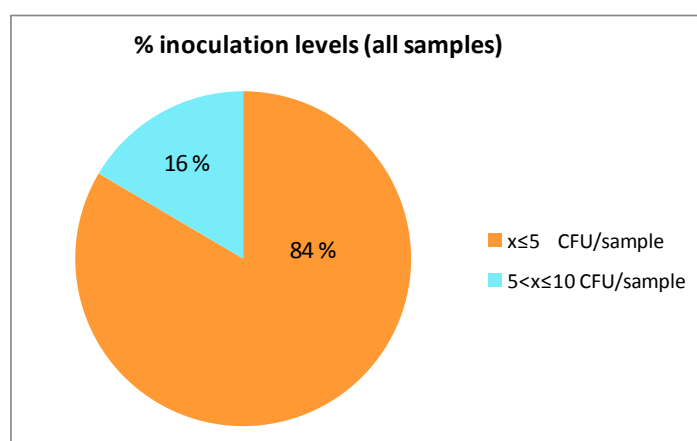
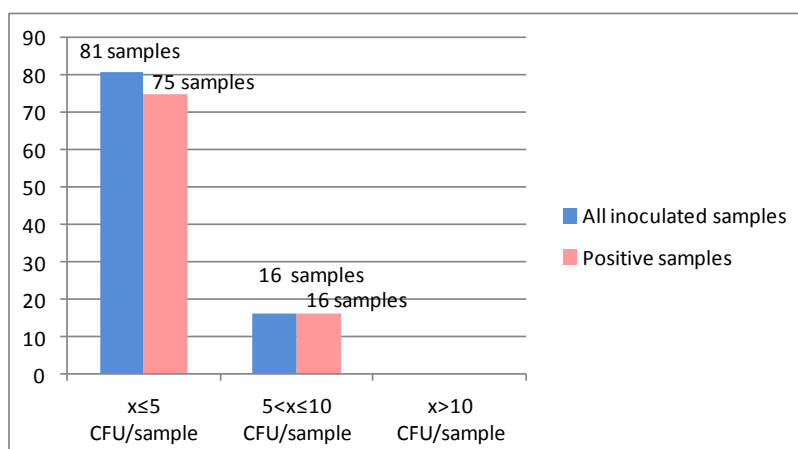


Figure 2 – Inoculated and positive samples according to the level of contamination



2.2 % of the samples were naturally contaminated.

2.1.1.3 Confirmation protocol

During the validation study, confirmations were realized by streaking 50 µl of enrichment broth on CT SMAC. An IMS step was performed prior to streaking if the simple tests did not confirm the results.

2.1.1.4 Test results

Raw data per category are provided in **Appendix 4**. A summary of the results is given in the following tables.

Table 2 – Paired results of the reference and alternative methods (all products)

BPW incubation time	Responses	Reference method positive (R+)	Reference method negative (R-)
8 h (beef meats)	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 50	Positive deviation (R-/A+) PD = 37
18 h (other products)	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 5	Negative agreement (A-/R-) NA = 93 (PPNA = 1)
18 h	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 51	Positive deviation (R-/A+) PD = 38
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 4	Negative agreement (A-/R-) NA = 92

PP: positive presumptive non confirmed samples

Results per category of sample

Table 3 – Raw beef meats

BPW incubation time	Responses	Reference method positive (R+)	Reference method negative (R-)
8 h	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 10	Positive deviation (R-/A+) PD = 16
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 3	Negative agreement (A-/R-) NA = 33 (PPNA = 1)
18 h	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 11	Positive deviation (R-/A+) PD = 17
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 2	Negative agreement (A-/R-) NA = 32 (PPNA = 1)

Table 4 – Dairy products

BPW incubation time	Responses	Reference method positive (R+)	Reference method negative (R-)
18 h	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 24	Positive deviation (R-/A+) PD = 7
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 1	Negative agreement (A-/R-) NA = 30

Table 5 – Fruits and vegetables products

BPW incubation time	Responses	Reference method positive (R+)	Reference method negative (R-)
18 h	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 16	Positive deviation (R-/A+) PD = 14
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 1	Negative agreement (A-/R-) NA = 30

2.1.1.5 Calculation of relative accuracy (AC), relative sensitivity (SE) and relative specificity (SP) (See Appendix 5)

The calculations of relative accuracy (AC), relative sensitivity (SE) and relative specificity (SP) are given in table 6.

Table 6 – Calculation of relative accuracy (AC), relative sensitivity (SE) and relative specificity (SP)

Category	PA	NA	ND	PD	N	Relative accuracy AC (%) [100x(PA+NA)]/N	N+ PA + ND	Relative sensitivity SE (%) [100xPA]/N+	N- NA + PD	Relative specificity SP (%) [100xNA]/N-
Raw beef meat 8 h incubation time	10	33	3	16	62	69.4	13	76.9	49	67.3
Raw beef meat 18 h incubation time	11	32	2	17	62	69.4	13	84.6	49	65.3
Dairy products	24	30	1	7	62	87.1	25	96.0	37	81.1
Fruits and vegetables	16	30	1	14	61	75.4	17	94.1	44	68.2
All products (8 h meat - 18h other products)	50	93	5	37	185	77.3	55	90.9	130	71.5
All products -18 h	51	92	4	38	185	77.3	55	92.7	130	70.8

PD = positive deviation (R-/A+)

ND = negative deviation (A-/R+)

The alternative method percentage values are:

	8 h incubation time (meat products)	8 h incubation time (meat products) and 18 h incubation time (other products)	18 h incubation time for all products
Relative accuracy : AC	69.4	77.3	77.3
Relative specificity : SP	67.3	71.5	70.8
Relative sensitivity : SE	76.9	90.9	92.7

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

	8 h incubation time (meat products)	8 h incubation time (meat products) and 18 h incubation time (other products)	18 h incubation time for all products
Alternative method	89.7	94.6	95.7
Reference method	44.8	59.8	59.1

2.1.1.6 Analysis of discordant results

Negative deviations

3 negative deviations were observed for 8 h incubation time and 4 for 18 h incubation time. The results are given in Table 7.

Table 7 – Negative deviations

Category	n° sample	Product	8 h incubation time			18 incubation time			Contamination (inoculation level)
			MDS test	Confirmatory test	Final result	MDS test	Confirmatory test	Final result	
Meat products	4180	Frozen ground beef	-/+ / +	+	-	+	+	+	Artificial <i>Escherichia coli</i> O157:H7 Ad 683 (5.0)
	4186	Beef meat piece	-	-	-	-	-	-	Artificial <i>Escherichia coli</i> O157:H7 Ad 486 (2.2)
	4213	Frozen ground beef with onions	-/+ / +	-	-	-/+ / +	+	-	Artificial <i>Escherichia coli</i> O157:H7 Ad 976 (1.2)
Dairy products	3271	Raw milk cheese	/	/	/	-	-	-	Artificial <i>Escherichia coli</i> O157:H7 Ad 573 (2.4)
Fruits and vegetables	3315	Spinach leaves	/	/	/	-	-	-	Artificial <i>Escherichia coli</i> O157:H7 Ad 557 (3.0)
Total negative deviations			3			4			

Among the 3 negative deviations observed on raw meat samples after 8 h incubation time, the presence of *Escherichia coli* O157:H7 was confirmed for only one sample (n° 4180); this sample gave a positive result after a 18 h incubation time.

For sample n° 4213, the confirmatory tests concluded to the presence of *Escherichia coli* O157:H7 only after 18 h incubation time.

Note that for these 2 samples (n° 4180 and 4213), 3 MDS replicates from the same lysis tube were done, and only the first result was negative.

This is an unpaired data study: when the MDS and the confirmatory tests are all negative; the observed deviations were probably due to the sampling heterogeneity between the reference and the alternative methods.



Positive deviations

38 positive deviations were observed; they are listed in Table 8.

Table 8 – Positive deviations

Category	Sample n°	Product	8 h incubation time		18 h incubation time		Contamination (inoculation level)
			MDS result	Confirmatory test	MDS result	Confirmatory test	
Meat products	4197	Beef meat piece	+	+	+	+	<i>E. coli</i> O157:H7 Ad 684 (1.2)
	4199	Beef meat piece	+	+	+	+	<i>E. coli</i> O157:H7 Ad 684 (1.2)
	4203	Beef meat piece	-	-	+	+	<i>E. coli</i> O157:H7 Ad 976 (3.2)
	4205	Beef meat piece	+	+	+	+	<i>E. coli</i> O157:H7 Ad 976 (3.2)
	4209	Carpaccio with basil	+	+	+	+	<i>E. coli</i> O157:H7 Ad 976 (3.2)
	4211	Bolognaise ground beef	+	+	+	+	<i>E. coli</i> O157:H7 Ad 976 (3.2)
	4215	Ground beef	+	+	+	+	<i>E. coli</i> O157:H7 Ad 490 (2.8)
	4219	Ground beef	+	+	+	+	<i>E. coli</i> O157:H7 Ad 922 (1.0)
	4221	Ground beef	+	+	+	+	<i>E. coli</i> O157:H7 Ad 922 (1.0)
	4804	Ground beef with ketchup	+	+	+	+	<i>E. coli</i> O157:H7 Ad 486 (1.0)
	4805	Ground beef with cheese	+	+	+	+	<i>E. coli</i> O157:H7 Ad 486 (1.0)
	4806	Beef meat piece with shallots	+	+	+	+	<i>E. coli</i> O157:H7 Ad 486 (1.0)
	4809	Ground beef with ketchup	+	+	+	+	<i>E. coli</i> O157:H7 Ad 486 (1.0)
	4810	Beef meat piece	+	+	+	+	<i>E. coli</i> O157:H7 Ad 922 (1.0)
	4812	Beef meat piece	+	+	+	+	<i>E. coli</i> O157 Ad 490 (2.2)
	4813	Ground beef	+	+	+	+	<i>E. coli</i> O157 Ad 490 (2.2)
	4814	Ground beef	+	+	+	+	<i>E. coli</i> O157 Ad 525 (1.0)
Total PD			16		17		

Category	Sample n°	Product	8 h incubation time		18 h incubation time		Contamination (inoculation level)
			MDS result	Confirmatory test	MDS result	Confirmatory test	
Dairy products	3239	Fermented milk	/	/	+	+	<i>E. coli</i> O157:H7 Ad 572 (2.8)
	3240	Fermented milk	/	/	+	+	<i>E. coli</i> O157:H7 Ad 576 (4.6)
	3243	Fermented milk	/	/	+	+	<i>E. coli</i> O157:H7 Ad 572 (2.8)
	3247	Fermented milk	/	/	+	+	<i>E. coli</i> O157:H7 Ad 572 (2.8)
	3251	Fermented milk	/	/	+	+	<i>E. coli</i> O157:H7 Ad 572 (2.8)
	3400	Raw milk	/	/	+	+	Natural
	3631	Fermented milk	/	/	+	+	<i>E. coli</i> O157:H7 Ad 688 (3.4)
	Total PD		/		7		
Fruits and vegetables	3307	Salad	/	/	+	+	<i>E. coli</i> O157:H7 Ad 575 (3.6)
	3323	Cider	/	/	+	+	<i>E. coli</i> O157:H7 Ad 556 (2.8)
	3325	Cider	/	/	+	+	<i>E. coli</i> O157:H7 Ad 557 (4.4)
	3326	Apple juice	/	/	+	+	<i>E. coli</i> O157:H7 Ad 556 (2.8)
	3645	Grated carrots	/	/	+	+	<i>E. coli</i> O157:H7 Ad 571 (2.8)
	3646	Vegetables mix	/	/	+	+	<i>E. coli</i> O157:H7 Ad 571 (2.8)
	3656	Banana juice	/	/	+	+	<i>E. coli</i> O157:H7 Ad 580 (4.0)
	3657	Cider	/	/	+	+	<i>E. coli</i> O157:H7 Ad 580 (4.0)
	3658	Cider	/	/	+	+	<i>E. coli</i> O157:H7 Ad 580 (4.0)
	4315	Deli salad	/	/	+	+	<i>E. coli</i> O157 Ad 525 (2.4)
	4316	Deli salad	/	/	+	+	<i>E. coli</i> O157 Ad 525 (2.4)
	4317	Deli salad	/	/	+	+	<i>E. coli</i> O157 Ad 525 (2.4)
	4320	Soy beans	/	/	+	+	<i>E. coli</i> O157 Ad 525 (2.4)
	4324	Link Alfalfa	/	/	+	+	<i>E. coli</i> O157 Ad 525 (2.4)
	Total PD		/		14		

For samples 4315, 4316, 4317 and 4324, the positive deviations were due to the presence of *Escherichia coli* O157 sorbitol positive strains in the enrichment broth. These strains gave atypical colonies on CT SMAC and Chromagar O157 according to the EN ISO 16654 technical rules. These samples gave MDS positive results and the observed atypical colonies (pink to red colonies) were confirmed as O157 strains.

The analysis of discordant results is:

	Alternative method protocol		
	8 h incubation time (meat products)	8 h (meat products) and 18 h (other products)	18 h (all products)
Y = ND + PD	Y = 3 + 16 = 19	Y = 5 + 37 = 42	Y = 4 + 38 = 42
m	3	/	/
M	4	/	/
$\chi^2 = d^2/y$	/	$\chi^2 = 24.38$	$\chi^2 = 27.52$
Conclusion	m < M The two methods are different at α 0.05.	$\chi^2 > 3.841$ The two methods are different at α 0.05.	$\chi^2 > 3.841$ The two methods are different at α 0.05.

The statistic tests conclude to difference between the two methods. The alternative method shows better performances than the reference method.

2.1.1.7 Confirmations

The confirmations were performed by streaking 50 µl of the enrichment broth onto CT SMAC plate agar or by proceeding to an IMS step prior to streaking if needed.

For the 8 h incubation time, 2 of the 26 positive results gave negative confirmatory tests by direct streaking. An IMS step was necessary to confirm the presence of *Escherichia coli* in these samples.

For 3 samples (n° 4812, 4813 and 4814), complementary tests were used: a subculture in BHI at 41.5°C after an IMS step was performed, before proceeding to a second IMS step and streaking onto plates.

All the other MDS positive results were confirmed by direct streaking onto CT SMAC plates.

The confirmatory tests allow the confirmation of all the positive deviations observed by the MDS tests.

2.1.1.8 Enrichment broth and lysate storage at 2 - 8°C for 72 h



Raw beef meats

The enrichment broth and lysates for the positive samples of the alternative method were stored at 2 - 8°C for 72 h and analyzed a second time according to 3M request while the study was already started.

Only one modification was observed after lysate storage for sample n° 4180 (8 h incubation time) which was a negative deviation sample before storage and became a positive agreement sample after lysate storage. This enhances no change in the discordant analysis:

$$Y = ND + PD = 2 + 16 = 18$$

$$m = 2$$

$$M = 4$$

$$m < M$$

The two methods are different at α 0.05.

The performances of the MDS method are not modified after storage at 2 – 8°C.



Raw dairy products, raw fruits and vegetables

For information, only 12 raw dairy samples as well as 11 raw vegetables and fruits were tested. No change occurred.

Enrichment broths and lysis can be stored up to 72 h at 2 – 8°C before proceeding to amplification.

2.1.2 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.

2.1.2.1 Matrices

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

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

The following matrices were tested:

- ground beef, inoculated with *Escherichia coli* O157:H7 Ad486, isolated from ground beef,
- Raw milk, inoculated with *Escherichia coli* O157:H7 Ad686 isolated from dairy environment,
- Raw spinach, inoculated with *Escherichia coli* O157:H7 Ad556, isolated from environmental sample.

2.1.2.2 Contamination protocol

Contaminations and enumerations were realized according to the AFNOR technical rules (weak level inoculation protocol). The contamination's levels are presented below:

- 0 CFU/ g or ml,
- Level necessary to obtain 0 to 50 % positives,
- Level necessary to obtain 50 to 75 % positives,
- Level necessary to obtain 75 to 100 % positives.

The matrices were analyzed by the ISO 16654 standard prior to inoculation in order to verify the absence of *E. coli* O157:H7. The total aerobic flora was enumerated.

2.1.2.3 Results

Raw data are given in **Appendix 6**. Detection levels are presented table 9.

Table 9 – Relative detection level results

Incubation time	Strain / matrix pairs	Relative detection level (CFU / 25 g) according to Spearman-Kärber test ¹	
		Reference method	Alternative method
8 h	Ground beef / <i>Escherichia coli</i> O157:H7 Ad486	0.4 [0.2; 0.7]	0.5 [0.3; 0.8]
18 h	Ground beef / <i>Escherichia coli</i> O157:H7 Ad486	0.4 [0.2; 0.7]	0.4 [0.2; 0.7]
	Raw milk / <i>Escherichia coli</i> O157:H7 Ad686	0.6 [0.3; 1.2]	0.5 [0.2; 0.8]
	Raw spinach / <i>Escherichia coli</i> O157:H7 Ad556	0.7 [0.4; 1.3]	0.6 [0.3; 1.0]

2.1.2.4 Conclusion

The relative detection level varies from 0.2 to 1.3 CFU/25 g for the reference method and from 0.2 to 1.0 for the alternative method. The alternative and the standard methods show similar detection levels.

2.1.3 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.

2.1.3.1 Test protocols

- **Inclusivity:** 50 *E. coli* O157:H7 and 7 *E. coli* O157 strains cultures were performed in BHI medium at 37°C. Dilutions were done in order to inoculate 10 to 100 cells/225 ml of prewarmed BPW. The alternative protocol was then performed after an enrichment time of 8 hours at 41.5°C.
- **Exclusivity:** 31 negative strains cultures were performed in BHI at 37°C. Dilutions were realized in order to inoculate 10⁵ cells/ml of BPW. The alternative protocol was then performed after an enrichment time of 24 hours at 37°C.

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

2.1.3.2 Results

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

- **Inclusivity:** All the strains gave a positive MDS result. Note that all the tested *E. coli* O157 strains were sorbitol positive strains.
- **Exclusivity:** No cross reaction was observed with the 31 non target strains.

2.2 Practicability

The 3M™ Molecular *E. coli* O157 (including H7) method practicability was evaluated according to the AFNOR criteria relative to method comparison study.

