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**Validation study according to the ISO 16140 standard:
Summary report**

**ISO 16140 validation of
the MicroSEQ[®] *E. coli* O157:H7
for the detection of *Escherichia coli* O157:H7**

Qualitative methods

This report includes 53 pages, with 4 annexes.

Only copies including the totality of this report are authorised.

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

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MicroSEQ[®] *E. coli* O157:H7 (Version 0)

April 13, 2011

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Quality Assurance documents related to this study can be consulted upon request by Life Technologies.

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

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- ✓ **Expert laboratory :** ADRIA Développement
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- ✓ **Studied method:** Validation of the MicroSEQ® *E. coli* O157:H7
for the detection of *Escherichia coli* O157:H7

- ✓ **Validation standard:** 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
Escherichia coli O157

- ✓ **Products categories:** Raw beef meat and raw vegetables

♦ Analysis performed according to the COFRAC accreditation

1 AIM OF THE STUDY

The validation study of MicroSEQ® *E. coli* O157:H7 method for the detection of *E. coli* O157:H7 in raw ground beef and raw vegetables were performed according to the ISO 16140 protocol.

Five criteria were evaluated during the validation study:

method comparison study:

- practicability,
- inclusivity and the exclusivity,
- relative detection limit,
- relative accuracy, relative sensitivity and relative specificity.

interlaboratory study

2 INTRODUCTION

2.1 Alternative method protocol

The alternative method protocols were adapted to the food product being analysed, the flow diagrams are presented in annex 1:

Category	Sample amount	Enrichment broth	Incubation time	Sample preparation ¹
Raw beef meat	25 g d 1/10	Prewarmed BHI	6 h – 8 h 42°C	PrepSEQ NA Extraction Kit (automated) (= MagMAX) &
	25 g d 1/10	BPW	16 h – 20 h 42°C	
Vegetables	25 g d 1/10	Prewarmed BHI	6 h - 8 h 42°C	PrepSEQ Rapid Spin Sample Preparation kit (manual)* ²
	25 g d 1/10	BPW	16 h – 20 h 42°C	

¹ Both sample prep protocols were evaluated during the expert study, PrepSEQ NA and PrepSEQ Rapid Spin.

² Proteinase K was added during the sample prep step for the raw beef meat analyses

The results were confirmed by streaking the enrichment broth on CT-SMAC or ChromID O157:H7 agar plates, and by performing latex tests on characteristic colonies isolated on Nutrient Agar. If the results were not confirmed using these simple tests, an IMS step was performed before streaking 50 µl on CT-SMAC or ChromID O157:H7.

During the study, for the protocol based on a 6 h – 8 h enrichment time, fifteen to thirty minutes were required between the two following steps:

- addition of the prewarmed enrichment broth to the stomacher bag,
- incubation of the enrichment at 42°C.

2.2 Application

Raw beef meat and raw vegetables

2.3 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 Annex 2).

3 METHODS COMPARISON STUDY

3.1 Relative accuracy, relative specificity and relative sensitivity

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

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

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

3.1.1 Number and nature of samples

Two categories were studied, with the following types:

- raw beef meat: fresh, frozen, with and without additives,
- raw vegetable: fresh, frozen, under modified atmosphere.

The breakdown per food category, food type and protocol is shown below:

Enrichment protocol	Extraction protocol	Category	Type	Positive	Negative	Total	
A	Rapid Spin	Beef meat products	Fresh	11	13	24	
			Frozen	9	16	25	
			with additives	10	10	20	
			Total	30	39	69	
		Vegetables	Fresh	11	11	22	
			Frozen	9	11	20	
			under MAP	10	10	20	
			Total	30	32	62	
	Total				60	71	131
	MagMAX	Beef meat products	Fresh	11	13	24	
			Frozen	10	15	25	
			with additives	11	9	20	
			Total	32	37	69	
		Vegetables	Fresh	11	11	22	
Frozen			9	11	20		
under MAP			10	10	20		
Total			30	32	62		
Total				62	69	131	
B	Rapid Spin	Beef meat products	Fresh	11	13	24	
			Frozen	11	14	25	
			with additives	10	9	19	
			Total	32	36	68	
		Vegetables	Fresh	12	10	22	
			Frozen	8	11	19	
			under MAP	10	10	20	
			Total	30	31	61	
	Total				62	67	129
	MagMAX	Beef meat products	Fresh	11	13	24	
			Frozen	11	14	25	
			with additives	10	9	19	
			Total	32	36	68	
		Vegetables	Fresh	12	10	22	
Frozen			8	11	19		
under MAP			10	10	20		
Total			30	31	61		
Total				62	67	129	

131 samples were analysed with the protocol A: 69 beef meat samples and 62 vegetables samples.

129 samples were analysed with the protocol B: 68 beef meat samples and 61 vegetables samples.

3.1.2 Artificial contamination of samples

Artificial contaminations were realised by spiking. For sample spiking, various injury protocols were used. Cell injury efficiency was evaluated by enumerating the pure culture on non selective and selective agars, i.e. TSYEA and CT-SMAC agar.

Note that naturally contaminated samples were analysed, given three positive results by the protocol A and four by the protocol B. The difference is probably due to sampling heterogeneity.

3.1.3 Confirmatory tests

During the validation study, confirmations were realised by streaking 50 µl of enrichment broths on CT SMAC and ChromID O157:H7 agar plates and by performing IMS with 1 ml enrichment broth and then streaking 50 µl on CT SMAC and ChromID O157:H7. All the confirmatory tests were performed for beef meat analyses. During the analyses of vegetables, the IMS step was done only when necessary, i.e. when negative confirmatory test results were observed by streaking.

3.1.4 Test results

Raw data per category are given in annex 3.

Note that 3 PCR replicates were done when disagreements were observed between the tested alternative method protocols.

**Table 1 – Paired results of the reference and alternative methods
(taking into account all the confirmation protocols)**

BEEF MEAT			
Protocol A (25 g / 6 hour enrichment)			
	Responses	Reference method positive (R+)	Reference method negative (R-)
Rapid Spin	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 11	Positive deviation (R-/A+) PD = 11
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 8	Negative agreement (A-/R-) NA = 39
MagMAX	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 14	Positive deviation (R-/A+) PD = 13
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 5 (PPNC = 1)	Negative agreement (A-/R-) NA = 37 (PPNC = 2)
Protocol B (25 g / 16 hour enrichment)			
	Responses	Reference method positive (R+)	Reference method negative (R-)
Rapid Spin	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 17	Positive deviation (R-/A+) PD = 14
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 1	Negative agreement (A-/R-) NA = 36
MagMAX	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 17	Positive deviation (R-/A+) PD = 14
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 1	Negative agreement (A-/R-) NA = 36

PPNC: positive presumptive non confirmed sample (samples n° 5155, 5157, 5193)

VEGETABLE PRODUCTS			
Protocol A (25 g)			
	Responses	Reference method positive (R+)	Reference method negative (R-)
Rapid Spin	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 17	Positive deviation (R-/A+) PD = 13
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 0	Negative agreement (A-/R-) NA = 32
MagMAX	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 16	Positive deviation (R-/A+) PD = 13
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 1	Negative agreement (A-/R-) NA = 32
Protocol B (25 g)			
	Responses	Reference method positive (R+)	Reference method negative (R-)
Rapid Spin	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 = 0	Negative agreement (A-/R-) NA = 31
MagMAX	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 = 0	Negative agreement (A-/R-) NA = 31

ALL PRODUCTS			
Protocol A (25 g)			
	Responses	Reference method positive (R+)	Reference method negative (R-)
Rapid Spin	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 28	Positive deviation (R-/A+) PD = 24
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 8	Negative agreement (A-/R-) NA = 71
MagMAX	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 30	Positive deviation (R-/A+) PD = 26
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 6 (PPNC = 1)	Negative agreement (A-/R-) NA = 69 (PPNC=2)
Protocol B (25 g)			
	Responses	Reference method positive (R+)	Reference method negative (R-)
Rapid Spin	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 33	Positive deviation (R-/A+) PD = 28
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 1	Negative agreement (A-/R-) NA = 67
MagMAX	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 33	Positive deviation (R-/A+) PD = 28
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 1	Negative agreement (A-/R-) NA = 67

