



NordVal Certificate

Issued for:	RAPID' <i>L. mono</i>
NordVal No:	022
First approval date:	20 November 2005
Extension date:	10 December 2009
Valid until:	10 December 2011

RAPID'*L. mono*

Manufactured and supplied by:
Bio-Rad Laboratories,
3 Blvd Raymond Poincare,
92430 Marnes-la-Coquette,
France


fulfils the requirements of the NordVal validation protocol. The reference method was EN ISO 11290-1(1996/ amendment 2004):Food microbiology - Horizontal method for the detection and enumeration of *Listeria monocytogenes* -- Part 1: Detection method and Part 2: Enumeration method.

NordVal has studied the enclosures to the application and evaluated the results obtained in the validations conducted by the expert laboratory l'Institut Pasteur de Lille, France, in accordance to EN ISO 16140. NordVal has concluded that it has been satisfactorily demonstrated that the requirements of the NordVal validation protocol are fulfilled for RAPID'*L. mono*, there are no statistical differences in the performances of *RAPID' L. mono* and the reference method for the detection and the enumeration of *Listeria monocytogenes* and the detection of *Listeria spp.* in foods and environmental samples. Further, it was demonstrated that confirmation is not necessary.

Date: 28 October 2009

Yours sincerely


Sven Qvist
Chair of NordVal


Hilde Skaar Norli
NMKL Secretary General



PRINCIPLE OF THE METHOD:

The principle of the RAPID'L. *Mono* medium relies on the chromogenic detection of the *Listeria monocytogenes* phosphatidylinositol-specific phospholipase C (PIPLC) and on the inability of these species to metabolise xylose. After 24 ± 2 hours of incubation, *Listeria monocytogenes* forms characteristics blue (pale blue, grey blue to dark blue) colonies without a yellow halo. Colonies formed by other species of *Listeria* are white, with or without a yellow halo. The particularity of *Listeria ivanovii* species, infrequently found in food matrices, should be noted: it presents blue-green colonies with a yellow halo (xylose positive character). This halo can appear after 24 to 48 hours of incubation. The selective mixture in the medium allow the inhibition of most interfering flora (Gram- positive and Gram-negative bacteria, yeast and mould) Thus RAPID' L mono detects *Listeria monocytogenes* in 24 hours and other *Listeria* species in 24 and 48 hours.

FIELD OF APPLICATION:

The method is applicable for the detection and the enumeration of *Listeria monocytogenes* and for the detection of other *Listeria* species in food and environmental samples.

COMPARISON STUDY

Relative accuracy, relative sensitivity, relative specificity and agreement between results, kappa.

Detection of *Listeria monocytogenes* after 24 ± 2 hours of incubation

In 2002 and 2006, 483 product samples were analysed: 202 were naturally contaminated, 47 artificially contaminated and 234 non-contaminated. All the samples were analysed once by the two methods. The following results were obtained:

Matrices	*P A	NA	ND	PD	Sum	Relative AC	Relative SE	Relative SP	Kappa
Meat products	40	39	2	2	83	95.2%	95.2%	95.1%	0.91
Fish products	38	62	0	1	101	99.0%	100%	98.4%	0.98
Dairy products	51	48	2	1	102	97.1%	96.2%	98.0%	0.94
Vegetable products	40	37	0	0	77	100%	100%	100%	1.00
Environment samples	70	49	1	0	120	99.2%	98.6%	100%	0.98
Total	239	235	5	4	483	98.1%	98.0%	98.3%	0.96

* PA = number of obtained results that are positive with both the alternative and the reference method

NA = number of obtained results that are negative with both the alternative and the reference method.

ND = number of obtained results that are negative with the alternative method and positive with the reference method (possible false negative)

PD = number of obtained results that are positive with the alternative method and negative with the reference method (possible false positive)

Relative AC = The relative accuracy; the degree of correspondence between the response obtained by the alternative method and the reference method.