<i>Packaging and reagents</i>	The 3M Molecular Detection Assay <i>Escherichia coli</i> O157 (including H7) test kit contains 96 tests and includes the following components: Lysis Solution (LS) tubes <i>Escherichia coli</i> O157 (including H7) Reagent tubes Extra caps Negative Control (NC) Reagent Control (RC) Quick Start Guide
<i>Storage conditions and shelf-life</i>	The storage temperature is: 2 – 8°C. The shelf-life is given on the package. All the reagents must be stored at the temperature mentioned on the package.
<i>Specific equipment</i>	Provided by 3M: 3M Molecular Detection Instrument 3M Molecular Detection Speed Loader Tray 3M Molecular Detection Software 3M Molecular Detection Chill Block insert 3M Molecular Detection Chill Block Tray 3M Molecular Detection Heat Block insert 3M Molecular Detection Cap/Decap Tool – Lysis 3M Molecular Detection Cap/Decap Tool – Reagent Not provided by 3M: Dry block heater or water bath
<i>Reagents</i>	All the reagents are ready-to-use.
<i>Training</i>	Less than half a day is required for technicians with microbiology knowledge.

Workflow (in minutes) for 24 samples	Steps	Reference Method	Alternative method
	Negative samples		
	Sampling	60	60
	Stomach	36	36
	Lysate	/	20
	MDS test	/	20
	IMS 6 h	72	/
	IMS 24 (if necessary)	72	/
	Selective plates reading	30	/
	Total for negative samples analyses	270	136
	Total/negative sample	11.3	5.7
	Presumptive samples or positive samples		
	Streaking onto CT SMAC	/	20
	Streaking onto nutrient agar	20	8
	Confirmatory tests	64	24
	Total for positive samples	354	196
	Total/positive sample	14.8	8.2

Time to result	Steps	Reference Method	Alternative method	
			Protocol 8 h	Protocol 18 h
	Negative samples			
	Sampling / pre-enrichment	Day 0	/	Day 0
	IMS 6 h	Day 0	/	/
	IMS 24 h	Day 1	/	/
	MDS test	/	Day 0	Day 1
	Selective plate reading	Day 2	/	/
	Final negative result	Day 2	Day 0	Day 1
Presumptive positive or positive results				
Streaking onto CT SMAC	/	Day 0	Day 1	
Streaking onto nutrient agar	Day 1 – Day 2	Day 1	Day 1	
Indole test	Day 2 – Day 3	/	Day 2	
Latex test	Day 3 – Day 4	Day 1	Day 2	
Technician background	Technician qualified in molecular microbiology or in food microbiology			
Common step with the reference method	Pre-enrichment step and confirmatory tests when Protocol 1 is applied. No common step if Protocol 2 is applied.			
Traceability of the results	/			
Maintenance	Clean and decontaminate the instrument immediately if any spills occur. Clean the external surfaces of the instrument regularly once per month. Decontaminate the interior of the instrument regularly once per month. Details are in the 3M Molecular Detection System User Manual.			

Negative results are available within the day of initializing the analysis with the 3M™ Molecular *Escherichia coli* O157 (including H7) Test Kit. Depending on the confirmation protocol, the positive results are available between 1 and 2 days.

The workflow of the 3M™ Molecular *Escherichia coli* O157 (including H7) method allows shortening the handling time in comparison to the reference method.

2.3 Inter-laboratory study: organization and results

2.3.1 Study organization

Collaborators number

Samples were sent to 17 laboratories.

Matrix and strain used

The study was run with ground beef samples contaminated with *Escherichia coli* O157:H7.

Samples

The samples were inoculated and sent on Monday 21th January 2013, as described below:

- 24 codified samples (25 g) (red labelled) for *Escherichia coli* O157 research by the 3MTM Molecular *Escherichia coli* O157 (including H7) Test Kit,
- 24 codified samples (25 g) (blue labelled) for analyses by the ISO 16654 (2001) reference method,
- 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 shall be incubated simultaneously with the samples during analysis (storage and alternative enrichment incubation).**

The analyses were started on Wednesday 23th January 2013.

 Inoculation

The targeted inoculation levels were:

- 0 CFU/25 ml,
- 1 – 10 CFU/25 ml,
- 5 – 50 CFU/25 ml.

8 samples were prepared per inoculation level, per method and per laboratory. Each laboratory received 24 samples to analyse by the reference method and 24 samples to analyse by the alternative method.

 Labelling and shipping

Blinded 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.

Samples were shipped in 24 h to 72 h to the involved laboratories. The temperature conditions had to stay lower or equal to 8.4°C during transport, and between 0°C – 8.4°C in the labs.

 Analyses

Collaborators and ADRIA Développement carried out the analyses with the alternative and reference methods at Day 2.

 Expedition conditions

The collaborative study instructions were sent on 21th December 2012.

2.3.2 Experimental parameters control

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

Before inoculation

In order to detect *Escherichia coli* O157, the ISO 16654 method was performed on five ground beef test portions (25 g) before the inoculation. All the results were negative.

Sample stability

Sample stability was checked by inoculating the matrix at 25 CFU/25 g and 3 CFU/g. Enumerations were performed for the high contamination level and detection analyses were performed for the low contamination level. *Triplicata* were analysed, and the results were the following:

Table 10

Day	Reference method (research)			CFU/g (XLD)			Aerobic mesophilic flora (CFU/g)
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	
Day 0	+	+	+	102	100	126	2.0 10 ³
Day 1	-	+	+	92	106	186	2.4 10 ³
Day 2	-	-	+	100	112	126	1.5 10 ³

No evolution was observed during storage at 4°C.

Contamination levels

The contamination levels and the confidence intervals were:

Table 11

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	4 – 6 – 9 – 12 – 13 – 17 – 22 – 23	/	/	/	/
Low level	1 – 3 – 8 – 10 – 16 – 20 – 21 – 24	3	3	2.4	3.4
High level	2 – 5 – 7 – 11 – 14 – 15 – 18 – 19	25	20.4	17.7	23.5

2.3.2.2 Logistic conditions

Temperature conditions are given below:

Table 12 - Sample temperatures at receipt

Laboratories	Temperature measured by the sensor (°C)	Temperature measured at receipt (°C)	Receipt date and time	
A	<i>Register problem encountered with the probe</i>	5.0	22 January 2013	10h00
B	0.5	3.0	22 January 2013	14h00
C	0.	2.1	22 January 2013	11h00
D	1.0	2.3	22 January 2013	09h13
E	1.0	3.0	23 January 2013	10h30
F	0.0	2.1	22 January 2013	07h30
G	0.5	3.9	22 January 2013	09h40
H	1.0	1.5	22 January 2013	11h00
I	0.0	4.0	22 January 2013	11h00
J	0.5	2.7	22 January 2013	09h30
K	1.0	3.8	22 January 2013	11h00
L	<i>Did not participate to the ring trial</i>			
M	<i>Received the samples but did not participate to the ring trial</i>			
N	0.5	1.0	22 January 2013	11h20
O	1.5	3.8	22 January 2013	12h30
P	1.0	1.6	22 January 2013	12h00
Q	2.0	7.7	22 January 2013	09h15

2.3.2.3 Conclusion

No problem was encountered during the transport or at receipt for 14 labs.

One Lab (E) received its package at Day 2, but in cold conditions (register during shipment, storage and analysis).

Lab L decided to not take part to the ring trial.

Lab M received the samples, but did not proceed to the analysis.

Finally, 15 Labs participated to the ring trial.

2.3.3 Results analysis

2.3.3.1 Alternative method incubation time

Two labs (B and N) forgot to get back the probe from the incubator at the end of the incubation step of this alternative stomacher bag, but they certified that the bags were incubated from 7h00 to 15h00 (Lab N) and from 8h20 to 16h25 (Lab B).

2.3.3.2 Aerobic mesophilic flora enumeration

Depending on the Lab results, the enumeration levels varied from $1.9 \cdot 10^2$ to $2.5 \cdot 10^3$ CFU/g.

2.3.3.3 Expert lab results

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

Table 13 – Results obtained by the expert Lab.

Level	Reference method	Alternative method
L0	0/8	0/8
L1	4/8	8/8
L2	8/8	8/8

2.3.3.4 Collaborator lab results

15 Labs participated to the study and analysed the samples by the two methods. All the raw data are given in **Appendix 9**.

Table 14 – Results obtained by the collaborator Labs.

Laboratory	Reference method		
	L0	L1	L2
A	0/8	6/8	8/8
B	0/8	8/8	8/8
C	0/8	8/8	8/8
D	0/8	8/8	8/8
E	0/8	8/8	8/8
F	2/8	7/8	8/8
G	1/8	7/8	8/8
H	0/8	7/8	8/8
I	1/8	7/8	8/8
J	1/8	8/8	8/8
K	2/8	8/8	8/8
N	0/8	7/8	8/8
O	1/8	8/8	8/8
P	1/8	5/8	8/8
Q	0/8	7/8	8/8

Laboratory	Alternative method		
	L0	L1	L2
A	0/8	7/8	8/8
B	0/8	7/8	8/8
C	0/8	7/8	8/8
D	0/8	8/8	8/8
E	0/8	7/8	8/8
F	0/8	2/8	8/8
G	0/8	8/8	8/8
H	0/8	8/8	8/8
I	0/8	8/8	8/8
J	0/8	6/8	8/8
K	0/8	4/8	6/8
N	0/8	8/8	8/8
O	0/8	4/8	8/8
P	0/8	8/8	8/8
Q	0/8	7/8	8/8

Unexpected data were observed for the Labs F, K and O. Since it was not possible to run additional testing to explain the observed data and/or due to the observed results of the additional testing, it was decided to exclude the data of these three Labs.

Finally, the data interpretation was done with 12 Labs.

2.3.4 Results interpretation

2.3.4.1 Specificity and sensitivity for each method

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

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

with :N- = total number of all L0 assays

FP = number of false positive results

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

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

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

TP = number of true positive results

Results (See **Appendix 10**) are reported in Table 15.

Table 15 – Interpretation

Level	Reference method		Alternative method	
	SP/SE %	LCL%	SP/SE %	LCL%
Lo(SP)	95.8	91.8	100.0	98.0
L1(SE)	89.6	83.3	92.7	87.4
L2(SE)	100.0	98.0	100.0	98.0
L1+L2(SE)	94.8	91.6	96.4	93.6

LCL: confidence interval

2.3.4.2 Relative accuracy (AC)

Results for all levels (See **Appendix 11**) are given below:

Table 16 - Paired results of the alternative and reference methods

Alternative method	Reference method		Total
	+	-	
+	PA = 175	PD = 10	185
-	ND = 11	NA = 92 (PPNA = 2)	103
Total	N+ = 186	N- = 102	N = 288

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

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

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

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

Table 17 – Interpretation

Level	AC %	LCL %
L0	95.8	91.8
L1	82.3	74.5
L2	100.0	98.0
L1 + L2	91.1	87.0
Total	92.7	89.6

LCL: Lower confidence limit at 95 %

2.3.4.3 Discordant results

The analysis of discordant result is:

$$Y = ND + PD = 11 + 10 = 21$$

$$m = 10$$

$$M = 5$$

$$m > M$$

The two methods are not different at $\alpha < 0.05$

2.3.5 Interpretation

2.3.5.1 Comparison of the relative accuracy, specificity and sensitivity values

The values obtained for the two parts of the validation study (comparative and inter-laboratory studies) are reported in Table 18.

Table 18 - Alternative method values calculated during the comparative and inter-laboratory studies

	Inter-laboratory study	Methods comparative study
Relative accuracy (AC)	92.7	77.3
Sensitivity (SE)	96.4	90.9
Specificity (SP)	100.0	71.5

2.3.5.2 Accordance (DA)

The accordance is the percentage chance of finding the same result (i.e. both negative or both positive) from two identical test portions analysed in the same laboratory, under repeatability conditions (i.e. one operator using the same apparatus and same reagents within the shortest feasible time interval). To derive the accordance from the results of an inter-laboratory study, the probability that two samples give the same result is calculated for each participating laboratory in turn, and this probability is then averaged over all laboratories.

Accordance values for both methods are:

Table 19 – Interpretation

Level	Reference method (DA)	Alternative method (DA)
L0	92.7	100.0
L1	83.9	87.8
L2	100.0	100.0

2.3.5.3 Concordance

The concordance is the percentage chance of finding the same result for two identical samples analysed in two different laboratories. To calculate the concordance from the results of an interlaboratory study, each replicate is taken in turn in each participating laboratory, and is paired with identical results of all the other laboratories. The concordance is the percentage of all pairings giving the same results on all the possible pairings of data.

Both methods concordance values are:

Table 20 – Interpretation

Level	Reference method	Alternative method
L0	92.0	100.0
L1	81.1	86.4
L2	100.0	100.0

2.3.5.4 Odds Ratio (COR)

The odds ratio value is determined according to:

$$COR = \frac{\text{Accordance} \times (100 - \text{condorcance})}{\text{Concordance} \times (100 - \text{accordance})}$$

Both method odds ratio values are:

Table 21 – Interpretation

Level	Reference method (COR)	Alternative method (COR)
L0	1.01	1.00
L1	1.21	1.13
L2	1.00	1.00

2.4 Conclusion

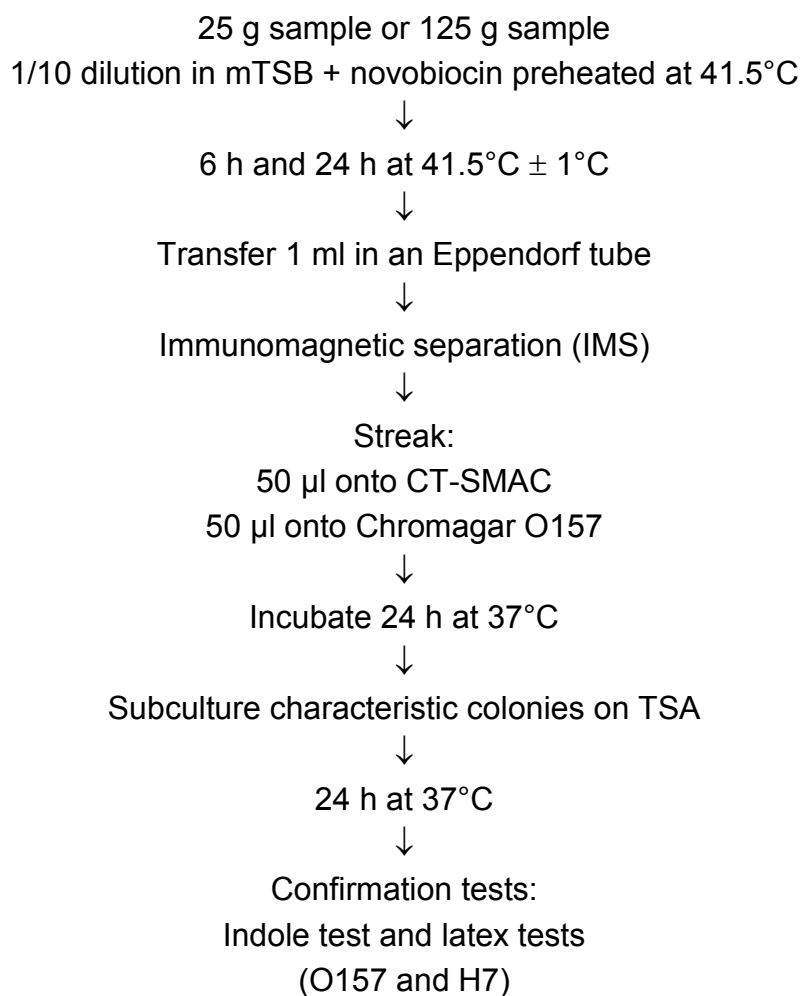
The **methods comparative study conclusions** are:

- ☒ The 3M™ Molecular *Escherichia coli* O157 (including H7) method shows very satisfying results for the relative accuracy, relative specificity and relative sensitivity.
- ☒ The relative detection limits of the alternative method and the ISO standard are similar.
- ☒ The 3M™ Molecular *E. coli* O157 (including H7) Test Kit shows clearly satisfying inclusivity and exclusivity results.

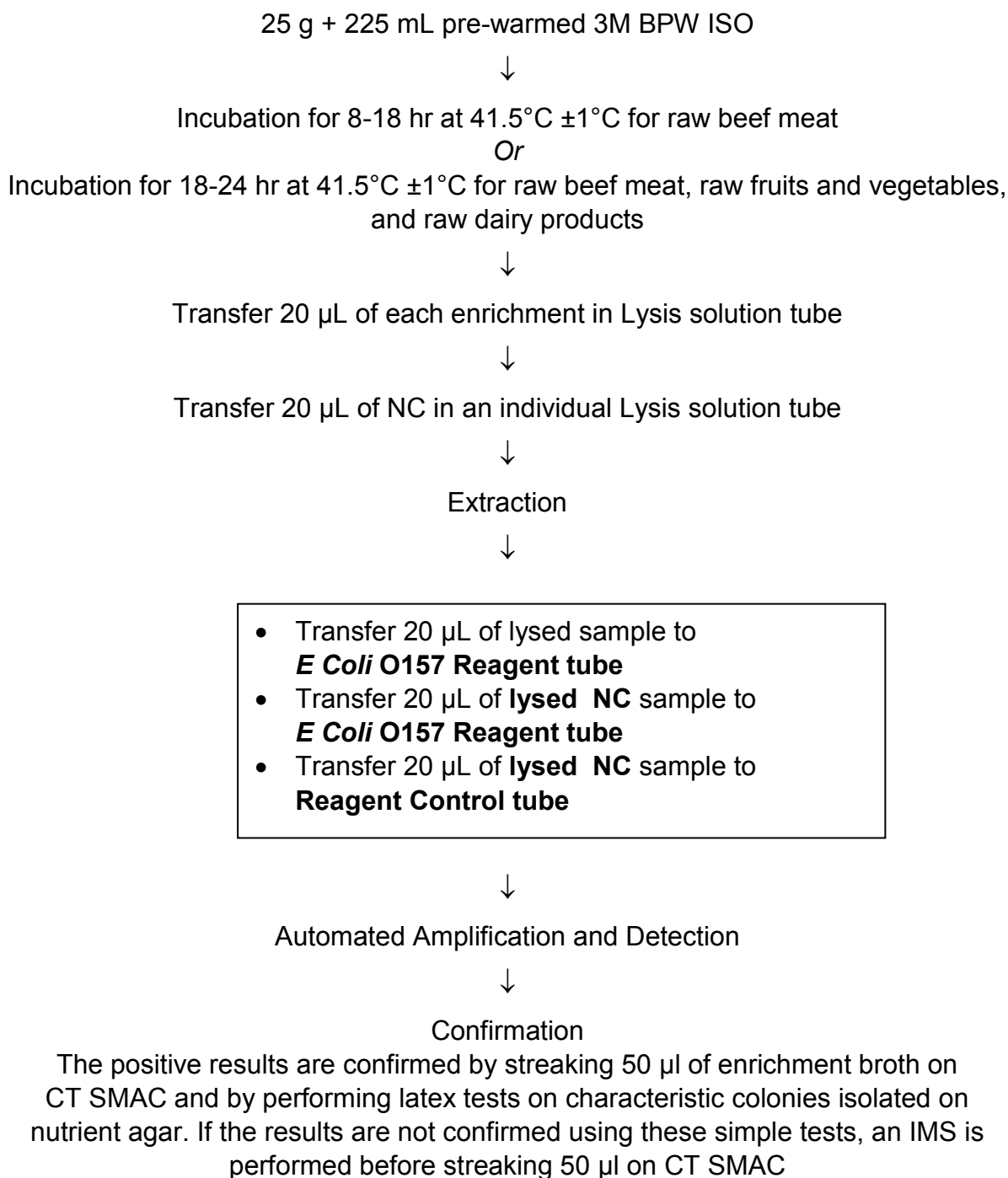
The **inter-laboratory study conclusions** are:

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

**Appendix 1 – ISO 16654: Horizontal method for the detection
of *Escherichia coli* O157**



Appendix 2 – Flow diagram of the alternative method



Appendix 3 – Artificial contamination of samples

N° Sample	Product	Artificial contaminations (spiking protocol)					Global result
		Strain	Origin	Injury protocol	Injury measurement	Inoculation level/25g	
4215	Ground beef	E. coli O157 Ad 490	Ground beef	4°C - 6 days	3,60	4-3-1-2-4 (2,8)	+
4217	Ground beef	E. coli O157 Ad 490	Ground beef	4°C- 6 days	3,60	4-3-1-2-4 (2,8)	-
4811	Beef meat piece	E. coli O157 Ad 490	Ground beef	4°C - 25 days	1,78	3-3-1-4-0 (2,2)	-
4812	Beef meat piece	E. coli O157 Ad 490	Ground beef	4°C - 25 days	1,78	3-3-1-4-0 (2,2)	+
4813	Ground beef	E. coli O157 Ad 490	Ground beef	4°C - 25 days	1,78	3-3-1-4-0 (2,2)	+
4315	Deli salad	E. coli O157 Ad 525	Bovine faeces	4°C - 7 days	0,56	1-4-4-2-1 (2,4)	+
4316	Deli salad	E. coli O157 Ad 525	Bovine faeces	4°C - 7 days	0,56	1-4-4-2-1 (2,4)	+
4317	Deli salad	E. coli O157 Ad 525	Bovine faeces	4°C - 7 days	0,56	1-4-4-2-1 (2,4)	+
4320	Soybeans	E. coli O157 Ad 525	Bovine faeces	4°C - 7 days	0,56	1-4-4-2-1 (2,4)	+
4324	Link alfalfa	E. coli O157 Ad 525	Bovine faeces	4°C - 7 days	0,56	1-4-4-2-1 (2,4)	+
4814	Ground beef	E. coli O157 Ad 525	Bovine faeces	4°C - 25 days	1,40	0-1-2-0-2 (1,0)	+
4185	Beef meat piece	E. coli O157:H7 Ad 486	Ground beef	4°C - 62 days	1,23	2-3-0-2-4 (2,2)	+
4186	Beef meat piece	E. coli O157:H7 Ad 486	Ground beef	4°C - 62 days	1,23	2-3-0-2-4 (2,2)	+
4804	Ground beef with ketchup	E. coli O157:H7 Ad 486	Ground beef	4°C - 82 days	1,45	2-1-0-1-1 (1,0)	+
4805	Carpaccio with cheese	E. coli O157:H7 Ad 486	Ground beef	4°C - 82 days	1,45	2-1-0-1-1 (1,0)	+
4806	Beef meat piece with shallot	E. coli O157:H7 Ad 486	Ground beef	4°C - 82 days	1,45	2-1-0-1-1 (1,0)	+
4807	Beef meat piece with pepper	E. coli O157:H7 Ad 486	Ground beef	4°C - 82 days	1,45	2-1-0-1-1 (1,0)	-
3323	Cider	E. coli O157:H7 Ad 556	Environmental sample	pH 4 - 9 days	0,75	3-3-2-3-3(2,8)	+
3324	Cider	E. coli O157:H7 Ad 556	Environmental sample	pH 4 - 9 days	0,75	3-3-2-3-3(2,8)	+
3326	Apple juice	E. coli O157:H7 Ad 556	Environmental sample	pH 4 - 9 days	0,75	3-3-2-3-3(2,8)	+
3327	Apple juice	E. coli O157:H7 Ad 556	Environmental sample	pH 4 - 9 days	0,75	3-3-2-3-3(2,8)	+
3313	Parsley	E. coli O157:H7 Ad 557	Environmental sample	4°C - 9 days	0,52	6-3-0-2-4(3,0)	+
3314	Salad	E. coli O157:H7 Ad 557	Environmental sample	4°C - 9 days	0,52	6-3-0-2-4(3,0)	+
3315	Spinach leaves	E. coli O157:H7 Ad 557	Environmental sample	4°C - 9 days	0,52	6-3-0-2-4(3,0)	+

N° Sample	Product	Artificial contaminations (spiking protocol)					Global result
		Strain	Origin	Injury protocol	Injury measurement	Inoculation level/25g	
3325	Cider	E. coli O157:H7 Ad 557	Environmental sample	pH 4 - 9 days	0,56	3-6-4-5-4(4,4)	+
3328	Pineapple juice	E. coli O157:H7 Ad 557	Environmental sample	pH 4 - 9 days	0,56	3-6-4-5-4(4,4)	+
3308	Salad	E. coli O157:H7 Ad 558	Environmental sample	4°C - 9 days	1,12	3-5-5-1-2(3,2)	+
3309	Salad	E. coli O157:H7 Ad 558	Environmental sample	4°C - 9 days	1,12	3-5-5-1-2(3,2)	+
3310	Salad	E. coli O157:H7 Ad 558	Environmental sample	4°C - 9 days	1,12	3-5-5-1-2(3,2)	+
3316	Carrot	E. coli O157:H7 Ad 558	Environmental sample	4°C - 9 days	1,12	3-5-5-1-2(3,2)	+
3317	Beetroot	E. coli O157:H7 Ad 558	Environmental sample	4°C - 9 days	1,12	3-5-5-1-2(3,2)	+
3655	Pear juice	E. coli O157:H7 Ad 558	Treatment plant	pH4 - 15 days	0,77	2-0-3-2-4 (2,2)	+
3311	White cabbage	E. coli O157:H7 Ad 571	Faeces	4°C - 9 days	0,71	3-4-1-4-5(3,4)	+
3312	Salad	E. coli O157:H7 Ad 571	Faeces	4°C - 9 days	0,71	3-4-1-4-5(3,4)	+
3645	Grated carrots	E. coli O157:H7 Ad 571	Faeces	4°C - 15 days	0,44	2-4-2-6-1 (2,8)	+
3646	Vegetables mix	E. coli O157:H7 Ad 571	Faeces	4°C - 15 days	0,44	2-4-2-6-1 (2,8)	+
3239	Fermented milk	E. coli O157:H7 Ad 572	Faeces	4°C - 8 days	0,59	4-6-3-0-1(2,8)	+
3243	Fermented milk	E. coli O157:H7 Ad 572	Faeces	4°C - 8 days	0,59	4-6-3-0-1(2,8)	+
3247	Fermented milk	E. coli O157:H7 Ad 572	Faeces	4°C - 8 days	0,59	4-6-3-0-1(2,8)	+
3251	Fermented milk	E. coli O157:H7 Ad 572	Faeces	4°C - 8 days	0,59	4-6-3-0-1(2,8)	+
3269	Raw milk cheese	E. coli O157:H7 Ad 573	Faeces	4°C - 8 days	0,92	2-5-1-0-4(2,4)	+
3270	Raw milk cheese	E. coli O157:H7 Ad 573	Faeces	4°C - 8 days	0,92	2-5-1-0-4(2,4)	+
3271	Raw milk cheese	E. coli O157:H7 Ad 573	Faeces	4°C - 8 days	0,92	2-5-1-0-4(2,4)	+
3272	Raw milk cheese	E. coli O157:H7 Ad 573	Faeces	4°C - 8 days	0,92	2-5-1-0-4(2,4)	+
3273	Raw milk cheese	E. coli O157:H7 Ad 573	Faeces	4°C - 8 days	0,92	2-5-1-0-4(2,4)	+
3255	Raw milk	E. coli O157:H7 Ad 574	Faeces	4°C - 8 days	0,46	7-9-7-9-7(7,8)	+
3258	Raw milk	E. coli O157:H7 Ad 574	Faeces	4°C - 8 days	0,46	7-9-7-9-7(7,8)	+
3261	Raw milk	E. coli O157:H7 Ad 574	Faeces	4°C - 8 days	0,46	7-9-7-9-7(7,8)	+
3306	Deli salad	E. coli O157:H7 Ad 575	Faeces	4°C - 9 days	0,89	3-2-3-5-5(3,6)	+
3307	Salad	E. coli O157:H7 Ad 575	Faeces	4°C - 9 days	0,89	3-2-3-5-5(3,6)	+

N° Sample	Product	Artificial contaminations (spiking protocol)					Global result
		Strain	Origin	Injury protocol	Injury measurement	Inoculation level/25g	
3319	Pineapple salad	E. coli O157:H7 Ad 575	Faeces	4°C - 9 days	0,89	3-2-3-5-5(3,6)	+
3320	Fruice salad	E. coli O157:H7 Ad 575	Faeces	4°C - 9 days	0,89	3-2-3-5-5(3,6)	+
3240	Fermented milk	E. coli O157:H7 Ad 576	Environmental sample	pH4 - 8 days	0,51	7-4-4-6-2(4,6)	+
3244	Fermented milk	E. coli O157:H7 Ad 576	Environmental sample	pH4 - 8 days	0,51	7-4-4-6-2(4,6)	+
3248	Fermented milk	E. coli O157:H7 Ad 576	Environmental sample	pH4 - 8 days	0,51	7-4-4-6-2(4,6)	+
3252	Fermented milk	E. coli O157:H7 Ad 576	Environmental sample	pH4 - 8 days	0,51	7-4-4-6-2(4,6)	+
3392	Raw milk	E. coli O157:H7 Ad 577	Faeces	4°C - 15 days	0,39	5-5-5-6-5(5,2)	+
3393	Raw milk	E. coli O157:H7 Ad 577	Faeces	4°C - 15 days	0,39	5-5-5-6-5(5,2)	+
3394	Raw milk	E. coli O157:H7 Ad 578	Faeces	4°C - 15 days	0,41	5-7-6-8-7(6,6)	+
3395	Raw milk	E. coli O157:H7 Ad 578	Faeces	4°C - 15 days	0,41	5-7-6-8-7(6,6)	+
3396	Raw milk	E. coli O157:H7 Ad 578	Faeces	4°C - 15 days	0,41	5-7-6-8-7(6,6)	+
3397	Raw milk	E. coli O157:H7 Ad 578	Faeces	4°C - 15 days	0,41	5-7-6-8-7(6,6)	+
3398	Raw milk	E. coli O157:H7 Ad 578	Faeces	4°C - 15 days	0,41	5-7-6-8-7(6,6)	+
3656	Banana juice	E. coli O157:H7 Ad 580	Faeces	pH 4 - 15 days	0,89	2-5-7-3-3 (4,0)	+
3657	Cider	E. coli O157:H7 Ad 580	Faeces	pH 4 - 15 days	0,89	2-5-7-3-3 (4,0)	+
3658	Cider	E. coli O157:H7 Ad 580	Faeces	pH 4 - 15 days	0,89	2-5-7-3-3 (4,0)	+
4179	Frozen ground beef	E. coli O157:H7 Ad 683	Frozen beef balls	20°C - 61 days	1,03	3-6-4-7-3 (5,0)	+
4180	Frozen ground beef	E. coli O157:H7 Ad 683	Frozen beef balls	20°C - 61 days	1,03	3-6-4-7-3 (5,0)	+
4181	Frozen beef balls	E. coli O157:H7 Ad 683	Frozen beef balls	20°C - 61 days	1,03	3-6-4-7-3 (5,0)	+
4195	Beef meat piece	E. coli O157:H7 Ad 684	Carpaccio	4°C - 65 days	0,83	0-2-2-2-0 (1,2)	-
4197	Beef meat piece	E. coli O157:H7 Ad 684	Carpaccio	4°C - 65 days	0,83	0-2-2-2-0 (1,2)	+
4199	Beef meat piece	E. coli O157:H7 Ad 684	Carpaccio	4°C - 65 days	0,83	0-2-2-2-0 (1,2)	+
4201	Beef meat piece	E. coli O157:H7 Ad 684	Carpaccio	4°C - 65 days	0,83	0-2-2-2-0 (1,2)	-
4808	Bolognaise ground beef	E. coli O157:H7 Ad 684	Carpaccio	4°C - 82 days	1,02	1-1-2-0-5 (1,8)	+
4809	Ground beef with ketchup	E. coli O157:H7 Ad 684	Carpaccio	4°C - 82 days	1,02	1-1-2-0-5 (1,8)	+
3264	Raw milk cheese	E. coli O157:H7 Ad 686	Environmental sample	10% NaCl - 8 days	1,13	2-5-3-0-1(2,2)	+

N° Sample	Product	Artificial contaminations (spiking protocol)					Global result
		Strain	Origin	Injury protocol	Injury measurement	Inoculation level/25g	
3265	Raw milk cheese	E. coli O157:H7 Ad 686	Environmental sample	10% NaCl - 8 days	1,13	2-5-3-0-1(2,2)	+
3266	Raw milk cheese	E. coli O157:H7 Ad 686	Environmental sample	10% NaCl - 8 days	1,13	2-5-3-0-1(2,2)	+
3267	Raw milk cheese	E. coli O157:H7 Ad 686	Environmental sample	10% NaCl - 8 days	1,13	2-5-3-0-1(2,2)	+
3268	Raw milk cheese	E. coli O157:H7 Ad 686	Environmental sample	10% NaCl - 8 days	1,13	2-5-3-0-1(2,2)	+
3630	Fermented milk	E. coli O157:H7 Ad 688	Environmental sample	pH4 - 15 days	0,51	4-4-3-7-5 (3,4)	+
3631	Fermented milk	E. coli O157:H7 Ad 688	Environmental sample	pH4 - 15 days	0,51	4-4-3-7-5 (3,4)	+
4187	Beef meat piece	E. coli O157:H7 Ad 922	Ground beef with onions	4°C - 62 days	1,22	2-5-3-6-3 (3,8)	+
4188	Beef meat piece	E. coli O157:H7 Ad 922	Ground beef with onions	4°C - 62 days	1,22	2-5-3-6-3 (3,8)	-
4219	Ground beef	E. coli O157:H7 Ad 922	Ground beef with onions	4°C - 65 days	1,02	2-0-1-2-0 (1,0)	+
4221	Ground beef	E. coli O157:H7 Ad 922	Ground beef with onions	4°C - 65 days	1,02	2-0-1-2-0 (1,0)	+
4223	Ground beef	E. coli O157:H7 Ad 922	Ground beef with onions	4°C - 65 days	1,02	2-0-1-2-0 (1,0)	+
4810	Beef meat piece	E. coli O157:H7 Ad 922	Ground beef with onions	4°C - 82 days	0,76	2-2-0-0-1 (1,0)	+
4203	Beef meat piece	E. coli O157:H7 Ad 976	Beef meat	4°C - 65 days	1,02	5-2-4-3-2 (3,2)	+
4205	Beef meat piece	E. coli O157:H7 Ad 976	Beef meat	4°C - 65 days	1,02	5-2-4-3-2 (3,2)	+
4207	Carpaccio	E. coli O157:H7 Ad 976	Beef meat	4°C - 65 days	1,02	5-2-4-3-2 (3,2)	+
4209	Carpaccio with basil	E. coli O157:H7 Ad 976	Beef meat	4°C - 65 days	1,02	5-2-4-3-2 (3,2)	+
4211	Bolognaise ground beef	E. coli O157:H7 Ad 976	Beef meat	4°C - 65 days	1,02	5-2-4-3-2 (3,2)	+
4213	Frozen ground beef with onions	E. coli O157:H7 Ad 976	Beef meat	20°C - 65 days	1,44	3-0-2-0-1 (1,2)	+
4182	Frozen ground beef	E. coli O157:H7 Ad 1174	Frozen ground beef	20°C - 65 days	0,95	10-5-9-5-10 (7,8)	+
4183	Frozen beef carpaccio	E. coli O157:H7 Ad 1174	Frozen ground beef	20°C - 65 days	0,95	10-5-9-5-10 (7,8)	+
4184	Frozen ground beef	E. coli O157:H7 Ad 1174	Frozen ground beef	20°C - 65 days	0,95	10-5-9-5-10 (7,8)	+

Appendix 4 – Relative accuracy: raw data

Bold typing: artificial contaminations

+/-: doubtful colonies

ox: oxidase test

st: plate without colonies

+1/2: 50% target bacteria

+m: minority of target bacteria

+M: majority of target bacteria

p: pure culture of target bacteria

O: *E. coli* O157

A: Autoagglutination

MEAT PRODUCTS																		
N° Sample	Product	NF EN ISO 16654 method♦					3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method											
							8 H at 41,5°C						18 H at 41,5°C					
		IMS 6h		IMS 24h		Result	Detection result	Confirmation			Final result	Agreement /NF EN ISO 16654	Detection result	Confirmation			Final result	Agreement /NF EN ISO 16654
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157			CT SMAC streaking	CT SMAC IMS	Latex (O157/H7)				CT SMAC streaking	CT SMAC IMS	Latex (O157/H7)		
4179	Frozen ground beef	st	+/- m	+/- m	-	+	+	+/-m	/	+/+	+	PA	+	+m	/	+/+	+	PA
4180	Frozen ground beef	st	st	+p	+p	+	-/+	+p	/	+/+	-	ND	+	+p	/	+/+	+	PA
4181	Frozen beef balls	st	st	+p	+p	+	+	+m	/	+/+	+	PA	+	+M	/	+/+	+	PA
4182	Frozen ground beef	+M	+M	/	/	+	+	+M	/	+/+	+	PA	+	+M	/	+/+	+	PA
4183	Frozen beef Carpaccio	+p	+p	/	/	+	+	+p	/	+/+	+	PA	+	+p	/	+/+	+	PA
4184	Frozen ground beef	+p	+p	/	/	+	+	+M	/	+/+	+	PA	+	+M	/	+/+	+	PA
4185	Beef meat piece	+p	+1/2	/	/	+	+	+M	/	+/+	+	PA	+	+M	/	+/+	+	PA
4186	Beef meat piece	+p	+p	/	/	+	-	-	/	/	-	ND	-	-	/	/	-	ND
4187	Beef meat piece	+p	+p	/	/	+	+	+m	/	+/+	+	PA	+	+M	/	+/+	+	PA
4188	Beef meat piece	st	-	-	-	-	-	-	/	/	-	NA	-	+M	/	-	-	NA
4195	Beef meat piece	-	+/- m	-	+/- m	-	-	-	/	/	-	NA	-	-	/	/	-	NA
4196	Beef meat piece	-	-	-	+m	-	-	+/-m	/	-	-	NA	-	+m	/	-	-	NA
4197	Beef meat piece	+/- m	-	+/-m	+/-m	-	+	+m (1col)	/	+/+	+	PD	+	-	+/-	+/+	+	PD
4198	Beef meat piece	+/- (1col)	-	+/- (1col)	-	-	-	+/-m	/	-	-	NA	-	+m	/	-	-	NA