Results per category of sample**Table 2 – Beef products**

Protocol A (25 g)			
	Responses	Reference method positive (R+)	Reference method negative (R-)
Rapid Spin	Streiking - CT SMAC		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 10	Positive deviation (R-/A+) PD = 9
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 9	Negative agreement (A-/R-) NA = 41
	Streiking - ChromID O157:H7		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 10	Positive deviation (R-/A+) PD = 7
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 9	Negative agreement (A-/R-) NA = 43
	IMS CT SMAC		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 11	Positive deviation (R-/A+) PD = 11
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 8	Negative agreement (A-/R-) NA = 39
	IMS ChromID		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 11	Positive deviation (R-/A+) PD = 10
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 8	Negative agreement (A-/R-) NA = 40
	All confirmation methods		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 11	Positive deviation (R-/A+) PD = 11
Alternative method negative (A-)	Negative deviation (A-/R+) ND = 8	Negative agreement (A-/R-) NA = 39	
Mag MAX	Streiking - CT SMAC		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 13	Positive deviation (R-/A+) PD = 9
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 6	Negative agreement (A-/R-) NA = 41
	Streiking - ChromID O157:H7		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 12	Positive deviation (R-/A+) PD = 7
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 7	Negative agreement (A-/R-) NA = 43
	IMS CT SMAC		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 14	Positive deviation (R-/A+) PD = 13
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 5	Negative agreement (A-/R-) NA = 37
	IMS ChromID		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 14	Positive deviation (R-/A+) PD = 12
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 5	Negative agreement (A-/R-) NA = 38
	All confirmation methods		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 14	Positive deviation (R-/A+) PD = 13
Alternative method negative (A-)	Negative deviation (A-/R+) ND = 5	Negative agreement (A-/R-) NA = 37	

Protocol B (25 g)			
	Responses	Reference method positive (R+)	Reference method negative (R-)
Rapid Spin	Streaking - CT SMAC		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 16	Positive deviation (R-/A+) PD = 11
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 2	Negative agreement (A-/R-) NA = 39
	Streaking - ChromID O157:H7		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 9	Positive deviation (R-/A+) PD = 5
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 9	Negative agreement (A-/R-) NA = 45
	IMS CT SMAC		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 17	Positive deviation (R-/A+) PD = 14
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 1	Negative agreement (A-/R-) NA = 36
	IMS ChromID		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 16	Positive deviation (R-/A+) PD = 13
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 2	Negative agreement (A-/R-) NA = 37
	All confirmation methods		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 17	Positive deviation (R-/A+) PD = 14
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 1	Negative agreement (A-/R-) NA = 36
	MagMAX	Streaking - CT SMAC	
Alternative method positive (A+)		Positive agreement (A+/R+) PA = 16	Positive deviation (R-/A+) PD = 11
Alternative method negative (A-)		Negative deviation (A-/R+) ND = 2	Negative agreement (A-/R-) NA = 39
Streaking - ChromID O157:H7			
Alternative method positive (A+)		Positive agreement (A+/R+) PA = 8	Positive deviation (R-/A+) PD = 5
Alternative method negative (A-)		Negative deviation (A-/R+) ND = 10	Negative agreement (A-/R-) NA = 45
IMS CT SMAC			
Alternative method positive (A+)		Positive agreement (A+/R+) PA = 17	Positive deviation (R-/A+) PD = 14
Alternative method negative (A-)		Negative deviation (A-/R+) ND = 1	Negative agreement (A-/R-) NA = 36
IMS ChromID			
Alternative method positive (A+)		Positive agreement (A+/R+) PA = 16	Positive deviation (R-/A+) PD = 13
Alternative method negative (A-)		Negative deviation (A-/R+) ND = 2	Negative agreement (A-/R-) NA = 37
All confirmation methods			
Alternative method positive (A+)		Positive agreement (A+/R+) PA = 17	Positive deviation (R-/A+) PD = 14
Alternative method negative (A-)		Negative deviation (A-/R+) ND = 1	Negative agreement (A-/R-) NA = 36

Table 3 – Vegetable products

Protocol A (25 g)			
	Responses	Reference method positive (R+)	Reference method negative (R-)
Rapid Spin	Streaking - CT SMAC		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 16	Positive deviation (R-/A+) PD = 12
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 1	Negative agreement (A-/R-) NA = 33
	Streaking - ChromID O157:H7		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 16	Positive deviation (R-/A+) PD = 11
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 1	Negative agreement (A-/R-) NA = 34
	All confirmation methods		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 17	Positive deviation (R-/A+) PD = 13
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 0	Negative agreement (A-/R-) NA = 32
	MagMAX	Streaking - CT SMAC	
Alternative method positive (A+)		Positive agreement (A+/R+) PA = 15	Positive deviation (R-/A+) PD = 12
Alternative method negative (A-)		Negative deviation (A-/R+) ND = 2	Negative agreement (A-/R-) NA = 33
Streaking - ChromID O157:H7			
Alternative method positive (A+)		Positive agreement (A+/R+) PA = 15	Positive deviation (R-/A+) PD = 10
Alternative method negative (A-)		Negative deviation (A-/R+) ND = 2	Negative agreement (A-/R-) NA = 35
All confirmation methods			
Alternative method positive (A+)		Positive agreement (A+/R+) PA = 16	Positive deviation (R-/A+) PD = 13
Alternative method negative (A-)		Negative deviation (A-/R+) ND = 1	Negative agreement (A-/R-) NA = 32

Protocol B (25 g)			
	Responses	Reference method positive (R+)	Reference method negative (R-)
Rapid Spin	Streaking - CT SMAC		
	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 = 0	Negative agreement (A-/R-) NA = 31
	Streaking - ChromID O157:H7		
	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 14	Positive deviation (R-/A+) PD = 11
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 2	Negative agreement (A-/R-) NA = 34
	All confirmation methods		
	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 = 0	Negative agreement (A-/R-) NA = 31
	MagMAX	Streaking - CT SMAC	
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 = 0	Negative agreement (A-/R-) NA = 31
Streaking - ChromID O157:H7			
Alternative method positive (A+)		Positive agreement (A+/R+) PA = 14	Positive deviation (R-/A+) PD = 11
Alternative method negative (A-)		Negative deviation (A-/R+) ND = 2	Negative agreement (A-/R-) NA = 34
All confirmation methods			
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 = 0	Negative agreement (A-/R-) NA = 31

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

Rapid Spin

Category	Enrichment Protocol	Confirmation protocol	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-
Beef meat	A	Streaking-CT SMAC	10	41	9	9	69	73,9	19	52,6	50	82,0
		Streaking-ChromID O157:H7	10	43	9	7	69	76,8	19	52,6	50	86,0
		IMS-CT SMAC	11	39	8	11	69	72,5	19	57,9	50	78,0
		IMS-ChromID O157:H7	11	40	8	10	69	73,9	19	57,9	50	80,0
		All confirmation protocols	11	39	8	11	69	72,5	19	57,9	50	78,0
	B	Streaking-CT SMAC	16	39	2	11	68	80,9	18	88,9	50	78,0
		Streaking-ChromID O157:H7	9	45	9	5	68	79,4	18	50,0	50	90,0
		IMS-CT SMAC	17	36	1	14	68	77,9	18	94,4	50	72,0
		IMS-ChromID O157:H7	16	37	2	13	68	77,9	18	88,9	50	74,0
		All confirmation protocols	17	36	1	14	68	77,9	18	94,4	50	72,0
Vegetables	A	Streaking-CT SMAC	16	33	1	12	62	79,0	17	94,1	45	73,3
		Streaking-ChromID O157:H7	16	34	1	11	62	80,6	17	94,1	45	75,6
		IMS-CT SMAC	/	/	/	/	/	/	/	/	/	/
		IMS-ChromID O157:H7	/	/	/	/	/	/	/	/	/	/
		All confirmation protocols	17	32	0	13	62	79,0	17	100,0	45	71,1
	B	Streaking-CT SMAC	16	31	0	14	61	77,0	16	100,0	45	68,9
		Streaking-ChromID O157:H7	14	34	2	11	61	78,7	16	87,5	45	75,6
		IMS-CT SMAC	/	/	/	/	/	/	/	/	/	/
		IMS-ChromID O157:H7	/	/	/	/	/	/	/	/	/	/
		All confirmation protocols	16	31	0	14	61	77,0	16	100,0	45	68,9
All products	A	All confirmation protocols	28	71	8	24	131	75,6	36	77,8	95	74,7
	B	All confirmation protocols	33	67	1	28	129	77,5	34	97,1	95	70,5

PA = positive agreement (R+/A+)
PD = positive deviation (R-/A+)

NA = negative agreement (R-/A-)
ND = negative deviation (A-/R+)

