



# NordVal Certificate

Issued for:	iQ-Check™ <i>Listeria monocytogenes</i> II
NordVal No:	037
First approval date:	15 November 2009
Valid until:	15 November 2011

## **iQ-Check™ *Listeria monocytogenes* II**

Manufactured and supplied by:

Bio-Rad Laboratories,  
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92430 Marnes-la-Coquette,  
France

fulfils the requirements of the NordVal validation protocol. The reference method was EN ISO 11290-1(1996/ amendment 2004) Part 1:– Food microbiology - Horizontal method for the detection and enumeration of *Listeria monocytogenes* -- Part 1: Detection 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 ISO 16140. NordVal has concluded that it has been satisfactorily demonstrated that iQ-Check *Listeria monocytogenes* II performs satisfactorily for the three described procedures:


- Enrichment in half Fraser followed by the standard lysis protocol
- Enrichment in *Listeria* Specific Broth (LSB) followed by the standard lysis protocol
- Enrichment in *Listeria* Specific Broth (LSB) followed by a simplified extraction protocol, no longer requiring the first centrifugation step

The results document no statistical difference in the performances between the iQ-Check™ *Listeria monocytogenes* II using enrichment in half Fraser followed by the standard lysis protocol and the reference method. iQ-Check™ *Listeria monocytogenes* II using enrichment in *Listeria* Specific Broth provides better sensitivity than the reference method. Further, it was demonstrated that confirmation is not necessary.

Date: 12 November 2009

Yours sincerely

  
Sven Qvist  
Chair of NordVal

  
Hilde Skaar Norli  
NMKL Secretary General



## PRINCIPLE OF THE METHOD:

The iQ-Check *Listeria monocytogenes* II method is a qualitative method allowing the detection of *Listeria monocytogenes* in environmental samples and food products. It is a culture method, by enrichment for 23 h ± 1 h in a *Listeria* Specific Broth (LSB) at 30 °C ± 2 °C or for 25 h ± 1 h in a half Fraser broth at 30 °C ± 2 °C. The method is based upon polymerase chain reaction and real time detection using fluorescent probes.

iQ-Check *Listeria* II describes the following three procedures, differing from each other in preliminary enrichment and lysis steps:

- Enrichment in half Fraser followed by the standard lysis protocol
- Enrichment in *Listeria* Specific Broth (LSB) followed by the standard lysis protocol
- Enrichment in *Listeria* Specific Broth (LSB) followed by a simplified extraction protocol, no longer requiring the first centrifugation step

## FIELD OF APPLICATION:

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

## COMPARISON STUDY

The comparison study using half Fraser broth and standard lysis was carried out in 2005. In 2006 the protocols using LSB and standard lysis and easy lysis, respectively, were validated.

The expert laboratory has analysed a number of samples belonging to the following main food categories: Meat products, dairy products, fish-based and vegetable products and environmental samples. All samples were analysed in single by both the alternative and the reference method.

### Accuracy, sensitivity, specificity

#### Enrichment in half Fraser followed by the standard lysis protocol

Table 1: Results after screening

Matrices	PA	NA	N D	PD	Sum	Relative AC	Relative SE	Relative SP	Kappa
Meat	29	44	1	2	76	96.1%	96.7%	95.7%	0.92
Seafood	29	29	3	2	63	92.1%	90.6%	93.5%	0.84
Dairy	30	35	0	1	66	98.5%	100.0%	97.2%	0.97
Vegetables	30	26	0	5	61	91.8%	100.0%	83.9%	0.84
Environmental	37	39	0	1	77	98.7%	100.0%	97.5%	0.97
Total	155	173	4	11	343	<b>95.6%</b>	<b>97.5%</b>	<b>94.0%</b>	<b>0.91</b>

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.

Table 2: Results after confirmation

Matrices	PA	NA	FN	TP	FP	Sum	Relative AC	Relative SE	Relative SP	Kappa
Meat	29	45	1	1	0	76	97.4%	100.0%	97.8%	0.94
Seafood	29	31	3	0	0	63	95.2%	90.6%	100.0%	0.90
Dairy	30	36	0	0	0	66	100.0%	100.0%	100.0%	1.00
Vegetables	30	30	0	1	0	61	98.4%	100.0%	96.8%	0.97
Environmental	37	40	0	0	0	77	100.0%	100.0%	100.0%	1.00
<b>Total</b>	<b>155</b>	<b>182</b>	<b>4</b>	<b>2</b>	<b>0</b>	<b>343</b>	<b>98.3%</b>	<b>97.5%</b>	<b>98.9%</b>	<b>0.96</b>

TP = True positive

FP = False positive

FN = False negative

For the other abbreviations, see table 1.

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

### Enrichment in *Listeria* Specific Broth (LSB) followed by the standard lysis protocol

Table 3: Results after screening

Matrices	PA	NA	ND	PD	Sum	Relative AC	Relative SE	Relative SP	Kappa
Meat	23	30	4	13	70	75.7%	85.2%	69.8%	0.52
Seafood	30	31	1	6	68	89.7%	96.8%	83.8%	0.80
Dairy	31	31	2	3	67	92.5%	93.9%	91.2%	0.85
Vegetables	26	31	2	3	62	91.9%	92.9%	91.2%	0.84
Environmental	29	27	1	4	61	91.8%	96.7%	87.1%	0.84
<b>Total</b>	<b>139</b>	<b>150</b>	<b>10</b>	<b>29</b>	<b>328</b>	<b>88.1%</b>	<b>93.3%</b>	<b>83.8%</b>	<b>0.76</b>

Explanations to the abbreviations are given under table 1.

