NMKL - NordVal International
c/o Institute of Marine Research
P.O. box 1870 Nordnes, N-5817 Bergen, Norway
www.nmkl.org

NordVal International Certificate

Issued for:	**iQ-Check® Listeria monocytogenes II**
NordVal No:	037
First approval date:	15 November 2009
Renewal date:	01 October 2024
Valid until:	01 October 2026

iQ-Check® Listeria monocytogenes II

Manufactured and supplied by:

Bio-Rad Laboratories,
3 Blvd Raymond Poincare,
92430 Marnes-la-Coquette,
France

fulfils the requirements of the NordVal Validation Protocol / ISO 16140-2:2016. The reference method was EN ISO 11290-1: 2017 Horizontal method for the detection and enumeration of *Listeria monocytogenes* -- Part 1: Detection method.

NordVal International has studied the enclosures to the application and evaluated the results obtained in the validations. The first validation was conducted in 2005 by Institut Scientifique d'Hygiéne et d'Analyse, France. The latest validations, i.e. renewals and extensions are carried out by ADRIA Développement, France, according to ISO 16140-2:2016. NordVal International has concluded that it has been satisfactorily demonstrated that iQ-Check *Listeria monocytogenes* II performs satisfactorily for the described method protocols.

The results document equivalent performances of the iQ-Check® *Listeria monocytogenes* II and the reference method for a broad range of foods and environmental samples.

Date: 01. October 2024

Yours sincerely,

Hrólfur Sigurðsson

Hrólfur Sigurðsson
Chair of NordVal International

Eystein Oveland
NMKL Executive Director

PRINCIPLE OF THE METHOD

The iQ-Check *Listeria monocytogenes* II method is a qualitative method allowing the detection of *Listeria monocytogenes* in a broad range of foods and in environmental samples. The method is based on real-time PCR using florescent probes.

The samples are prepared according to one of the following protocols:

Protocol 1 (P1) - enrichment in Half-Fraser broth and Standard II lysis

- Enrichment: 25 ± 1 hours at 30 °C ± 2 °C in Half-Fraser broth (1/10)
- Lysis of bacteria to release bacterial DNA
- Amplification
- Detection with the thermal cycler

Protocol 2 (P2) - use of specific LSB broth and Standard II lysis protocol

- Enrichment: 23 ± 1 hours at 30 °C ± 2 °C in LSB for the standard lysis protocol
- Lysis of bacteria to release bacterial DNA – standard protocol
- Amplification
- Detection with the CFX96 and CFX96 Touch DeepWell real-time PCR system

Protocol 3 (P3) - use of specific LSB broth and Easy II lysis protocol

- Enrichment: 25 ± 1 hours at 30 °C ± 2 °C LSB
- Simplified lysis protocol - no longer requiring the first centrifugation step
- Amplification
- Detection with, the CFX96 and CFX96 Touch DeepWell real-time PCR system

Protocol 4 (P4) – environmental samples

- Enrichment: 18-26 hours at 30 °C ± 1 °C LSB
- Easy II lysis protocol - no longer requiring the first centrifugation step
- Amplification
- Detection with, the CFX96 and CFX96 Touch DeepWell real-time PCR system, with or without using APF Fast

Protocol 5 (P5) – environmental samples with FDRS

- Enrichment: 18-26 hours at 30 °C ± 1 °C LSB
- Easy II lysis protocol - no longer requiring the first centrifugation step – with preceding Free DNA removal treatment.
- Amplification
- Detection with, the CFX96 and CFX96 Touch DeepWell real-time PCR system, with or without using APF Fast

Samples detected as positive sample with the iQ-Check ® *Listeria monocytogenes* II test can be confirmed by:

- using standard tests described in the NF EN ISO 11290-1 (2017) standard method (including confirmations).
- using the chromogenic medium RAPID'*L.mono* and/or AL agar

The presence of characteristic *Listeria monocytogenenes* colonies is sufficient to confirm the presence of *Listeria monocytogenenes*.

FIELD OF APPLICATION

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

HISTORY

In 2005, a comparison study and an interlaboratory study were carried out for iQ-Check *Listeria monocytogenes* II using enrichment in half Fraser followed by the Standard II lysis protocol (P1).

In 2006, the protocols using LSB and Standard II lysis and Easy II lysis (P1, P2 and P3), respectively, were validated.

The validations in 2005 and 2006 were carried out according to ISO 16140:2003
In 2017, results from earlier validations studies are included and additional samples analysed and evaluated according to ISO 16140-2:2016.

In 2019/2020, an extension for environmental samples protocol including for the Easy II lysis protocol a new enrichment protocol and a protocol for use of Free DNA Removal Solution (FDRS) as well as a new application protocol APF Fast was carried out (P4 and P5).