MagMAX

Category	Enrichment Protocol	Confirmation protocol	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-
Beef meat	A	Streaking-CT SMAC	13	41	6	9	69	78,3	19	68,4	50	82,0
		Streaking-ChromID O157:H7	12	43	7	7	69	79,7	19	63,2	50	86,0
		IMS-CT SMAC	14	37	5	13	69	73,9	19	73,7	50	74,0
		IMS-ChromID O157:H7	14	38	5	12	69	75,4	19	73,7	50	76,0
		All confirmation protocols	14	37	5	13	69	73,9	19	73,7	50	74,0
	B	Streaking-CT SMAC	16	39	2	11	68	80,9	18	88,9	50	78,0
		Streaking-ChromID O157:H7	8	45	10	5	68	77,9	18	44,4	50	90,0
		IMS-CT SMAC	17	36	1	14	68	77,9	18	94,4	50	72,0
		IMS-ChromID O157:H7	16	37	2	13	68	77,9	18	88,9	50	74,0
		All confirmation protocols	17	36	1	14	68	77,9	18	94,4	50	72,0
Vegetables	A	Streaking-CT SMAC	15	33	2	12	62	77,4	17	88,2	45	73,3
		Streaking-ChromID O157:H7	15	35	2	10	62	80,6	17	88,2	45	77,8
		IMS-CT SMAC	/	/	/	/	/	/	/	/	/	/
		IMS-ChromID O157:H7	/	/	/	/	/	/	/	/	/	/
		All confirmation protocols	16	32	1	13	62	77,4	17	94,1	45	71,1
	B	Streaking-CT SMAC	16	31	0	14	61	77,0	16	100,0	45	68,9
		Streaking-ChromID O157:H7	14	34	2	11	61	78,7	16	87,5	45	75,6
		IMS-CT SMAC	/	/	/	/	/	/	/	/	/	/
		IMS-ChromID O157:H7	/	/	/	/	/	/	/	/	/	/
		All confirmation protocols	16	31	0	14	61	77,0	16	100,0	45	68,9
All products	A	All confirmation protocols	30	69	6	26	131	75,6	36	83,3	95	72,6
	B	All confirmation protocols	33	67	1	28	129	77,5	34	97,1	95	70,5

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

The alternative method relative accuracy, specificity and sensitivity values are:

	Rapid Spin					
	Beef meats		Vegetables		All products	
	Protocol A	Protocol B	Protocol A	Protocol B	Protocol A	Protocol B
Relative accuracy	72,5	77,9	79,0	77,0	75,6	77,5
Relative specificity	78,0	72,0	71,1	68,9	74,7	70,5
Relative sensitivity	57,9	94,4	100,0	100,0	77,8	97,1

	MagMAX					
	Beef meats		Vegetables		All products	
	Protocol A	Protocol B	Protocol A	Protocol B	Protocol A	Protocol B
Relative accuracy	73,9	77,9	77,4	77,0	75,6	77,5
Relative specificity	74,0	72,0	71,1	68,9	72,6	70,5
Relative sensitivity	73,7	94,4	94,1	100,0	83,3	97,1

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

	Rapid Spin - Sensitivity calculated with alternative positive deviations					
	Beef meats		Vegetables		All products	
	Protocol A	Protocol B	Protocol A	Protocol B	Protocol A	Protocol B
Alternative	73,3	96,9	100,0	100,0	86,7	98,4
Reference	63,3	56,3	56,7	53,3	60,0	54,8

	MagMAX - Sensitivity calculated with alternative positive deviations					
	Beef meats		Vegetables		All products	
	Protocol A	Protocol B	Protocol A	Protocol B	Protocol A	Protocol B
Alternative	84,4	96,9	96,7	100,0	90,3	98,4
Reference	59,4	56,3	56,7	53,3	58,1	54,8

3.1.6 Analysis of discordants

According to the observed PD and ND, the ISO 16140 statistical tests conclude:

All products	A	Rapid Spin	$24 + 8 = 32$	$Y > 22$ $d = 16$ $x^2 = d^2/y = 8$	Mc Nemar test: \neq
		MagMAX	$26 + 6 = 32$	$Y > 22$ $d = 20$ $x^2 = d^2/y = 12,5$	Mc Nemar test: \neq
	B	Rapid Spin	$28 + 1 = 29$	$Y > 22$ $d = 27$ $x^2 = d^2/y = 25,1$	Mc Nemar test: \neq
		MagMAX	$28 + 1 = 29$	$Y > 22$ $d = 27$ $x^2 = d^2/y = 25,1$	Mc Nemar test: \neq

The Mc Nemar tests concluded a significant difference between both methods, alternative and reference. These differences were justified by the high number of positive deviations for the alternative method. Consequently, the alternative method showed superior performances to the reference method.

The **negative deviations** are the following:

Beef meat – Negative deviations Rapid Spin – Protocol A		
N° Sample	PCR result	Confirmation
5152 (A)	-	+
5159 (A)	-/- (Ct > 35)	+
5162 (A)	-/- (Ct > 35)	-
5192 (A)	-	-
5193 (A)	-	-
5196 (A)	-	-
5419 (N)	-	-
5588 (A)	-/- (Ct > 35)	+

A: artificially contaminated sample

N: naturally contaminated sample

Beef meat – Negative deviations MagMAX – Protocol A		
N° Sample	PCR result	Confirmation
5162 (A)	-	-
5192 (A)	-	-
5193 (A)	+/- (Ct > 35) / - (Ct > 35)	-
5196 (A)	-	-
5419 (N)	-	-
Beef meat – Negative deviations Rapid Spin – Protocol B		
N° Sample	PCR result	Confirmation
5419 (N)	-	-

Beef meat – Negative deviations MagMAX – Protocol B		
N° Sample	PCR result	Confirmation
5419 (N)	-	-

Vegetables – Negative deviations MagMAX – Protocol A		
N° Sample	PCR result	Confirmation
5248	- (Ct > 35)	+

The **positive deviations** are the following:

Beef meat – Positive deviations	
Protocol A	
Rapid Spin	MagMAX
N° Sample	N° Sample
5153 (A)	5153 (A)
5156 (A)	5156 (A)
5160 (A)	5160 (A)
5161 (A)	5161 (A)
5163 (A)	5163 (A)
5164 (A)	5164 (A)
5194 (A)	5191 (A)
5202 (A)	5194 (A)
5203 (A)	5200 (A)
5403 (A)	5202 (A)
5424 (A)	5203 (A)
	5423 (A)
	5424 (A)

Vegetables – Positive deviations	
Protocol A	
Rapid Spin	MagMAX
N° Sample	N° Sample
5252 (A)	5252 (A)
5253 (A)	5253 (A)
5254 (A)	5254 (A)
5255 (A)	5255 (A)
5256 (A)	5257 (A)
5257 (A)	5258 (A)
5282 (A)	5282 (A)
5283 (A)	5283 (A)
5286 (A)	5286 (A)
5291 (A)	5291 (A)
5292 (A)	5292 (A)
5293 (A)	5293 (A)
5296 (A)	5296 (A)

Beef meat – Positive deviations	
Protocol B	
Rapid Spin	MagMAX
N° Sample	N° Sample
5153 (A)	5153 (A)
5155 (A)	5155 (A)
5156 (A)	5156 (A)
5160 (A)	5160 (A)
5163 (A)	5163 (A)
5164 (A)	5164 (A)
5191 (A)	5191 (A)
5199 (A)	5199 (A)
5200 (A)	5200 (A)
5202 (A)	5202 (A)
5203 (A)	5203 (A)
5420 (N)	5420 (N)
5423 (N)	5423 (N)
5424 (N)	5424 (N)

Vegetables – Positive deviations	
Protocol B	
Rapid Spin	MagMAX
N° Sample	N° Sample
5252 (A)	5252 (A)
5253 (A)	5253 (A)
5254 (A)	5254 (A)
5255 (A)	5255 (A)
5256 (A)	5256 (A)
5257 (A)	5257 (A)
5258 (A)	5258 (A)
5282 (A)	5282 (A)
5283 (A)	5283 (A)
5286 (A)	5286 (A)
5291 (A)	5291 (A)
5292 (A)	5292 (A)
5293 (A)	5293 (A)
5296 (A)	5296 (A)

3.2 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.

3.2.1 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 analysing the different matrix/strain pairs. Four levels were tested. Six replicates of each combination were prepared.

The following matrices were tested:

- frozen ground beef,
- frozen spinach.

3.2.2 Contamination protocol

Contaminations and enumerations were realised according to the AFNOR technical rules (protocol for low level inoculations). The contamination 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 samples were analysed by both methods, and the background microflora were enumerated.

3.2.3 Results

Detection levels are presented in Table 5.

**Table 5 – Relative detection level results
(Rapid Spin and MagMAX - Extraction protocols)**

Strain / matrix pairs	Relative detection level (CFU / 25 g) according to Spearman-Kärber test ³	
	Reference method	Alternative method
Frozen ground beef / <i>E. coli</i> O157:H7 Ad 683	Protocol A	0,785 [0,488 ; 1,261]
	Protocol B	0,785 [0,488 ; 1,261]
Frozen spinach / <i>E. coli</i> O157:H7 Ad 557	Protocol A	0,284 [0,091 ; 0,886]
	Protocol B	0,284 [0,091 ; 0,886]

Strain / matrix pairs	Relative detection level (CFU / 25 g) according to Spearman-Kärber test	
	Reference method	Alternative method
Frozen ground beef / <i>E. coli</i> O157:H7 Ad 683	Protocol A	0,8 [0,5 ; 1,3]
	Protocol B	0,8 [0,5 ; 1,3]
Frozen spinach / <i>E. coli</i> O157:H7 Ad 557	Protocol A	0,3 [0,1 ; 0,9]
	Protocol B	0,3 [0,1 ; 0,9]

3.2.4 Conclusion

The alternative method shows lower detection levels than the reference method, comprised between 0,1 to 0,9 CFU/25 g.