Relative SE = The relative sensitivity; the ability of the alternative method to detect the analyte compared to the reference method

Relative SP = The relative specificity is the ability of the alternative method not to detect the target microorganism when it is not detected by the reference method.

Kappa = The degree of agreement between the alternative method and the reference method, kappa of 0,80 or higher is considered to be very good agreement.

For the response to *Listeria monocytogenes* alone, after 24 hours (+/- 2 hours) of incubation of the agar plates, the relative accuracy obtained is 98.1%, the relative sensitivity is 98.0% and the relative specificity is 98.3%. Nine discordant results were obtained: 4 supplemental

positive results and 5 false negative results. The positive samples by the alternative method being confirmed positive samples, the sensitivities and specificities were recalculated with respect to all of the positive results and are:

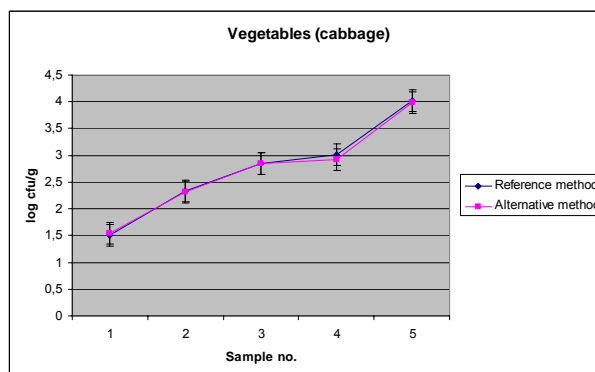
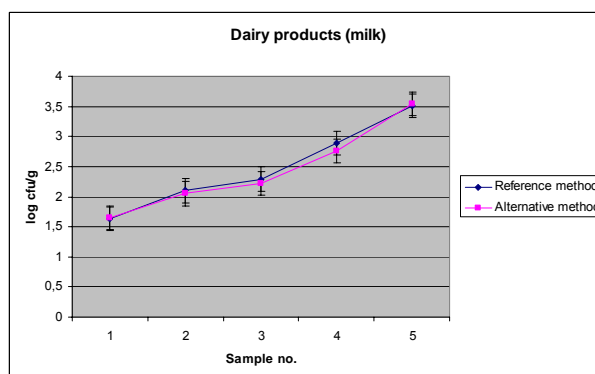
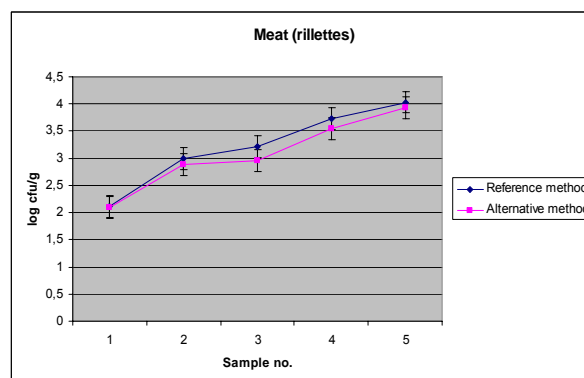
- 98.0% sensitivity for the alternative method,
- 98.4% sensitivity for the reference method.

The agreement between the two methods is very good ($Kappa > 0.80$) for all matrices. The sensitivity is satisfactory ($\geq 95\%$) for all matrices, confirmation is not considered necessary.

Enumeration of *Listeria monocytogenes* after 24 ± 2 hours of incubation

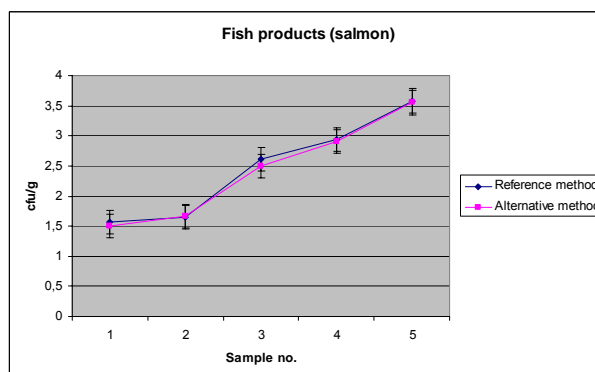
In 2005 tests were performed on 5 food product/strain combinations. The samples were analysed in duplicate by each of the two methods. The following results were obtained:

Product/ Matrix / Strain	Reference method		Alternative method	
	Mean cfu/g	std. cfu/g	Mean cfu/g	std. cfu/g
Meat product Rillettes <i>Listeria monocytogenes</i> 1/2b	2.11	0.064	2.09	0.127
	2.99	0.035	2.89	0.057
	3.22	0.106	2.96	0.021
	3.73	0.014	3.55	0.057
	4.03	0.092	3.94	0.134
Dairy products Untreated Milk <i>Listeria monocytogenes</i> 1/2b	1.63	0.212	1.65	0.071
	2.10	0.205	2.05	0.212
	2.29	0.007	2.22	0.085
	2.89	0.028	2.76	0.071
	3.51	0.042	3.55	0.007
Vegetables Cabbage <i>Listeria monocytogenes</i> 4b	1.50	0.445	1.54	0.339
	2.33	0.049	2.31	0.014
	2.85	0.042	2.85	0.064
	3.01	0.042	2.92	0.163
	4.02	0.049	3.96	0.064

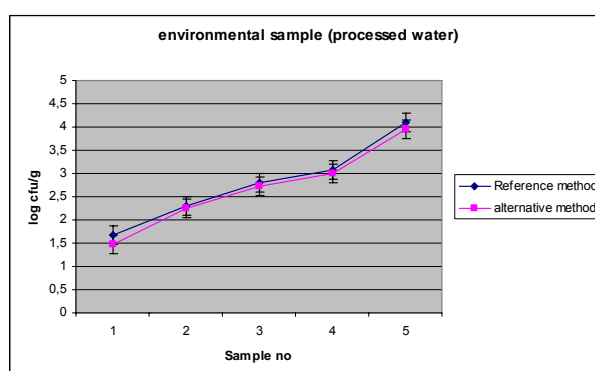




Product/ Matrix / Strain	Reference method		Alternative method	
	Mean	std.	Mean	std.
	cfu/g	cfu/g	cfu/g	cfu/g
Fish products	1.57	0.042	1.50	0.283
Salmon	1.65	0.071	1.67	0.262
<i>Listeria monocytogenes</i> 1/2b	2.61	0.134	2.50	0.021
	2.94	0.000	2.91	0.014
	3.58	0.028	3.56	0.106



Environmental samples	1.67	0.099	1.48	0.000
Process water	2.30	0.141	2.26	0.000
<i>Listeria monocytogenes</i> 1/2b	2.80	0.057	2.74	0.064
	3.07	0.007	2.96	0.007
	4.09	0.035	3.94	0.085



The mean of the standard deviation, std.	0.094	0.093
The uncertainty of the method, U	0.19	0.19

The results of the comparison study shows that the estimated measurement uncertainty is identical for both the reference and the alternative method, $U = 0.19$. The diagrams show that all the mean values of the results are overlapping when taking the uncertainty of the method into account. Hence there is no statistical difference between the methods and both methods perform satisfactorily - the precision is good.

Detection of *Listeria* other than *moncotygenes* after 48 hours of incubation

In 2006, 420 product samples were analysed: 99 were naturally contaminated, 87 artificially contaminated and 234 non-contaminated. All the samples were analysed once by the two methods. The following results were obtained:

Matrices	*P A	NA	ND	PD	Sum	Relative AC	Relative SE	Relative SP	Kappa
Meat products	41	38	1	0	80	98.8%	97.6%	100%	0.97
Fish products	28	62	1	2	93	96.8%	96.9%	96.9%	0.93
Dairy products	29	48	1	2	80	96.3%	96.7%	96.0%	0.92
Vegetable products	33	37	3	0	73	95.9%	91.7%	100%	0.92
Environment samples	43	49	1	1	94	97.9%	97.7%	98.0%	0.96
Total	174	234	7	5	420	97.1%	96.1%	97.9%	0.94

* PA = number of obtained results that are positive with both the alternative and the reference method

NA = number of obtained results that are negative with both the alternative and the reference method.