♦ Analysis performed according to the COFRAC accreditation

ADRIA Développement

Summary Report (Version 0)

3M MDA *E. coli* O157 (including H7)

MEAT PRODUCTS																		
N° Sample	Product	NF EN ISO 16654 method [♦]					3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method											
							8 H at 41,5°C						18 H at 41,5°C					
		IMS 6h		IMS 24h		Result	Detection result	Confirmation			Final result	Agreement /NF EN ISO 16654	Detection result	Confirmation			Final result	Agreement /NF EN ISO 16654
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157			CT SMAC streaking	CT SMAC IMS	Latex (O157/H7)				CT SMAC streaking	CT SMAC IMS	Latex (O157/H7)		
4199	Beef meat piece	-	-	-	-	-	+	+m	/	+/+	+	PD	+	+m	/	+/+	+	PD
4200	Beef meat piece	-	+/- (1col)	-	-	-	-	-	/	/	-	NA	-	-	/	/	-	NA
4201	Beef meat piece	st	-	-	+/- m	-	-	-	/	/	-	NA	-	-	/	/	-	NA
4202	Beef meat piece	st	-	-	-	-	-	+/-m	/	-	-	NA	-	-	/	/	-	NA
4203	Beef meat piece	st	+/- (2col)	-	+m	-	-/-	+/-m	+/-m	-	-	NA	+	+M	/	+/+	+	PD
4204	Beef meat piece	st	-	-	-	-	-	-	/	/	-	NA	-	-	/	/	-	NA
4205	Beef meat piece	st	-	st	-	-	+	+1/2	/	+/+	+	PD	+	+/-m	/	+/+	+	PD
4206	Beef meat piece	st	-	st	-	-	-	+/- 1/2	/	-	-	NA	-	-	/	/	-	NA
4207	Carpaccio	+p	+p	/	/	+	+	+M	/	+/+	+	PA	+	+M	/	+/+	+	PA
4208	Carpaccio	-	st	-	-	-	-	-	/	/	-	NA	-	-	/	/	-	NA
4209	Carpaccio with basil	st	-	-	-	-	+	+1/2	/	+/+	+	PD	+	+M	/	+/+	+	PD
4210	Carpaccio with basil	st	-	-	-	-	-	-	/	/	-	NA	-	-	/	/	-	NA
4211	Bolognaise ground beef	-	-	+m	-	-	+	+M	/	+/+	+	PD	+	+M	/	+/+	+	PD
4212	Bolognaise ground beef	-	-	-	-	-	-	-	/	/	-	NA	-	-	/	/	-	NA
4213	Frozen ground beef with onions	+p	+m	/	/	+	-/+	-	- ChromID O157:H7:-	/	-	ND	-/+	-	+m (2)	+/+	-	ND
4214	Ground beef with onions	-	-	-	-	-	-	-	/	/	-	NA	-	-	/	/	-	NA
4215	Ground beef	- (sorbitol +)	- (purple blue)	- (sorbitol +)	- (purple blue)	-	+	+/-m (sorbitol +)	+ (sorbitol +) Chromagar+	+/-	+	PD	+	+/-m (sorbitol +)	+ (sorbitol +) Chromagar:+	+/-	+	PD
4216	Ground beef	-	-	-	-	-	-	-	/	/	-	NA	-	-	/	/	-	NA

MEAT PRODUCTS																		
N° Sample	Product	NF EN ISO 16654 method [♦]					3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method											
							8 H at 41,5°C					18 H at 41,5°C						
		IMS 6h		IMS 24h		Result	Detection result	Confirmation			Final result	Agreement /NF EN ISO 16654	Detection result	Confirmation			Final result	Agreement /NF EN ISO 16654
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157			CT SMAC streaking	CT SMAC IMS	Latex (O157/H7)				CT SMAC streaking	CT SMAC IMS	Latex (O157/H7)		
4217	Ground beef	- (sorbitol +)	- (purple blue)	- (sorbitol +)	- (purple blue)	-	-	+/-m	/	-	-	NA	-/-	+/-m	/	-	-	NA
4218	Ground beef	st	-	-	-	-	-	-	/	/	-	NA	-	+/-M	/	-	-	NA
4219	Ground beef	-	-	-	+/- m	-	+	+/-m	+/-m	+/+	+	PD	+	+/-m	/	+/+	+	PD
4220	Ground beef	-	-	-	+m	-	-	-	/	/	-	NA	+/-/-	-	/	/	-	PPNA
4221	Ground beef	st	-	-	-	-	+	+M	/	+/+	+	PD	+	+M	/	+/+	+	PD
4222	Ground beef	-	st	-	-	-	-	-	/	/	-	NA	-	-	/	/	-	NA
4223	Ground beef	+p	+M	/	/	+	+	+m	/	+/+	+	PA	+	+M	/	+/+	+	PA
4224	Ground beef	-	-	-	-	-	-	-	/	/	-	NA	-	-	/	/	-	NA
4804	Ground beef with ketchup	st	-	+/- M	-	-	+	+p	/	+/+	+	PD	+	+M	/	+/+	+	PD
4805	Carpaccio with cheese	st	st	-	-	-	+	+M	/	+/+	+	PD	+	+p	/	+/+	+	PD
4806	Beef meat piece with shallot	st	-	st	-	-	+	+1/2	/	+/+	+	PD	+	+M	/	+/+	+	PD
4807	Beef meat piece with pepper	st	st	st	-	-	-	st	/	/	-	NA	-	st	/	/	-	NA
4808	Bolognaise ground beef	st	st	+p	+p	+	+	+p	/	+/+	+	PA	+	+p	/	+/+	+	PA
4809	Ground beef with ketchup	st	-	-	-	-	+	+M	/	+/+	+	PD	+	+M	/	+/+	+	PD
4810	Beef meat piece	-	-	-	-	-	+	+m	/	+/+	+	PD	+	+M	/	+/+	+	PD

♦ Analysis performed according to the COFRAC accreditation

MEAT PRODUCTS																		
N° Sample	Product	NF EN ISO 16654 method [♦]				3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method												
						8 H at 41,5°C							18 H at 41,5°C					
		IMS 6h		IMS 24h		Result	Detection result	Confirmation			Final result	Agreement /NF EN ISO 16654	Detection result	Confirmation			Final result	Agreement /NF EN ISO 16654
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157			CT SMAC streaking	CT SMAC IMS	Latex (O157/H7)				CT SMAC streaking	CT SMAC IMS	Latex (O157/H7)		
4811	Beef meat piece	- (sorbitol +)	- (purple blue)	- (sorbitol +)	- (purple blue)	-	-	-	/	/	-	NA	-	-	/	/	-	NA
4812	Beef meat piece	st	+ m (purple blue)	- (sorbitol +)	+ M (purple blue)	-	+	+/-m (sorbitol +)	+/- (sorbitol +) Chromagar:-	+ (after subculture in BHI)	+	PD	+	+/-m (sorbitol +)	+/- (sorbitol +) Chromagar:-	+ (after subculture in BHI)	+	PD
4813	Ground beef	st	st	+ M	-	-	+	+/-m (sorbitol +)	-	+ (after subculture in BHI)	+	PD	+	+/-m (sorbitol +)	+/- (sorbitol +)	+/-	+	PD
4814	Ground beef	st	+/- M (purple blue)	+ m	+/- M (purple blue)	-	+	+/-m (sorbitol +)	+/- (sorbitol +) Chromagar:-	+ (after subculture in BHI)	+	PD	+	+/-m (sorbitol +)	+/- (sorbitol +) Chromagar:-	+ (after subculture in BHI)	+	PD
4815	Ground beef with ketchup	st	st	st	-	-	-	-	/	/	-	NA	-	+/-	/	/	-	NA
4816	Carpaccio with cheese	st	st	-	-	-	-	-	/	/	-	NA	-	-	/	/	-	NA
4817	Beef meat piece with shallot	st	-	st	-	-	-	-	/	/	-	NA	-	-	/	/	-	NA
4818	Beef meat piece with pepper	st	st	st	st	-	-	st	/	/	-	NA	-	st	/	/	-	NA
4819	Bolognaise ground beef	st	st	st	-	-	-	-	/	/	-	NA	-	+/-	/	/	-	NA
4820	Frozen beef balls	st	st	-	-	-	+/-/-	-	-	/	- (after subculture in BHI)	PPNA	-/-/+	-	-	/	- (after subculture in BHI)	NA

♦ Analysis performed according to the COFRAC accreditation

MEAT PRODUCTS																		
N° Sample	Product	NF EN ISO 16654 method [♦]					3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method											
							8 H at 41,5°C					18 H at 41,5°C						
		IMS 6h		IMS 24h		Result	Detection result	Confirmation			Final result	Agreement /NF EN ISO 16654	Detection result	Confirmation			Final result	Agreement /NF EN ISO 16654
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157			CT SMAC streaking	CT SMAC IMS	Latex (O157/H7)				CT SMAC streaking	CT SMAC IMS	Latex (O157/H7)		
4821	Beef meat piece	-	+ m	-	+M	-	-	-	/	/	-	NA	-	-	/	/	-	NA
4822	Ground beef	-	-	-	-	-	-	-	/	/	-	NA	-	-	/	/	-	NA
4823	Frozen ground beef	st	st	st	-	-	-	-	/	/	-	NA	-	-	/	/	-	NA
4824	Ground beef	-	+ M	-	-	-	-	-	/	/	-	NA	-	-	/	/	-	NA
4825	Ground beef	st	st	-	-	-	-	st	/	/	-	NA	-	-	/	/	-	NA

♦ Analysis performed according to the COFRAC accreditation

MEAT PRODUCTS																				
N° sample	Products	NF EN ISO 16654 method♦ Result	3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method																	
			8H at 41,5°C + 72 H at 4°C									18 H at 41,5°C + 72 H at 4°C								
			Detection result Enrichment storage	Detection result- Lysate storage	Confirmation			Final result- BPW storage	Final result- Lysate storage	Agreement 8H+72H BPW /NF EN ISO 16654	Agreement 8H+72H lysate /NF EN ISO 16654	Detection result- Enrichment storage	Detection result- Lysate storage	Confirmation			Final result- BPW storage	Final result- Lysate storage	Agreement 18H+72H BPW /NF EN ISO 16654	Agreement 18H+72H lysate /NF EN ISO 16654
					CT-SMAC streaking	CT-SMAC IMS	Latex (O157/ H7)							CT SMAC streaking	CT SMAC IMS	Latex (O157 /H7)				
4179	Frozen ground beef	+	+	+	+/-m	/	+/+	+	+	PA	PA	+	-/+/-	+/- (2col) ni/+	/	+/+	+	+	PA	PA
4180	Frozen ground beef	+	-/+/-	+	+M	/	+/+	-	+	ND	PA	+	+	+/-M	/	+/+	+	+	PA	PA
4181	Frozen beef balls	+	+	+	+m	/	+/+	+	+	PA	PA	+	+	+p	/	+/+	+	+	PA	PA
4182	Frozen ground beef	+	+	+	+m	/	+/+	+	+	PA	PA	+	+	+/-M	/	+/+	+	+	PA	PA
4183	Frozen beef carpaccio	+	+	+	+1/2	/	+/+	+	+	PA	PA	+	+	+p	/	+/+	+	+	PA	PA
4184	Frozen ground beef	+	+	+	+M	/	+/+	+	+	PA	PA	+	+	+/-M	/	+/+	+	+	PA	PA
4185	Beef meat piece	+	+	+	+M	+/-m	+/+	+	+	PA	PA	+	+	+M	/	+/+	+	+	PA	PA
4186	Beef meat piece	+	-	-	-	/	/	-	-	ND	ND	-	-	-	/	/	-	-	ND	ND
4187	Beef meat piece	+	+	+	+M	+m	+/+	+	+	PA	PA	+	+	+M	+M	+/+	+	+	PA	PA
4188	Beef meat piece	-	-	-	+M	/	-	-	-	NA	NA	-	-	+M	/	-	-	-	NA	NA
4197	Beef meat piece	-	+	+	+m	+	+/+	+	+	PD	PD	+	+	-	+	+/+	+	+	PD	PD
4199	Beef meat piece	-	+	+	+m	/	+/+	+	+	PD	PD	+	+	+m	/	+/+	+	+	PD	PD
4203	Beef meat piece	-	-	-	+m	/	-	-	-	NA	NA	+	+	+/-m	/	+/+	+	+	PD	PD
4205	Beef meat piece	-	+	+	+m	+	+/+	+	+	PD	PD	+	+	+/-m	+	+/+	+	+	PD	PD

♦ Analysis performed according to the COFRAC accreditation

ADRIA Développement

Summary Report (Version 0)

3M MDA *E. coli* O157 (including H7)

MEAT PRODUCTS																				
N° sample	Products	NF EN ISO 16654 method♦ Result	3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method																	
			8 H at 41,5°C + 72 H at 4°C									18 H at 41,5°C + 72 H at 4°C								
			Detection result Enrichment storage	Detection result- Lysate storage	Confirmation			Final result- BPW storage	Final result- Lysate storage	Agreement 8H+72H BPW /NF EN ISO 16654	Agreement 8H+72H lysate /NF EN ISO 16654	Detection result- Enrichment storage	Detection result- Lysate storage	Confirmation			Final result- BPW storage	Final result- Lysate storage	Agreement 18H+72H BPW /NF EN ISO 16654	Agreement 18H+72H lysate /NF EN ISO 16654
					CT-SMAC streaking	CT-SMAC IMS	Latex (O157/ H7)							CT SMAC streaking	CT SMAC IMS	Latex (O157/ H7)				
4207	Carpaccio	+	+	+	+m	/	+/+	+	+	PA	PA	+	+	+M	/	+/+	+	+	PA	PA
4209	Carpaccio with basil	-	+	+	+m	/	+/+	+	+	PD	PD	+	+	+/-M	/	+/+	+	+	PD	PD
4211	Bolognaise ground beef	-	+	+	+m	/	+/+	+	+	PD	PD	+	+	+M	/	+/+	+	+	PD	PD
4213	Frozen ground beef with onions	+	-	+	-	-	-	-	-	ND	PPND	-	-	-	+m (2)	+/+	-	-	ND	ND
4215	Ground beef	-	+	+	+/-m (sorbitol +)	+(sorbitol +) Chromagar:+	+/-	+	+	PD	PD	+	+	+/-m (sorbitol +)	+(sorbitol +) Chromagar:+	+/-	+	+	PD	PD
4217	Ground beef	-	-	-	-	/	/	-	-	NA	NA	-	-	+/-	/	-	-	-	NA	NA
4219	Ground beef	-	+	+	+/-m (2col)	+	+/+	+	+	PD	PD	+	+	+m	/	+/+	+	+	PD	PD
4221	Ground beef	-	+	+	+/-m	/	+/+	+	+	PD	PD	+	+	+M	/	+/+	+	+	PD	PD
4223	Ground beef	+	+	+	+m	/	+/+	+	+	PA	PA	+	+	+m	/	+/+	+	+	PA	PA
4804	Ground beef with ketchup	-	+	+	+M	/	+/+	+	+	PD	PD	+	+	+M	/	+/+	+	+	PD	PD
4805	Carpaccio with cheese	-	+	+	+m	/	+/+	+	+	PD	PD	+	+	+p	/	+/+	+	+	PD	PD
4806	Beef meat piece with shallot	-	+	+	+m	/	+/+	+	+	PD	PD	+	+	+M	/	+/+	+	+	PD	PD
4808	Bolognaise ground beef	+	+	+	+p	/	+/+	+	+	PA	PA	+	+	+p	/	+/+	+	+	PA	PA

MEAT PRODUCTS																				
N° sample	Products	NF EN ISO 16654 method♦ Result	3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method																	
			8H at 41,5°C + 72 H at 4°C									18 H at 41,5°C + 72 H at 4°C								
			Detection result Enrichment storage	Detection result- Lysate storage	Confirmation			Final result- BPW storage	Final result- Lysate storage	Agreement 8H+72H BPW /NF EN ISO 16654	Agreement 8H+72H lysate /NF EN ISO 16654	Detection result- Enrichment storage	Detection result- Lysate storage	Confirmation			Final result- BPW storage	Final result- Lysate storage	Agreement 18H+72H BPW /NF EN ISO 16654	Agreement 18H+72H lysate /NF EN ISO 16654
					CT- SMAC streaking	CT- SMAC IMS	Latex (O157/ H7)							CT SMAC streaking	CT SMAC IMS	Latex (O157 /H7)				
4809	Ground beef with ketchup	-	+	+	+m	+M	+/+	+	+	PD	PD	+	+	+M	+M	+/+	+	+	PD	PD
4810	Beef meat piece	-	+	+	+m	/	+/+	+	+	PD	PD	+	+	+1/2	/	+/+	+	+	PD	PD
4811	Beef meat piece	-	-	-	-	/	/	-	-	NA	NA	-	-	-	/	/	-	-	NA	NA
4812	Beef meat piece	-	+	+	+/-m (sorbitol +)	/	+/- (after subculture in BHI)	+	+	PD	PD	+	+	+/-m (sorbitol +)	/	+/- (after subculture in BHI)	+	+	PD	PD
4813	Ground beef	-	+	+	+/-m (sorbitol +)	/	+/- (after subculture in BHI)	+	+	PD	PD	+	+	+/-m (sorbitol +)	/	+/-	+	+	PD	PD
4814	Ground beef	-	+	+	+/-m (sorbitol +)	/	+/- (after subculture in BHI)	+	+	PD	PD	+	+	+/-m (sorbitol +)	/	+/- (after subculture in BHI)	+	+	PD	PD
4820	Frozen beef balls	-	-	+	- (+/-m sorbitol +)	/	/	- (after subculture in BHI)	- (after subculture in BHI)	NA	PPNA	-	-	- (+/-m sorbitol +)	/	/	- (after subculture in BHI)	- (after subculture in BHI)	NA	NA

