ipl santé, environnement durables
Nord
filiale de l'Institut Pasteur de Lille

BIO-RAD

cofrac
ESSAIS

ACCREDITATION
N°1-0264
PORTEE
DISPONIBLE SUR
WWW.COFRAC.FR

AFNOR Certification of the AL Listeria monocytogenes enumeration method according to EN ISO 16140 standard

Certificate number: BRD 07/17 – 01/09

SUMMARY REPORT

Validation date:	26/01/2009
End validation date:	26/01/2013

IPL santé, environnement durables Nord – 1 rue du Professeur Calmette –59046 LILLE cedex

ipl santé, environnement durables
Nord
filiale de l'Institut Pasteur de Lille

Study realized by:

IPL santé, environnement durables Nord
Secteur Alimentaire
1 rue du Professeur Calmette
59046 LILLE cedex

for:

BIO-RAD
3, Bd Raymond Poincaré
92430 MARNES-LA-COQUETTE

PLAN

1 Introduction

1.1 Certification references and scope

The A.L. detection method has been certified with the certificate number BRD 07/17 – 01/09 in January 2009 for human food products and environmental samples.

1.2 Protocol and principle of the alternative method

1.2.1 Principle of the A.L. medium

The A.L. medium is specific for *Listeria monocytogenes*.
The principle of A.L. medium (Agar Listeria according to Ottaviani and Agosti) is based on the simultaneous detection of 2 enzyme activities: β-glucosidase and phosphatidylinositol-specific phospholipase C (PI-PLC).

- β-D-glucosidase activity, common to all *Listeria* genus bacteria is detected using a chromogenic substrate (X-glucoside). Its hydrolysis induces the formation of a blue to blue-green colour in all Listeria colonies.
- PI-PLC is an enzyme only detected in pathogenic Listeria species: *L. monocytogenes* and *L. ivanovii*. A.L. medium contains phosphatidylinositol which, when it breaks down, produces an opaque halo around colonies of bacteria of these 2 species.

The formula of the A.L. medium is the same as the reference method.

1.2.2 Protocol

The diagram summarising the method is shown in appendix A.

From a mother suspension (1 :10) :

- In buffered peptone water (BPW), with 1 hour of revivification at 20℃ +/- 2℃,

or

- In buffered peptone water (BPW), without revivification,

or

- in Half Fraser both, with 1 hour of revivification at 20℃ +/- 2℃

the A.L. plates are inoculated by:

- spreading 0,1 mL on the surface of one plate per dilution, with the possibility to inoculate 1ml on 3 plate to enumerate low contaminations of *Listeria monocytogenes*,

or

- pouring 1 mL in an A.L. plate.

The inoculated plates are incubated at 37℃ for 24 hours and 48 hours.

The characteristic colonies of ***Listeria monocytogenes*** on A.L. plates (blue with halo) have to be confirmed:

1) by the conventional tests described in the methods standardized by the CEN or ISO, including a purification step,
2) by the conventional tests described in the methods standardized by the CEN or ISO, without prior purification if the colony is sufficiently isolated,
3) by spot sub-culture on RAPID *L.Mono* agar, without prior purification if the colony is sufficiently isolated,
4) by PCR assay, specific of *Listeria monocytogenes*, directly from the colony.

The A.L. plates could also be stored up to 48 hours at 3℃ +/- 2℃ before interpretation.
Assays to confirm this possibility were made during the initial certification study.

1.3 Reference method

The validation study was carried out by reference to the EN ISO 11290-2/A1:2004 (#) standard method.
The diagram summarizing the method is shown in appendix A.

2 Comparative study of methods

The following criteria were determined:
- linearity
- relative accuracy
- inclusivity and exclusivity
- practicability

2.1 Relative accuracy

The relative accuracy is the closeness of agreement between a test result and the accepted reference value.

2.1.1 Nature of the tests

Food products have been analysed in duplicate according to the 2 methods:
- reference method EN ISO 10290-2, using Listeria Ottaviani and Agosti agar medium,
- and A.L. medium, within the different conditions (surface and depth, after dilution in BPW or Half Fraser)

In total, 56 products were analysed so as to obtain at least 10 usable results in each food category.

The categories and the types of samples studied are the following :

Categories	Analysed samples	Exploited results
Meat products	17	11
Dairy products	10	10
Seafood products	12	11 ou 10 *
Vegetables	12	10
Environment samples	10	10
TOTAL	**61**	**51 ou 52 ***

* depending on the alternative method conditions, results for sample n°23 are or not included.

The 10 samples the results of which were not used, exhibited :
- Colony counts below 10 CFU/g or 100 CFU/g with both methods in 8 cases,
- Colony counts below 10 CFU/g or 100 CFU/g with one method in 2 cases,

2.1.2 Artificial contamination of the samples and percentage

Artificial contamination was achieved by using stressed bacterial suspensions, the stress treatment and efficiency of which have been determined according to EN ISO 16140 and AFNOR validation rules.
37 positive results were obtained after artificial contamination.

Finally, 72% of positive results were obtained as a result of artificial contamination.

2.1.3 Raw Data

Each sample was analysed in duplicate by the alternative method and the reference method. The log (N(CFU/g)) results are presented in appendix B.

Following the EN ISO 16140 standard, the values for each sample were plotted on a two-dimensional graph. The vertical axis (y) is used for the alternative method and the horizontal axis (x) for the reference method.
The data were then tested by a linear regression program in order to determine the intercept value (a) and the slope value (b).
The relative accuracy relationship is evaluated according to the model: $y = bx + a$.
For each of the two methods, robust repeatability standard deviations were calculated (Rob.sr(x) and Rob.sr(y)).
As a function of the ratio of these standard deviations, Rob.R = Rob.sr(y)/Rob.sr(x), the linear regression to be used for the interpretation is defined in the EN ISO 16140 standard.

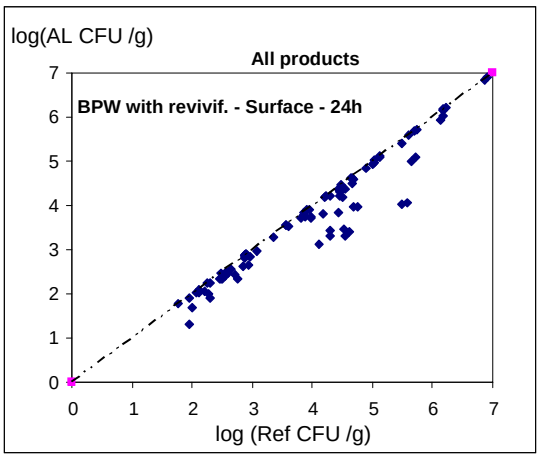
The following graphs represent the raw values obtained for the samples analysed.
The straight line represented is the first bisector (y = x).

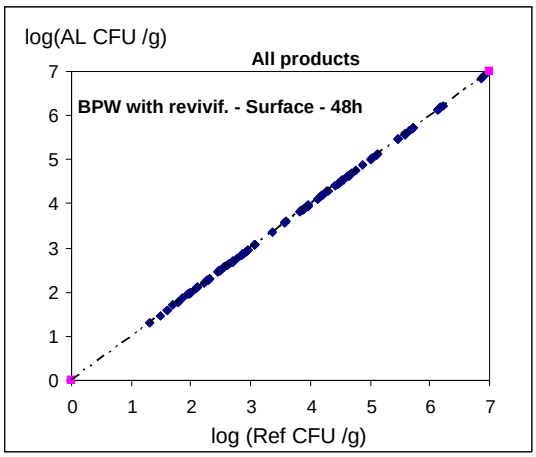
The graphs represent the values obtained after 48 hours incubation for the reference method and after 24 or 48 heures incubation for the alternative method.
But, the statistical interpretations have been done with the values obtained after 48 heures d'incubation for the both methods.
For information, the values after 24 hours incubation were considered for two samples (a raw milk cheese and a sample of beef minced balls) because the plates were not interpretable after 48 hours incubation.

No difference was observed between the plates after 48 hours incubation and the plates stored 72 hours at 3℃ +/- 2℃.

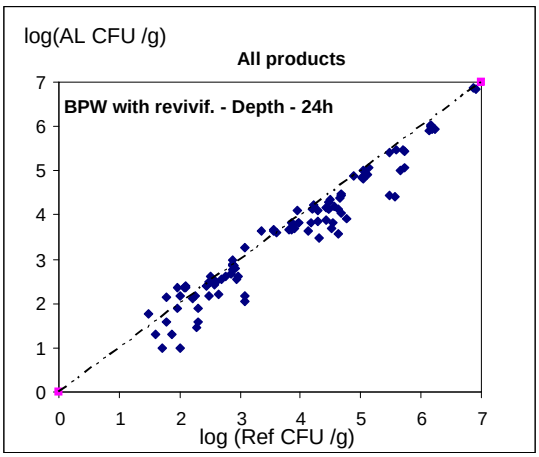
2.1.3.1 A.L. enumeration method – BPW with revivification –surface inoculation

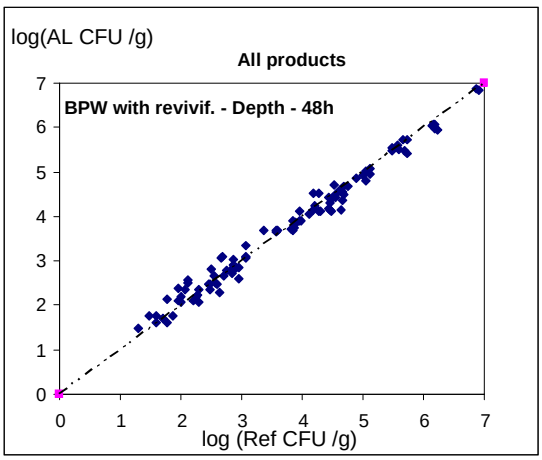
The conditions are the same as those of the reference method. The evolution between the 24 hours incubation period and the 48 hours incubation period is showed in the following graphs.



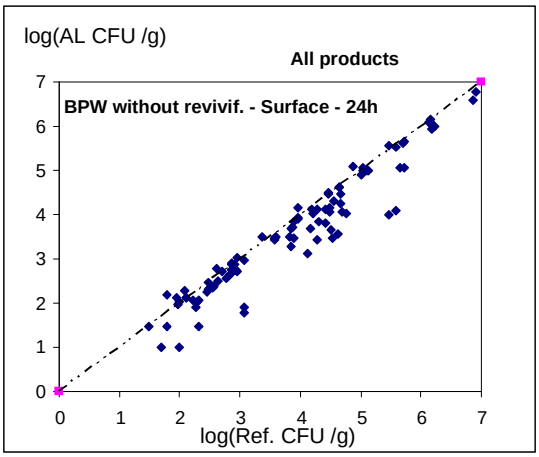


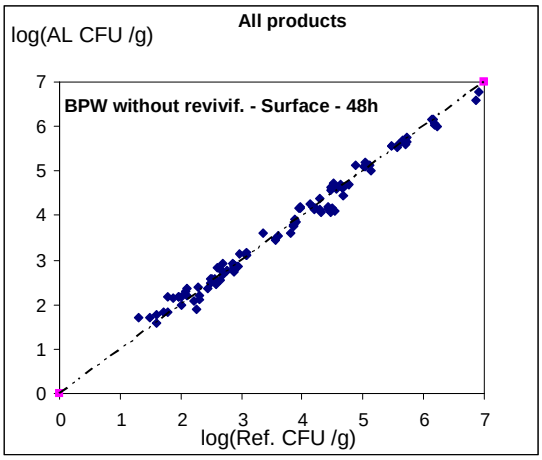
2.1.3.2 A.L. enumeration method – BPW with revivification –depth inoculation



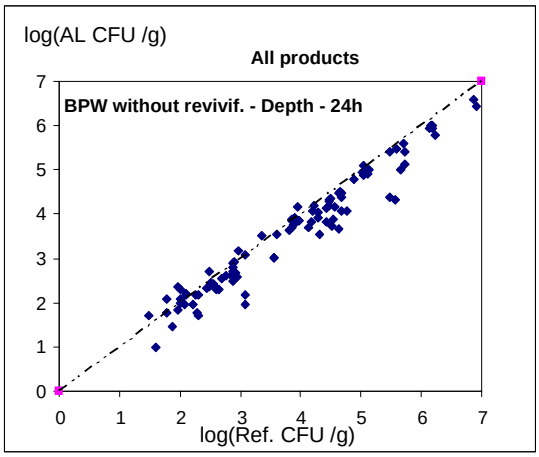


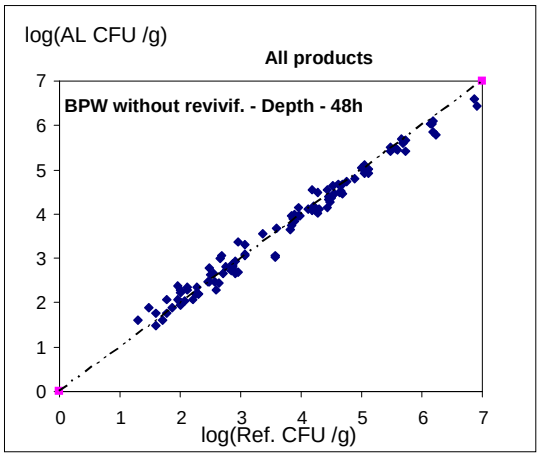
2.1.3.3 A.L. enumeration method – BPW without revivification –surface inoculation



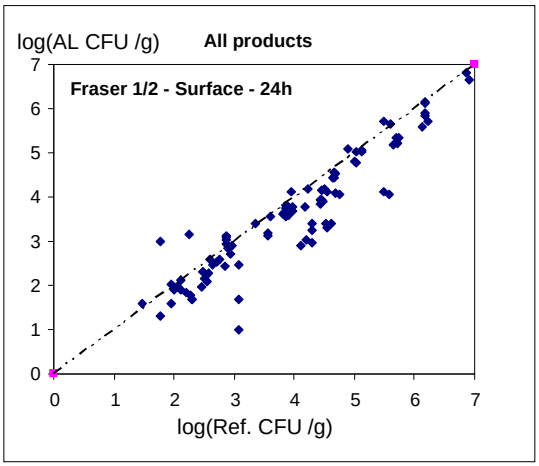


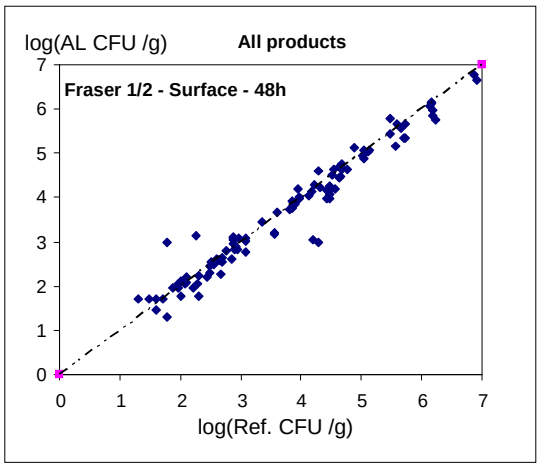
2.1.3.4 A.L. enumeration method – BPW without revivification –depth inoculation