ND = number of obtained results that are negative with the alternative method and positive with the reference method (possible false negative)

PD = number of obtained results that are positive with the alternative method and negative with the reference method (possible false positive)

Relative AC = The relative accuracy; the degree of correspondence between the response obtained by the alternative method and the reference method.

Relative SE = The relative sensitivity; the ability of the alternative method to detect the analyte compared to the reference method

Relative SP = The relative specificity is the ability of the alternative method not to detect the target microorganism when it is not detected by the reference method.

Kappa = The degree of agreement between the alternative method and the reference method, kappa of 0,80 or higher is considered to be very good agreement.

For the response to *Listeria* other than *monocytogenes*, the results demonstrate that the agar plates must be incubated for 24 hours, and for 24 additional hours in the case of absence of colonies or of weak growth. After 48 hours of incubation, the relative accuracy obtained is 97.1%, the relative sensitivity is 96.1% and the relative specificity is 97.9%. Twelve discordant results were obtained: 5 supplemental positive results and 7 false negative results. The positive samples by the alternative method being confirmed positive samples, the sensitivities and specificities were recalculated with respect to all of the positive results and are:

- 96.2% sensitivity for the alternative method,
- 97.3% sensitivity for the reference method.

The agreement between the two methods is very good (Kappa > 0.80) for all matrices. The sensitivity is satisfactory ($\geq 95\%$) for all matrices, confirmation is not considered necessary.

Detection Level

The different matrices have been analysed 6 times at 4 different contamination levels by both methods. The detection level was found to be 1-10 cfu in a sample of 25g or 25 ml for all matrices.

Inclusivity /exclusivity

Inclusivity: 50 strains of *Listeria monocytogenes* were detected out of the 50 tested.
30 strains of *Listeria* other than *monocytogenes* were detected out of the 30 tested.

Exclusivity: The study conducted in 1998 and 1999 of 47 strains not belonging to the genus *Listeria monocytogenes* did not detect presence of any cross-reaction, even with strains listed in the bibliography as having PLPLC activity: *Bacillus cereus*, *Clostridium perfringens* and *Staphylococcus aureus*.

For the accuracy study performed in 2006, some strains were identified because they produced colonies that looked similar to *Listeria* other than *monocytogenes*; these were *Bacillus*, *Enterococcus faecium*, *Oekskovia xanthineolytica*, *Gardnerella vaginalis* and *Lactobacillus*. However, these few strains have a different appearance from *Listeria* in the GRAM test.



COLLABORATIVE STUDY

DETECTION OF *LISTERIA MONOCYTOGENES*:

The collaborative study was conducted in 2006.

Number of participating laboratories: 15. Results from one laboratory were excluded

The analyses were performed on samples of pasteurized milk, artificially contaminated with a strain of *Listeria monocytogenes* (L37), originating in "raw-milk cheese) at the following three contamination levels:

- 0 cfu/25 ml
- Low level, about the detection level, 1-10 cfu/25 ml
- High level, about 10 times the detection level, 10-100 cfu/25 ml

The laboratories analysed 24 samples, 8 replicates for each level using both the alternative and the reference method. The following results were obtained:

- Sensitivity: 96%
- Specificity: 100%
- Relative accuracy: 100%
- Kappa: 0,99

Thus, the collaborative study showed no statistical difference between the results obtained by the two methods.

ENUMERATION OF *LISTERIA MONOCYTOGENES*.

The collaborative study was conducted in 2006.