SENSITIVITY STUDIES

Sensitivity studies have been carried out for the three different method procedures on the following matrixes (6 categories each with 3 different types):

- meat products: raw products, ready-to-eat and processed meat products, fermented or dried meat products
- dairy products: raw milk cheese, other raw milk products, heat-processed milk and dairy products
- seafood products: raw products, smoked products, marinated products, processed products
- vegetal products: raw vegetal products, ready-to-eat and ready-to-cook raw vegetal products, precooked vegetal products, processed vegetal products
- composite foods: ready-to-eat foods, ready-to-reheat foods, pastries, egg products
- environmental samples: process waters, dust and residues, surface sample.

Half Fraser broth enrichment followed by the Standard II lysis protocol (P1)

A total of 425 samples are included in the study, whereof 292 samples from the previous validations. The samples were analyzed with both the alternative method and the reference method. The results are given in **Table 1**.

Table 1. Sensitivity study - Enrichment in half Fraser followed by the Standard II lysis protocol (P1)

Category	PA	NA	ND	PD	N	PPND	PPNA	SE_{alt} (%)	SE_{ref} (%)	RT (%)	FPR (%)	Kappa
Meat	29	45	1	1	76	0	1	96.8	96.8	97.4	2.2	0.94
Dairy	34	38	0	0	72	0	1	100	100	100	2.6	1.0
Seafood	33	37	3	0	73	0	3	91.7	100	95.9	8.1	0.92
Vegetal	35	30	1	0	66	0	2	97.2	100	98.5	6.7	0.97
Composite food	34	30	0	0	64	0	2	100	100	100	6.7	1.0
Environmental	31	42	1	0	74	0	0	96.9	100	98.6	0	0.97
Total	**196**	**222**	**6**	**1**	**425**	**0**	**9**	**97.0**	**99.5**	**98.4**	**4.1**	**0.97**

PA = number of obtained results that are positive with the alternative and the reference method that are confirmed positives

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 (false negative)

PD = number of obtained results that are positive with the alternative method and negative with the reference method and confirmed as positive.

PPNA = presumptive positive of the alternative method which after confirmation was found to be negative – the reference method was negative (false positive without confirmation)

PPND = presumptive positive with both method but confirmed as negative

SEalt = sensitivity for the alternative method

SEref = sensitivity for the reference method

RT = relative trueness

FPR = false positive ratio for the alternative method

There are 6 negative deviations (ND); results that were negative with the alternative method and positive with the reference method, which were confirmed as positives. There were one positive deviation (PD), i.e. the result was positive with the alternative method and negative with the reference method. The differences in the results are within the acceptability limits.

Further, the statistical entity Kappa shows that there is a very good agreement between the results obtained by the alternative method and the reference method.

Enrichment in *Listeria* Specific Broth (LSB) followed by the Standard II lysis protocol (P2)

A total of 422 samples are included in the study, whereof 287 samples from the previous validations. The samples were analysed with both the alternative method and the reference method. The results are given in **Table 2**.

Table 2. Sensitivity study - Enrichment in *Listeria* Specific Broth (LSB) followed by the Standard II lysis protocol (P2)

Category	PA	NA	ND	PD	N	PPND	PPNA	SE_{alt} (%)	SE_{ref} (%)	RT (%)	FPR (%)	Kappa
Meat	27	31	4	10	72	0	2	90.2	75.6	80.6	6.5	0.61
Dairy	36	40	0	4	80	0	1	100	90.0	95.0	2.5	0.90
Seafood	29	30	0	4	63	0	1	100	87.9	93.7	3.3	0.87
Vegetal	30	31	3	0	64	0	2	90.9	100	95.3	0	0.91
Composite food	28	32	3	0	64	0	0	94.1	91.2	92.4	3.1	0.90
Environmental	37	38	2	0	77	1	4	94.9	100	97.4	13.2	0.95
Total	**188**	**202**	**11**	**21**	**422**	**1**	**9**	**95.0**	**90.5**	**92.4**	**5.0**	**0.85**

Explanations to the abbreviations are given under Table 1.

There were 11 negative deviations (ND); results that were negative with the alternative method and positive with the reference method. There were 21 positive deviations (PD), i.e. the results that were positive with the alternative method and negative with the reference method.

As the PD is higher than ND, the (ND-PD) is less than the acceptability limit. According to Kappa there is not a very good agreement between the methods for meat products, as Kappa is less than 0.80. However, the alternative method performs better than the reference method for these samples as the sensitivity of the alternative method is better than the sensitivity of the reference method. In total the agreement between the methods, Kappa is satisfactory (>0.80).