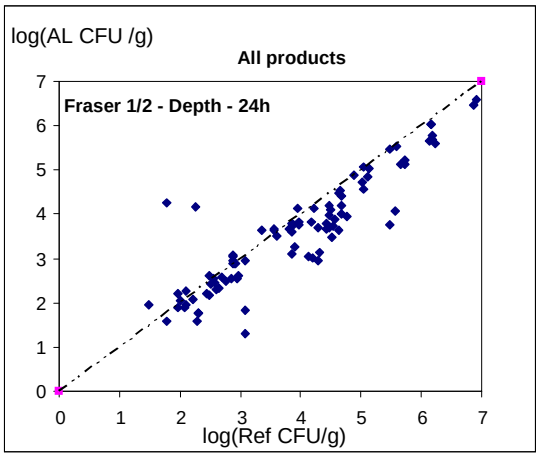


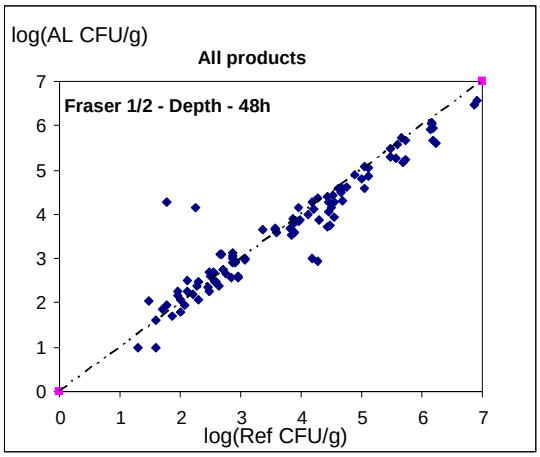
2.1.3.5 A.L. enumeration method – Half Fraser broth with revivification –surface inoculation





2.1.3.6 A.L. enumeration method – Half Fraser broth with revivification –depth inoculation





2.1.4 Statistical Interpretation

In order to check whether the relative accuracy is satisfactory, the two following hypotheses must be verified for a risk α = 5%:

- **Ordinate at the origin (or intercept) {a = 0}**
  The alternative method exhibits a systematic bias compared with the reference method:
  - If the value t = a / Sa with (q-2) degrees of freedom is higher than the T-critical value, obtained in Student's table, or
  - If the probability p{a = 0} < α (=0.05 or 5%), p{a = 0} being defined by Student's law.

- **Slope {b = 1}**
  If the alternative method does not yield the same values as the reference method:
  - If the value t = (b-1) / Sb with (q-2) degrees of freedom is higher than the T-critical value, obtained in Student's table, or
  - If the probability p{b = 1} < α (=0,05 or 5%), p{b = 1} being defined by Student's law.

Different values needed in the EN ISO 16140 standard are clarified in table below. It allowed to compare A.L. enumeration with reference method for all products tested.

Protocol	Rob.R	Regression used	a	p(t ;a=0) %	b	p(t ;b=1) %	Conclusion
BPW with revivification – surface inoculation	1.000	GMFR	0.000	100	1.000	100	{a=0} accepted {b=1} accepted
BPW with revivification – depth inoculation	0.819	GMFR	0.165	1	0.954	0	{a=0} not accepted {b=1} not accepted
BPW without revivification – surface inoculation	1.316	GMFR	0.142	0	0.965	0	{a=0} not accepted {b=1} not accepted
BPW without revivification – depth inoculation	0.963	GMFR	0.184	0	0.945	0	{a=0} not accepted {b=1} not accepted
Half Fraser broth with revivification – surface inoculation	1.643	GMFR	0.039	73	0.970	29	{a=0} accepted {b=1} accepted
Half Fraser broth with revivification – depth inoculation	1.306	GMFR	0.202	11	0.920	1	{a=0} accepted {b=1} not accepted

The equations for the regression lines between the alternative method and the reference method, for all products are the following:

BPW with revivification –surface inoculation	log(Alt.) = log(Ref.)
BPW with revivification –depth inoculation	log(Alt.) = 0.954 log(Ref.) + 0.165
BPW without revivification –surface inoculation	log(Alt.) = 0.965 log(Ref.) + 0.142
BPW without revivification –depth inoculation	log(Alt.) = 0.945 log(Ref.) + 0.184
Half Fraser broth with revivification –surface inoculation	log(Alt.) = 0.970 log(Ref.) + 0.039
Half Fraser broth with revivification –depth inoculation	log(Alt.) = 0.920 log(Ref.) + 0.202

Other parameters were presented in the following tables :

- the limits of robust repeatability (log values) obtained for the alternative method and the reference method
- the bias between the two methods (alternative method –reference method)

Protocol	Robust repeatability		Bias (D) (log CFU/g) (alternative – reference)		Contamination range (log)
	Réf.	Alt.	average	median	
BPW with revivification – surface inoculation	0.170	0.170	0.000	0.000	1.30 – 6.90
BPW with revivification – depth inoculation	0.173	0.142	- 0.010	- 0.026	
BPW without revivification – surface inoculation	0.173	0.228	+ 0.012	+ 0.010	
BPW without revivification – depth inoculation	0.170	0.163	- 0.019	- 0.012	
Half Fraser broth with revivification – surface inoculation	0.173	0.284	- 0.073	- 0.057	
Half Fraser broth with revivification – depth inoculation	0.173	0.226	- 0.096	- 0.087	1.00 – 6.90

2.1.5 Conclusion

When the reference and the alternative methods use the BPW as diluent, the correlation between the both methods is quite satisfactory for all the conditions, surface or depth, with or without revivification.
The ordinate at the origin and the slope are satisfactory. The repeatability log values are quite close to those obtained with the reference method.
The bias calculated between the alternative method and the reference method is in the order of D = +/- 0.01 log CFU/g (alternative method –reference method).

When the alternative method uses the Half Fraser broth as diluent, the two hypotheses {a=0} and {b=1} are accepted.
The repeatability log values obtained with the alternative method are higher than with the reference method and the bias are in the order of D = – 0.1 log CFU/g.
It should nevertheless remember that the test samples between the reference method and the alternative method are different.

2.2 Linearity

Linearity is the ability of the method when used with a given matrix to give results that are in proportion to the amount of analyte present in the sample, that is an increase in analyte corresponds to a linear or proportional increase in results.

2.2.1 Nature of the tests

Five food products were contaminated, at five contamination levels. For each product and each contamination level, the alternative and the reference methods were performed with two repetitions.

The analysed products were:

- rillettes,
- raw milk,
- vegetables,
- smoked fish,
- process water.

The contamination levels were:

- 50 to 100 CFU/g
  100 to 500 CFU/g
  500 to 1000 CFU/g
  1000 to 5 000 CFU/g
  5 000 to 10 000 CFU/g

Different strains of *Listeria monocytogenes* were used, as presented in the following table:

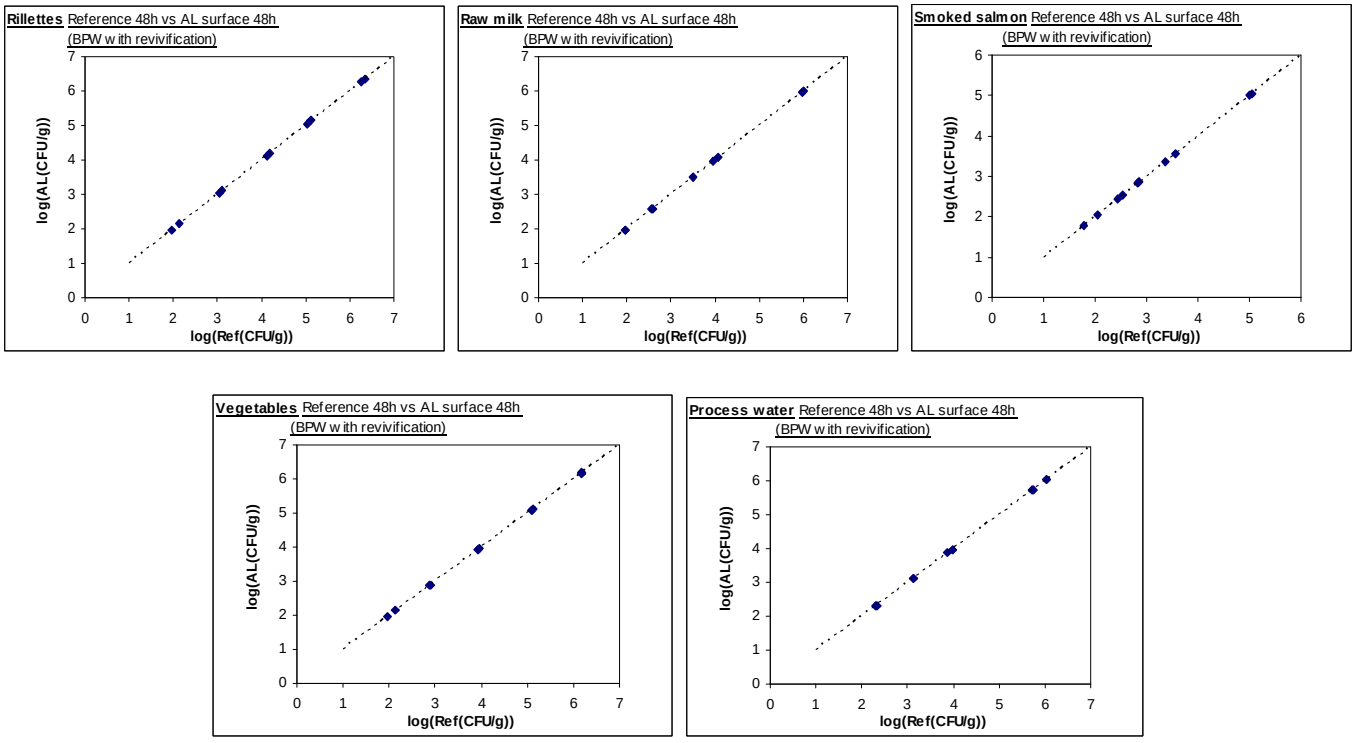
Product	Strain	Origin
rillettes	*Listeria monocytogenes* 1/2b	Delicatessen
raw milk	*Listeria monocytogenes* 4b	Munster
vegetables	*Listeria monocytogenes* 1/2a	Smoked salmon
smoked fish	*Listeria monocytogenes* 4b	Salad
process water	*Listeria monocytogenes* 1/2c	Environment

2.2.2 Raw Data

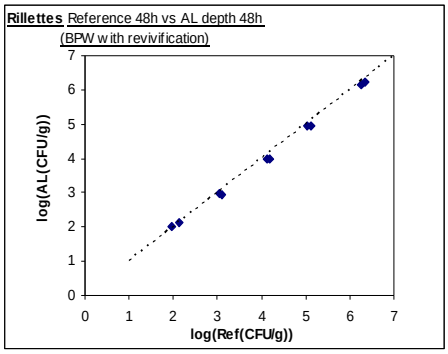
Following the EN ISO 16140 standard, the values for each sample were plotted on a two-dimensional graph. The vertical axis (y) is used for the alternative method and the horizontal axis (x) for the reference method.
The data were then tested by a linear regression program in order to determine the intercept value (a) and the slope value (b), like in the relative accuracy part.

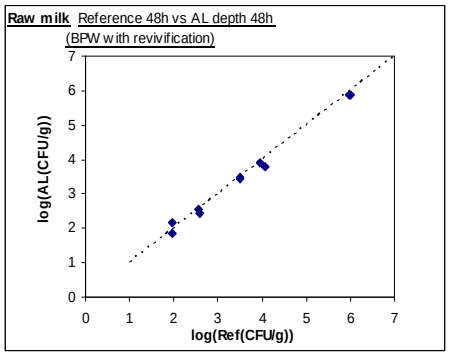
The following graphs represent the raw values obtained for each product and each protocol.