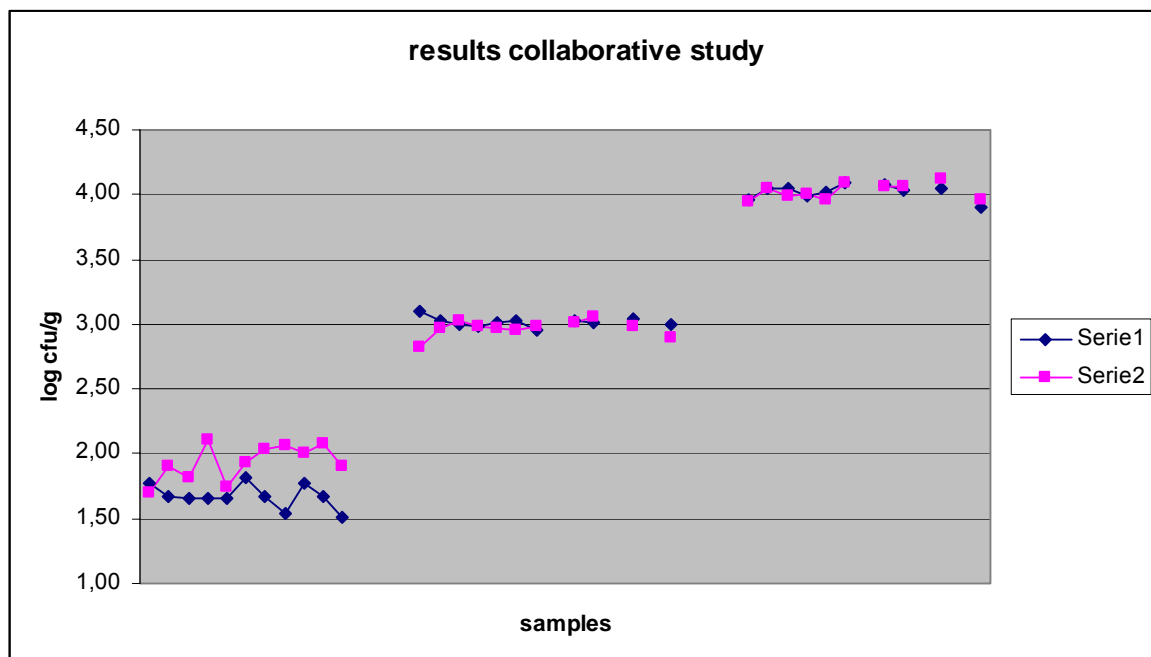
Number of participating laboratories: 15. Four laboratories were omitted, one due to late receipt and the three others because the temperatures of the samples were $> 8^{\circ}\text{C}$

Matrix: Pasteurized milk

Strain: *Listeria monocytogenes* L37

No of samples: 8, 4 levels (blind, low, medium, high), 2 sample each level

The diagram below shows the results for the low, medium and high level analysed with the reference (Serie1) and the alternative method (Serie 2) respectively.





The precision is given in the table below:

Level (log cfu/g)	Reference method		Alternative method	
	Repeatability limit, r (log cfu/g)	Reproducibility limit, R (log cfu/g)	Repeatability limit, r (log cfu/g)	Reproducibility limit, R (log cfu/g)
50 - 500	0.37	0.37	0.56	0.65
500 - 5000	0.14	0.14	0.15	0.16
5000 - 5000	0.050	0.14	0.082	0.19

The repeatability limit is the value less than or equal to which the absolute difference between two tests results obtained under repeatability conditions is expected to be with a probability of 95%.

The reproducibility limit is the value less than or equal to which the absolute difference between two test results obtained under reproducibility conditions is expected to be with

The precision is satisfactory, even for low levels. For the lowest level, the precision is better for the reference method than for the alternative method. However, it is not statistical significant.

CONCLUSION

The studies have shown that there are no statistical differences in the performances of *RAPID¹ L. mono* and the reference method for the detection and the enumeration of *Listeria monocytogenes* and the detection of *Listeria spp.* in foods and environmental samples. Further, it has been demonstrated that confirmation is not necessary.