Enrichment in *Listeria* Specific Broth (LSB) followed by the Easy II DNA extraction protocol (P3)

A total of 422 samples are included in the study, whereof 287 samples are from the previous validations. The samples were analysed with both the alternative method and the reference method. The results are given in **Table 3**.

Table 3. Sensitivity study - Enrichment in *Listeria* Specific Broth (LSB) followed by the Easy II DNA extraction protocol (P3).

Category	PA	NA	ND	PD	N	PPND	PPNA	SE_{alt} (%)	SE_{ref} (%)	RT (%)	FPR (%)	Kappa
Meat	27	31	4	10	72	0	1	90.2	75.6	80.6	3.2	0.61
Dairy	34	40	2	4	80	0	1	95.0	90.0	92.5	2.5	0.90
Seafood	28	30	1	4	63	0	1	97.0	87.9	92.1	3.3	0.84
Vegetal	30	31	3	0	64	0	1	90.9	100	95.3	3.2	0.91
Composite food	29	32	2	3	66	0	1	94.1	91.2	92.4	3.1	0.85
Environmental	36	38	3	0	77	0	2	92.3	100	96.1	5.3	0.92
Total	**184**	**202**	**15**	**21**	**422**	**0**	**7**	**93.2**	**90.5**	**91.5**	**3.5**	**0.83**

Explanations to the abbreviations are given under Table 1.

There were 15 negative deviations (ND); results that were negative with the alternative method and positive with the reference method. There were 21 positive deviations (PD), i.e. results that were positive with the alternative method and negative with the reference method.

The deviations of the results fall within the acceptability limit. There is not a very good agreement between the methods for meat products, as Kappa is less than 0.80. However, the alternative method performs better than the reference method for these samples as the sensitivity of the alternative method is higher than the sensitivity of the reference method. Kappa for total agreement between the methods is satisfactory (>0.80).

Enrichment in *Listeria* Specific Broth (LSB) followed by the Easy II DNA extraction protocol (P4) with or without APF Fast

A total of 60 environmental samples are included in the study. The samples were analysed using both the alternative method (with and without APF Fast) and the reference method. The results are given in **Table 4**.

Table 4. Sensitivity study - Enrichment in *Listeria* Specific Broth (LSB) followed by the Easy II DNA extraction protocol (P4) with or without APF Fast on environmental samples

Environmetal samples	PA	NA	ND	PD	N	PPND	PPNA	SE_{alt} (%)	SE_{ref} (%)	RT (%)	FPR (%)	Kappa
Without APF Fast	15	29	7	9	60	1	1	76.7	73.3	75.0	6.7	0.61
With APF Fast	15	30	7	8	60	0	0	76.7	73.3	75.0	0.0	0.60

Explanations to the abbreviations are given under Table 1.

There were 7 negative deviations (ND); In addition, there were 8 positive deviations (PD) i.e. results that were positive with the alternative method and negative with the reference method, all in naturally contaminated samples.

The deviations of the results fall within the acceptability limit. There is not a very good agreement between the methods for meat products, as Kappa is less than 0.80. However, the alternative method performs somewhat better than the reference method for these samples as the sensitivity of the alternative method is higher than the sensitivity of the reference method. The sensitivity is poor for both the alternative method and the reference method, most probably as the levels in the samples are on the level of detection for the method.

Enrichment in *Listeria* Specific Broth (LSB) followed by the Easy II DNA extraction protocol and using Free DNA removal treatment (P5) and with or without APF Fast

A total of 60 environmental samples are included in the study. The samples were analysed using both the alternative method (with and without APF Fast) and the reference method. The results are given in **Table 5**.

Table 5. Sensitivity study - Enrichment in *Listeria* Specific Broth (LSB) followed by the Easy II DNA extraction protocol using free DNA removal treatment (P5) with or without APF Fast on environmental samples.

Environmental samples	PA	NA	ND	PD	N	PPND	PPNA	SE_{alt} (%)	SE_{ref} (%)	RT (%)	FPR (%)	Kappa
Without APF Fast	16	30	6	8	60	0	1	80.0	73.3	76.7	3.3	0.58
With APF Fast	16	30	6	8	60	0	0	80.0	73.3	76.7	0.0	0.58

Explanations to the abbreviations are given under Table 1.

There were 6 negative deviations (ND). In addition, there were 8 positive deviations (PD) i.e. results that were positive with the alternative method and negative with the reference method, all in naturally contaminated samples.

The deviations of the results fall within the acceptability limit. There is not a very good agreement between the methods for meat products, as Kappa is less than 0.80. However, the alternative method performs somewhat better than the reference method for these samples as the sensitivity of the alternative method is higher than the sensitivity of the reference method.