DAIRY PRODUCTS												
N° sample	Products	NF EN ISO 16654 method♦					3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method 18 H at 41,5°C					
		IMS 6h		IMS 24h		Result	Detection result	Confirmation			Final result	Agreement / NF EN ISO 16654
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157			CT SMAC streaking	CT SMAC IMS	Latex (O157/H7)		
3239	Fermented milk	st	st	st	st	-	+	+p	/	+/+	+	PD
3240	Fermented milk	st	st	st	st	-	+	+p	/	+/+	+	PD
3241	Fermented milk	st	st	st	st	-	-	st	/	/	-	NA
3243	Fermented milk	st	st	st	+/-m	-	+	+p	/	+/+	+	PD
3244	Fermented milk	+p	+p	/	/	+	+	+p	/	+/+	+	PA
3245	Fermented milk	st	st	st	st	-	-	st	/	/	-	NA
3247	Fermented milk	st	st	st	st	-	+	+p	/	+/+	+	PD
3248	Fermented milk	+p	+p	/	/	+	+	+p	/	+/+	+	PA
3249	Fermented milk	st	st	st	st	-	-	st	/	/	-	NA
3251	Fermented milk	st	st	-	+m	-	+	+p	/	+/+	+	PD
3252	Fermented milk	+p	+p	/	/	+	+	+p	/	+/+	+	PA
3253	Fermented milk	st	st	st	-	-	-	st	/	/	-	NA
3255	Raw milk	+M	+M	/	/	+	+	+M	/	+/+	+	PA
3256	Raw milk	+/-M	+/-m	-	+/-m	-	-	-	/	/	-	NA
3258	Raw milk	+M	+M	/	/	+	+	+M	/	+/+	+	PA
3259	Raw milk	+/-M	+/-m	+/-M	+/-M	-	-	-	/	/	-	NA
3261	Raw milk	+p	+m	/	/	+	+	+M	/	+/+	+	PA
3262	Raw milk	st	-	-	-	-	-	-	/	/	-	NA
3264	Raw milk cheese	st	st	+m	+m	+	+	+M	/	+/+	+	PA
3265	Raw milk cheese	st	-	+m	+m	+	+	-	+m	+/+	+	PA
3266	Raw milk cheese	+3col	-	+m	+m	+	+	+M	/	+/+	+	PA
3267	Raw milk cheese	st	-	+m	+m	+	+	+M	/	+/+	+	PA
3268	Raw milk cheese	+1col	-	+1col	-	+	+	+1/2	/	+/+	+	PA
3269	Raw milk cheese	+p	+p	/	/	+	+	+M	/	+/+	+	PA
3270	Raw milk cheese	st	st	+M	+M	+	+	+M	/	+/+	+	PA

♦ Analysis performed according to the COFRAC accreditation

ADRIA Développement

Summary Report (Version 0)

3M MDA *E. coli* O157 (including H7)

DAIRY PRODUCTS												
N° sample	Products	NF EN ISO 16654 method [♦]					3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method 18 H at 41,5°C					
		IMS 6h		IMS 24h		Result	Detection result	Confirmation			Final result	Agreement / NF EN ISO 16654
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157			CT SMAC streaking	CT SMAC IMS	Latex (O157/H7)		
3271	Raw milk cheese	+p	+M	/	/	+	-	-	/	/	-	ND
3272	Raw milk cheese	+p	+p	/	/	+	+	+M	/	+/+	+	PA
3273	Raw milk cheese	+M	+m	/	/	+	+	+M	/	+/+	+	PA
3382	Raw milk cheese	st	-	-	-	-	-	-	/	/	-	NA
3383	Raw milk cheese	st	st	st	st	-	-	st	/	/	-	NA
3384	Raw milk cheese	-	-	-	-	-	-	-	/	/	-	NA
3385	Raw milk cheese	st	-	-	-	-	-	-	/	/	-	NA
3386	Raw milk cheese	st	st	st	st	-	-	st	/	/	-	NA
3387	Raw milk cheese	-	-	-	-	-	-	-	/	/	-	NA
3388	Raw milk cheese	st	st	-	-	-	-	-	/	/	-	NA
3389	Raw milk cheese	st	-	st	-	-	-	-	/	/	-	NA
3390	Raw milk cheese	st	st	st	st	-	-	-	/	/	-	NA
3391	Raw milk cheese	st	-	st	-	-	-	st	/	/	-	NA
3392	Raw milk	+m	+m	/	/	+	+	+m	/	+/+	+	PA
3393	Raw milk	+M	+m	/	/	+	+	-	+M	+/+	+	PA
3394	Raw milk	+1/2	+m	/	/	+	+	+m	/	+/+	+	PA
3395	Raw milk	+m	+m	/	/	+	+	-	+m	+/+	+	PA
3396	Raw milk	+M	+m	/	/	+	+	+m	/	+/+	+	PA
3397	Raw milk	+p	-	/	/	+	+	+m	/	+/+	+	PA
3398	Raw milk	+p	+m	/	/	+	+	+m	/	+/+	+	PA
3399	Raw milk	-	-	-	-	-	-	-	/	/	-	NA
3400	Raw milk	-	-	-	-	-	+/+/+	-	+ (after subculture in BHI)	+/-	+ (after subculture in BHI)	PD
3401	Raw milk	-	-	-	-	-	-	-	/	/	-	NA
3402	Raw milk	-	-	-	-	-	-	-	/	/	-	NA
3403	Raw milk	-	-	-	-	-	-	-	/	/	-	NA

DAIRY PRODUCTS												
N° sample	Products	NF EN ISO 16654 method♦					3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method 18 H at 41,5°C					
		IMS 6h		IMS 24h		Result	Detection result	Confirmation			Final result	Agreement / NF EN ISO 16654
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157			CT SMAC streaking	CT SMAC IMS	Latex (O157/H7)		
3404	Raw milk	-	-	-	-	-	-	-	/	/	-	NA
3405	Raw milk	-	-	-	-	-	-	-	/	/	-	NA
3630	Fermented milk	+p	+p	/	/	+	+	+	/	+/+	+	PA
3631	Fermented milk	st	st	st	st	-	+	+	/	+/+	+	PD
3632	Fermented milk	st	st	-	st	-	-	-	/	/	-	NA
3633	Fermented milk	st	st	st	st	-	-	-	/	/	-	NA
3634	Fermented milk	st	st	st	st	-	-	-	/	/	-	NA
3635	Fermented milk	st	st	-	st	-	-	-	/	/	-	NA
3636	Fermented milk	st	-	-	-	-	-	-	/	/	-	NA
3637	Fermented milk	st	st	-	st	-	-	st	/	/	-	NA
3663	Raw milk cheese	+M	+M	/	/	+	+	-	+ m	+/-	+	PA
4314	Raw milk	-	+m	-	+/- m	-	-	-	/	/	-	NA

DAIRY PRODUCTS															
N° Sample	Products	NF EN ISO 16654 method [♦]					3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method 18 H at 41,5°C + 72 H at 4°C								
		IMS 6h		IMS 24h		Result	Detection result- Enrichment storage	Detection result- Lysate storage	Confirmation			Final result- BPW storage	Final result- Lysate storage	Agreement 18H+72H BPW /NF EN ISO 16654	Agreement 18H +72H lysate/ NF EN ISO 16654
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157				CT SMAC streaking	CT SMAC IMS	Latex (O157 /H7)				
3392	Raw milk	+m	+m	/	/	+	+	+	-	+ M	+/+	+	+	PA	PA
3393	Raw milk	+M	+m	/	/	+	+	+	+/-	+ M	+/+	+	+	PA	PA
3394	Raw milk	+1/2	+m	/	/	+	+	+	+/-	+/- m	+/+	+	+	PA	PA
3395	Raw milk	+m	+m	/	/	+	+	+	-	+/- m	+/+	+	+	PA	PA
3396	Raw milk	+M	+m	/	/	+	+	+	+	/	+/+	+	+	PA	PA
3397	Raw milk	+p	-	/	/	+	+	+	+	/	+/+	+	+	PA	PA
3398	Raw milk	+p	+m	/	/	+	+	+	-	+ (after subculture in BHI)	+/+	+	+	PA	PA
3400	Raw milk	-	-	-	-	-	+	+	+/-	+ (after subculture in BHI)	+/-	+	+	PD	PD
3630	Fermented milk	+p	+p	/	/	+	+	+	+ M	/	+/+	+	+	PA	PA
3631	Fermented milk	st	st	st	st	-	+	+	+ M	/	+/+	+	+	PD	PD
3663	Raw milk cheese	+M	+M	/	/	+	+	+	+ m (2)	+ M	+/-	+	+	PA	PA
4314	Raw milk	-	+m	-	+/- m	-	-	-	-	/	/	-	-	NA	NA

[♦] Analysis performed according to the COFRAC accreditation

FRUITS AND VEGETABLES												
N° Sample	Products	NF EN ISO 16654 method♦					3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method 18 H at 41,5°C					
		IMS 6h		IMS 24h		Result	Detection result	Confirmation			Final result	Agreement / NF EN ISO 16654
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157			CT SMAC streaking	CT SMAC IMS	Latex (O157/H7)		
3306	Deli salad	+p	+p	/	/	+	+	+M	/	+/+	+	PA
3307	Salad	st	st	-	-	-	+	+p	/	+/+	+	PD
3308	Salad	+1col	st	+p	+p	+	+	+1/2	/	+/+	+	PA
3309	Salad	+p	+p	/	/	+	+	+m	/	+/+	+	PA
3310	Salad	+p	+m	/	/	+	+	+m	/	+/+	+	PA
3311	White cabbage	+p	+p	/	/	+	+	+p	/	+/+	+	PA
3312	Salad	+p	+p	/	/	+	+	+1/2	/	+/+	+	PA
3313	Parsley	+p	+m	/	/	+	+	+m	/	+/+	+	PA
3314	Salad	+M	+m	/	/	+	+	+/-m (4 O- 1 A+)	+/- 1/2	+/+	+	PA
3315	Spinach leaves	+m	-	+1/2	+	+	-	-	/	/	-	ND
3316	Carrot	+p	+m	/	/	+	+	+p	/	+/+	+	PA
3317	Beetroot	+M	+1/2	/	/	+	+	+p	/	+/+	+	PA
3319	Pineapple salad	+p	+p	/	/	+	+	+p	/	+/+	+	PA
3320	Fruice salad	+M	+M	/	/	+	+	+1/2	/	+/+	+	PA
3321	Pineapple salad	st	st	-	-	-	-	st	/	/	-	NA
3322	Fruice salad	st	st	-	-	-	-	st	/	/	-	NA
3323	Cider	st	st	-	-	-	+	+p	/	+/+	+	PD
3324	Cider	+p	+p	/	/	+	+	+p	/	+/+	+	PA
3325	Cider	st	st	st	st	-	+	+p	/	+/+	+	PD
3326	Apple juice	st	st	st	st	-	+	+p	/	+/+	+	PD
3327	Apple juice	+p	+p	/	/	+	+	+p	/	+/+	+	PA
3328	Pineapple juice	+p	+p	/	/	+	+	+p	/	+/+	+	PA
3329	Cider	st	st	st	st	-	-	st	/	/	-	NA
3330	Cider	st	st	st	st	-	-	st	/	/	-	NA
3331	Cider	st	st	st	st	-	-	st	/	/	-	NA
3332	Apple juice	st	st	st	st	-	-	st	/	/	-	NA

♦ Analysis performed according to the COFRAC accreditation

ADRIA Développement

Summary Report (Version 0)

3M MDA *E. coli* O157 (including H7)

FRUITS AND VEGETABLES												
N° Sample	Products	NF EN ISO 16654 method [♦]					3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method 18 H at 41,5°C					
		IMS 6h		IMS 24h		Result	Detection result	Confirmation			Final result	Agreement / NF EN ISO 16654
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157			CT SMAC streaking	CT SMAC IMS	Latex (O157/H7)		
3333	Apple juice	st	st	st	st	-	-	st	/	/	-	NA
3334	Pineapple juice	st	st	st	st	-	-	st	/	/	-	NA
3408	Celeri	st	st	st	st	-	-	st	/	/	-	NA
3409	Green salad	st	st	-	-	-	-	-	/	/	-	NA
3410	Lettuce	st	st	-	-	-	-	-	/	/	-	NA
3411	Green salad	st	st	st	st	-	-	-	/	/	-	NA
3638	Parsley	st	st	-	-	-	-	-	/	/	-	NA
3639	Lettuce	st	-	-	-	-	-	-	/	/	-	NA
3640	Salad	-	-	-	-	-	-	-	/	/	-	NA
3641	Green salad	st	st	st	-	-	-	-	/	/	-	NA
3642	Green salad	st	st	-	-	-	-	-	/	/	-	NA
3643	Leafy greens	-	-	-	-	-	-	-	/	/	-	NA
3644	Water cress	-	-	-	-	-	-	-	/	/	-	NA
3645	Grated carrots	st	st	st	-	-	+	+	/	+/+	+	PD
3646	Vegetables mix	st	st	st	-	-	+	+	/	+/+	+	PD
3655	Pear juice	+p	+p	/	/	+	+	+	/	+/+	+	PA
3656	Banana juice	st	st	st	-	-	+	+	/	+/+	+	PD
3657	Cider	st	st	st	st	-	+	+	/	+/+	+	PD
3658	Cider	st	st	st	st	-	+	+	/	+/+	+	PD
3659	Banana juice	st	st	st	st	-	-	st	/	/	-	NA
3660	Pear juice	st	st	st	st	-	-	st	/	/	-	NA
3661	Cider	st	st	st	st	-	-	st	/	/	-	NA
3662	Cider	st	st	st	st	-	-	st	/	/	-	NA
4315	Deli salad	+/-m	-	- (sorbitol +)	- (purple blue)	-	+	+/- (sorbitol +)	/	+/-	+	PD
4316	Deli salad	st	- (purple blue)	- (sorbitol +)	- (purple blue)	-	+	-	+/- (sorbitol +) Chromagar: +/- (purple blue)	- +/-	+	PD
4317	Deli salad	-	- (purple blue)	- (sorbitol +)	- (purple blue)	-	+	+/- (sorbitol +)	/	+/-	+	PD

FRUITS AND VEGETABLES												
N° Sample	Products	NF EN ISO 16654 method [♦]					3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method 18 H at 41,5°C					
		IMS 6h		IMS 24h		Result	Detection result	Confirmation			Final result	Agreement / NF EN ISO 16654
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157			CT SMAC streaking	CT SMAC IMS	Latex (O157/H7)		
4318	Deli salad	-	+/-m	-	+/- m	-	-	-	/	/	-	NA
4319	Deli salad	-	-	-	-	-	-	-	/	/	-	NA
4320	Soybeans	-	-	- (sorbitol +)	+/- (1col)	-	+	+/- (sorbitol +)	+/- (sorbitol +) Chromagar: +/- (purple blue)	- +/-	+	PD
4321	Soybeans	-	-	-	-	-	-	-	/	/	-	NA
4322	Deli salad	-	+/-m	-	+/-m	-	-	-	/	/	-	NA
4323	Deli salad	-	-	-	-	-	-	-	/	/	-	NA
4324	Link alfalfa	-	-	-	- (1col) (purple blue)	-	+	-	+/- (sorbitol +) Chromagar: +/- (purple blue)	- -	+ (after subculture in BHI)	PD
4325	Link alfalafa	-	-	-	-	-	-	-	/	/	-	NA
4826	Deli salad	-	-	-	-	-	-	-	/	/	-	NA

FRUITS AND VEGETABLES

N° Sample	Products	NF EN ISO 16654 method [♦]					3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method 18 H at 41,5°C + 72 H at 4°C								
		IMS 6h		IMS 24h		Result	Detection result- Enrichment storage	Detection result- Lysate storage	Confirmation			Final result- BPW storage	Final result- Lysate storage	Agreement 18H+72H BPW /NF EN ISO 16654	Agreement 18H+72H lysate /NF EN ISO 16654
		CT SMAC	Chromagar O157	CT SMAC	Chromagar O157				CT SMAC streaking	CT SMAC IMS	Latex (O157/ H7)				
3645	Grated carrots	st	st	st	-	-	+	+	+ M	/	+/+	+	+	PD	PD
3646	Vegetables mix	st	st	st	-	-	+	+	+ M	/	+/+	+	+	PD	PD
3655	Pear juice	+p	+p	/	/	+	+	+	+ M	/	+/+	+	+	PA	PA
3656	Banana juice	st	st	st	-	-	+	+	+ M	/	+/+	+	+	PD	PD
3657	Cider	st	st	st	st	-	+	+	+ M	/	+/+	+	+	PD	PD
3658	Cider	st	st	st	st	-	+	+	+ M	/	+/+	+	+	PD	PD
4315	Deli salad	+/-m	-	- (sorbitol +)	- (purple blue)	-	+	+	+/- (sorbitol +)	/	+/-	+	+	PD	PD
4316	Deli salad	st	- (purple blue)	- (sorbitol +)	- (purple blue)	-	+	+	+/- (sorbitol +)	+/- (sorbitol +) Chromagar: +/- (purple blue)	- +/-	+	+	PD	PD
4317	Deli salad	-	- (purpleblue)	- (sorbitol +)	- (purple blue)	-	+	+	+/- (sorbitol +)	/	+/-	+	+	PD	PD
4320	Soybeans	-	-	- (sorbitol +)	+/- (1col)	-	+	+	+/- (sorbitol +)	+/- (sorbitol +)	+/-	+	+	PD	PD
4324	Link alfalfa	-	-	-	- (1col) (purple blue)	-	+	+	+/- (sorbitol +)	+/- (sorbitol +) Chromagar: +/- (purple blue)	- -	+	+	PD	PD

♦ Analysis performed according to the COFRAC accreditation

ADRIA Développement

Summary Report (Version 0)

3M MDA *E. coli* O157 (including H7)

**Appendix 5 – Calculation of relative accuracy (AC), relative sensitivity (SE) and relative specificity (SP)
integrating LCL (lower confidence limit)**