³ "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".

3.3 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.

3.3.1 Test protocols

- **Protocol for inclusivity**: 50 *E. coli* O157:H7 strains were tested. Cultures were grown in BHI medium at 37°C. Dilutions were done in order to inoculate between 10 to 100 cells/225 ml BHI. The alternative method protocol A was then performed (BHI incubation time: 6 h at 42°C). The Rapid Spin extraction protocol was used.
- **Protocol for exclusivity** : 37 negative strains were tested. Cultures were grown in BHI, incubated at 37°C. Dilutions were performed in order to inoculate 10⁵ cell/ml BPW. The alternative method protocol B was then performed (BPW incubation time: 16 h at 42°C). The MagMAX extraction protocol was then used.

3.3.2 Results

Raw data are given in Annex 4.

The 50 target strains showed positive results.

The 37 tested non-target strains showed negative results.

The inclusivity and exclusivity studies give satisfying results.

3.4 Practicability

MicroSEQ[®] *E. coli* O157:H7 method practicability was evaluated according to the AFNOR criteria relative to preliminary study.

<i>Packaging and volume of reagents</i>	<p>The components needed for the analyses are:</p> <ul style="list-style-type: none"> - Rapid Spin: <ul style="list-style-type: none"> * lysis buffer: lysis buffer is available in a pouch. Each pouch contains one flask, * Proteinase K= 1 tube (1,25 ml) * Nuclease free water : 1 box of 10 flasks (1 flask= 50 ml) * MicroSEQ <i>E. coli</i> O157:H7 detection kit (the kit contains PCR reagents for 96 reactions) * pathogen negative control: 1 tube (1,5 ml) - MagMAX: <ul style="list-style-type: none"> * Box MagMAX: PK buffer (50 ml), elution buffer (25 ml), wash water, lysis buffer (50 ml), binding solution (empty flask) * Proteinase K: 1 tube (1,25 ml) * Magnetic particles: 2 tubes x 1,5 ml * MicroSEQ <i>E. coli</i> O157:H7 detection kit (the kit contains PCR reagents for 96 reactions) * pathogen negative control: 1 tube (1,5 ml)
<i>Storage conditions and shelf-life</i>	<p>Rapid Spin:</p> <ul style="list-style-type: none"> - Lysis buffer : 2 – 8°C - Proteinase K: - 15 – 25°C - Nuclease free water: room temperature <p>MagMAX:</p> <ul style="list-style-type: none"> - Box MagMAX: room temperature - Magnetic particles: 2 – 8°C <p>MicroSEQ:</p> <ul style="list-style-type: none"> - Pathogen negative control: 2 – 8°C <p>The shelf life is given on the package</p> <ul style="list-style-type: none"> - Box MagMAX: 12 months after manufacturing - MicroSEQ <i>E. coli</i> O157:H7 detection kit: 18 months after manufacturing
<i>Modalities after opening</i>	<p>All the reagents must be stored at the temperature mentioned on the package.</p>
<i>Specific equipment</i>	<ul style="list-style-type: none"> - Applied Biosystems 7500 Fast Real-Time PCR system - Consumables: <ul style="list-style-type: none"> * PrepSEQ[™] Nucleic Acid Extraction kit * PrepSEQ[™] Rapid Spin sample preparation kit * Aerosol-resistant pipette tips (200, 1 000 µl) * Disposable gloves * Pipettors (200, 1 000 µl) * MicroAmp Fast 8 – tube strip 0,1 ml * MicroAmp Optical 8 – capstrip - MagMAX[™] Express 96
<i>Reagents</i>	<p>All the reagents are ready to use except:</p> <ul style="list-style-type: none"> - wash buffer: add 74 ml Ethanol 95 % - binding solution: the lab must fill the flask with its own isopropanol.
<i>Training</i>	<p>One day is required for technicians with microbiology and molecular biology knowledge</p>

Workflow (in minutes)	Steps	Reference Method ISO 16654	Alternative method MicroSEQ® <i>E. coli</i> O157:H7 method	
		25 g	Protocols A & B (25 g)	
Negative samples				
	Sampling	45	45	
	IMS 6 h	90	/	
	IMS 24 h if necessary	90	/	
	DNA Extraction	/	120	
	PCR	/	15	
	Selective agar reading	30	/	
	Total for negative samples analyses	255	180	
	Total/negative sample	8,5	6	
Presumptive sample or positive sample				
	Streaking on selective agars	/	7	
	Selective agar reading	/	10	
	Streaking on nutrient agar	25	10	
	Indol test	30	/	
	Latex test	50	30	
	Total for positive samples	340	237	
	Total/positive sample	11,3	7,9	
Time to result	Steps	Reference Method ISO 16654	Alternative method MicroSEQ® <i>E. coli</i> O157:H7 method	
			Protocol A	Protocol B
Negative samples				
	Sampling	D0	D0	D0
	IMS 6 h	D0	/	/
	IMS 24 h	D1	/	/
	PCR	/	D0	D1
	Final negative result	D1	D0	D1
Presumptive positive or positive results				
	Sampling	D0	D0	D0
	IMS 6 h	D0	/	/
	IMS 24 h	D1	/	/
	PCR	/	D0	D1
	Streak on selective agars	/	D0	D1
	Streak on nutritive agar	D1 – D2	D1	D2
	Indol test	D2 – D3	/	/
	Latex test	D3 – D4	D2	D3
Negative results are obtained at D0 for the alternative method for protocol A and at D1 for the reference method and for protocol B of the alternative method. For positive samples, the results are obtained at D3-D4 for the reference method and at D2 or D3 for the alternative method depending on the enrichment used protocol.				

<i>Technician background</i>	Technician qualified in microbiology and molecular biology
<i>Common step with the reference method</i>	No comment step
<i>Traceability of the results</i>	All data obtained with the <i>E. coli</i> O157:H7 method are traced over time by computer archive means
<i>Maintainance</i>	The periodicity for maintainance is once every year when a service contract is established

4 INTERLABORATORY STUDY ORGANISATION AND RESULTS

4.1 Study organisation

Number of Collaborative laboratories

Initially 15 labs consented to collaborate in the Interlaboratory Study. One lab (lab H) finally decided not to participate in the study. Samples were shipped to the 14 remaining laboratories.

Matrix and strain used

The study was carried out with ground beef samples contaminated by *Escherichia coli* O157:H7 ATCC 43888.

Samples

Samples were inoculated and shipped on Monday 7 February 2011, as described below:

- 24 codified samples (25 g) (red labelled) for analysis by the alternative method MicroSEQ[®] *E. coli* O157:H7
- 24 codified samples (25 g) (blue labelled) for analyses by the reference method ISO 16654 (2001),
- 1 ground beef sample (labelled “Sample for Total Count enumeration”) for aerobic mesophilic flora enumeration by ISO 4833 method,
- **1 water flask labelled “Temperature Control” with a temperature probe, which must be incubated simultaneously with the samples during analysis (storage and alternative enrichment incubation).**

The collaborative labs started the analyses on Wednesday 9 February 2011.

Inoculation

The targeted inoculation levels were:

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

At least, each laboratory received 24 samples of 25 g, i.e. 8 samples per inoculation level and method.

Labelling and shipping

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

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

Samples were shipped within 24 h to 48 h to the different laboratories. Sample temperature had to stay lower or equal to 8,4°C during transport, and between 0°C – 8,4°C at arrival.

Analyses

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

Expedition conditions

The collaborative study instructions were sent to the different labs on January 27, 2011.

4.2 Control of the experimental parameters

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

Before inoculation

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

Sample stability

Sample stability was performed by inoculating the matrix at 150 CFU/25 g and 5 CFU/25 g. Enumerations were performed on 1,0 g of ground beef samples for the high contamination level and detection analyses were performed for the low contamination level. *Triplicates* were analysed, and the results were the following:

Table 6

Day	Reference method (research)			CFU/25 g (CT SMAC)			Aerobic mesophilic flora (CFU/g)
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	
Day 0	+	+	+	175	175	175	2,2.10 ⁴
Day 1	+	+	+	200	225	225	1,2.10 ⁴
Day 2	+	+	+	225	175	175	8,1.10 ⁴

No evolution was observed during storage at 4°C.