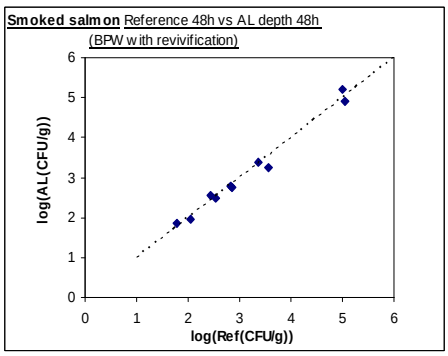
2.2.2.1 A.L. enumeration method – BPW with revivification –surface inoculation

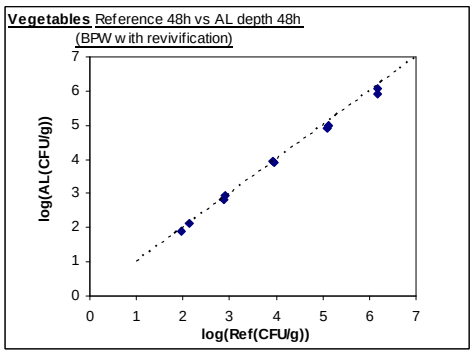


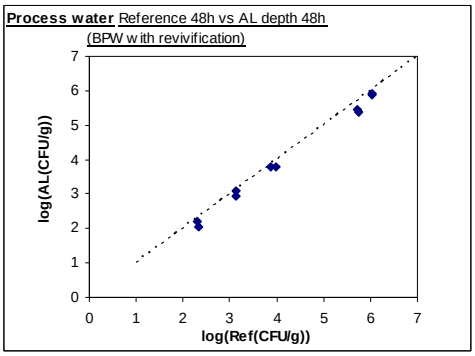
2.2.2.2 A.L. enumeration method – BPW with revivification –depth inoculation



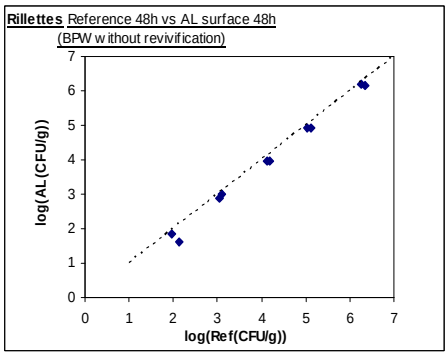


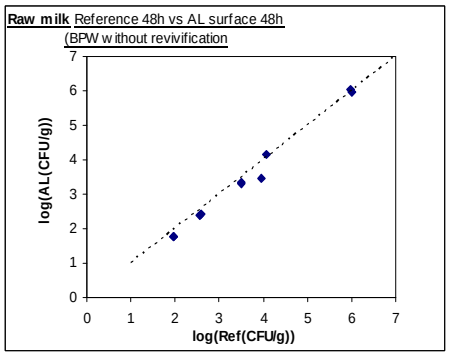


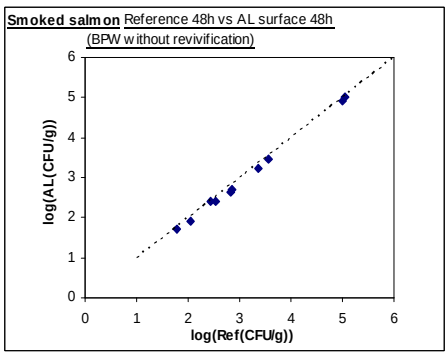


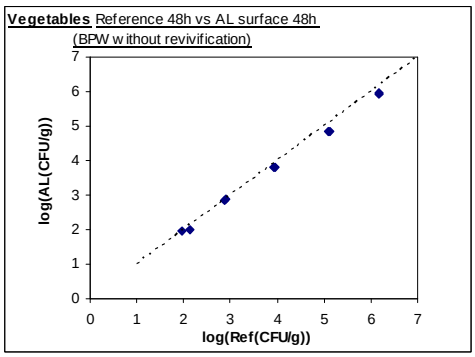


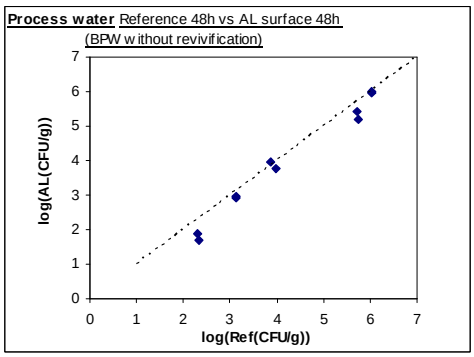
2.2.2.3 A.L. enumeration method – BPW without revivification –surface inoculation



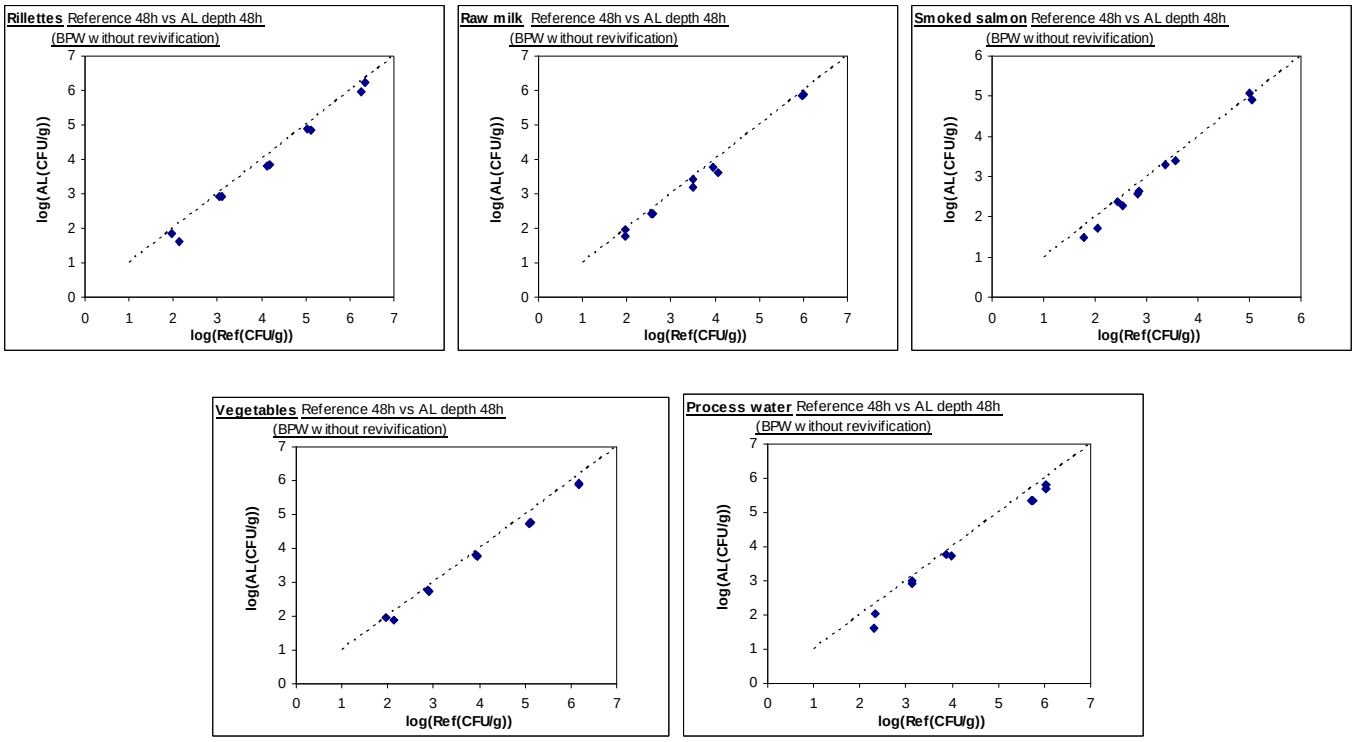




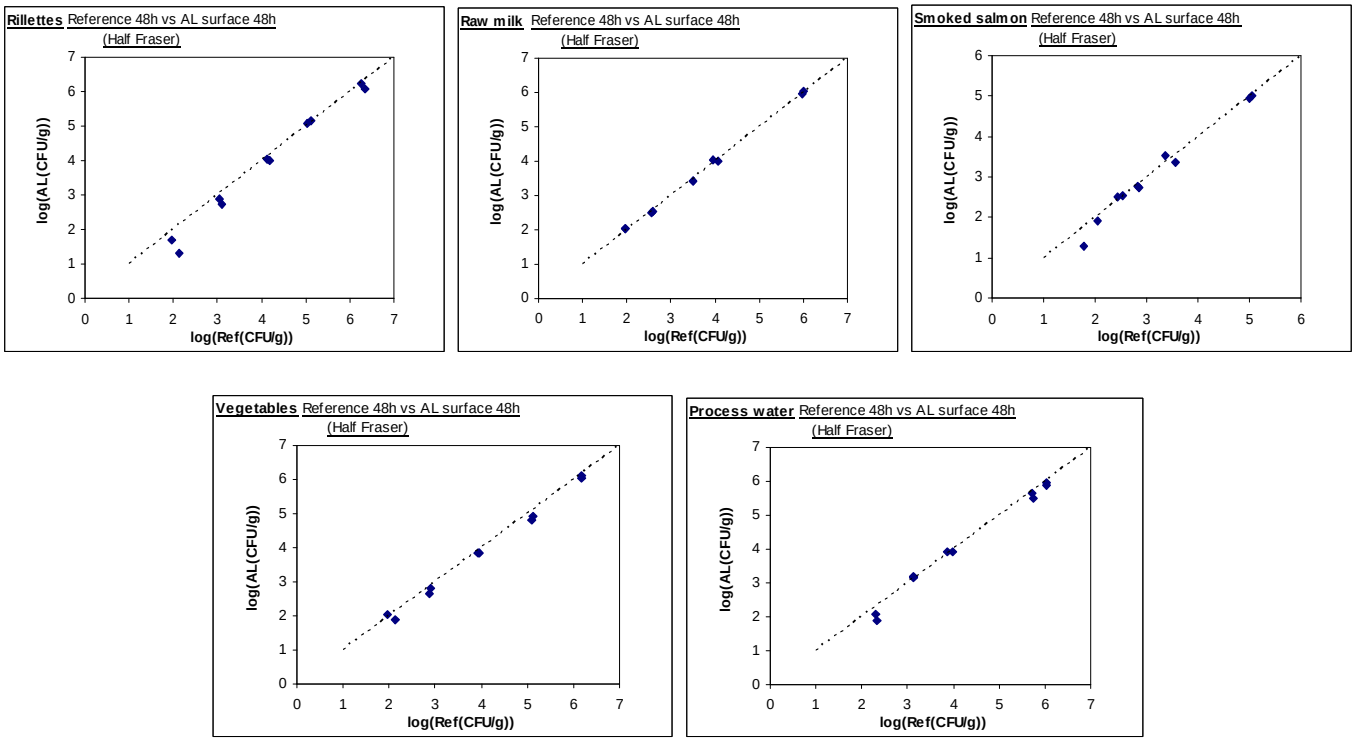




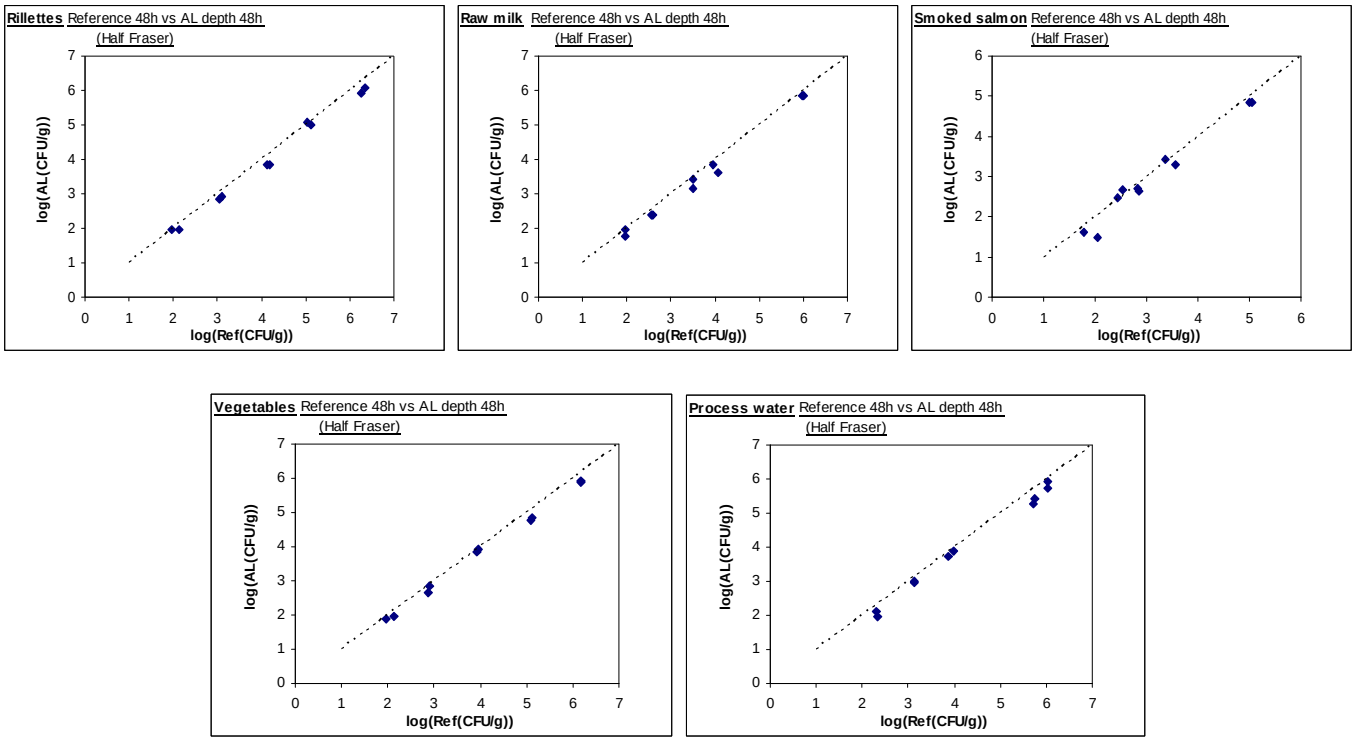
2.2.2.4 A.L. enumeration method – BPW without revivification –depth inoculation



2.2.2.5 A.L. enumeration method – Half Fraser broth with revivification –surface inoculation



2.2.2.6 *A.L. enumeration method – Half Fraser broth with revivification –depth inoculation*



2.2.3 Statistical Interpretation

The linearity is evaluated with the probability of lack-of-fit.
The value Rob.F is calculated as follow :

$$Rob.F = \frac{(N-2)\,(s^2y{:}x \,/\, Rob.sr(y)^2) - q\,(n-1)}{q-2}$$

with q, number of levels (q = 5)
n, number of repetitions (n = 2)
N, number of samples (N = nq)

The relationship is non linear if :
- [Rob.F > Fcrit (νnum, νden)]
or
- p(F, νnum, νden).< α (=0.05)

The type of regression and the Rob.F values are detailed in the following table:

Protocol	Product	Rob.R	Régression used	Rob.F	p (Rob.F) %	Conclusion
BPW with revivification – surface inoculation	rillettes	1.000	GMFR	0.000	100	linear
	raw milk					
	smoked salmon					
	raw vegetables					
	process water					
BPW with revivification – depth inoculation	rillettes	0.448	OLS 2	34.250	0	non linear
	raw milk	5.540	OLS	2.700	16	linear
	smoked salmon	1.063	GMFR	1.612	16	linear
	raw vegetables	4.965	OLS	0.000	100	linear
	process water	4.102	OLS	5.608	4	non linear
BPW without revivification – surface inoculation	rillettes	0.578	GMFR	8.959	2	non linear
	raw milk	2.459	OLS	29.460	0	non linear
	smoked salmon	0.944	GMFR	0.000	100	linear
	raw vegetables	1.572	GMFR	7.630	3	non linear
	process water	10.394	OLS	3.438	11	linear
BPW without revivification – depth inoculation	rillettes	0.678	GMFR	9.414	2	non linear
	raw milk	2.781	OLS	0.650	62	linear
	smoked salmon	1.217	GMFR	0.000	100	linear
	raw vegetables	2.202	OLS	9.564	2	non linear
	process water	4.382	OLS	16.993	4	non linear