Matrices	Types	PA	NA	ND	PD	N	Relative accuracy AC (%) [100x(PA+NA)]/N	LCL	N+ PA + ND	Relative sensitivity SE (%) [100xPA]/N+	LCL	N- NA + PD	Relative specificity SP (%) [100xNA]/N-	LCL
Raw beef meat 8 h incubation time	Raw meat	2	12	1	5	20	69.4	57.6	3	76.9	53.6	17	67.3	53.9
	Minced meat	4	11	1	6	22			5			17		
	Ground beef	4	10	1	5	20			5			15		
	Total	10	33	3	16	62			13			49		
Raw beef meat 18 h incubation time	Raw meat	2	11	1	6	20	69.4	57.6	3	84.6	64.6	17	65.3	51.7
	Minced meat	4	11	1	6	22			5			17		
	Ground beef	5	10	0	5	20			5			15		
	Total	11	32	2	17	62			13			49		
Dairy products	Raw milks	10	10	0	1	21	87.1	78.6	10	96.0	88.2	11	81.1	68.2
	Raw fermented or acidified milks	4	10	0	6	20			4			16		
	Raw milk cheeses	10	10	1	0	21			11			10		
	Total	24	30	1	7	62			25			37		
Fruits and vegetables	Produce and leafy greens	10	15	1	5	31	75.4	64.4	11	94.1	82.7	20	68.2	54.1
	RTE foods	2	5	0	3	10			2			8		
	Juices and fermented products	4	10	0	6	20			4			16		
	Total	16	30	1	14	61			17			44		
All products: 8 h (Meat) 18 h (other products)		50	93	5	37	185	77,3	71.1	55	90.9	83.2	130	71.5	63.6
All products 18 h (All products)		51	92	4	38	185	77,3	71.1	76	67.1	56.3	161	57.1	49.3

Appendix 6 – Relative detection levels: raw data

Raw meat – 8 H incubation time

E.coli O157:H7 Ad486

Level	Inoculation level	I.C.	Method	Negative	Positive	Total
0	/	/	Reference	6	0	6
			Alternative	6	0	6
			Total	12	0	12
1	0,2	[0;1]	Reference	4	2	6
			Alternative	4	2	6
			Total	8	4	12
2	0,4	[0;2]	Reference	3	3	6
			Alternative	3	3	6
			Total	6	6	12
3	0,8	[0;3]	Reference	2	4	6
			Alternative	3	3	6
			Total	5	7	12
4	2,1	[0;5]	Reference	0	6	6
			Alternative	0	6	6
			Total	0	12	12

Raw meat – 18 H incubation time

E.coli O157:H7 Ad486

Level	Inoculation level	I.C.	Method	Negative	Positive	Total
0	/	/	Reference	6	0	6
			Alternative	6	0	6
			Total	12	0	12
1	0,2	[0;1]	Reference	4	2	6
			Alternative	4	2	6
			Total	8	4	12
2	0,4	[0;2]	Reference	3	3	6
			Alternative	3	3	6
			Total	6	6	12
3	0,8	[0;3]	Reference	2	4	6
			Alternative	2	4	6
			Total	4	8	12
4	2,1	[0;5]	Reference	0	6	6
			Alternative	0	6	6
			Total	0	12	12

Raw milk

E.coli O157:H7 Ad686

Level	Inoculation level	I.C.	Method	Negative	Positive	Total
0	/	/	Reference	6	0	6
			Alternative	6	0	6
			Total	12	0	12
1	0,8	[0;3]	Reference	3	3	6
			Alternative	1	5	6
			Total	4	8	12
2	1,6	[0;4]	Reference	1	5	6
			Alternative	1	5	6
			Total	2	10	12
3	3,1	[0;7]	Reference	0	6	6
			Alternative	1	5	6
			Total	1	11	12
4	7,8	[3;4]	Reference	0	6	6
			Alternative	0	6	6
			Total	0	12	12

Raw spinach

E. coli O157:H7 Ad556

Level	Inoculation level	I.C.	Method	Negative	Positive	Total
0	/	/	Reference	6	0	6
			Alternative	6	0	6
			Total	12	0	12
1	0,3	[0;2]	Reference	4	2	6
			Alternative	4	2	6
			Total	8	4	12
2	0,6	[0;3]	Reference	2	4	6
			Alternative	4	2	6
			Total	6	6	12
3	1,3	[0;4]	Reference	4	2	6
			Alternative	0	6	6
			Total	4	8	12
4	3,2	[0;7]	Reference	1	5	6
			Alternative	1	5	6
			Total	2	10	12

Ground beef – 8 H incubation time
 Strain : E.coli O157:H7 Ad486
 Total flora enumeration: 5,8.10³ CFU/g

N° Sample	Level	Inoculation level	NF EN ISO 16654 method♦					3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method Pre-warmed BPW – 8 H at 41.5°C						
			IMS 6h		IMS 24h		Result	Positive /Total	Detection result MDS (MC)	Confirmation			Final result	Positive /Total
			CT SMAC	Chromagar O157	CT SMAC	Chromagar O157				CT SMAC streaking	CT SMAC IMS	Latex		
3164	0	/	st	st	-	-	-	0/6	-	-	/	/	-	0/6
3165			st	st	-	-	-		-	-	/	/	-	
3166			st	st	-	-	-		-	-	/	/	-	
3167			-	-	-	-	-		-	-	/	/	-	
3168			st	st	-	-	-		-	-	/	/	-	
3169			-	st	-	-	-		-	-	-	/	/	
3170	1	0,2	st	st	-	-	-	2/6	-	-	/	/	-	2/6
3171			st	st	-	-	-		+	+M	/	+/+	+	
3172			st	st	-	-	-		+	+m	/	+/+	+	
3173			-	-	-	-	-		-	-	/	/	-	
3174			+p	+p	/	/	+		-	-	/	/	-	
3175			+p	+M	/	/	+		-	-	/	/	-	
3176	2	0,4	st	st	-	-	-	3/6	+	+m	/	+/+	+	3/6
3177			-	-	-	-	-		+	+M	/	+/+	+	
3178			+p	+p	/	/	+		-	-	/	/	-	
3179			+p	+m	/	/	+		-(i)	-	/	/	-	
3180			-	-	-	-	-		-	-	/	/	-	
3181			+p	+M	/	/	+		+	+M	/	+/+	+	
3182	3	0,8	+p	+m	/	/	+	4/6	-	-	/	/	-	3/6
3183			-	-	-	-	-		+	+1/2	/	+/+	+	
3184			+p	+m	/	/	+		-	-	/	/	-	
3185			+p	+p	/	/	+		+	+1/2	/	+/+	+	
3186			-	-	-	-	-		+	+1/2	/	+/+	+	
3187			+p	+M	/	/	+		-/+	+m	/	+/+	-	
3188	4	2,1	+p	+M	/	/	+	6/6	+	+M	/	+/+	+	6/6
3189			+p	+M	/	/	+		+	+M	/	+/+	+	
3190			+p	+M	/	/	+		+	+M	/	+/+	+	
3191			+p	+M	/	/	+		+	+M	/	+/+	+	
3192			+p	+M	/	/	+		+	+M	/	+/+	+	
3193			+p	+M	/	/	+		+	+M	/	+/+	+	

Ground beef - - 18 H incubation time

Strain : E.coli O157:H7 Ad486

Total flora enumeration: 5,8.10³ CFU/g

N° Sample	Level	Inoculation level	NF EN ISO 16654 method♦					3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method Pre-warmed BPW – 18 H at 41.5°C						
			IMS 6h		IMS 24h		Result	Positive /Total	Detection result MDS(MC)	Confirmation			Final result	Positive /Total
			CT SMAC	Chromagar O157	CT SMAC	Chromagar O157				CT SMAC streaking	CT SMAC IMS	Latex		
3164	0	/	st	st	-	-	-	0/6	-	-	/	/	-	0/6
3165			st	st	-	-	-		-	-	/	/	-	
3166			st	st	-	-	-		-	-	/	/	-	
3167			-	-	-	-	-		-	-	/	/	-	
3168			st	st	-	-	-		-	-	/	/	-	
3169			-	st	-	-	-		-	-	-	/	/	
3170	1	0,2	st	st	-	-	-	2/6	-	-	/	/	-	2/6
3171			st	st	-	-	-		+	+m	/	+/+	+	
3172			st	st	-	-	-		+	+M	/	+/+	+	
3173			-	-	-	-	-		-	-	/	/	-	
3174			+p	+p	/	/	+		-	-	/	/	-	
3175			+p	+M	/	/	+		-	-	/	/	-	
3176	2	0,4	st	st	-	-	-	3/6	+	+M	/	+/+	+	3/6
3177			-	-	-	-	-		+	+M	/	+/+	+	
3178			+p	+p	/	/	+		-	-	/	/	-	
3179			+p	+m	/	/	+		-	-	/	/	-	
3180			-	-	-	-	-		-	-	/	/	-	
3181			+p	+M	/	/	+		+	+M	/	+/+	+	
3182	3	0,8	+p	+m	/	/	+	4/6	-	-	/	/	-	4/6
3183			-	-	-	-	-		+	+M	/	+/+	+	
3184			+p	+m	/	/	+		-	-	/	/	-	
3185			+p	+p	/	/	+		+	+M	/	+/+	+	
3186			-	-	-	-	-		+	+M	/	+/+	+	
3187			+p	+M	/	/	+		+	+M	/	+/+	+	
3188	4	2,1	+p	+M	/	/	+	6/6	+	+M	/	+/+	+	6/6
3189			+p	+M	/	/	+		+	+M	/	+/+	+	
3190			+p	+M	/	/	+		+	+M	/	+/+	+	
3191			+p	+M	/	/	+		+	+M	/	+/+	+	
3192			+p	+M	/	/	+		+	+M	/	+/+	+	
3193			+p	+M	/	/	+		+	+m	/	+/+	+	

♦ Analysis performed according to the COFRAC accreditation

ADRIA Développement

58/82

July 19, 2016

Summary Report (Version 0)**3M MDA E. coli O157 (including H7)**

Raw milk

Strain : E.coli O157:H7 Ad686

Total flora enumeration: 2,2.10⁵ CFU/g

N° Sample	Level	Level inoculation	NF EN ISO 16654 method♦					3M™ Molecular detection Assay <i>E. coli</i> O150 (including H7) method Pre-warmed BPW – 18 H at 41.5°C						
			IMS 6h		IMS 24h		Result	Positive /Total	Detection result	Confirmation			Final result	Positive /Total
			CT SMAC	Chromagar O157	CT SMAC	Chromagar O157				CT SMAC streaking	CT SMAC IMS	Latex (O157/H7)		
3064	0	/	-	-	-	-	-	0/6	-	-	/	/	-	0/6
3065			st	-	-	-	-		-	-	/	/	-	
3066			st	-	st	-	-		-	-	/	/	-	
3067			st	-	st	-	-		-	-	/	/	-	
3068			-	-	-	-	-		-	-	/	/	-	
3069			-	-	-	-	-		-	-	/	/	-	
3070	1	0,8	-	-	-	-	-	3/6	+	+m	/	+/+	+	5/6
3071			+p	+m	/	/	+		-	-	/	/	-	
3072			st	-	-	-	-		+	+m	/	+/+	+	
3073			+p	+p	/	/	+		+	+m	/	+/+	+	
3074			-	-	-	-	-		+	+1/2	/	+/+	+	
3075			+p	+M	/	/	+		+	+m	/	+/+	+	
3076	2	1,6	+m	+M	/	/	+	5/6	-	-	/	/	-	5/6
3077			+p	+M	/	/	+		+	+2col ni/+	/	+/+	+	
3078			st	st	+M	+M	+		+	+m	/	+/+	+	
3079			+p	+M	/	/	+		+	+m	/	+/+	+	
3080			st	-	-	-	-		+	+1/2	/	+/+	+	
3081			+p	+M	/	/	+		+	+m	/	+/+	+	
3082	3	3,1	+p	+M	/	/	+	6/6	-	-	/	/	-	5/6
3083			+m	+M	/	/	+		+	-	+m	+/+	+	
3084			+p	+M	/	/	+		+	+M	/	+/+	+	
3085			+p	+M	/	/	+		+	+m	/	+/+	+	
3086			+p	+p	/	/	+		+	+M	/	+/+	+	
3087			+p	+p	/	/	+		+	+M	/	+/+	+	
3088	4	7,8	+p	+M	/	/	+	6/6	+	+M	/	+/+	+	6/6
3089			+M	+M	/	/	+		+	+M	/	+/+	+	
3090			+M	+M	/	/	+		+	+M	/	+/+	+	
3091			+M	+M	/	/	+		+	+M	/	+/+	+	
3092			+p	+M	/	/	+		+	+M	/	+/+	+	
3093			+p	+M	/	/	+		+	+M	/	+/+	+	

♦ Analysis performed according to the COFRAC accreditation

ADRIA Développement

59/82

July 19, 2016

Summary Report (Version 0)**3M MDA E. coli O157 (including H7)**

Mesclun (mix of young shoots of vegetables)

Strain: E. coli O157:H7 Ad556

Total flora enumeration: 5,2.10⁶ CFU/g

N° Sample	Level	Inoculation level	NF EN ISO 16654 method♦						3M™ Molecular detection Assay <i>E. coli</i> O157 (including H7) method					
			IMS 6h		IMS 24h		Result	Positive /Total	Detection result	Confirmation			Final result	Positive /Total
			CT SMAC	Chromagar O157	CT SMAC	Chromagar O157				CT SMAC direct streaking	CT SMAC IMS	Latex		
888	0	0,0	+	-	-	-	-	0/6	-	-	/	/	-	0/6
889			-	-	-	-	-		-	-	/	/	-	
890			-	-	-	-	-		-	-	/	/	-	
891			-	-	-	-	-		-	-	/	/	-	
892			+	-	-	-	-		-	-	/	/	-	
893			-	-	-	-	-		-	-	/	/	-	
928	1	0,3	-	-	-	-	-	2/6	+	+	/	+	+	2/6
929			+	+	/	/	+		-	/	/	/	-	
930			-	-	-	-	-		-	/	/	/	-	
931			-	-	-	-	-		-	/	/	/	-	
932			+	+	/	/	+		+	+	/	+	+	
933			-	-	-	-	-		-	/	/	/	-	
934	2	0,6	-	-	-	-	-	4/6	-	/	/	/	-	2/6
935			+	+	/	/	+		-	/	/	/	-	
936			+	+	/	/	+		-	/	/	/	-	
937			-	-	-	-	-		+	+	/	+	+	
938			+	+	/	/	+		+	+	/	+	+	
939			+	+	/	/	+		-	/	/	/	-	
940	3	1,3	-	-	-	-	-	2/6	+	+	/	+	+	6/6
941			+	+	/	/	+		+	+	/	+	+	
942			-	-	-	-	-		+	+	/	+	+	
943			-	-	-	-	-		+	+	/	+	+	
944			-	-	-	-	-		+	+	/	+	+	
945			+	+	/	/	+		+	+	/	+	+	
946	4	3,2	+	+	/	/	+	5/6	+	+	/	+	+	5/6
947			+	+	/	/	+		-	/	/	/	-	
948			+	+	/	/	+		+	+	/	+	+	
949			+	+	/	/	+		+	+	/	+	+	
950			-	-	-	-	-		+	+	/	+	+	
951			+	+	/	/	+		+	+	/	+	+	

♦ Analysis performed according to the COFRAC accreditation

ADRIA Développement

60/82

July 19, 2016

Summary Report (Version 0)**3M MDA E. coli O157 (including H7)**

Appendix 7 – Inclusivity and exclusivity: raw data

POSITIVE STRAINS					Inoculation level (cfu/225ml BPW pre-warmed)	3M Molecular Detection System 8h at 41.5°C	Confirmatory tests		
Strains				Origin			CT-SMAC Characteristic colonies		
								O157	H7
1.	<i>Escherichia coli</i>	O157:H7	Ad552	Slaughterhouse	10	+	+	+	+
2.	<i>Escherichia coli</i>	O157:H7	Ad553	Slaughterhouse	11	+	+	+	+
3.	<i>Escherichia coli</i>	O157:H7	Ad554	Slaughterhouse	22	+	+	+	+
4.	<i>Escherichia coli</i>	O157:H7	Ad555	Slaughterhouse	15	+	+	+	+
5.	<i>Escherichia coli</i>	O157:H7	Ad556	Water purification	6	+	+	+	+
6.	<i>Escherichia coli</i>	O157:H7	Ad557	Water purification	1	+	+	+	+
7.	<i>Escherichia coli</i>	O157:H7	Ad558	Water purification	4	+	+	+	+
8.	<i>Escherichia coli</i>	O157:H7	Ad559	Ground beef	6	+	+	+	+
9.	<i>Escherichia coli</i>	O157:H7	Ad560	Ground beef	13	+	+	+	+
10.	<i>Escherichia coli</i>	O157:H7	Ad561	Ground beef	14	+	+	+	+
11.	<i>Escherichia coli</i>	O157:H7	Ad562	Ground beef	12	+	+	+	+
12.	<i>Escherichia coli</i>	O157:H7	Ad563	Ground beef	14	+	+	+	+
13.	<i>Escherichia coli</i>	O157:H7	Ad564	Ground beef	46	+	+	+	+
14.	<i>Escherichia coli</i>	O157:H7	Ad565	Ground beef	2	+	+	+	+
15.	<i>Escherichia coli</i>	O157:H7	Ad566	Ground beef	2	+	+	+	+
16.	<i>Escherichia coli</i>	O157:H7	Ad567	Slaughterhouse	10	+	+	+	+
17.	<i>Escherichia coli</i>	O157:H7	Ad568	Slaughterhouse	16	+	+	+	+
18.	<i>Escherichia coli</i>	O157:H7	Ad569	Slaughterhouse	20	+	+	+	+
19.	<i>Escherichia coli</i>	O157:H7	Ad570	Slaughterhouse	18	+	+	+	+
20.	<i>Escherichia coli</i>	O157:H7	Ad571	Feces	16	+	+	+	+
21.	<i>Escherichia coli</i>	O157:H7	Ad572	Feces	6	+	+	+	+
22.	<i>Escherichia coli</i>	O157:H7	Ad573	Feces	7	+	+	+	+
23.	<i>Escherichia coli</i>	O157:H7	Ad574	Feces	5	+	+	+	+
24.	<i>Escherichia coli</i>	O157:H7	Ad575	Feces	5	+	+	+	+
25.	<i>Escherichia coli</i>	O157:H7	Ad576	Feces	9	+	+	+	+
26.	<i>Escherichia coli</i>	O157:H7	Ad577	Feces	11	+	+	+	+
27.	<i>Escherichia coli</i>	O157:H7	Ad578	Feces	18	+	+	+	+
28.	<i>Escherichia coli</i>	O157:H7	Ad579	Feces	17	+	+	+	+
29.	<i>Escherichia coli</i>	O157:H7	Ad580	Feces	9	+	+	+	+
30.	<i>Escherichia coli</i>	O157:H7	Ad581	Feces	12	+	+	+	+