□ *Contamination levels*

The contamination levels and the confidence intervals were:

Table 7

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	2 - 7 - 8 - 12 - 14 - 17 - 18 - 24	/	/	/	/
Low level	1 - 3 - 6 - 10 - 15 - 19 - 22 - 23	5	5,8	5,0	6,8
High level	4 - 5 - 9 - 11 - 13 - 16 - 20 - 21	25	30,8	26,7	35,5

4.2.2 *Logistic conditions*

Temperature conditions are given below:

Table 8 - Sample temperatures at receipt

Laboratories	Temperature measured by the sensor (°C)	Temperature measured at receipt (°C)	Receipt delay
A	0,0	3,7	07/02/2011 - 15h15
B	1,0	1,9	08/02/2011 - 11h30
C	1,0	3,4	08/02/2011 - 08h30
D	1,5	6,2	08/02/2011 - 09h15
E	2,0	4,0	08/02/2011 - 11h25
F	1,5	3,8	08/02/2011 - 07h30
G	1,0	3,2	08/02/2011 - 08h30
I	0,5	1,9	08/02/2011 - 09h08
J	2,0	4,4	08/02/2011 - 10h30
K	0,5	5,1	08/02/2011 - 09h30
L	2,0	7,4	08/02/2011 - 13h05
M	2,0	4,0	08/02/2011 - 11h00
N	2,0	0,5	08/02/2011 - 13h00
O	3,0	3,7	08/02/2011 - 07h35

4.2.3 *Conclusion*

No problem was encountered during the transport or at receipt.

4.3 Results analysis

4.3.1 *Aerobic mesophilic flora enumeration*

Two Labs did not realise this analysis.

Depending on the Lab results, the enumeration levels varied from $1,8 \cdot 10^3$ to $3,6 \cdot 10^6$ CFU/g.

4.3.2 *Expert lab results*

For the low inoculation level, one sample (P23) gave a negative result by the reference method. For the high inoculation level, all the samples gave positive results by the reference and the alternative methods.

4.3.3 *Collaborator lab results*

14 Labs participated in the study.

One lab (Lab O) obtained 4 positive results for non inoculated samples by the reference method. This is probably due to cross contamination.

The incubation times for the alternative method are shown in Table 9.

Table 9

Laboratories	Incubation hours		Incubation time
	Beginning	End	
A	09h53	15h38	05h45
B	08h24	14h09	05h45
C	08h39	14h39	06h00
D	08h54	14h24	06h00
E	08h25	14h25	06h00
F	08h40	14h55	06h15
G	08h40	14h40	06h00
I	08h26	14h11	05h45
J	08h11	14h26	06h15
K	08h56	14h41	06h15
L	11h12	16h57	05h45
M	11h12	17h00	06h00
N	07h58	13h43	05h45
O	08h28	14h13	05h45
P (ADRIA)	08h28	14h43	06h15

Two curves indicate an incubation temperature close to 37°C, whereas the Labs confirmed that the enrichment broths were really incubated in an incubator at 41,5°C. These two Labs provided the temperature records from the incubators. For Lab B, the curve shows clearly that the incubator was at 41,5°C. The Lab O didn't provide a curve, but only temperature measurements of the incubator that were done on an ad'hoc basis.

Regarding the facts that (i) the Lab O was not able to provide a record of the temperature incubator, (ii) that cross contaminations were observed, it has been decided to not take into account the Lab O results.

The Lab M incubated the probe with the enrichments of the reference method. This Lab confirmed that a 6 h incubation time was done for the alternative method.

Finally, 12 Labs were retained for the interpretation: A, B, C, D, E, F, G, I, J, K, L and M.

Two Labs (J and N) encountered difficulties to confirm PCR positive results:

- Lab J did'nt obtain typical colonies with positive latex tests (O157 and H7) by direct streaking onto CT SMAC for 11 samples. An immunoseparation was then applied (on Tuesday 15 February 2011) prior to streaking. For all the samples, typical colonies confirmed as *E. coli* O157:H7 were then isolated.
- Lab N isolated the typical colonies from the reference method and the alternative method on blood agar instead of TSA prior to proceed to latex tests. All the latex tests gave negative results. We asked them to subculture the colonies on TSA as detailed in the instructions. Positive latex tests were then observed for some of these colonies, but some PCR tests were not still confirmed. The Lab proceeded then to immunoseparation on the alternative enrichment broth for samples n° N3, N6, N10, N15, N20 and N21. This protocol was probably applied too late (on Monday 21 February 2011, 2 weeks after enrichment) to permit the recovery of the *E. coli* O157:H7 strains among a high level of background microflora. We decided to discard the results obtained by this Lab.

4.4 Results interpretation

4.4.1 Specificity and sensitivity for each method

For the L0 level and for each method, the specificity percentages have been calculated according to the following formula:

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

with :N- = total number of all L0 assays

FP = number of false positive results

For each contamination level and each method, the sensitivity percentages have been calculated according to the following formula:

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

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

TP = number of true positive results

Results are reported in the following table:

Table 10

Level	Reference method		Alternative method	
	SP/SE %	LCL%	SP/SE %	LCL%
L0	SP = 100,0	100,0	SP = 100,0	100,0
L1	SE = 99,0	96,9	SE = 99,0	96,9
L2	SE = 100,0	100,0	SE = 100,0	100,0
L1+L2	SE = 99,5	98,5	SE = 99,5	98,5

4.4.2 Relative accuracy (AC)

Results for all levels are shown below:

Table 11 - Paired results of the alternative and reference methods

Alternative method	Reference method		Total
	+	-	
+	PA = 190	PD = 1	191
-	ND = 1	NA = 96	97
Total	N+ = 191	N- = 97	N = 288

Relative accuracy (AC) is calculated according to: $AC = \frac{(PA + NA)}{N} \times 100\%$

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

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

Table 12

Level	AC %	LCL %
L0	100,0	100,0
L1	97,9	95,0
L2	100,0	100,0
L1 + L2	99,0	97,5
Total	99,3	98,3

4.4.3 *Discordant results*

Two discordant results were observed between the reference and the alternative methods:

- one negative deviation for Lab E (sample n° E15),
- one positive deviation for Lab L (sample n° L1).

$$Y = ND + PD = 2$$

$Y < 6$, no test is available.

4.5 Interpretation

4.5.1 *Comparison of the relative accuracy, specificity and sensibility values*

The values obtained for the two parts of the validation study (comparative and inter-laboratory studies) are reported in table 13.

Table 13 - Alternative method values calculated during the comparative and inter-laboratory studies

	Interlaboratory study	Methods comparative study	
		Beef meat	All products
Relative accuracy (AC)	99,3	72,5	77,5
Sensibility (SE)	99,5	57,9	97,1
Specificity (SP)	100,0	78,0	70,5

4.5.2 *Accordance (DA)*

Accordance values for both methods are:

Table 14

Level	Reference method (DA)	Alternative method (DA)
L0	100,0 %	100,0 %
L1	98,2 %	98,2 %
L2	100,0 %	100,0 %

4.5.3 Concordance

Both methods concordance values are:

Table 15

Level	Reference method	Alternative method
L0	100,0 %	100,0 %
L1	97,9 %	97,9 %
L2	100,0 %	100,0 %

4.5.4 Odds Ratio (COR)

The odds ratio value is determined according to the following formula:

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

Both method odds ratio values are:

Table 16

Level	Reference method (COR)	Alternative method (COR)
L0	1,00	1,00
L1	1,00	1,00
L2	1,00	1,00

5 CONCLUSION

The methods comparative study conclusions are:

The MicroSEQ[®] *E. coli* O157:H7 method shows satisfying relative accuracy, specificity and sensitivity whatever the tested protocol and food type.

The detection limit of the MicroSEQ[®] *E. coli* O157:H7 method are better than the ISO 16654 method, whatever the tested protocol and food type.

The MicroSEQ[®] *E. coli* O157:H7 method is selective and specific.