Protocol	Product	Rob.R	Régression used	Rob.F	p (Rob.F) %	Conclusion
Half Fraser broth with revivification – surface inoculation	rillettes	1.884	GMFR	3.410	11	linear
	raw milk	1.733	GMFR	14.517	1	non linear
	smoked salmon	0.538	GMFR	25.967	0	non linear
	raw vegetables	5.379	OLS	1.469	33	linear
	process water	4.286	OLS	12.333	1	non linear
Half Fraser broth with revivification – depth inoculation	rillettes	1.000	GMFR	12.317	1	non linear
	raw milk	8.310	OLS	0.771	56	linear
	smoked salmon	1.217	GMFR	8.962	2	non linear
	raw vegetables	3.844	OLS	2.273	20	linear
	process water	7.727	OLS	1.169	41	linear

The equations for the regression lines between the alternative method and the reference method, are the following:

Protocol	Product	Equation	Correlation coefficient
BPW with revivification – surface inoculation	rillettes	log(Alt.) = log(Ref.)	$R^2 = 1.000$
	raw milk		
	smoked salmon		
	raw vegetables		
	process water		
BPW with revivification – depth inoculation	rillettes	log(Ref.) = 1.031 log(Alt.) – 0.229	$R^2 = 0.998$
	raw milk	log(Alt.) = 0.967 log(Ref.) + 0.046	$R^2 = 0.994$
	smoked salmon	log(Alt.) = 1.009 log(Ref.) – 0.068	$R^2 = 0.995$
	raw vegetables	log(Alt.) = 0.958 log(Ref.) + 0.092	$R^2 = 0.997$
	process water	log(Alt.) = 0.978 log(Ref.) – 0.087	$R^2 = 0.996$
BPW without revivification – surface inoculation	rillettes	log(Alt.) = 1.032 log(Ref.) – 0.322	$R^2 = 0.999$
	raw milk	log(Alt.) = 1.042 log(Ref.) – 0.295	$R^2 = 0.989$
	smoked salmon	log(Alt.) = 1.013 log(Ref.) – 0.153	$R^2 = 0.998$
	raw vegetables	log(Alt.) = 0.952 log(Ref.) + 0.054	$R^2 = 0.999$
	process water	log(Alt.) = 1.044 log(Ref.) – 0.426	$R^2 = 0.983$
BPW without revivification – depth inoculation	rillettes	log(Alt.) = 1.016 log(Ref.) – 0.309	$R^2 = 0.998$
	raw milk	log(Alt.) = 0.988 log(Ref.) – 0.140	$R^2 = 0.994$
	smoked salmon	log(Alt.) = 1.084 log(Ref.) – 0.443	$R^2 = 0.999$
	raw vegetables	log(Alt.) = 0.952 log(Ref.) – 0.013	$R^2 = 0.999$
	process water	log(Alt.) = 1.008 log(Ref.) – 0.327	$R^2 = 0.987$
Half Fraser broth with revivification – surface inoculation	rillettes	log(Alt.) = 1.106 log(Ref.) – 0.645	$R^2 = 0.995$
	raw milk	log(Alt.) = 0.995 log(Ref.) + 0.012	$R^2 = 0.999$
	smoked salmon	log(Alt.) = 1.064 log(Ref.) – 0.286	$R^2 = 0.991$
	raw vegetables	log(Alt.) = 0.993 log(Ref.) – 0.100	$R^2 = 0.997$
	process water	log(Alt.) = 1.013 log(Ref.) – 0.163	$R^2 = 0.991$
Half Fraser broth with revivification – depth inoculation	rillettes	log(Alt.) = 0.974 log(Ref.) – 0.083	$R^2 = 0.995$
	raw milk	log(Alt.) = 0.991 log(Ref.) – 0.150	$R^2 = 0.994$
	smoked salmon	log(Alt.) = 1.024 log(Ref.) – 0.227	$R^2 = 0.983$
	raw vegetables	log(Alt.) = 0.956 log(Ref.) – 0.002	$R^2 = 0.990$
	process water	log(Alt.) = 0.979 log(Ref.) – 0.134	$R^2 = 0.993$

2.2.4 Conclusion

Considering the different graphs and regression equations, the **linearity is satisfactory**.

Note that the correlation coefficients are very high, about 99%, so the significance of the non-linearity test could be failed.

2.3 Specificity / selectivity (inclusivity / exclusivity)

The aim of this study is to check that all *Listeria monocytogenes* strains are detected, and that no cross reaction exists with other species of *Listeria* or with other genus strains.
The inclusivity and the exclusivity of the method are defined by analysis, respectively, of 30 positive strains and 20 negative strains.
These strains were studied during the comparative study of the A.L. detection method and the results were satisfactory. So no complementary assay was made in this study.

The results are presented in appendix C.
All the 60 *Listeria monocytogenes* strains were detected with the A.L. detection method (blue colony with halo).
The 19 *Listeria* other than *monocytogenes* strains were blue without halo on A.L. medium, except the *Listeria ivanovii* strains, which gave blue colonies with halo at 24 hours incubation. These *Listeria ivanovii* strains were characteristic when they were tested with the reference method (after 48 hours incubation on PALCAM plates and 24 hours incubation on A.L. plates).
However, the size of halo for the *Listeria ivanovii* strains is smaller than the size of halo for the *Listeria monocytogenes* strains.
No cross reaction was observed with the 18 non-*Listeria* strains, tested on A.L. medium.

3 Interlaboratory study

3.1 Study organization

- Number of participating laboratories

16 laboratories received samples.

- Matrix used

Pasteurized milk".

- Strain used

Listeria monocytogenes (origin « raw milk cheese »).

- Number of samples per laboratory

8 samples were prepared per laboratory, two flasks par inoculation level.

- Analyses day and performed protocol

Start of analyses at D2 with the protocol “BPW without revivification in depth”

3.2 Control of experimental parameters

3.2.1 Contamination levels obtained after artificial contamination

The four contamination levels are presented in the following table:

Level	Sample	Targeted level (CFU/ml)	Real level(CFU/ml))
Level 0	5 and 8	0	0
Level 1	4 and 7	100	91
Level 2	2 and 6	1 000	786
Level 3	1 and 3	10 000	8400

3.2.2 Strain stability during transport

To evaluate the *Listeria monocytogenes* strain variability during transport, bacterial count of flasks inoculating at differents levels, have been checked at different time, during storage at 6°C.
Results (CFU/ml) are reported in following table:

	J0	**J1**	**J2**
Sample 1	110	100	130
Sample 2	1200	1500	1400
Sample 3	13000	10000	12000

No evolution of the strain has been observed after 48 h of storage at 6°C.

3.2.3 Problems of temperature recorded during transport, temperature on reception and reception times

3.2.3.1 Analysis of temperature monitoring curves during transport

Temperatures registered by thermo button during shipment were comprised between 0,1℃ et 5,0℃ for all laboratories.

3.2.3.2 Temperatures on reception and reception times

The temperatures obtained are recorded in the following tables:

Laboratory	Reception Temperatures (℃)		Comments
	communicated by the laboratory	indicated by the thermo button	
A	2.2	1.1	/
B	1.9	1.0	/
C	3.9	1.1	/
D	3.1	4.1	/
E	5.9	5.2	/
F	3.1	3.0	/
G	1.1	0.5	/
H	7.0	4.7	/
I	3.8	1.1	/
J	Not communicated	1.6	/
K	2.0	1.1	/
L	4.7	1.6	/
M	Not communicated	2.5	/
N	Not communicated	5.1	/
O	5.3	3.6	Delivery at D2
P	Not communicated	1.0	/

The laboratory O received the samples at D2, but the temperature at reception and the temperature curve during the transport (between 0.1 et 4.0℃) confirmed its participation.

3.2.4 Conclusion

Due to the delivery conditions, all the laboratories could perform the analyses at D2.

3.3 Results

3.3.1 Expert laboratory

Results obtained by the expert laboratory with EN ISO 11290-2 method and A.L. enumeration method are presented in the following table:

	EN ISO 11290-2		A.L. method BPW without revivification - depth	
	Duplicate 1	**Duplicate 2**	**Duplicate 1**	**Duplicate 2**
Level 0	<10	<10	<10	<10
Level 1	270	190	130	130
Level 2	3200	3800	1400	1800
Level 3	40000	15000	12000	11000

Results according to standard EN ISO 11290-2 and alternative method were in agreement.

3.3.2 Results obtained by cooperating laboratories

Due to delivery problems of A.L. dehydrated medium, 3 laboratories (I, M and O) could'nt realize the analyses. The laboratory J didn't send its results.

So, results of the 12 laboratories which realised the analysis were:
(see appendix D for detailed results)

Level 1 (results in CFU/ml)

Lab	EN ISO 11290-2		A.L. method BPW without revivification - depth	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
A	140	40	150	160
B	90	60	120	60
C	120	140	110	90
D	20	10	110	180
E	80	30	70	90
F	60	80	110	200
G	140	100	80	109
H	140	200	220	70
K	140	70	210	160
L	80	100	100	110
N	190	130	110	240
P	91	91	100	110

Level 2 (results in CFU/ml)

Lab	EN ISO 11290-2		A.L. method BPW without revivification - depth	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
A	1300	1100	1100	1300
B	1200	1400	970	1000
C	790	790	880	820
D	360	200	840	890
E	780	630	1200	1300
F	610	470	1300	880
G	1100	1100	1300	1300
H	2700	1400	1400	1300
K	1500	1100	1400	1500
L	1100	980	1000	1100
N	1200	1100	1200	1200
P	1100	770	910	860

Level 3 (results in CFU/ml)

Lab	EN ISO 11290-2		A.L. method BPW without revivification - depth	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
A	11000	9800	11000	12000
B	15000	13000	10000	11000
C	9200	11000	7900	9900
D	6400	5300	11000	9400
E	10000	6600	11000	10000
F	14000	10000	16000	8500
G	11000	9100	11000	13000
H	8600	16000	12000	11000
K	12000	11000	14000	13000
L	11000	7600	9600	8500
N	14000	13000	14000	15000
P	13000	8700	12000	7800

3.3.3 Conclusion

The obtained results were in accordance with those of the expert laboratory.

However, the results obtained by the laboratory F, for the reference method, were inconsistent if we review the counts obtained for each decimal dilution. *Therefore, its results were not exploited*.

Eventually, results of 11 laboratories have been statistically exploited.

3.4 Calculations

Statistical interpretations have been calculated according to standard EN ISO 16140, per level of contamination. Results were converted in log for the calculations.

3.4.1 Bias calculation

For each level, difference between duplicate means (d_i) obtained by the alternative method and reference method has been calculated, that allows the determination of (=MED{d_i}), and the **robust standard deviation** s{d_i}) (=$k_1 S_n$).

In order to verify if the relative accuracy is correct, the hypothesis **{D = 0}** was tested for each level, with calculating the statistic as:

$t(d) = MED\{d_i\} \sqrt{n} / s\{d_i\}$	for n-1 df (degrees of freedom) (n = number of labs) with α = 5%.

The bias is significant if the t(d) > critical $t_{0.05,\ df}$ value, i.e; the alternative method lacks accuracy, relative to the reference method for the considered level.

The bias D (alternative – reference) values, the robust standard deviation values (s{d_i}) and the t(d) values obtained by level are reported in the following table :

	Bias D (log)	s{d_i}	t(d)	$t_{0.05,\ df}$ value	Conclusion
Level 1	0.060	0.204	0.978	2.228	{D=0} accepted
Level 2	0.024	0.077	1.035		{D=0} accepted
Level 3	0.026	0.106	0.822		{D=0} accepted

Reminder :
The bias value (alternative – reference) obtained in the comparative study was (– 0.01 log).

Conclusion :
The **{D=0}** hypothesis is statistically accepted for all levels. So, the alternative method accuracy. relative to the reference method is satisfactory.

3.4.2 Repeatability calculation

3.4.2.1 Repeatability limits

For each method and each level, the repeatability limits, r, have been computed : $r = 2.8\ S_r$,
with S_r : repeatability standard deviation.

Values obtained for the repeatability limit are reported in the following table :

	r (log CFU/mL) - Reference method	r (log CFU/mL) - Alternative method
Level 1	0.513	0.320
Level 2	0.231	0.079
Level 3	0.232	0.122

3.4.2.2 Interpretation

The repeatability of the alternative and reference method were compared with a F-distribution: $F = (S_{r,alt} / S_{r,réf})^2$ with n and n degrees of freedom. If F (ou 1/F) > critical $F_{\alpha;n,n}$ value, then the compared methods have different repeatability, for the considered level.
F Values obtained are reported in the following table:

	F (ou 1/F*)	F (0.05 ;n ;n)	n	Conclusion
Level 1	2,569	2.85	11	Comparable repeatability
Level 2	8.562			Different repeatability
Level 3	3.659			Different repeatability

3.4.2.3 Conclusion
The alternative method and reference method repeatability limits are statistically comparable for level 1.
For the levels 2 and 3, the repeatability limits are less important for the alternative method.

Reminder :
The repeatability limits obtained during the comparative study were:
- 0.17 log for the reference method,
- 0.16 log for the alternative method.

3.4.3 Reproducibility calculation

3.4.3.1 Reproducibility limits

For each method and each level, the reproducibility limits, R, have been computed : $R = 2.8\ S_R$,
with S_R : reproducibility standard deviation.

Values obtained for the reproducibility limit are reported in the following table :

	R (log CFU/mL) - Reference method	R (log CFU/mL) - Alternative method
Level 1	0.727	0.489
Level 2	0.427	0.265
Level 3	0.302	0.235

3.4.3.2 Interpretation

The reproducibilities of the alternative and reference method were compared with a F-distribution: $F = (S_{R,alt} / S_{R,réf})^2$ with n-1 et n-1 degrees of freedom. If F (ou 1/F) > critical $F_{\alpha;n-1,n-1}$ value, then the compared methods have different repeatability, for the considered level.
F Values obtained are reported in the following table :

	F	F (0.05 ;n-1 ;n-1)	n	Conclusion
Level 1	2.209	2.98	11	Comparable reproducibilities
Level 2	2.603			Comparable reproducibilities
Level 3	1.645			Comparable reproducibilities

3.4.3.3 Conclusion
The alternative method and reference method reproducibility limits are statistically comparable for all levels.