Table 4: Results after confirmation

Matrices	PA	NA	FN	TP	FP	Sum	Relative AC	Relative SE	Relative SP	Kappa
Meat	23	30	4	11	2	70	78.6 %	125.9 %	93.8 %	0.59
Seafood	30	31	1	5	1	68	91.2 %	112.9 %	96.9 %	0.82
Dairy	31	31	2	3	0	67	92.5 %	103.0 %	100.0 %	0.85
Vegetables	26	31	2	3	0	62	91.9 %	103.6 %	100.0 %	0.84
Environmental	28	27	2	0	4	61	96.7 %	93.3 %	87.1 %	0.93
<b>Total</b>	<b>138</b>	<b>150</b>	<b>11</b>	<b>22</b>	<b>6</b>	<b>328</b>	<b>89.9 %</b>	<b>107.4 %</b>	<b>95.5 %</b>	<b>0.80</b>

Explanations to the abbreviations are given under table 1 and 2 respectively.

After screening, for meat and seafood products, a number of samples were positive with the alternative method but negative with the reference method (high PD). The relative sensitivity and agreement between the methods were therefore not found acceptable. When these samples were confirmed, the positive results obtained by the alternative method were true positive, i.e. the reference method failed to detect the bacteria (were false negative). This



also means that 11 meat samples were false negative with the reference method.

For meat products, 11 of the 13 samples, which were found positive with the alternative method, were confirmed positive, and hence the relative sensitivity, SE, would be above 100% (122%). Only two of the samples were false positive.

For seafood products, 5 of the 6 false positive (PD) were true positives, only one sample was false positive and hence the relative sensitivity would be above 100% (113%).

The agreement between the methods, expressed as kappa, is not improved after confirmation, as the methods give different results. The alternative method performed better than the reference method.

### Enrichment in *Listeria* Specific Broth (LSB) followed by the easy DNA extraction protocol.

Table 5: Results after screening

Matrices	PA	NA	N D	P D	Sum	Relative AC	Relative SE	Relative SP	Kapp a
Meat	23	32	4	11	70	78.6%	85.2%	74.4%	0.57
Seafood	29	31	2	6	68	88.2%	93.5%	83.8%	0.77
Dairy	29	31	4	3	67	89.6%	87.9%	91.2%	0.79
Vegetables	26	31	2	3	62	91.9%	92.9%	91.2%	0.84
Environmental	28	29	2	2	61	93.4%	93.3%	93.5%	0.87
Total	135	154	14	25	328	<b>88.1%</b>	<b>90.6%</b>	<b>86.0%</b>	<b>0.76</b>

Explanations to the abbreviations are given under table 1.

Table 6: Results after confirmation

Matrices	PA	NA	FN	TP	FP	Sum	Relative AC	Relative SE	Relative SP	Kappa
Meat	23	32	4	10	1	70	80.0 %	122.2 %	97.0 %	0.59
Seafood	29	31	2	5	1	68	89.7 %	109.7 %	96.9 %	0.77
Dairy	29	31	4	3	0	67	89.6 %	97.0 %	100.0 %	0.79
Vegetables	26	31	2	3	0	62	91.9 %	103.6 %	100.0 %	0.84
Environmental	28	29	2	0	2	61	96.7 %	93.3 %	93.5 %	0.93
Total	135	158	14	21	4	328	<b>89.3 %</b>	<b>104.7 %</b>	<b>97.5 %</b>	<b>0.79</b>

Explanations to the abbreviations are given under table 1 and 2.

For meat and seafood products, a number of samples were positive with the alternative method but negative with the reference method (high PD). The relative sensitivity and agreement between the methods were therefore not found acceptable. When these samples were confirmed, the positive results obtained by the alternative method were true positive, i.e. the reference method failed to detect the bacteria (false negative).

For meat products, 10 of the 11 samples, which were found positive with the alternative method, were confirmed positive, and hence the relative sensitivity, SE, would be above 100% (122%). Only one of the samples was false positive.

For seafood products, 5 of the 6 false positive (PD) were true positives, only one sample was a false positive and hence the relative sensitivity would be above 100% (110%).

The agreement between the methods, expressed as kappa, is not improved after confirmation, as the methods give different results. The alternative method performed better than the reference method.



## **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 25 g or 25 ml for all matrices.

## **Inclusivity /exclusivity**

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

Exclusivity: The study of 33-non-*Listeria monocytogenes* showed one cross-reaction with one strain of *Enterococcus faecium* grown in nutrient broth, but transfer to selective medium failed to confirm this result.

## **COLLABORATIVE STUDY:**

The collaborative study was conducted in 2005.

Number of participating laboratories: 15

Number of laboratories reporting results: 14

The analyses were performed on samples of pasteurized milk, artificially contaminated with a strain of *Listeria monocytogenes* at the following three contamination levels:

- 0 cfu/25 ml
- 1-10 cfu/25 ml
- 10-50 cfu/25 ml

The laboratories analysed 8 replicates for each level using both the alternative by enrichment in half Fraser and the reference method. The following results were obtained:

- Sensitivity: 99.1%
- Specificity: 99.1%
- Relative accuracy: 99.7%
- Kappa: 0.99

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

## **CONCLUSION:**

The comparison study and the collaborative study showed that iQ-Check *Listeria monocytogenes* II performs satisfactorily. Further, it was demonstrated that confirmation is not necessary.