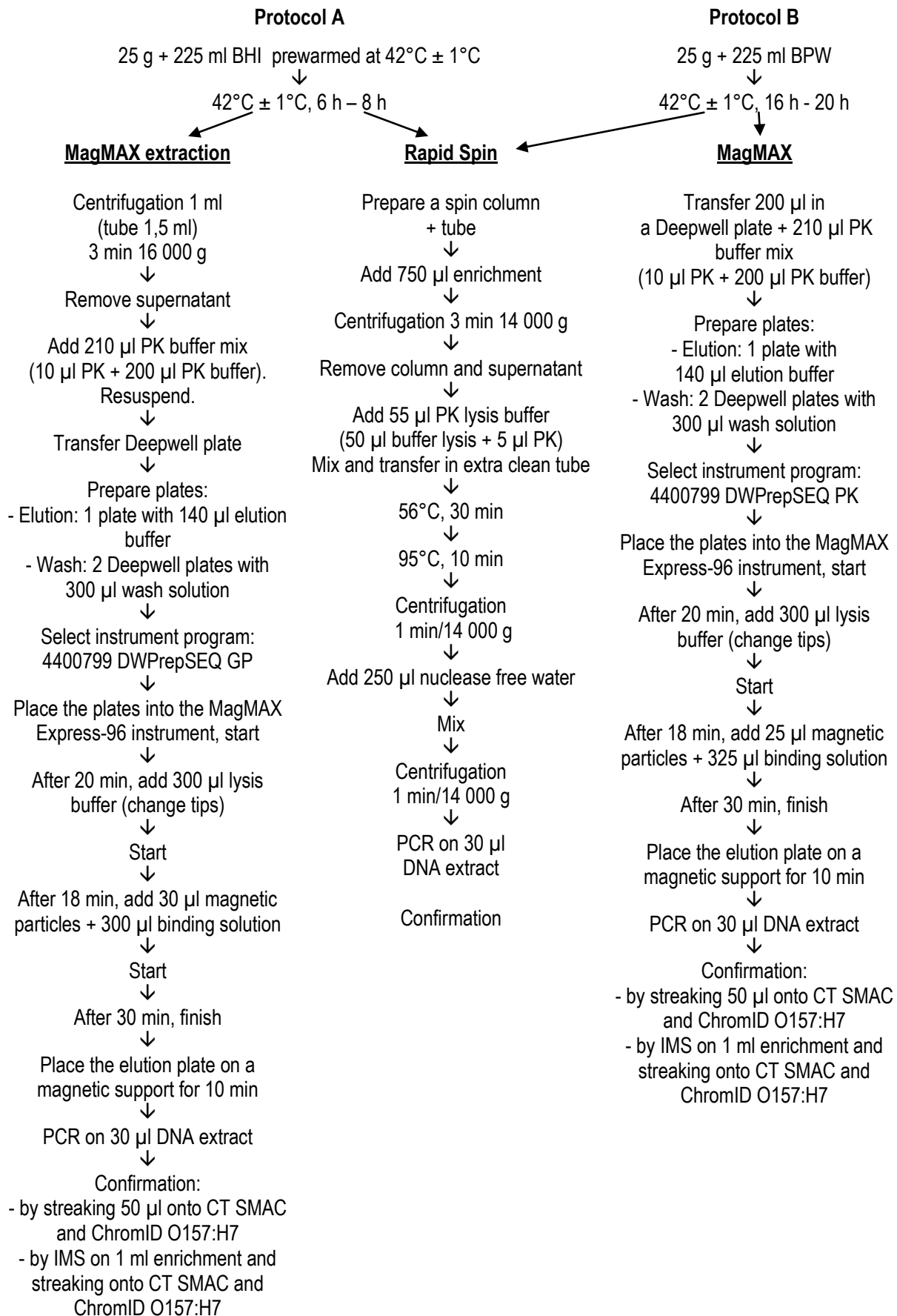
Negative results could be available within one day using the MicroSEQ[®] *E. coli* O157:H7 method.

The interlaboratory study conclusions are:

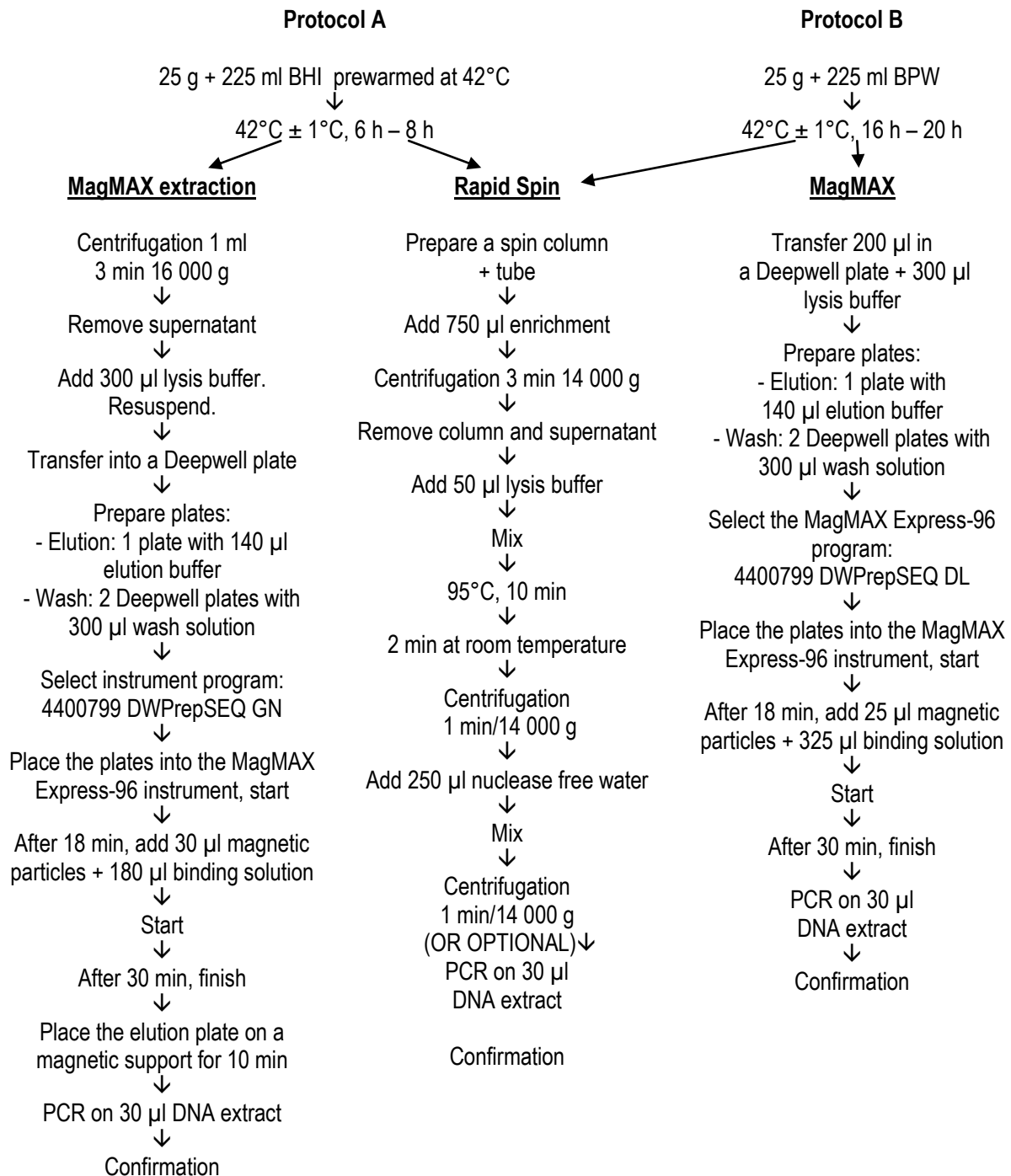
The alternative method and reference method show equivalent performances (accordance, concordance, odds ratio).

Annex 1 – Alternative method

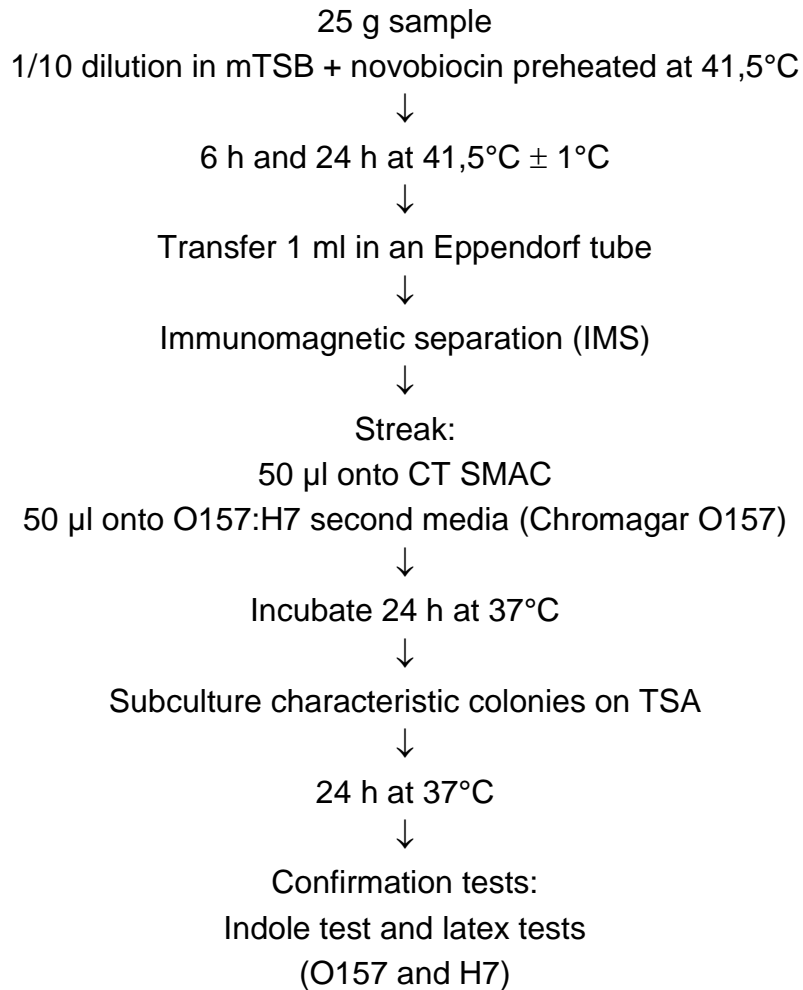
□ Beef meat (sampling: 25 g)