4 Practicability

Practicability is studied according to the 13 criteria defined by the AFNOR technical board. comparing the EN ISO 11290 reference method to the A.L. enumeration method.

1. *Packaging mode of the components of the method (see package insert)* 2. *Reagent volumes (see package insert and vial packaging)*	Agar plates are packaged in boxes of 20 boxes of 90 mm
3. *Storage conditions (see package insert)*	The plates must be stored between entre +2℃ and +8 ℃. protected from light
4. Modalities of use after first use (see package insert)	The conservation outside the boxes, in started cellophane bag. is 1 month at 2–8℃
5. Equipment or necessary specific premises (see package insert)	Among the required equipment. - an air incubator at 30℃ ± 1℃ - an air incubator at 37℃ ± 1℃
6. Ready-to-use reagents or requiring reconstitution (see package insert)	/
7. Training of the operator	For an operator trained in standard techniques of microbiology. training in the technique requires less than 1 day.

8. Real time handling - Flexibility of the technique relative to the number of samples to be analyzed

Steps	Average time for one sample (min)		
	ISO 11290-2	**AL – depth**	**AL – surface**
Preparation. weighing. dilution and stomaching (and media preparation)	7	7	5
Transfert and inoculations (average time depending on number od considered dilutions)	3	1,5	3
Plates reading, interpretation and calculation	5	5	5
Average total time (per sample)	**15 minutes**	**13.5 minutes**	**15 minutes**

When confirmations are necessary, time is more important, depending on the number of confirmed colonies.

9. Time-to-result

Steps	**Time-to-result Reference method**	**Time-to-result Alternative method**
Realization of first dilution and decimal dilutions	D0	D0
Media inoculation	D0	D0
Plates reading, interpretation and calculation	D1 et D2	D1 et D2
Obtaining "negative" result	**D2**	**D2**
Obtaining"positive" result Confirmations - by reference method tests (including purification) - by spot sub-culture on RAPID'*L.mono* - by PCR test	**D4 à D9**	**D4 à D9** **D3** **D2**

10. Type of qualification of the operator:	level identical to that necessary for the reference method
11. Steps common to the reference method	Dilutions and surface inoculation Characteristic colonies interprétation
12. Traceability of the analysis results	/
13. Maintenance by the laboratory	/

5 Conclusion

The validation study of the methods was conducted according to the reference document EN ISO 16140.

The **comparison** of A.L. enumeration method (with these different protocols) with EN ISO 11290-2 standard allows to conclude that the alternative method gives **accurate results** with regard to standard method.
The **linearity** of the alternative method is satisfactory.
The method is *Listeria monocytogenes* **specific** as for standard method.

Collaborative study (accuracy) gave **satisfactory values of repeatability and of reproducibility**.

- for all the contamination levels, the alternative method accuracy, relative to the reference method is satisfactory. The bias **{D = 0}** hypothesis is statistically accepted for the first level and the bias obtained for the other levels are similar.
  The obtained bias values (alternative– reference) varied between (0.02 log) to (0.06 log).
  The bias value (alternative – reference) obtained in the comparative study varied between (-0.09 log) to (0.01 log).
- the repeatability limits varied by 0.23 to 0.51 log CFU/ml for the reference method and by 0.08 to 0.32 log CFU /ml for the alternative method
  And the repeatability limits obtained during the comparative study were 0.17 log for the reference method and [0.14 – 0.28] log for the alternative method.
- the reproducibility limits varied by 0.30 to 0.73 log CFU/ml for the reference method and by 0.27 to 0.49 log CFU /ml for the alternative method. They are statistically comparable for all the levels.

Set of results led to **AFNOR certification** according to ISO 16140 of the A.L. enumeration method (certificate n° BRD 07/17 – 01/09). for the enumeration of *Listeria monocytogenes* in human food product and environmental samples. **for a 4 years period**.

Lille. January 18th 2010

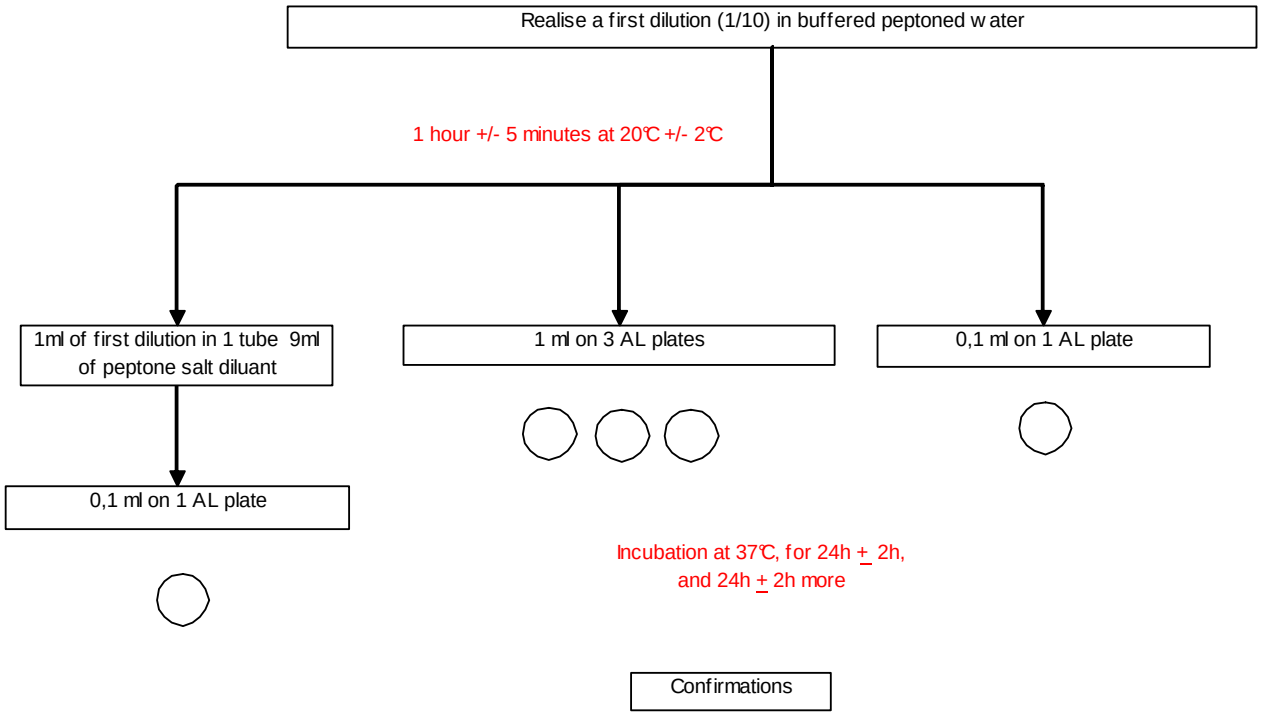
Virginie Ewe
Technical Manager

APPENDICES

APPENDIX A

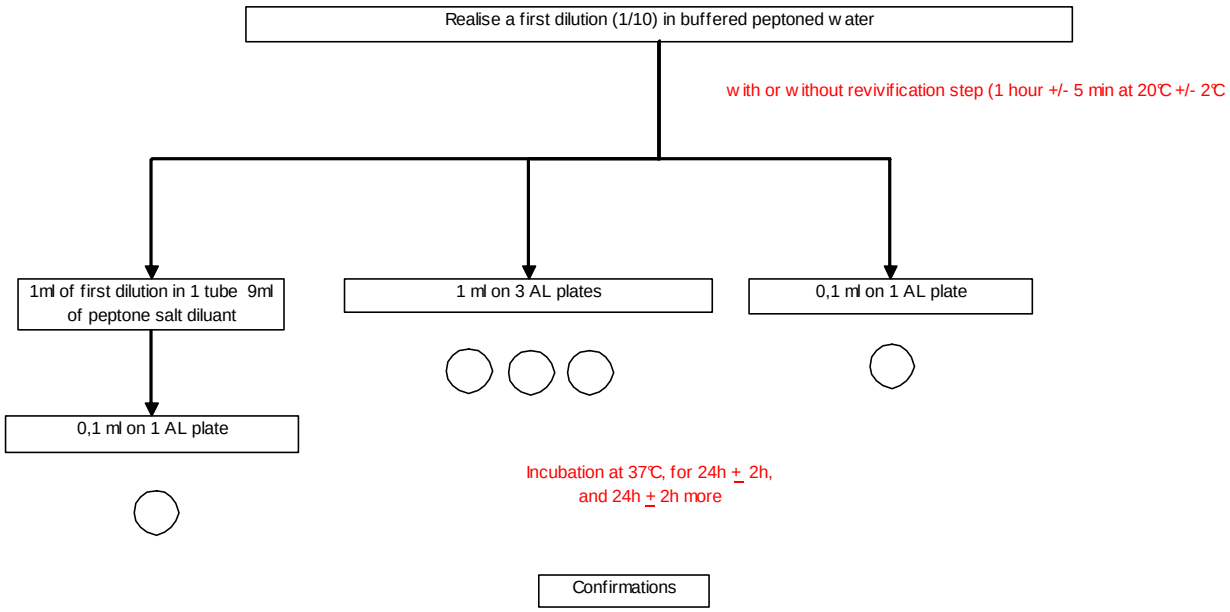
ANALYTICAL PROTOCOLS

EN ISO STANDARD 11290-2/A1: 2004 (#)