POSITIVE STRAINS					Inoculation level (cfu/225ml BPW pre-warmed)	3M Molecular Detection System 8h at 41.5°C	Confirmatory tests		
Strains				Origin			CT-SMAC Characteristic colonies		
								O157	H7
31.	<i>Escherichia coli</i>	O157:H7	Ad582	Feces	12	+	+	+	+
32.	<i>Escherichia coli</i>	O157:H7	Ad583	Ground beef	3	+	+	+	+
33.	<i>Escherichia coli</i>	O157:H7	Ad584	Ground beef	13	+	+	+	+
34.	<i>Escherichia coli</i>	O157:H7	Ad585	Ground beef	11	+	+	+	+
35.	<i>Escherichia coli</i>	O157:H7	Ad586	Ground beef	24	+	+	+	+
36.	<i>Escherichia coli</i>	O157:H7	Ad587	Ground beef	25	+	+	+	+
37.	<i>Escherichia coli</i>	O157:H7	Ad588	Ground beef	10	+	+	+	+
38.	<i>Escherichia coli</i>	O157:H7	Ad589	Ground beef	11	+	+	+	+
39.	<i>Escherichia coli</i>	O157:H7	Ad590	Ground beef	3	+	+	+	+
40.	<i>Escherichia coli</i>	O157:H7	Ad591	Ground beef	6	+	+	+	+
41.	<i>Escherichia coli</i>	O157:H7	EF190	Feces	18	+	+	+	+
42.	<i>Escherichia coli</i>	O157:H7	Ad686	Slaughterhouse	24	+	+	+	+
43.	<i>Escherichia coli</i>	O157:H7	CIP103571 (ATCC 35150)	Clinical origin	25	+	+	+	+
44.	<i>Escherichia coli</i>	O157:H7	ATCC 43888	/	15	+	+	+	+
45.	<i>Escherichia coli</i>	O157:H7	Ad485	Ground beef	3	+	+	+	+
46.	<i>Escherichia coli</i>	O157:H7	Ad486	Ground beef	7	+	+	+	+
47.	<i>Escherichia coli</i>	O157:H7	Ad487	Ground beef	10	+	+	+	+
48.	<i>Escherichia coli</i>	O157:H7	Ad488	Ground beef	10	+	+	+	+
49.	<i>Escherichia coli</i>	O157:H7	Ad489	Ground beef	23	+	+	+	+
50.	<i>Escherichia coli</i>	O157:H7	ATCC 700728	/	21	+	+	+	+
51.	<i>Escherichia coli</i>	O157	Ad524	Environment (dairy products)	8	+	- (sorbitol +)	+	-
52.	<i>Escherichia coli</i>	O157	Ad525	Feces	9	+	- (sorbitol +)	+	-
53.	<i>Escherichia coli</i>	O157	Ad526	Feces	5	+	- (sorbitol +)	+	-
54.	<i>Escherichia coli</i>	O157	Ad527	Clinical origin	4	+	- (sorbitol +)	+	-
55.	<i>Escherichia coli</i>	O157:H-	Ad535	/	12	+	- (sorbitol +)	+	-
56.	<i>Escherichia coli</i>	O157:H-	Ad536	/	8	+	- (sorbitol +)	+	-
57.	<i>Escherichia coli</i>	O157:H43	Ad517	Pork (USA)	14	+	- (sorbitol +)	+	-

NEGATIVE STRAINS					Inoculation level (cfu/ml BPW non pre-warmed)	3M Molecular Detection System 24h - 37°C	Confirmatory tests	
Strains				Origin			CT-SMAC Characteristic colonies	O157 & H7 latex test
1.	<i>Escherichia coli</i>	O92:H33	Ad503	Clinical origin (Mexico)	6,60E+05	-	-	/
2.	<i>Escherichia coli</i>	O3:H2	Ad504	Clinical origin (Chilli)	1,00E+05	-	-	/
3.	<i>Escherichia coli</i>	O78:H11	ATCC 35401	Human origin	3,40E+05	-	-	/
4.	<i>Escherichia coli</i>	O6:H6	Ad506	Human origin	3,10E+05	-	-	/
5.	<i>Escherichia coli</i>	O6:H10	Ad507	Clinical origin (Sweden)	4,80E+05	-	-	/
6.	<i>Escherichia coli</i>	O111:H21	Ad508	Clinical origin (USA)	3,30E+05	-	-	/
7.	<i>Escherichia coli</i>	O86:H43	Ad509	Animal origin (elephant USA)	3,40E+05	-	-	/
8.	<i>Escherichia coli</i>	O26:H11	Ad510	Clinical origin (USA)	4,80E+05	-	-	/
9.	<i>Escherichia coli</i>	O111:H8	Ad511	Clinical origin (USA)	3,60E+05	-	-	/
10.	<i>Escherichia coli</i>	O128:H2	Ad512	Clinical origin (USA)	6,00E+05	-	-	/
11.	<i>Escherichia coli</i>	O111:H2	Ad513	Clinical origin (UK)	7,70E+05	-	-	/
12.	<i>Escherichia coli</i>	O128:H7	Ad514	Clinical origin (USA)	6,40E+05	-	-	/
13.	<i>Escherichia coli</i>	O78:K80:H12	ATCC 43896	Human origin	3,80E+05	-	-	/
14.	<i>Escherichia coli</i>	O104:H21	Ad516	Clinical origin (USA)	3,70E+05	-	-	/
15.	<i>Escherichia coli</i>	O26	Ad980	Clinical origin	4,40E+05	-	-	/
16.	<i>Escherichia coli</i>	O55:H7	Ad518	Clinical origin (Sri Lanka)	4,30E+05	-	-	/
17.	<i>Escherichia coli</i>	O44:H18	Ad519	Clinical origin (Peru)	4,00E+05	-	-	/
18.	<i>Escherichia coli</i>	O127:H6	Ad520	Clinical origin (UK)	5,20E+05	-	+	O157-
19.	<i>Escherichia coli</i>	O55:H6	Ad521	Clinical origin (USA)	4,50E+05	-	+	O157-
20.	<i>Escherichia coli</i>	O18:K1:H7	Ad522	Clinical origin	4,80E+05	-	-	/
21.	<i>Citrobacter freundii</i>	/	25	Frozen raw spinach	4,50E+05	-	-	/
22.	<i>Citrobacter freundii</i>	/	104	Ground beef	3,30E+05	-	-	/
23.	<i>Escherichia vulneris</i>	/	127	Raw milk	7,30E+05	-	-	/
24.	<i>Hafnia alvei</i>	/	88	Bakery	4,80E+05	-	-	/

NEGATIVE STRAINS					Inoculation level (cfu/ml BPW non pre-warmed)	3M Molecular Detection System 24h - 37°C	Confirmatory tests	
Strains				Origin			CT-SMAC Characteristic colonies	O157 & H7 latex test
25.	<i>Hafnia alvei</i>	/	167	Sausage	2,60E+05	-	-	/
26.	<i>Pantoea spp.</i>	/	134	Pork	4,00E+04	-	-	/
27.	<i>Salmonella</i> Landau		Ad499	/	2,90E+05	-	-	/
28.	<i>Salmonella</i> Sternhauze		Ad500	/	2,60E+05	-	-	/
29.	<i>Salmonella</i> Urbana		Ad501	/	4,00E+05	-	-	/
30.	<i>Salmonella</i> Wayne		Ad502	/	3,20E+05	-	-	/
31.	<i>Escherichia coli</i>	O145	Ad983	Clinical origin	3,10E+05	-	-	/

Appendix 8 – Results obtained by the expert laboratory

Laboratory: R (ADRIA)

Aerobic mesophilic flora 1,5.10³/g

N°Sample	Reference method ISO 16654♦				Alternative method- 3M MDS E. coli O157 (including H7)				Agreement
	CT SMAC	CHROMagar O157	Confirmation	Final result	MDS result	CT SMAC	Latex	Final result	
R4	st	-	/	-	-	st	/	-	NA
R6	-	-	/	-	-	-	/	-	NA
R9	st	-	/	-	-	st	/	-	NA
R12	st	-	/	-	-	-	/	-	NA
R13	st	-	/	-	-	st	/	-	NA
R17	-	-	/	-	-	st	/	-	NA
R22	st	-	/	-	-	st	/	-	NA
R23	-	-	/	-	-	-	/	-	NA
R1	st	-	/	-	+	+	+	+	PD
R3	st	-	/	-	+	+	+	+	PD
R8	+	+	+	+	+	+	+	+	PA
R10	-	-	/	-	+	+	+	+	PD
R16	+	+	+	+	+	+	+	+	PA
R20	+	+	+	+	+	+	+	+	PA
R21	+	+	+	+	+	+	+	+	PA
R24	st	st	/	-	+	+	+	+	PD
R2	+	+	+	+	+	+	+	+	PA
R5	+	+	+	+	+	+	+	+	PA
R7	+	+	+	+	+	+	+	+	PA
R11	+	+	+	+	+	+	+	+	PA
R14	+	+	+	+	+	+	+	+	PA
R15	+	+	+	+	+	+	+	+	PA
R18	+	+	+	+	+	+	+	+	PA
R19	+	+	+	+	+	+	+	+	PA

st: sterile

♦ Analysis performed according to the COFRAC accreditation

Appendix 9 – Results obtained by each collaborator

Laboratory: **A**
 Aerobic mesophilic flora 1,9.10³/g

N°Sample	Reference method ISO 16654				Alternative method 3M MDS E.coli O157 (including H7)				Agreement
	CT SMAC	CHROMagar O157	Confirmation	Final result	MDS result	CT SMAC	Latex	Final result	
A4	-	-	/	-	-	/	/	-	NA
A6	-	-	/	-	-	/	/	-	NA
A9	-	-	/	-	-	/	/	-	NA
A12	-	-	/	-	-	/	/	-	NA
A13	-	-	/	-	-	/	/	-	NA
A17	-	-	/	-	-	/	/	-	NA
A22	-	-	/	-	-	/	/	-	NA
A23	-	-	/	-	-	/	/	-	NA
A1	+	+	+	+	+	+	+	+	PA
A3	-	-	/	-	+	+	+	+	PD
A8	-	-	/	-	+	+	+	+	PD
A10	+	+	+	+	+	+	+	+	PA
A16	+	+	+	+	+	+	+	+	PA
A20	+	+	+	+	-	- *	/	-	ND
A21	+	+	+	+	+	+	+	+	PA
A24	+	+	+	+	+	+	+	+	PA
A2	+	+	+	+	+	+	+	+	PA
A5	+	+	+	+	+	+	+	+	PA
A7	+	+	+	+	+	+	+	+	PA
A11	+	+	+	+	+	+	+	+	PA
A14	+	+	+	+	+	+	+	+	PA
A15	+	+	+	+	+	+	+	+	PA
A18	+	+	+	+	+	+	+	+	PA
A19	+	+	+	+	+	+	+	+	PA

* Sample A20(Alt): The streaking of BPW after one week storage didn't allow to recover the inoculated strain.

Laboratory:

B

Aerobic mesophilic flora

1,9.10²/g

N°Sample	Reference method ISO 16654				Alternative method -3M MDS E.coli O157 (including H7)				Agreement
	CT SMAC	CHROMagar O157	Confirmation	Final result	MDS result	CT SMAC	Latex	Final result	
B4	-	-	/	-	-	/	/	-	NA
B6	-	-	/	-	-	/	/	-	NA
B9	-	-	/	-	-	/	/	-	NA
B12	-	-	/	-	-	/	/	-	NA
B13	-	-	/	-	-	/	/	-	NA
B17	-	-	/	-	-	/	/	-	NA
B22	-	-	/	-	-	/	/	-	NA
B23	-	-	/	-	-	/	/	-	NA
B1	+	+	+	+	+	+	+	+	PA
B3	+	+	+	+	-	-	/	-	ND
B8	+	+	+	+	+	+	+	+	PA
B10	+	+	+	+	+	+	+	+	PA
B16	+	+	+	+	+	+	+	+	PA
B20	+	+	+	+	+	+	+	+	PA
B21	+	+	+	+	+	+	+	+	PA
B24	+	+	+	+	+	+	+	+	PA
B2	+	+	+	+	+	+	+	+	PA
B5	+	+	+	+	+	+	+	+	PA
B7	+	+	+	+	+	+	+	+	PA
B11	+	+	+	+	+	+	+	+	PA
B14	+	+	+	+	+	+	+	+	PA
B15	+	+	+	+	+	+	+	+	PA
B18	+	+	+	+	+	+	+	+	PA
B19	+	+	+	+	+	+	+	+	PA

Laboratory:

C

Aerobic mesophilic flora

1,3.10³/g

N°Sample	Reference method ISO 16654				Alternative method 3M MDS E.coli O157 (including H7)				Agreement
	CT SMAC	CHROMagar O157	Confir- mation	Final result	MDS result	CT SMAC	Latex	Final result	
C4	-	-	/	-	+/-/-/-/ (low ct values)	-	/	-	PPNA
C6	-	-	/	-	-	/	/	-	NA
C9	-	-	/	-	-	/	/	-	NA
C12	-	-	/	-	-	/	/	-	NA
C13	-	-	/	-	-	/	/	-	NA
C17	-	-	/	-	-	/	/	-	NA
C22	-	-	/	-	-	/	/	-	NA
C23	-	-	/	-	-	/	/	-	NA
C1	+	+	+	+	+	+	+	+	PA
C3	+	+	+	+	+	+	+	+	PA
C8	+	+	+	+	-/-/-	-	/	-	ND
C10	+	+	+	+	+	+	+	+	PA
C16	+	+	+	+	+	+	+	+	PA
C20	+	+	+	+	+	+	+	+	PA
C21	+	+	+	+	+	+	+	+	PA
C24	+	+	+	+	+	+	+	+	PA
C2	+	+	+	+	+	+	+	+	PA
C5	+	+	+	+	+	+	+	+	PA
C7	+	+	+	+	+	+	+	+	PA
C11	+	+	+	+	+	+	+	+	PA
C14	+	+	+	+	+	+	+	+	PA
C15	+	+	+	+	+	+	+	+	PA
C18	+	+	+	+	+	+	+	+	PA
C19	+	+	+	+	+	+	+	+	PA

Laboratory:

D

Aerobic mesophilic flora

7,8.10²/g

N°Sample	Reference method ISO 16654				Alternative method- 3M MDS E.coli O157 (including H7)				Agreement
	CT SMAC	CHROMagar O157	Confir- mation	Final result	MDS result	CT SMAC	Latex	Final result	
D4	-	-	/	-	-	/	/	-	NA
D6	-	-	/	-	-	/	/	-	NA
D9	-	-	/	-	-	/	/	-	NA
D12	-	-	/	-	-	/	/	-	NA
D13	-	-	/	-	-	/	/	-	NA
D17	-	-	/	-	-	/	/	-	NA
D22	-	-	/	-	-	/	/	-	NA
D23	-	-	/	-	-	/	/	-	NA
D1	+	+	+	+	+	+	+	+	PA
D3	+	+	+	+	+	+	+	+	PA
D8	+	+	+	+	+	+	+	+	PA
D10	+	+	+	+	+	+	+	+	PA
D16	+	+	+	+	+	+	+	+	PA
D20	+	+	+	+	+	+	+	+	PA
D21	+	+	+	+	+	+	+	+	PA
D24	+	+	+	+	+	+	+	+	PA
D2	+	+	+	+	+	+	+	+	PA
D5	+	+	+	+	+	+	+	+	PA
D7	+	+	+	+	+	+	+	+	PA
D11	+	+	+	+	+	+	+	+	PA
D14	+	+	+	+	+	+	+	+	PA
D15	+	+	+	+	+	+	+	+	PA
D18	+	+	+	+	+	+	+	+	PA
D19	+	+	+	+	+	+	+	+	PA

Laboratory:

E

Aerobic mesophilic flora

2,3.10³/g

N° Sample	Reference method ISO 16654				Alternative method 3M MDS E.coli O157(including H7)				Agreement
	CT SMAC	CHROMagar O157	Confirmation	Final result	MDS result	CT SMAC	Latex	Final result	
E4	-	-	/	-	-	/	/	-	NA
E6	-	-	/	-	-	/	/	-	NA
E9	+	+	+ (Latex Auto ADRIA) **	+(-)	-	/	/	-	NA
E12	-	-	/	-	-	/	/	-	NA
E13	-	-	/	-	+	+	+(ADRIA: Latex -) **	-	PPNA
E17	-	-	/	-	-	/	/	-	NA
E22	-	-	/	-	-	/	/	-	NA
E23	-	-	/	-	-	/	/	-	NA
E1	+	+	+	+	+	+	+	+	PA
E3	+	+	+	+	+	+	+	+	PA
E8	+	+	+	+	-	- *	/	-	ND
E10	+	+	+	+	+	+	+(ADRIA: Latex+) **	+	PA
E16	+	+	+	+	+	+	+	+	PA
E20	+	+	+	+	+	+	+	+	PA
E21	+	+	+	+	+	+	+	+	PA
E24	+	+	+	+	+	+	+	+	PA
E2	+	+	+	+	+	+	+	+	PA
E5	+	+	+	+	+	+	+	+	PA
E7	+	+	+	+	+	+	+(Latex+ ADRIA) **	+	PA
E11	+	+	+	+	+	+	+	+	PA
E14	+	+	+	+	+	+	+	+	PA
E15	+	+	+	+	+	+	+	+	PA
E18	+	+	+	+	+	+	+	+	PA
E19	+	+	+	+	+	+	+	+	PA

* Sample E8: The streaking of BPW after BPW storage didn't allow to recover the inoculated strains.

** Samples E9, E13, E10 and E7: The isolates were tested twice in ADRIA.

Laboratory:

F

Aerobic mesophilic flora

4,7.10²/g

N° Sample	Reference method ISO 16654				Alternative method 3M MDS E. coli O157(including H7)				Agreement
	CT SMAC	CHROMagar O157	Confirmation	Final result	MDS result	CT SMAC	Latex	Final result	
F4	-	-	/	-	-	+ (ADRIA: +/-)	+ (ADRIA: auto+)	-	NA
F6	+(ADRIA:-)	+	+ (ADRIA: +)	+	-	-	/	-	ND
F9	+(ADRIA:-)	-	+ (+ADRIA: +)	+	-	-	/	-	ND
F12	-	-	/	-	-	-	/	-	NA
F13	-	-	/	-	-	+ (few colonies)	+ (ADRIA:-)	-	NA
F17	-	-	/	-	-	-	/	-	NA
F22	-	-	/	-	-	-	/	-	NA
F23	-	-	/	-	-	-	/	-	NA
F1	+	+	+	+	+	+	+	+	PA
F3	+	+	+	+	+	+	+	+	PA
F8	+	+	+	+	-/+ *	+	+	-	ND
F10	+	+	+	+	-/- *	+ (few colonies)	+	-	ND
F16	-	-	/	-	-/+ *	+	+	-	NA
F20	+	+	+	+	-/- *	-	/	-	ND
F21	+	+	+	+	-/+ *	-	/	-	ND
F24	+	+	+	+	-/- *	+	+	-	ND
F2	+	+	+	+	+	+	+	+	PA
F5	+	+	+	+	+	+	+	+	PA
F7	+	+	+	+	+	+	+	+	PA
F11	+	+	+	+	+	+	+	+	PA
F14	+	+	+	+	+	+	+	+	PA
F15	+	+	+	+	+	+	+	+	PA
F18	+	+	+	+	+	+	+	+	PA
F19	+	+	+	+	+	+	+	+	PA

* Samples F8-F10-F16-F20-F21-F24: New lysates were tested after 1 week storage of BPW at 4°C. Confirmations were also run by streaking onto CT-SMAC

Streaking performed at ADRIA on BPW provided by the Lab

Due to the observed unexpected data and the additional testing results, it was decided to exclude this lab.