□ **Vegetables (sampling: 25 g)**



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



+/-:doutbful colonies I : inhibition *: result of 6 IMS NC: non characteristic colonies O: O157 A:autoagglutination ⚠ : see SDS files

N° Sample	Product	ISO 16654 method♦					Alternative method – PROTOCOL B																														
		IMS 6h		IMS 24h		Result	Enrichment B:BPW 16h - 42°C(25g +225ml BPW) (25g + 225ml BPW)																														
		CT-SMAC	Chromagar O157	CT-SMAC	Chromagar O157		Confirmation by streaking										Confirmation by IMS										All confirmation protocols										
						Rapid Spin	MagMAX	CT-SMAC	Latex		Chrom ID O157:H7	Latex		Rapid Spin		MagMAX		CT-SMAC	Latex		chrom ID O157:H7	Latex		Rapid Spin		MagMAX		Result	Agreement	Result	Agreement						
O157	H7	O157	H7	Final result	Agreement				Final result	Agreement		Final result	Agreement	Final result	Agreement	O157	H7		O157:H7	O157		H7	Final result	Agreement	Final result	Agreement	Final result					Agreement					
5245	Frozen mixed vegetables	+	+	/	/	+	+	+	+	+	+	+	+	+	+	=	+	=	+	=	+	=	/	/	/	/	/	/	/	/	/	/	/	+	=	+	=
5246	Frozen peas	+	+	/	/	+	+	+	+ 1 col	+	+	-	/	/	+	=	-	ND	+	=	-	ND	-	/	/	-	/	/	/	/	/	/	/	+	=	+	=
5247	Frozen spinaches	+	+	/	/	+	⚠	+	+	+	+	+	+	+	+	=	+	=	+	=	+	=	/	/	/	/	/	/	/	/	/	/	+	=	+	=	
5248	Frozen mixed vegetables	+	+	/	/	+	+	+	+	+	+	+	+	+	+	=	+	=	+	=	+	=	/	/	/	/	/	/	/	/	/	/	+	=	+	=	
5249	Sliced links	-	-	+	+	+	+	+	+	+	+	+	+	+	+	=	+	=	+	=	+	=	/	/	/	/	/	/	/	/	/	/	+	=	+	=	
5250	Frozen flower cabbages	+	+	/	/	+	+	+	+	+	+	+	+	+	+	=	+	=	+	=	+	=	/	/	/	/	/	/	/	/	/	+	=	+	=		
5251	Frozen vegetables	+	+	/	/	+	+	+	+	+	+	+	+	+	+	=	+	=	+	=	+	=	/	/	/	/	/	/	/	/	/	+	=	+	=		
5252	Fresh parsley	+	+	-	+	-	+	+	+	+	+	+	+	+	+	PD	+	PD	+	PD	+	PD	/	/	/	/	/	/	/	/	/	+	PD	+	PD		
5253	Fresh mushrooms	-	-	-	-	-	+	+	+	+	+	+	+	+	+	PD	+	PD	+	PD	+	PD	/	/	/	/	/	/	/	/	/	+	PD	+	PD		
5254	Cauliflower	-	-	-	-	-	+	+	+	+	+	+	+	+	+	PD	+	PD	+	PD	+	PD	/	/	/	/	/	/	/	/	+	PD	+	PD			
5255	Fresh Brussels cabbages	-	-	-	-	-	+	+	+	+	+	+	+	+	+	PD	+	PD	+	PD	+	PD	/	/	/	/	/	/	/	/	+	PD	+	PD			
5256	Turnip	-	-	-	-	-	+	+	+	+	+	+	+	+	+	PD	+	PD	+	PD	+	PD	/	/	/	/	/	/	/	/	+	PD	+	PD			
5257	Black radish	-	-	-	-	-	+	+	+	+	-	/	/	+	PD	-	=	+	PD	-	=	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
5258	Red radish	-	-	-	-	-	+	+	+	+	-	/	/	+	PD	-	=	+	PD	-	=	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
5259	Salad(Roquette)	+	+	/	/	+	+	+	+	+	-	/	/	+	=	-	ND	+	=	-	ND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
5282	Grated carrots(MAP)	-	-	+	+	-	+	+	+	+	+	+	+	+	+	PD	+	PD	+	PD	+	PD	/	/	/	/	/	/	/	/	+	PD	+	PD			
5283	Vegetables(MAP)	+	-	-	-	-	+	+	+	+	-	/	/	+	PD	-	=	+	PD	-	=	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
5284	Fresh mesclum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	=	+	=	+	=	+	=	/	/	/	/	/	/	/	/	+	=	+	=	+	=	
5285	Fresh spinach	+	-	+	+	+	+	+	+	+	+	+	+	+	+	=	+	=	+	=	+	=	/	/	/	/	/	/	/	/	+	=	+	=	+	=	
5286	Salad(Mache-MAP)	-	-	-	-	-	+	+	+	+	+	+	+	+	+	PD	+	PD	+	PD	+	PD	/	/	/	/	/	/	/	/	+	PD	+	PD			
5287	Red cabbage(MAP)	+	-	+	+	+	+	+	+	+	+	+	+	+	+	=	+	=	+	=	+	=	/	/	/	/	/	/	/	+	=	+	=	+	=		
5288	Vegetables mix(MAP)	+	+	/	/	+	+	+	+	+	+	+	+	+	+	=	+	=	+	=	+	=	/	/	/	/	/	/	/	+	=	+	=	+	=		
5289	Vegetables mix(MAP)	-	+	+	+	+	+	+	+	+	+	+	+	+	+	=	+	=	+	=	+	=	/	/	/	/	/	/	/	+	=	+	=	+	=		
5290	Fresh spinach growths	+	+	+	+	+	+	+	+	+	+	+	+	+	+	=	+	=	+	=	+	=	/	/	/	/	/	/	/	+	=	+	=	+	=		
5291	White cabbage(MAP)	-	-	+	+	-	⚠	+	+	+	+	+	+	+	+	PD	+	PD	+	PD	+	PD	/	/	/	/	/	/	/	+	PD	+	PD				
5292	Beans(MAP)	-	-	-	-	-	+	+	+	+	+	+	+	+	+	PD	+	PD	+	PD	+	PD	/	/	/	/	/	/	/	+	PD	+	PD				
5293	Fresh turnip	-	-	-	-	-	+	+	+	+	+	+	+	+	+	PD	+	PD	+	PD	+	PD	/	/	/	/	/	/	/	+	PD	+	PD				
5294	Salad(MAP)	+	+	+	+	+	⚠	+	+	+	+	+	+	+	+	=	+	=	+	=	+	=	/	/	/	/	/	/	+	=	+	=	+	=			
5295	Fresh spinach growths(MAP)	-	-	+	+	+	+	+	+	+	+	+	+	+	+	=	+	=	+	=	+	=	/	/	/	/	/	/	+	=	+	=	+	=			
5296	Fresh watercress	-	-	+	+	-	+	+	+	+	+	+	+	+	+	PD	+	PD	+	PD	+	PD	/	/	/	/	/	/	+	PD	+	PD					
5337	Fresh spinach growths	+	+	-	-	-	-	-	-	-	-	-	-	-	-	=	-	=	-	=	-	=	/	/	/	/	/	/	-	=	-	=	-	=			
5338	Fresh watercress	-	-	-	-	-	-	-	-	-	-	-	-	-	-	=	-	=	-	=	-	=	/	/	/	/	/	/	-	=	-	=	-	=			
5339	Fresh spinach	-	-	-	-	-	-	-	-	-	-	-	-	-	-	=	-	=	-	=	-	=	/	/	/	/	/	/	-	=	-	=	-	=			