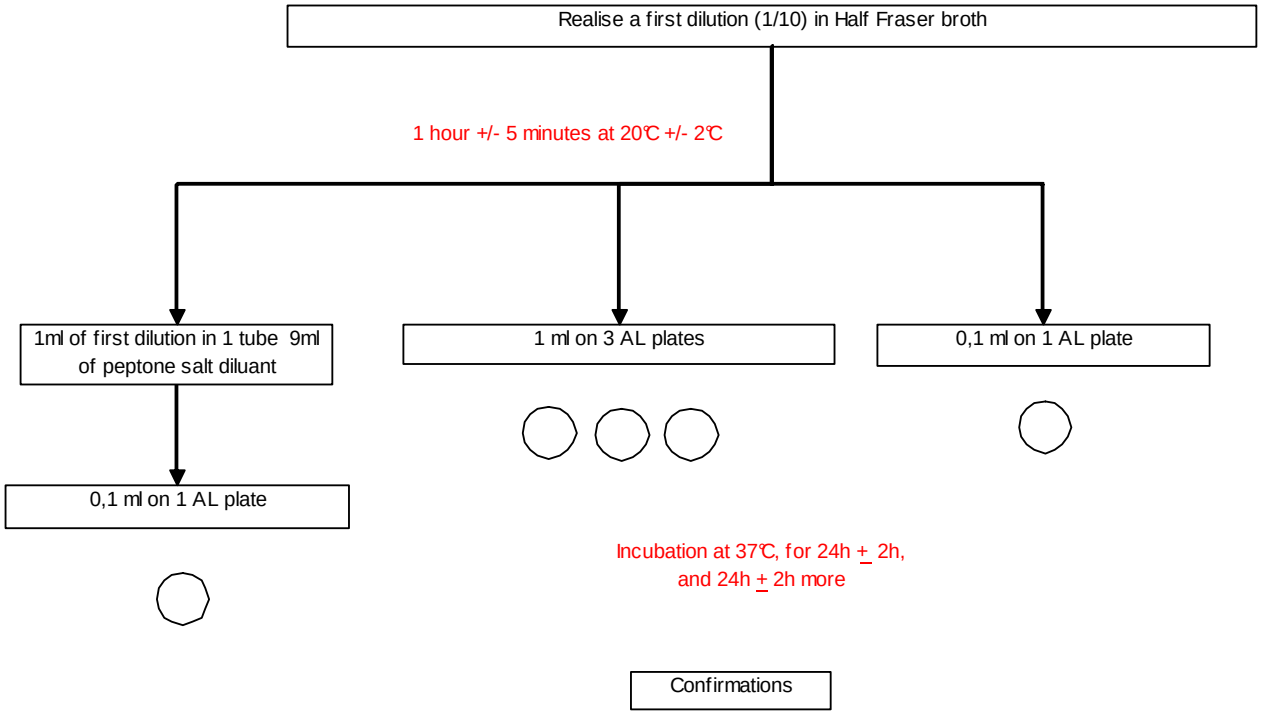


ALTERNATIVE METHOD PROTOCOL
Surface inoculation

- Buffered peptone water

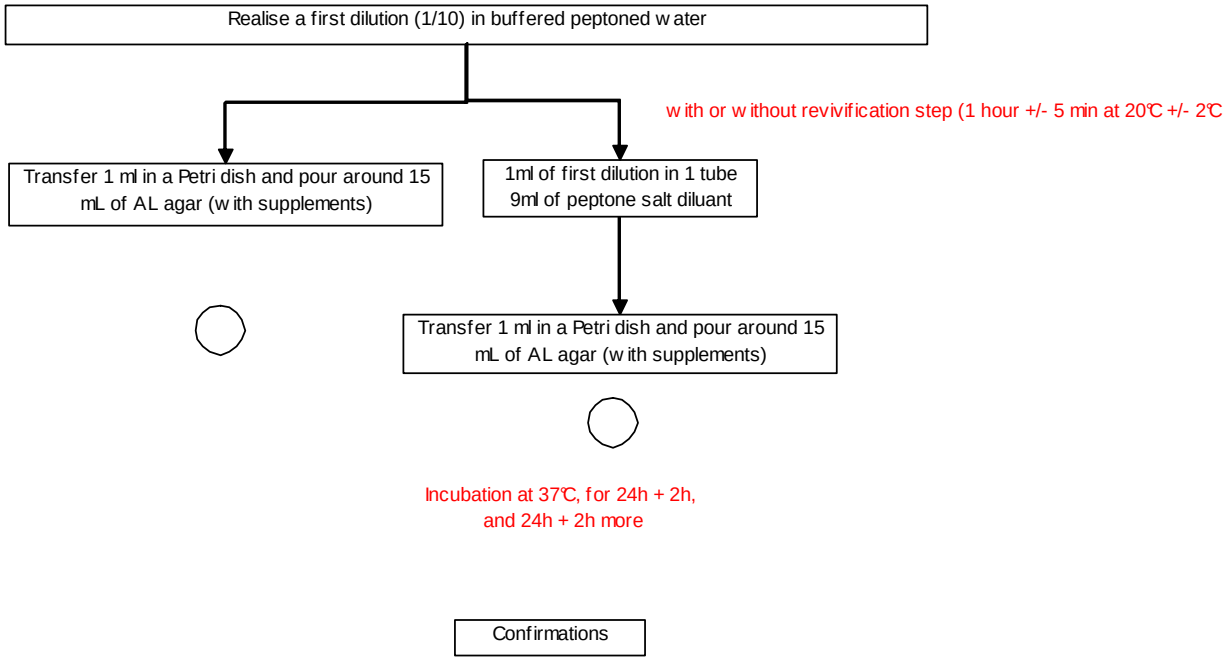


- Half Fraser broth

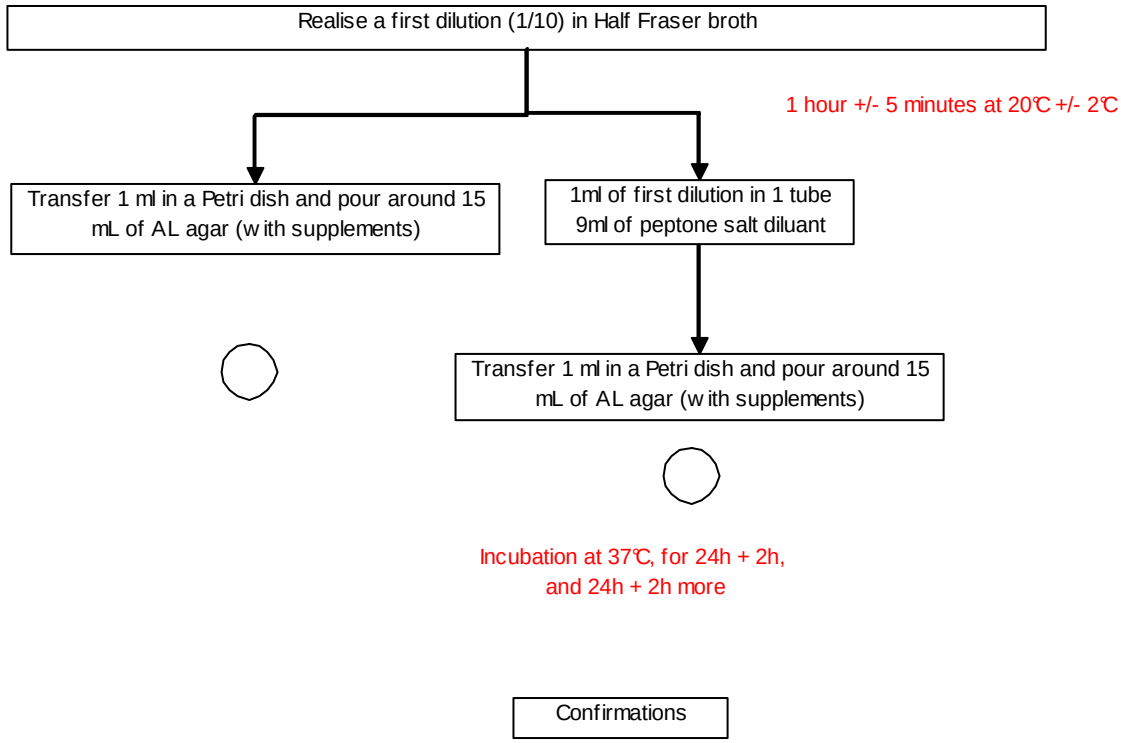


ALTERNATIVE METHOD PROTOCOL
Depth inoculation

- Buffered peptone water



- Half Fraser broth



APPENDIX B

ACCURACY
-
DETAILED RESULTS TABLES
FOR EACH SAMPLE CATEGORY

COUNTS RESULTS (log(CFU/g))

			ISO 11290-2		A.L. enumeration method - surface												A.L. enumeration method - depth											
			BPW with revivif		BPW with revivif				BPW without revivif				Half Fraser Ready-to-use				BPW with revivif				BPW without revivif				Half Fraser Ready-to-use			
			48H		24H		48H		24H		48H		24H		48H		24H		48H		24H		48H		24H		48H	
Produit	Cat.	Artif	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2
Mashed broccolis	VG	Yes	3,95	4,21	3,90	4,21	3,95	4,21	4,15	4,04	4,17	4,16	4,13	4,18	4,19	4,28	4,11	4,24	4,13	4,24	4,16	4,19	4,16	4,19	4,13	4,13	4,14	4,13
Grated carotts salad	VG	Yes	6,16	6,16	6,16	6,16	6,16	6,16	6,10	6,16	6,16	6,17	6,11	6,15	6,12	6,15	6,04	6,00	6,07	6,04	6,00	6,00	6,04	6,04	6,05	6,03	6,06	6,03
Whipped cream	DP	Yes	2,69	2,67	<10	<10	2,69	2,67	<10	<10	2,67	2,78	<10	<10	2,54	2,28	<10	<10	3,09	3,07	<10	<10	3,07	3,02	<10	<10	3,09	3,11
Peas	VG	Yes	5,72	5,65	5,09	4,99	5,72	5,65	5,06	5,07	5,76	5,69	5,23	5,19	5,67	5,58	5,07	5,00	5,74	5,74	5,13	5,01	5,67	5,71	5,13	5,12	5,68	5,72
Hamburger	MP	No	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Chicken meat with pasta and vegetables	MP	No	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Goat's milk cheese	DP	Yes	4,28	4,54	3,31	3,32	4,28	4,54	3,43	3,48	4,37	4,12	3,41	3,31	4,61	4,63	3,85	3,84	4,52	4,51	3,91	3,89	4,49	4,46	3,71	3,74	4,36	4,28
Fish fillet	SF	Yes	3,07	3,07	<10	<10	3,07	3,07	1,78	1,90	3,12	3,12	1,70	1,00	3,02	3,08	2,04	2,16	3,08	3,10	2,16	1,95	3,06	3,10	1,85	1,30	2,98	2,98
Salmon with sauce	SF	Yes	1,70	2,00	<10	<10	1,70	2,00	1,00	1,00	1,85	2,00	<10	<10	1,70	1,78	1,00	1,00	1,70	2,07	<10	1,00	1,60	1,95	<10	<10	1,85	1,80
Process water	EN	Yes	5,57	5,48	4,05	4,03	5,57	5,48	4,11	4,00	5,54	5,56	4,05	4,13	5,17	5,45	4,41	4,45	5,59	5,54	4,32	4,39	5,48	5,50	4,08	3,78	5,26	5,28
Dirty knife surface	EN	Yes	4,62	4,51	3,41	3,46	4,62	4,51	3,56	3,64	4,69	4,74	3,41	3,40	4,71	4,50	3,59	3,69	4,59	4,72	3,66	3,73	4,68	4,64	3,63	3,48	4,57	4,42
Andouillette	MP	Yes	4,17	4,42	3,82	3,85	4,17	4,42	3,69	3,83	4,21	4,19	3,78	3,83	4,13	4,17	3,83	3,90	4,53	4,44	3,82	3,83	4,55	4,56	3,82	3,80	4,28	4,39
Taboulleh	VG	Yes	4,46	4,49	4,32	4,18	4,46	4,49	4,51	4,15	4,56	4,18	4,16	4,19	4,26	4,19	4,30	4,36	4,30	4,42	4,30	4,36	4,39	4,36	4,21	4,12	4,26	4,14
Stuffed calamari	SF	No	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Foie gras	MP	No	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Shrimps	SF	No	5,69	5,73	5,69	5,73	5,69	5,73	5,61	5,66	5,61	5,66	5,36	5,34	5,36	5,34	5,48	5,44	5,48	5,44	5,61	5,42	5,61	5,42	5,16	5,24	5,16	5,24
Shrimps	SF	Yes	4,88	5,11	4,86	5,10	4,88	5,11	5,09	5,02	5,14	5,13	5,08	5,02	5,13	5,05	4,87	4,93	4,87	4,94	4,78	4,93	4,80	4,94	4,89	4,85	4,90	4,88
Reblochon cheese	DP	Yes	4,65	4,64	4,64	4,62	4,65	4,64	4,64	4,64	4,65	4,66	4,45	4,44	4,49	4,46	4,37	4,13	4,37	4,16	4,50	4,48	4,53	4,50	4,55	4,49	4,55	4,59
Strawberries tart	DP	Yes	5,48	5,59	5,41	5,58	5,48	5,59	5,55	5,54	5,57	5,59	5,72	5,65	5,78	5,65	5,42	5,49	5,48	5,50	5,41	5,46	5,41	5,46	5,46	5,54	5,48	5,56
Raw milk	DP	Yes	4,68	4,76	3,98	3,98	4,68	4,76	4,05	4,02	4,62	4,70	4,08	4,05	4,76	4,63	4,05	3,93	4,69	4,67	4,06	4,09	4,69	4,74	4,00	3,94	4,58	4,61
Raw milk	DP	Yes	2,28	2,30	2,00	1,90	2,28	2,30	2,04	1,48	2,39	2,21	1,78	1,70	2,04	2,24	1,48	1,60	2,24	2,34	1,78	1,70	2,36	2,19	1,60	1,78	2,39	2,49
Saint Jacques terrine	SF	Yes	3,07	2,95	2,95	2,85	3,07	2,95	2,96	3,04	3,16	3,13	2,48	2,90	2,78	3,07	3,26	2,60	3,36	2,85	3,07	3,16	3,30	3,37	2,95	2,60	3,00	2,60
Fish terrine	SF	Yes	2,00	2,00	2,00	<10	2,00	2,00	2,00	<10	2,00	<10	<10	<10	<10	<10	2,00	<10	2,00	<10	2,00	2,30	2,00	2,30	<10	2,30	<10	2,30
Process water (Candy plant)	EN	Yes	5,12	5,03	5,12	5,03	5,12	5,03	5,01	5,08	5,02	5,10	5,05	5,04	5,07	5,06	5,07	5,01	5,07	5,02	5,02	5,10	5,02	5,10	5,03	5,08	5,04	5,08
Raw milk cheese	DP	No	4,48	4,42	4,48	4,37	4,48	4,42	4,07	4,14	4,07	4,14	3,91	3,95	3,98	3,98	4,13	4,16	4,13	4,18	4,19	4,14	4,26	4,15	3,70	3,68	3,74	3,71
Raw milk	DP	No	2,70	2,76	2,45	2,36	2,70	2,76	2,72	2,55	2,92	2,78	2,54	2,59	2,65	2,80	2,54	2,62	2,66	2,79	2,54	2,62	2,66	2,82	2,58	2,50	2,76	2,67
Raw milk	DP	Yes	2,90	2,86	2,90	2,86	2,90	2,86	2,87	2,74	2,87	2,74	2,85	2,95	2,85	2,95	2,86	2,85	2,86	2,85	2,94	2,88	2,94	2,88	2,90	2,90	2,90	2,90
Corn, red beans and peppers	VG	Yes	2,10	2,10	2,10	2,04	2,10	2,10	2,13	2,13	2,36	2,21	1,91	2,13	2,07	2,21	2,37	2,41	2,50	2,58	2,19	2,21	2,34	2,30	1,96	2,26	2,26	2,50
Spinashes	VG	Yes	2,55	2,50	2,42	2,34	2,55	2,50	2,34	2,34	2,58	2,57	2,10	2,16	2,50	2,56	2,54	2,62	2,66	2,82	2,44	2,46	2,67	2,64	2,54	2,44	2,69	2,61
Ham	MP	No	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Roasted pork	MP	Yes	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Calf ground meat	MP	No	<10	<10	<10	<10	<10	<10	<10	<10	1,30	<10	<10	<10	1,00	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	1,30	<10	1,48
Liver	MP	Yes	2,48	2,60	2,48	2,48	2,48	2,60	2,30	2,78	2,48	2,85	2,30	2,60	2,30	2,60	2,48	2,48	2,48	2,48	2,70	2,30	2,78	2,30	2,60	2,30	2,70	2,48
Saucisson	MP	No	2,21	2,30	2,07	2,26	2,21	2,30	2,07	2,07	2,07	2,10	1,85	1,70	1,95	1,78	2,10	1,91	2,10	2,07	1,95	2,19	2,07	2,19	2,07	1,78	2,19	2,07
Cooked carotts	VG	Yes	3,88	3,86	3,76	3,79	3,88	3,86	3,83	3,71	3,93	3,79	3,82	3,82	3,89	3,91	3,79	3,70	3,86	3,74	3,75	3,87	3,85	3,91	3,75	3,80	3,82	3,89
Scraps from cheese retail outlet	EN	Yes	2,57	2,64	2,50	2,57	2,57	2,64	2,39	2,50	2,46	2,55	2,28	2,46	2,56	2,58	2,42	2,21	2,51	2,28	2,37	2,30	2,48	2,45	2,46	2,32	2,48	2,37
Process water	EN	Yes	3,36	3,60	3,28	3,53	3,36	3,60	3,51	3,49	3,61	3,54	3,41	3,57	3,46	3,66	3,64	3,61	3,70	3,67	3,51	3,55	3,57	3,67	3,63	3,51	3,66	3,60
Frozen french fries	VG	No	6,22	6,18	6,22	6,18	6,22	6,18	6,00	6,02	6,00	6,03	5,73	5,85	5,76	5,86	5,95	5,99	5,95	5,99	5,78	6,00	5,78	5,86	5,59	5,68	5,59	5,68
Scraps from cutting machine	EN	No	4,28	4,19	4,21	4,19	4,28	4,19	4,12	4,13	4,14	4,13	2,98	3,04	2,98	3,04	4,10	4,14	4,11	4,15	4,03	4,07	4,04	4,09	2,95	3,02	2,95	3,02
Beef meatballs	MP	No	2,87	2,87	2,87	2,87	2,87	2,87	2,89	2,87	2,89	2,88	3,09	3,12	3,09	3,12	2,90	3,00	2,90	3,04	2,80	2,48	2,80	2,85	3,07	3,04	3,07	3,13
Ossau Iraty cheese	DP	No	1,48	1,78	<10	1,00	1,48	1,78	1,48	1,48	1,70	1,85	1,60	1,30	1,70	1,30	1,78	1,60	1,78	1,60	1,70	1,78	1,90	1,78	1,95	1,60	2,04	1,95
Smoked salmon	SF	No	1,95	2,07	1,90	2,04	1,95	2,07	2,13	2,28	2,16	2,30	2,04	1,96	2,04	2,04	2,37	2,36	2,39	2,36	2,37	1,96	2,37	2,04	2,21	1,90	2,26	1,95
Sausage	MP	No	5,01	5,03	4,92	4,97	5,01	5,03	4,92	5,00	5,10	5,19	4,81	4,78	4,94	4,88	4,85	4,81	4,92	4,81	4,94	4,90	5,03	4,93	4,74	4,56	4,79	4,59
Rillettes	MP	No	4,55	4,46	4,37	4,21	4,55	4,46	4,32	4,48	4,59	4,64	4,13	3,95	4,21	4,07	4,20	4,13	4,42	4,16	4,18	4,34	4,45	4,34	3,89	3,98	3,94	4,06
Black pudding	MP	No	2,92	2,86	2,87	2,81	2,92	2,86	2,75	2,76	2,86	2,85	2,87	3,04	2,90	3,06	2,78	2,76	2,85	2,82	2,67	2,71	2,67	2,75	2,89	2,95	2,94	3,00
Hamburger	MP	No	3,56	3,57	3,55	3,55	3,56	3,57	3,46	3,44	3,47	3,46	3,11	3,18	3,17	3,21	3,64	3,66	3,64	3,67	3,01	3,00	3,02	3,06	3,64	3,66	3,64	3,67
Chipolatas	MP	No	2,26	1,78	2,26	1,78	2,26	1,78	1,90	2,19	1,90	2,19	3,15	2,99	3,15	2,99	2,19	2,13	2,19	2,13	2,19	2,07	2,19	2,07	3,16	3,26	3,16	3,26
Ham	MP	No	6,86	6,90	6,86	6,90	6,86	6,90	6,59	6,78	6,59	6,78	6,80	6,66	6,80	6,66	6,86	6,85	6,86	6,85	6,60	6,45	6,60	6,45	6,48	6,58	6,48	6,58
Smoked salmon	SF	Yes	3,85	3,89	3,85	3,89	3,85	3,89	3,28	3,48	3,77	3,87	3,57	3,60	3,77	3,86	3,82	3,71	3,89	3,84	3,88	3,91	3,98	3,99	3,10	3,26	3,53	3,60
Hoki fillet with sauce	SF	Yes	4,30	4,12	3,44	3,13	4,30	4,12	3,83	3,13	4,09	4,26	3,24	2,90	4,24	4,03	3,49	3,65	4,11	4,07	3,55	3,69	4,13	4,12	3,13	3,04	3,87	3,98
Surface fish weighing machine	EN	Yes	1,96	2,00	1,30	1,70	1,96	2,00	1,95	2,04	2,16	2,16	1,60	1,90	1,95	2,10	1,90	2,19	2,10	2,21	1,85	2,07	2,07	2,24	1,90	2,04	2,16	2,07
Floor	EN	Yes	2,48	2,45	2,34	2,36	2,48	2,45	2,48	2,24	2,58	2,36	2,32	1,96	2,45	2,21	2,16	2,41	2,34	2,49	2,37	2,34	2,49	2,48	2,19	2,21	2,26	2,36
Shrimps	SF	Yes	3,85	3,82	3,78	3,71	3,85	3,82	3,69	3,51	3,78	3,61	3,74	3,64	3,81	3,74	3,68	3,67	3,70	3,71	3,69	3,64	3,74	3,66	3,62	3,67	3,66	3,67
Smoked salmon	SF	Yes	4,67	4,67	4,59	4,50	4,67	4,67	4,48	4,24	4,64	4,45	4,53	4,57	4,62	4,67	4,44	4,49	4,49	4,54	4,39	4,48	4,45	4,50	4,21	4,42	4,32	4,48
Mixed vegetables	VG	Yes	1,60	1,86	1,30	<10	1,60	1,86	<10	1,30	1,78	2,13	<10	<10	1,70	1,95	1,30	1,30	1,78	1,78	1,00	1,48	1,78	1,90	1,00	<10	1,60	1,70
Mixed salads	VG	Yes	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Process water	EN	Yes	2,95	2,85	2,67	2,64	2,95	2,85	2,71	2,65	2,87	2,93	2,71	2,44	2,85	2,62	2,55	2,69	2,61	2,72	2,59	2,61	2,70	2,74	2,54	2,54	2,57	2,57
Scraps from delicatessen retail outlet	EN	Yes	3,96	3,97	3,71	3,74	3,96	3,97	3,89	3,93	4,17	4,21	3,77	3,67	4,01	3,97	3,82	3,84	3,90	3,91	3,86	3,86	3,97	3,95	3,75	3,82	3,85	3,87
Haù	MP	Yes	6,18	6,13	6,04	5,93	6,18	6,13	5,93	6,08	6,07	6,17	5,91	5,60	5,98	6,07	5,95	5,90	6,08	6,04	5,95	5,94	6,10	6,04	5,79	5,67	5,96	5,92
Salad	VG	Yes	1,30	1,60	<10	<10	1,30	1,60	1,00	<10	1,70	1,60	<10	<10	1,70	1,48	<10	<10	1,48	1,60	<10	<10	1,60	1,48	<10	<10	1,00	1,00
Mixed vegetables	VG	Yes	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10