Laboratory:

G

Aerobic mesophilic flora

2,4.10³/g

N°Sample	Reference method ISO 16654				Alternative method- 3M MDS E.coli O157 (including H7)				Agreement
	CT SMAC	CHROMagar O157	Confirmation	Final result	MDS result	CT SMAC	Latex	Final result	
G4	-	-	/	-	-	/	/	-	NA
G6	-	-	/	-	-	/	/	-	NA
G9	+	+	+(ADRIA: O157+/H7+) *	+	-	/	/	-	ND
G12	-	-	/	-	-	/	/	-	NA
G13	-	-	/	-	-	/	/	-	NA
G17	-	-	/	-	-	/	/	-	NA
G22	-	-	/	-	-	/	/	-	NA
G23	-	+	-	-	-	/	/	-	NA
G1	+	+	+	+	+	+	+	+	PA
G3	+	+	+	+	+	+	+	+	PA
G8	+	+	+	+	+	+	+	+	PA
G10	+	+	+	+	+	+	+	+	PA
G16	-	-	/	-	+	+	+	+	PD
G20	+	+	+	+	+	+	+	+	PA
G21	+	+	+	+	+	+	+	+	PA
G24	+	+	+	+	+	+	+	+	PA
G2	+	+	+	+	+	+	+	+	PA
G5	+	+	+	+	+	+	+	+	PA
G7	+	+	+	+	+	+	+	+	PA
G11	+	+	+	+	+	+	+	+	PA
G14	+	+	+	+	+	+	+	+	PA
G15	+	+	+	+	+	+	+	+	PA
G18	+	+	+	+	+	+	+	+	PA
G19	+	+	+	+	+	+	+	+	PA

* The additional test done in ADRIA showed as well a positive reaction.

Laboratory:

H

Aerobic mesophilic flora : $1,5 \cdot 10^3/g$

N°Sample	Reference method ISO 16654				Alternative method- 3M MDS E.coli O157(including H7)				Agreement
	CT SMAC	CHROMagar O157	Confirmation	Final result	MDS result	CT SMAC	Latex	Final result	
H4	-	-	/	-	-	/	/	-	NA
H6	-	-	/	-	-	/	/	-	NA
H9	-	-	/	-	-	/	/	-	NA
H12	-	-	/	-	-	/	/	-	NA
H13	-	-	/	-	-	/	/	-	NA
H17	-	-	/	-	-	/	/	-	NA
H22	-	-	/	-	-	/	/	-	NA
H23	-	-	/	-	-	/	/	-	NA
H1	+	+	+	+	+	+	+	+	PA
H3	+	+	+	+	+	+	+	+	PA
H8	+	+	+	+	+	+	+	+	PA
H10	+	+	+	+	+	+	+	+	PA
H16	+	-	+	+	+	+	+	+	PA
H20	-	-	/	-	+	+	+	+	PD
H21	+	+	+	+	+	+	+	+	PA
H24	+	+	+	+	+	+	+	+	PA
H2	+	+	+	+	+	+	+	+	PA
H5	+	+	+	+	+	+	+	+	PA
H7	+	+	+	+	+	+	+	+	PA
H11	+	+	+	+	+	+	+	+	PA
H14	+	+	+	+	+	+	+	+	PA
H15	+	+	+	+	+	+	+	+	PA
H18	+	+	+	+	+	+	+	+	PA
H19	+	+	+	+	+	+	+	+	PA

Laboratory: I

Aerobic mesophilic flora 1,7.10³/g

N°Sample	Reference method ISO 16654				Alternative method- 3M MDS E.coli O157 (including H7)				Agreement
	CT SMAC	CHROMagar O157	Confirmation	Final result	MDS result	CT SMAC	Latex	Final result	
I4	-	-	/	-	-	-	/	-	NA
I6	+	+	+	+	-	-	/	-	ND
I9	-	-	/	-	-	-	/	-	NA
I12	-	-	/	-	-	-	/	-	NA
I13	-	-	/	-	-	-	/	-	NA
I17	-	-	/	-	-	-	/	-	NA
I22	-	-	/	-	-	-	/	-	NA
I23	-	-	/	-	-	-	/	-	NA
I1	+	+	+	+	+	+	+	+	PA
I3	+	+	+	+	+	+	+	+	PA
I8	+	+	+	+	+	+	+	+	PA
I10	+	+	+	+	+	+	+	+	PA
I16	-	-	/	-	+	+	+	+	PD
I20	+	+	+	+	+	+	+	+	PA
I21	+	+	+	+	+	+	+	+	PA
I24	+	+	+	+	+	+	+	+	PA
I2	+	+	+	+	+	+	+	+	PA
I5	+	+	+	+	+	+	+	+	PA
I7	+	+	+	+	+	+	+	+	PA
I11	+	+	+	+	+	+	+	+	PA
I14	+	+	+	+	+	+	+	+	PA
I15	+	+	+	+	+	+	+	+	PA
I18	+	+	+	+	+	+	+	+	PA
I19	+	+	+	+	+	+	+	+	PA

Laboratory:**J**

Aerobic mesophilic flora

7,8.10²/g

N°Sample	Reference method ISO 16654				Alternative method- 3M MDS E.coli O157 (including H7)				Agreement
	CT SMAC	CHROMagar O157	Confirmation	Final result	MDS result	CT SMAC	Latex	Final result	
J4	+	-	-	-	-	/	/	-	NA
J6	-	-	/	-	-	/	/	-	NA
J9	-	-	/	-	-	/	/	-	NA
J12	+/- *	+/- *	+/- *	+	-	/	/	-	ND
J13	-	-	/	-	-	/	/	-	NA
J17	-	-	/	-	-	/	/	-	NA
J22	-	-	/	-	-	/	/	-	NA
J23	-	-	/	-	-	/	/	-	NA
J1	+	+	+	+	-	+ **	+ **	-	ND
J3	+	+	+	+	+	+	+	+	PA
J8	+	+	+	+	+	+	+	+	PA
J10	+	+	+	+	-	+ **	+ **	-	ND
J16	+	+	+	+	+	+	+	+	PA
J20	+	-	+	+	+	+	+	+	PA
J21	+	+	+	+	+	+	+	+	PA
J24	+	+	+	+	+	+	+	+	PA
J2	+	+	+	+	+	+	+	+	PA
J5	+	+	+	+	+	+	+	+	PA
J7	+	+	+	+	+	+	+	+	PA
J11	+	+	+	+	+	+	+	+	PA
J14	+	+	+	+	+	+	+	+	PA
J15	+	+	+	+	+	+	+	+	PA
J18	+	+	+	+	+	+	+	+	PA
J19	+	+	+	+	+	+	+	+	PA

* Sample J12 (Ref): A second IMS after 1 week storage of the mTSB gave a negative result..

** Samples J1 and J10 (Alt): Streaking were done twice according to ADRIA recommendations. When coming back again to the lab, it was then not possible anymore to run additional MDS.

Laboratory:

K

Aerobic mesophilic flora 2,5.10³/g

N°Sample	Reference method ISO 16654				Alternative method- 3M MDS E.coli O157 (including H7)				Agreement
	CT SMAC	CHROMagar O157	Confirmation	Final result	MDS result	CT SMAC	Latex	Final result	
K4	-	-	/	-	-	/	/	-	NA
K6	+	-	+**	+	-	+(IMS)*	+	-	FN
K9	+	-	+**	+	-	/	/	-	ND
K12	-	-	/	-	-	/	/	-	NA
K13	-	-	/	-	-	/	/	-	NA
K17	-	-	/	-	-	/	/	-	NA
K22	-	-	/	-	-	/	/	-	NA
K23	-	-	/	-	-	/	/	-	NA
K1	+	+	+	+	+	+	+	+	PA
K3	+	+	+	+	-	+*	+	-	ND
K8	+	+	+	+	+	+	+	+	PA
K10	+	+	+	+	+	+	+	+	PA
K16	+	+	+	+	-	/	/	-	ND
K20	+	+	+	+	-	+(IMS)*	+	-	ND
K21	+	+	+	+	-	+(IMS)*	+	-	ND
K24	+	+	+	+	+	+	+	+	PA
K2	+	+	+	+	+	+	+	+	PA
K5	+	+	+	+	+	+	+	+	PA
K7	+	+	+	+	+	+	+	+	PA
K11	+	+	+	+	+	+	+	+	PA
K14	+	+	+	+	+	+	+	+	PA
K15	+	+	+	+	+	+	+	+	PA
K18	+	+	+	+	-	+*	+	-	ND
K19	+	+	+	+	-	+*	+	-	ND

* Samples K3-K6-K18-K19-K20-K21: Confirmatory tests were realized after 1 week storage of BPW. When coming back to the lab, it was too late to repeat the assays related to the unexpected results.

** Samples K6 and K9 (ref: Isolates from were not available for additional testing by ADRIA.

Due to the observed unexpected data, and the impossibility to run further testing to explain the observed results, it was decided to exclude that Lab.

Laboratory:

N

Aerobic mesophilic flora

1,5.10³/g

N°Sample	Reference method ISO 16654				Alternative method- 3M MDS E.coli O157 (including H7)				Agreement
	CT SMAC	CHROMagar O157	Confirmation	Final result	MDS result	CT SMAC	Latex	Final result	
N4	-	-	/	-	-	/	/	-	NA
N6	-	-	/	-	-	/	/	-	NA
N9	-	-	/	-	-	/	/	-	NA
N12	-	-	/	-	-	/	/	-	NA
N13	-	-	/	-	-	/	/	-	NA
N17	-	-	/	-	-	/	/	-	NA
N22	-	-	/	-	-	/	/	-	NA
N23	-	-	/	-	-	/	/	-	NA
N1	+	+	+	+	+	+	+	+	PA
N3	+	+	+	+	+	+	+	+	PA
N8	+	+	+	+	+	+	+	+	PA
N10	+	+	+	+	+	+	+	+	PA
N16	-	-	/	-	+	+	+	+	PD
N20	+	+	+	+	+	+	+	+	PA
N21	+	+	+	+	+	+	+	+	PA
N24	+	+	+	+	+	+	+	+	PA
N2	+	+	+	+	+	+	+	+	PA
N5	+	+	+	+	+	+	+	+	PA
N7	+	+	+	+	+	+	+	+	PA
N11	+	+	+	+	+	+	+	+	PA
N14	+	+	+	+	+	+	+	+	PA
N15	+	+	+	+	+	+	+	+	PA
N18	+	+	+	+	+	+	+	+	PA
N19	+	+	+	+	+	+	+	+	PA

Laboratory: **O**Aerobic mesophilic flora 1,7.10³/g

N°Sample	Reference method ISO 16654				Alternative method- 3M MDS E.coli O157 (including H7)				Agreement
	CT SMAC	CHROMagar O157	Confirmation	Final result	MDS result	CT SMAC	Latex	Final result	
O4	-	-	/	-	-	/	/	-	NA
O6	+	+	+	+	+	-/- (IMS)*	/	-	PPND
O9	-	-	/	-	-	/	/	-	NA
O12	-	-	/	-	-	/	/	-	NA
O13	-	-	/	-	-	/	/	-	NA
O17	-	-	/	-	-	/	/	-	NA
O22	-	-	/	-	-	/	/	-	NA
O23	-	-	/	-	-	/	/	-	NA
O1	+	+	+	+	-	-	/	-	ND
O3	+	+	+	+	+	-/- (IMS)*	/	-	PPND
O8	+	+	+	+	+	+	+	+	PA
O10	+	+	+	+	+	+	+	+	PA
O16	+	+	+	+	+	+	+	+	PA
O20	+	+	+	+	-	-/- (IMS)*	/	-	ND
O21	+	+	+	+	-	-/- (IMS)*	/	-	ND
O24	+	+	+	+	+	+	+	+	PA
O2	+	+	+	+	+	+	+	+	PA
O5	+	+	+	+	+	+	+	+	PA
O7	+	+	+	+	+	+	+	+	PA
O11	+	+	+	+	+	+	+	+	PA
O14	+	+	+	+	+	+	+	+	PA
O15	+	+	+	+	+	+	+	+	PA
O18	+	+	+	+	+	+	+	+	PA
O19	+	+	+	+	+	+	+	+	PA

* Samples O3-O6-O20-O21: The Lab certified that the IMS were negative by running an IMS. But, despite many exchanges with them, the Lab. didn't provide additional test results, as well as the MDS reaction curves.

Due to the observed unexpected data, and the impossibility to run further testing to explain the observed results, it was decided to exclude that Lab.

Laboratory:

P

Aerobic mesophilic flora

1,4.10³/g

N°Sample	Reference method ISO 16654				Alternative method- 3M MDS E.coli O157 (including H7)				Agreement
	CT SMAC	CHROMagar O157	Confirmation	Final result	MDS result	CT SMAC	Latex	Final result	
P4	-	-	/	-	-	/	/	-	NA
P6	-	-	/	-	-	/	/	-	NA
P9	-	-	/	-	-	/	/	-	NA
P12	+	+	+	+	-	/	/	-	ND
P13	-	-	/	-	-	/	/	-	NA
P17	-	-	/	-	-	/	/	-	NA
P22	-	-	/	-	-	/	/	-	NA
P23	-	-	/	-	-	/	/	-	NA
P1	+	+	+	+	+	+	+	+	PA
P3	-	-	/	-	+	+	+	+	PD
P8	+	+	+	+	+	+	+	+	PA
P10	+	+	+	+	+	+	+	+	PA
P16	+	+	+	+	+	+	+	+	PA
P20	+	-	+	+	+	+	+	+	PA
P21	-	-	/	-	+	+	+	+	PD
P24	-	-	/	-	+	+	+	+	PD
P2	+	+	+	+	+	+	+	+	PA
P5	+	+	+	+	+	+	+	+	PA
P7	+	+	+	+	+	+	+	+	PA
P11	+	+	+	+	+	+	+	+	PA
P14	+	+	+	+	+	+	+	+	PA
P15	+	+	+	+	+	+	+	+	PA
P18	+	+	+	+	+	+	+	+	PA
P19	+	+	+	+	+	+	+	+	PA

Laboratory:

Q

Aerobic mesophilic flora

2,1.10³/g

N° Sample	Reference method ISO 16654				Alternative method 3M MDS E.coli O157(including H7)				Agree- ment
	CT SMAC	CHROMagar O157	Confirmation	Final result	MDS result	CT SMAC	Latex	Final result	
Q4	+	-	+ (- ADRIA) *	+ (-)*	-	-	/	-	NA
Q6	+	-	+ (- ADRIA) *	+ (-)*	-	-	/	-	NA
Q9	-	-	-	-	-	-	/	-	NA
Q12	+	+	+ (- ADRIA) *	+ (-)*	-	-	/	-	NA
Q13	-	-	-	-	-	-	/	-	NA
Q17	+	+	+ (- ADRIA) *	+ (-)*	-	-	/	-	NA
Q22	-	-	-	-	-	-	/	-	NA
Q23	-	-	-	-	-	-	/	-	NA
Q1	+	+	+	+	+	+	+	+	PA
Q3	-	-	-	-	+	+	+	+	PD
Q8	+	+	+	+	+	+	+	+	PA
Q10	+	+	+	+	+	+	+	+	PA
Q16	+	+	+	+	-	-	/	-	ND
Q20	+	+	+	+	+	+	+	+	PA
Q21	+	+	+	+	+	+	+	+	PA
Q24	+	+	+	+	+	+	+	+	PA
Q2	+	+	+	+	+	+	+	+	PA
Q5	+	+	+	+	+	+	+	+	PA
Q7	+	+	+	+	+	+	+	+	PA
Q11	+	+	+	+	+	+	+	+	PA
Q14	+	+	+	+	+	+	+	+	PA
Q15	+	+	+	+	+	+	+	+	PA
Q18	+	+	+	+	+	+	+	+	PA
Q19	+	+	+	+	+	+	+	+	PA

* Additional testing done in ADRIA provided negative reaction test.

Appendix 10 – Specificity and sensitivity

Reference method positive results

Laboratory	Contamination level					
	L0		L1		L2	
	Obtained	Samples number	Obtained	Samples number	Obtained	Samples number
A	0	8	6	8	8	8
B	0	8	8	8	8	8
C	0	8	8	8	8	8
D	0	8	8	8	8	8
E	1	8	8	8	8	8
G	1	8	7	8	8	8
H	0	8	7	8	8	8
I	1	8	7	8	8	8
J	1	8	8	8	8	8
N	0	8	7	8	8	8
O	1	8	8	8	8	8
P	1	8	5	8	8	8
Total	6	96	87	96	96	96

Alternative method positive results

Laboratory	Contamination level					
	L0		L1		L2	
	Obtained	Samples number	Obtained	Samples number	Obtained	Samples number
A	0	8	7	8	8	8
B	0	8	7	8	8	8
C	0	8	7	8	8	8
D	0	8	8	8	8	8
E	1	8	7	8	8	8
G	0	8	8	8	8	8
H	0	8	8	8	8	8
I	0	8	8	8	8	8
J	0	8	6	8	8	8
N	0	8	8	8	8	8
O	0	8	4	8	8	8
P	0	8	8	8	8	8
Total	1	96	86	96	96	96

**Appendix 11 – Paired results of the alternative
and reference methods for each level**

L0	Reference method		Total	PPNA = 2
Alternative method	+	-		
+	0	0	0	
-	4	92	96	
Total	4	92	96	
AC=	95,8	LCL=	91.8	

L1	Reference method		Total
Alternative method	+	-	
+	79	10	89
-	7	0	7
Total	86	10	96
AC=	82,3	LCL=	74.5

L2	Reference method		Total
Alternative method	+	-	
+	96	0	96
-	0	0	0
Total	96	0	96
AC=	100,0	LCL=	100.0

L1+L2	Reference method		Total
Alternative method	+	-	
+	175	10	185
-	7	0	7
Total	182	10	192
AC=	91,1	LCL=	87.0

L0+L1+L2	Reference method		Total	PPNA = 2
Alternative method	+	-		
+	175	10	185	
-	11	92	103	
Total	186	102	288	
AC=	92,7	LCL=	89.6	