♦ Analysis performed according to the COFRAC accreditation

Annex 4 – Inclusivity and exclusivity : raw data

POSITIVE STRAINS						
	Strain			Origin	Inoculation CFU/225ml BHI	PCR Result Protocol A Rapid Spin
1.	<i>Escherichia coli</i>	O157:H7	B177	Slaughterhouse	18	+
2.	<i>Escherichia coli</i>	O157:H7	BV2	Slaughterhouse	10	+
3.	<i>Escherichia coli</i>	O157:H7	BR3	Slaughterhouse	6	+
4.	<i>Escherichia coli</i>	O157:H7	BD4	Slaughterhouse	11	+
5.	<i>Escherichia coli</i>	O157:H7	ENV177	Water purification	7	+
6.	<i>Escherichia coli</i>	O157:H7	ET8	Water purification	4	+
7.	<i>Escherichia coli</i>	O157:H7	EK9	Water purification	6	+
8.	<i>Escherichia coli</i>	O157:H7	435	Ground beef	2	+
9.	<i>Escherichia coli</i>	O157:H7	670T	Ground beef	4	+
10.	<i>Escherichia coli</i>	O157:H7	730T	Ground beef	3	+
11.	<i>Escherichia coli</i>	O157:H7	226T	Ground beef	4	+
12.	<i>Escherichia coli</i>	O157:H7	42197-1	Ground beef	3	+
13.	<i>Escherichia coli</i>	O157:H7	A3612	Ground beef	5	+
14.	<i>Escherichia coli</i>	O157:H7	A4513	Ground beef	2	+
15.	<i>Escherichia coli</i>	O157:H7	A1075	Ground beef	3	+
16.	<i>Escherichia coli</i>	O157:H7	B68	Slaughterhouse	7	+
17.	<i>Escherichia coli</i>	O157:H7	AT40	Slaughterhouse	14	+
18.	<i>Escherichia coli</i>	O157:H7	AV36	Slaughterhouse	7	+
19.	<i>Escherichia coli</i>	O157:H7	AR15	Slaughterhouse	9	+
20.	<i>Escherichia coli</i>	O157:H7	LS3	Feces	8	+
21.	<i>Escherichia coli</i>	O157:H7	AMVT6	Feces	4	+
22.	<i>Escherichia coli</i>	O157:H7	ATKP8	Feces	5	+
23.	<i>Escherichia coli</i>	O157:H7	AZRS15	Feces	2	+
24.	<i>Escherichia coli</i>	O157:H7	R33-9	Feces	2	+
25.	<i>Escherichia coli</i>	O157:H7	AZ15-6	Feces	4	+
26.	<i>Escherichia coli</i>	O157:H7	AQ29-4	Feces	2	+
27.	<i>Escherichia coli</i>	O157:H7	AA18-3	Feces	9	+
28.	<i>Escherichia coli</i>	O157:H7	LS56	Feces	2	+
29.	<i>Escherichia coli</i>	O157:H7	A425TK	Feces	6	+
30.	<i>Escherichia coli</i>	O157:H7	A206RP	Feces	7	+
31.	<i>Escherichia coli</i>	O157:H7	A778EF	Feces	2	+
32.	<i>Escherichia coli</i>	O157:H7	MK41242	Ground beef	2	+
33.	<i>Escherichia coli</i>	O157:H7	AMK2608	Ground beef	10	+
34.	<i>Escherichia coli</i>	O157:H7	AMK1506	Ground beef	5	+

POSITIVE STRAINS						
	Strain			Origin	Inoculation CFU/225ml BHI	PCR Result Protocol A Rapid Spin
35.	<i>Escherichia coli</i>	O157:H7	AMK1311	Ground beef	1	+
36.	<i>Escherichia coli</i>	O157:H7	37006ID	Ground beef	3	+
37.	<i>Escherichia coli</i>	O157:H7	A1518ID	Ground beef	0	+
38.	<i>Escherichia coli</i>	O157:H7	A1512ID	Ground beef	3	+
39.	<i>Escherichia coli</i>	O157:H7	A1814ID	Ground beef	1	+
40.	<i>Escherichia coli</i>	O157:H7	A1989ID	Ground beef	1	+
41.	<i>Escherichia coli</i>	O157:H7	EF190	Feces	10	+
42.	<i>Escherichia coli</i>	O157:H7	Ad686	Slaughterhouse	5	+
43.	<i>Escherichia coli</i>	O157:H7	CIP103571 (ATCC 35150)	Clinical origin	9	+
44.	<i>Escherichia coli</i>	O157:H7	ATCC 43888		7	+
45.	<i>Escherichia coli</i>	O157:H7	Ad485	Ground beef	0	+
46.	<i>Escherichia coli</i>	O157:H7	Ad486	Ground beef	2	+
47.	<i>Escherichia coli</i>	O157:H7	Ad487	Ground beef	2	+
48.	<i>Escherichia coli</i>	O157:H7	Ad488	Ground beef	2	+
49.	<i>Escherichia coli</i>	O157:H7	Ad489	Ground beef	1	+
50.	<i>Escherichia coli</i>	O157:H7	ATCC 700728		14	+

NEGATIVE STRAINS						
Strain				Origin	Inoculation/ml BHI	PCR Result Protocol B MagMAX
1	<i>Escherichia coli</i>	O92:H33	JM221	Clinical origin (Mexico)	5,7.10 ⁵	-
2	<i>Escherichia coli</i>	O3:H2	38765	Clinical origin (Chilli)	3,4.10 ⁵	-
3	<i>Escherichia coli</i>	O78:H11	H10407	ATCC 35401	1,4.10 ⁵	-
4	<i>Escherichia coli</i>	O6:H6	EDL1493		3,3.10 ⁵	-
5	<i>Escherichia coli</i>	O6:H10	ECOR10	Clinical origin (Sweden)	4,9.10 ⁵	-
6	<i>Escherichia coli</i>	O111:H21	DEC6a	Clinical origin (USA)	9,6.10 ⁴	-
7	<i>Escherichia coli</i>	O86:H43	ECOR23	Animal origin (elephant USA)	4,0.10 ⁵	-
8	<i>Escherichia coli</i>	O26:H11	DEC9a	Clinical origin (USA)	3,6.10 ⁵	-
9	<i>Escherichia coli</i>	O111:H8	DEC8b	Clinical origin (USA)	8,6.10 ⁴	-
10	<i>Escherichia coli</i>	O128:H2	DEC11a	Clinical origin (USA)	4,9.10 ⁵	-
11	<i>Escherichia coli</i>	O111:H2	DEC12a	Clinical origin (UK)	5,5.10 ⁵	-
12	<i>Escherichia coli</i>	O128:H7	DEC13a	Clinical origin (USA)	3,0.10 ⁵	-
13	<i>Escherichia coli</i>	O78:K80:H12	TX-1	ATCC 43896	4,9.10 ⁵	-
14	<i>Escherichia coli</i>	O104:H21	ECOR26	Clinical origin (USA)	5,6.10 ⁵	-
15	<i>Escherichia coli</i>	O157:H43	DEC7a	Pork (USA)	6,1.10 ⁵	-
16	<i>Escherichia coli</i>	O55:H7	DEC5d	Clinical origin (Sri Lanka)	5,5.10 ⁵	-
17	<i>Escherichia coli</i>	O44:H18	42	Clinical origin (Peru)	3,7.10 ⁵	-
18	<i>Escherichia coli</i>	O127:H6	E2348/69	Clinical origin (UK)	3,6.10 ⁵	-
19	<i>Escherichia coli</i>	O55:H6	DEC1a	Clinical origin (USA)	6,4.10 ⁵	-
20	<i>Escherichia coli</i>	O18:K1:H7	RS218	Clinical origin	7,5.10 ⁵	-
21	<i>Salmonella</i>	Landau	Ad499		2,3.10 ⁵	-
22	<i>Salmonella</i>	Sternhauze	Ad500		2,4.10 ⁵	-
23	<i>Salmonella</i>	Urbana	Ad501		3,3.10 ⁵	-
24	<i>Salmonella</i>	Wayne	Ad502		3,2.10 ⁵	-
25	<i>Hafnia alvei</i>		88	Bakery	5,0.10 ⁵	-
26	<i>Hafnia alvei</i>		167	Sausage	5,2.10 ⁵	-

NEGATIVE STRAINS						
Strain				Origin	Inoculation/ml BHI	PCR Result Protocol B MagMAX
27	<i>Citrobacter freundii</i>		25	Frozen raw spinach	3,9.10 ⁵	-
28	<i>Citrobacter freundii</i>		104	Ground beef	5,2.10 ⁵	-
29	<i>Escherichia vulneris</i>		127	Raw milk	6,7.10 ⁵	-
30	<i>Pantoea spp.</i>		134	Pork	1,9.10 ⁵	-
31	<i>Escherichia coli</i>	O157	Ad524	Environment (dairy products)	6,6.10 ⁵	-
32	<i>Escherichia coli</i>	O157	Ad525	Feces	3,7.10 ⁵	-
33	<i>Escherichia coli</i>	O157	Ad526	Feces	3,0.10 ⁵	-
34	<i>Escherichia coli</i>	O157	Ad527	Clinical origin	3,3.10 ⁵	-
35	<i>Escherichia coli</i>	O157:H-	O1.12.903		7,5.10 ⁵	-
36	<i>Escherichia coli</i>	O157:H-	O1.12.905		5,5.10 ⁵	-
37	<i>Escherichia coli</i>	O145		Clinical origin	4,7.10 ⁵	-