APPENDIX C

INCLUSIVITY / EXCLUSIVITY

Inclusivity

Strain		Origin	Inoculation level in 225 mL Half Fraser	Colonies on AL medium after incubation for 22 hours at 37°C		Result
				Color	Presence of halo	
L 4	*Listeria monocytogenes* 1/2a	ATCC 35152	7,0E+00	blue	Yes	+MA
L5	*Listeria monocytogenes* 1/2a	Pieces of smoked salmon	9,5E+03	blue	Yes	+MA
L6	*Listeria monocytogenes* 1/2a	Pizza	1,0E+06	blue	Yes	+MA
L7	*Listeria monocytogenes* 1/2a	Munster cheese (rind)	7,0E+00	blue	Yes	+MA
L9	*Listeria monocytogenes* 1/2a	Munster cheese (rind)	8,0E+00	blue	Yes	+MA
L10	*Listeria monocytogenes* 1/2a	Rillettes	1,0E+01	blue	Yes	+MA
L11	*Listeria monocytogenes* 1/2a	Munster cheese (rind)	5,7E+05	blue	Yes	+MA
L12	*Listeria monocytogenes* 1/2a	Smoked salmon	1,2E+01	blue	Yes	+MA
L13	*Listeria monocytogenes* 1/2b	Pork ear	9,0E+00	blue	Yes	+MA
L14	*Listeria monocytogenes* 1/2c	Ground meat	8,0E+00	blue	Yes	+MA
L15	*Listeria monocytogenes* 1/2c	Beef meat	1,1E+04	blue	Yes	+MA
L16	*Listeria monocytogenes* 1/2c	Ground meat	8,0E+00	blue	Yes	+MA
L17	*Listeria monocytogenes* 1/2c	Bacon	1,5E+04	blue	Yes	+MA
L18	*Listeria monocytogenes* 1/2c	Munster cheese (rind)	7,0E+00	blue	Yes	+MA
L20	*Listeria monocytogenes* 1/2	Smoked salmon	1,5E+01	blue	Yes	+MA
L25	*Listeria monocytogenes* 1/2	Chicken	4,0E+00	blue	Yes	+MA
L28	*Listeria monocytogenes* 1/2c	Environment sample	1,2E+01	blue	Yes	+MA
L32	*Listeria monocytogenes* 4b	Munster cheese (rind)	6,0E+03	blue	Yes	+MA
L33	*Listeria monocytogenes* 4b	ATCC 19115	1,0E+04	blue	Yes	+MA
L37	*Listeria monocytogenes* 1/2b	Maroille cheese	3,2E+05	blue	Yes	+MA
L39	*Listeria monocytogenes*	Saucisson	1,0E+01	blue	Yes	+MA
L40	*Listeria monocytogenes* 1/2a	Munster cheese (rind)	4,2E+05	blue	Yes	+MA
L42	*Listeria monocytogenes* 1/2a	Chicken meat	6,0E+00	blue	Yes	+MA
L43	*Listeria monocytogenes* 1/2a	Ground meat	8,0E+00	blue	Yes	+MA
L44	*Listeria monocytogenes* 1/2a	Saucisson	7,0E+00	blue	Yes	+MA
L45	*Listeria monocytogenes* 1/2a	Wind terrine	4,0E+00	blue	Yes	+MA
L47	*Listeria monocytogenes* 1/2a	Browed potatoes	1,5E+01	blue	Yes	+MA
L48	*Listeria monocytogenes* 1/2b	Pork tongue	3,0E+00	blue	Yes	+MA
L49	*Listeria monocytogenes* 1/2b	Poultry pâté	9,0E+00	blue	Yes	+MA
L51	*Listeria monocytogenes* 1/2b	Germain cheese	1,5E+01	blue	Yes	+MA
L52	*Listeria monocytogenes* 1/2b	SLCC 2755	5,0E+00	blue	Yes	+MA
L53	*Listeria monocytogenes* 1/2c	Ground meat	8,0E+00	blue	Yes	+MA
L54	*Listeria monocytogenes* 1/2c	Meat product	8,0E+00	blue	Yes	+MA
L55	*Listeria monocytogenes* 3b	SLCC 2540	8,0E+00	blue	Yes	+MA
L56	*Listeria monocytogenes* 3c	SLCC 2479	5,0E+00	blue	Yes	+MA
L57	*Listeria monocytogenes* 4a	ATCC 19114	3,0E+00	blue	Yes	+MA
L58	*Listeria monocytogenes* 4b	Salad	1,0E+01	blue	Yes	+MA
L60	*Listeria monocytogenes* 4d	ATCC 19117	7,0E+00	blue	Yes	+MA
L61	*Listeria monocytogenes* 4e	ATCC 19118	4,0E+00	blue	Yes	+MA
L62	*Listeria monocytogenes* 4e	Reblochon cheese	3,0E+00	blue	Yes	+MA
L63	*Listeria monocytogenes* 4e	Munster cheese (rind)	7,0E+00	blue	Yes	+MA
L67	*Listeria monocytogenes* 7	SLCC 2482	7,0E+00	blue	Yes	+MA
L69	*Listeria monocytogenes*	Saucisson	1,0E+01	blue	Yes	+MA
L70	*Listeria monocytogenes*	Salmon from Ireland	8,0E+00	blue	Yes	+MA
L116	*Listeria monocytogenes* 1/2a	Fish meal	1,0E+01	blue	Yes	+MA
L117	*Listeria monocytogenes* 1/2c	Montbeliard sausage	8,0E+00	blue	Yes	+MA
L119	*Listeria monocytogenes*	Spinashes	1,0E+01	blue	Yes	+MA
L121	*Listeria monocytogenes*	Neufchatel cheese	9,0E+03	blue	Yes	+MA
L123	*Listeria monocytogenes*	Mozzarella cheese	1,2E+01	blue	Yes	+MA
L124	*Listeria monocytogenes*	Perch fillet	7,0E+00	blue	Yes	+MA
L125	*Listeria monocytogenes*	Vegetables pan fry	6,0E+00	blue	Yes	+MA
L128	*Listeria monocytogenes* 1/2a	Soya cattle cake	9,0E+03	blue	Yes	+MA
L129	*Listeria monocytogenes* 1/2a	Browed potatoes	7,0E+00	blue	Yes	+MA
L130	*Listeria monocytogenes*	Ground meat	5,0E+00	blue	Yes	+MA
L137	*Listeria monocytogenes*	Ground meat	1,0E+01	blue	Yes	+MA
L141	*Listeria monocytogenes*	Environmental sample	8,0E+00	blue	Yes	+MA
L149	*Listeria monocytogenes*	Environmental sample	5,0E+00	blue	Yes	+MA
L152	*Listeria monocytogenes*	Environmental sample	1,0E+04	blue	Yes	+MA
L156	*Listeria monocytogenes*	French pies	2,7E+04	blue	Yes	+MA
L176	*Listeria monocytogenes*	Beef meat	1,0E+04	blue	Yes	+MA

Exclusivity

Strain		Origin	Inoculation level in 225 mL non selective nutrient	Colonies on AL medium after incubation for 22 hours at 37°C		Result
				Color	Presence of halo	
L143	*Listeria grayi*	Frozen french fries	9,5E+03	blue	no	-
L146	*Listeria grayi*	CIP 103 213	1,0E+06	blue	no	-
L64	*Listeria innocua*	Epoisses cheese	5,7E+05	blue	no	-
L72	*Listeria innocua*	Boulettes d'Avesnes cheese	1,1E+04	blue	no	-
L108	*Listeria innocua*	Gorgonzola cheese	1,5E+04	blue	no	-
L76	*Listeria innocua* 6b	Ground meat	6,0E+03	blue	no	-
L80	*Listeria ivanovii*	Collection	1,0E+04	blue	yes	+
L133	*Listeria ivanovii*	Roquefort cheese	3,2E+05	blue	yes	+
L150	*Listeria ivanovii*	Dairy product	1,7E+05	blue	yes	+
L151	*Listeria ivanovii*	Ground meat	4,2E+05	blue	yes	+
L154	*Listeria ivanovii*	Sausage with herbs	2,4E+05	blue	yes	+
L161	*Listeria ivanovii spp. ivanovii*	Meat product	1,9E+05	blue	yes	+
L166	*Listeria ivanovii spp. londoniensis*	Collection	2,8E+08	blue	yes	+
L84	*Listeria seeligeri*	Beef ground meat	9,0E+03	blue	no	-
L142	*Listeria seeligeri*	Raw milk cheese	9,0E+03	blue	no	-
L83	*Listeria seeligeri* 1/2b	Beef tongue	1,4E+04	blue	no	-
L101	*Listeria welshimeri*	Ham	1,0E+04	blue	no	-
L91	*Listeria welshimeri*	Saucisson	2,7E+04	blue	no	-
L99	*Listeria welshimeri*	Sausages	1,0E+04	blue	no	-
BA1	*Bacillus cereus*	Eggproduct	9,0E+04	Ø	Ø	-
BA2	*Bacillus cereus*	Beet	7,0E+05	Ø	Ø	-
BA14	*Bacillus cereus*	Egg	6,0E+04	Ø	Ø	-
BA5	*Bacillus megaterium*	Collection	5,4E+05	Ø	Ø	-
BA6	*Bacillus mycoïdes*	Collection	4,3E+03	Ø	Ø	-
BA22	*Bacillus pumilus*	Taboulleh	1,3E+04	blue	no	-
BA4	*Bacillus stearothermophilus*	Collection	9,2E+06	Ø	Ø	-
BA29	*Bacillus thuringiensis*	Collection	1,2E+04	Ø	Ø	-
E10	*Enterococcus durans*	Collection	1,1E+05	Ø	Ø	-
E1	*Enterococcus faecalis*	Eggproduct	9,0E+05	Ø	Ø	-
E2	*Enterococcus faecium*	ATCC 3286	8,0E+05	Ø	Ø	-
E9	*Enterococcus faecium*	Tarama	8,0E+05	Ø	Ø	-
L139	*Jonesia denitrificans*	ATCC 55134	1,0E+04	blue	no	-
LAC5	*Lactobacillus reuteri*	Dairy product	3,0E+04	Ø	Ø	-
LAC22	*Lactobacillus plantarum*	Collection	5,4E+04	Ø	Ø	-
39	*Oekskovia xanthineolytica*	Reblochon cheese	1,8E+05	blue	no	-
32	*Rhodococcus equi*	Meat product	1,2E+05	blue	no	-
STA3	*Staphylococcus epidermidis*	Yoghurt	2,5E+05	blue	no	-

APPENDIX D

INTERLABORATORY STUDY
-
DETAILED RESULTS OF PARTICIPANT LABORATORIES

Laboratories datas

Level 0

Number of characteristic colonies

Laboratories (i)	Reference method ISO 11290-2															
	Sample 5								Sample 8							
	-1				-1	-2	-3	Result (CFU/ml)	-1				-1	-2	-3	Result (CFU/ml)
	1 ml on 3 plates				0,1 ml per plate				1 ml on 3 plates				0,1 ml per plate			
	plate 1	plate 2	plate 3	Total					plate 1	plate 2	plate 3	Total				
A	0	0	0	**0**	0	0	0	**<10**	0	0	0	**0**	0	0	0	**<10**
B	0	0	0	**0**	0	0	0	**<10**	0	0	0	**0**	0	0	0	**<10**
C	0	0	0	**0**	0	0	0	**<10**	0	0	0	**0**	0	0	0	**<10**
D	0	0	0	**0**	0	0	0	**<10**	0	0	0	**0**	0	0	0	**<10**
E	0	0	0	**0**	0	0	0	**<10**	0	0	0	**0**	0	0	0	**<10**
F	0	0	0	**0**	0	0	0	**<10**	0	0	0	**0**	0	0	0	**<10**
G	0	0	0	**0**	0	0	0	**<10**	0	0	0	**0**	0	0	0	**<10**
H	0	0	0	**0**	0	0	0	**<10**	0	0	0	**0**	0	0	0	**<10**
K	0	0	0	**0**	0	0	0	**<10**	0	0	0	**0**	0	0	0	**<10**
L	0	0	0	**0**	0	0	0	**<10**	0	0	0	**0**	0	0	0	**<10**
N	0	0	0	**0**	0	0	0	**<10**	0	0	0	**0**	0	0	0	**<10**
P	0	0	0	**0**	0	0	0	**<10**	0	0	0	**0**	0	0	0	**<10**
Expert lab	0	0	0	**0**	0	0	0	**<10**	0	0	0	**0**	0	0	0	**<10**

Laboratories (i)	Alternative method (AL depth without revivification) - 48h of incubation									
	Sample 5					Sample 8				
	-1	-2	-3	-4	Result (UFC/ml)	-1	-2	-3	-4	Result (UFC/ml)
	1 ml per Petri dish					1 ml per Petri dish				
A	0	0	0	0	**<10**	0	0	0	0	**<10**
B	0	0	0	0	**<10**	0	0	0	0	**<10**
C	0	0	0	0	**<10**	0	0	0	0	**<10**
D	0	0	0	0	**<10**	0	0	0	0	**<10**
E	0	0	0	0	**<10**	0	0	0	0	**<10**
F	0	0	0	0	**<10**	0	0	0	0	**<10**
G	0	0	0	0	**<10**	0	0	0	0	**<10**
H	0	0	0	0	**<10**	0	0	0	0	**<10**
K	0	0	0	0	**<10**	0	0	0	0	**<10**
L	0	0	0	0	**<10**	0	0	0	0	**<10**
N	0	0	0	0	**<10**	0	0	0	0	**<10**
P	0	0	0	0	**<10**	0	0	0	0	**<10**
Expert lab	0	0	0	0	**<10**	0	0	0	0	**<10**

Laboratories datas

Level 1

Number of characteristic colonies
Initial inoculation : 91/mL

Laboratories (i)	Reference method ISO 11290-2															
	Sample 4								Sample 7							
	-1				-1	-2	-3	Result (CFU/ml)	-1				-1	-2	-3	Result (CFU/ml)
	1 ml on 3 plates				0,1 ml per plate				1 ml on 3 plates				0,1 ml per plate			
	plate 1	plate 2	plate 3	Total					plate 1	plate 2	plate 3	Total				
A	4	7	2	**13**	2	0	0	**136**	2	1	1	**4**	1	0	0	**40**
B	4	4	1	**9**	1	0	0	**90**	0	3	3	**6**	0	0	0	**60**
C	2	4	4	**10**	3	0	0	**118**	2	7	5	**14**	1	0	0	**136**
D	1	1	0	**2**	1	0	0	**20**	1	0	0	**1**	0	0	0	**10**
E	4	2	2	**8**	0	2	0	**80**	2	1	0	**3**	0	0	0	**30**
F	2	2	2	**6**	1	1	0	**60**	4	2	2	**8**	2	1	0	**80**
G	9	2	1	**12**	3	0	0	**136**	1	7	3	**11**	0	0	0	**100**
H	8	4	3	**15**	0	0	0	**136**	0	0	1	**1**	2	0	0	**200**
K	6	5	3	**14**	1	0	0	**136**	2	3	2	**7**	1	0	0	**70**
L	5	2	1	**8**	1	0	0	**80**	4	4	2	**10**	1	0	0	**100**
N	7	8	4	**19**	2	0	0	**190**	4	5	5	**14**	0	0	0	**127**
P	2	3	5	**10**	0	0	0	**91**	2	0	8	**10**	0	0	0	**91**
Expert lab	6	10	12	**28**	2	0	0	**273**	3	8	8	**19**	2	0	0	**191**

Laboratories (i)	Alternative method (AL depth without revivification) - 48h of incubation									
	Sample 4					Sample 7				
	-1	-2	-3	-4	Result (UFC/ml)	-1	-2	-3	-4	Result (UFC/ml)
	1 ml per Petri dish					1 ml per Petri dish				
A	15	1	0	0	**145**	17	1	0	0	**164**
B	12	1	0	0	**118**	6	2	0	0	**60**
C	11	1	0	0	**109**	9	1	0	0	**90**
D	10	2	0	0	**109**	19	1	0	0	**182**
E	7	0	0	0	**70**	9	1	1	0	**90**
F	11	1	0	0	**109**	20	2	0	0	**200**
G	8	0	0	0	**80**	12	0	0	0	**109**
H	19	5	1	1	**218**	7	2	0	0	**70**
K	22	1	0	0	**209**	16	2	0	0	**164**
L	10	1	0	0	**100**	12	0	0	0	**109**
N	12	0	0	0	**109**	20	6	0	0	**236**
P	11	0	0	0	**100**	10	2	0	0	**109**
Expert lab	14	0	0	0	**127**	14	0	0	0	**127**

Laboratories datas

Level 2

Number of characteristic colonies
Initial inoculation : 786/mL

Laboratories (i)	Reference method ISO 11290-2															
	Sample 2								Sample 6							
	-1				-1	-2	-3	Result (CFU/ml)	-1				-1	-2	-3	Result (CFU/ml)
	1 ml on 3 plates				0,1 ml per plate				1 ml on 3 plates				0,1 ml per plate			
	plate 1	plate 2	plate 3	Total					plate 1	plate 2	plate 3	Total				
A	46	39	49	**134**	8	1	0	**1291**	47	38	30	**115**	9	1	0	**1127**
B	50	44	21	**115**	16	1	0	**1191**	42	49	45	**136**	21	2	0	**1427**
C	25	22	28	**75**	12	0	0	**791**	23	26	25	**74**	13	0	0	**791**
D	20	12	3	**35**	5	1	0	**364**	8	3	9	**20**	2	4	0	**200**
E	28	21	25	**74**	12	1	0	**782**	23	19	20	**62**	7	0	0	**627**
F	18	20	23	**61**	34	16	0	**610**	15	16	16	**47**	24	3	0	**470**
G	48	28	35	**111**	15	1	0	**1145**	32	30	53	**115**	7	0	0	**1109**
H	18	45	26	**89**	28	2	0	**2727**	19	39	31	**89**	13	2	0	**1364**
K	84	32	28	**144**	21	2	0	**1500**	57	28	20	**105**	15	0	0	**1091**
L	30	43	39	**112**	14	0	0	**1145**	38	31	32	**101**	7	0	0	**982**
N	32	49	41	**122**	15	4	0	**1245**	32	39	37	**108**	11	1	0	**1082**
P	44	28	33	**105**	11	2	0	**1055**	20	22	25	**67**	18	2	0	**773**
Expert lab	177	169	171	**517**	34	1	0	**3182**	178	240	184	**602**	40	2	0	**3818**

Laboratories (i)	Alternative method (AL depth without revivification) - 48h of incubation									
	Sample 2					Sample 6				
	-1	-2	-3	-4	Result (UFC/ml)	-1	-2	-3	-4	Result (UFC/ml)
	1 ml per Petri dish					1 ml per Petri dish				
A	112	13	1	0	**1136**	94	14	0	0	**1273**
B	102	5	0	0	**973**	101	9	0	0	**1000**
C	88	9	0	0	**882**	81	9	0	0	**818**
D	83	9	0	0	**836**	87	11	0	0	**891**
E	120	12	1	0	**1200**	127	11	2	0	**1255**
F	124	18	4	0	**1291**	81	16	2	0	**882**
G	128	11	0	0	**1264**	124	14	0	0	**1255**
H	148	2	0	0	**1364**	132	9	1	0	**1282**
K	132	22	2	0	**1400**	161	9	1	1	**1545**
L	105	5	0	0	**1000**	113	7	0	0	**1091**
N	116	17	0	0	**1209**	119	18	1	0	**1245**
P	92	8	0	0	**909**	86	8	0	0	**855**
Expert lab	141	17	0	0	**1436**	159	19	1	0	**1818**

Laboratories datas

Level 3

Number of characteristic colonies
Initial inoculation : 8400/mL

Laboratories (i)	Reference method ISO 11290-2															
	Sample 1								Sample 3							
	-1				-1	-2	-3	Result (CFU/ml)	-1				-1	-2	-3	Result (CFU/ml)
	1 ml on 3 plates				0,1 ml per plate				1 ml on 3 plates				0,1 ml per plate			
	plate 1	plate 2	plate 3	Total					plate 1	plate 2	plate 3	Total				
A	>150	>150	>150	>>	106	18	3	**11273**	>150	>150	>150	>>	96	12	2	**9818**
B	140	160	204	**504**	174	16	1	**15455**	298	133	138	**569**	140	8	2	**13455**
C	126	135	117	**378**	90	11	0	**9182**	145	158	234	**537**	110	10	0	**10909**
D	136	105	61	**302**	62	8	0	**6364**	73	84	41	**198**	51	7	0	**5273**
E	>150	>150	>150	>>	110	5	2	**10455**	163	139	132	**434**	63	10	0	**6636**
F	>150	>150	>150	>>	>150	77	14	**14000**	80	85	82	**247**	67	35	10	**10000**
G	>150	>150	>150	>>	154	12	0	**10909**	>150	>150	>150	>>	166	10	0	**9091**
H	112	135	>150	>>	74	21	2	**8636**	>150	125	>150	>>	>150	18	0	**16364**
K	181	>150	161	>>	121	12	1	**12091**	222	236	271	>>	131	12	0	**10909**
L	>150	>150	>150	>>	98	20	2	**10727**	>150	>150	>150	>>	77	7	0	**7636**
N	>150	>150	>150	>>	142	15	0	**14273**	>150	>150	>150	>>	150	13	1	**12727**
P	70	88	139	**297**	128	11	4	**12636**	229	155	117	**501**	89	7	1	**8727**
Expert lab	>150	>150	>150	>>	257	44	0	**40000**	>150	>150	>150	>>	264	17	0	**15455**

Laboratories (i)	Alternative method (AL depth without revivification) - 48h of incubation									
	Sample 1					Sample 3				
	-1	-2	-3	-4	Result (UFC/ml)	-1	-2	-3	-4	Result (UFC/ml)
	1 ml per Petri dish					1 ml per Petri dish				
A	>150	111	14	2	**11364**	>150	115	20	1	**12273**
B	>150	99	12	0	**10091**	>150	110	7	0	**10636**
C	>150	79	8	1	**7909**	>150	96	13	1	**9909**
D	>150	108	12	1	**10909**	>150	93	10	1	**9364**
E	>150	117	4	2	**11000**	>150	98	12	0	**10000**
F	>150	>150	17	1	**16364**	>151	82	12	1	**8545**
G	>150	115	11	0	**11455**	>150	135	6	0	**12818**
H	>150	119	12	0	**11909**	>150	118	8	0	**11455**
K	>150	140	16	3	**14182**	>150	128	17	0	**13182**
L	>150	99	7	1	**9636**	>150	83	11	0	**8545**
N	>150	136	18	2	**14000**	>150	146	15	5	**14636**
P	>150	110	19	2	**11727**	>150	79	7	1	**7818**
Expert lab	>150	118	12	0	**11818**	>150	118	7	2	**11364**