LEVEL OF DETECTION (LOD) AND RELATIVE LEVEL OF DETECTION (RLOD)

One type for the six different matrix categories were analysed at the following levels:

Level 1: 0 cfu/25 g of sample (5 replicates)
Level 2: 0.7 to 1.2 cfu/25g of sample (20 replicates)
Level 3: 2.8 to 3.2 cfu/25g of sample (5 replicates)

Wilrich&Wilrich complementary log-log model (the *EXCEL program for the estimation of the POD function and the LOD of a qualitative microbiological measurement method* according to Wilrich, C., and P.-Th. Wilrich: AOAC International **92** (2009) 1763 - 1772) was used to estimate both the LOD and the RLOD. The same data set, shown in **Table 4**, was used for both calculations given in **Table 6**.

Table 6. Determination of LOD and RLOD – the levels are given in cfu/25g

		Protocol I			Protocol 2			Protocol 3		
Category	Level	+/N *	LOD_{50} & 95% conf. level	RLOD	+/N	LOD_{50} & 95% conf. level	RLOD	+/N	LOD_{50} & 95% conf. level	RLOD
Ground beef (Meat product)	0	0/5	0.6 [0.3;1.0]	1.0	0/5	0.6 [0.3;1.0]	1.0	0/5	0.7 [0.4;1.1]	1.0
	1.2	15/20			15/20			14/20		
	2.8	5/5			5/5			5/5		
Raw milk (Dairy product)	0	0/5	0.5 [0.3;0.9]	1.0	0/5	0.7 [0.4;1.1]	1.3	0/5	0.7 [0.4;1.1]	1.3
	0.9	14/20			12/20			12/20		
	3.2	5/5			5/5			5/5		
Cod filet (Seafood)	0	0/5	0.5 [0.3;0.9]	1.0	0/5	0.6 [0.3;1.0]	1.1	0/5	0.6 [0.3;1.0]	1.1
	0.7	12/20			11/20			11/20		
	2.8	5/5			5/5			5/5		
Salad (Vegetal)	0	0/5	0.6 [0.4;1.1]	1.0	0/5	0.7 [0.4;1.2]	1.1	0/5	0.8 [0.5;1.4]	1.3
	1.0	13/20			12/20			11/20		
	3.2	5/5			5/5			5/5		
Pasta salad (Composite food)	0	0/5	0.4 [0.2;0.7]	1.0	0/5	0.5 [0.3;0.8]	1.2	0/5	0.5 [0.3;0.8]	1.2
	0.8	15/20			14/20			14/20		
	3.0	5/5			5/5			5/5		
Processed water (Environmental)	0	0/5	0.7 [0.4;1.2]	1.0	0/5	0.9 [0.5;1.6]	1.3	0/5	0.9 [0.5;1.6]	1.3
	1.0	12/20			10/20			10/20		
	3.4	5/5			5/5			5/5		
Combined			0.6 [0.4;0.7]	1.0		0.6 [0.5;0.8]	1.2		0.7 [0.5;0.8]	1.2

+/N = the number of positives divided by the number of replicates
The level of detection (LOD_{50}) is 0.5-0.9 log cfu/25 g for the iQ-Check® *Listeria monocytogenes* II method.

		ISO 11290-1/A1		Protocol 4			Protocol 5		
Category: Environmental	Level	+/N	LOD_{50}	+/N *	LOD_{50} & 95% conf. level	RLOD	+/N	LOD_{50} & 95% conf. level	RLOD
Without APF Fast	0	0/5	0.73	0/5	1.0 [0.56-1.9]	1.4	0/5	1.0 [0.56-1.9]	1.4
	0.7	11/20		6/20			6/20		
	2.7	4/5		5/5			5/5		
With APF Fast	0	0/5	0.73	0/5	0.98 [0.64-1.5]	1.3	0/5	0.98 [0.64-1.5]	1.3
	0.7	11/20		7/20			7/20		
	2.7	4/5		5/5			5/5		

The relative level of detection (RLOD) for the alternative method relative to the reference method should be no more than 2.5 for unpaired studies (different incubation steps) and no more than 1.5 for paired studies. The RLOD are satisfactory for Protocol 1, 2, 3, 4 and 5; there is no significant difference in detection level between the alternative methods and the reference method.

INCLUSIVITY / EXCLUSIVITY

Inclusivity: 50 strains of *Listeria monocytogenes* were detected out of the 50 tested. For the extension study in 2019, 50 *Listeria monocytogenes* strains using Protocol 4 and 5. All strains gave positive PCR results and were confirmed as positive.

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.

INTERLABORATORY 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:

- L0: 0 cfu/25 ml
- L1: 1-10 cfu/25 ml
- L2: 10-50 cfu/25 ml

The laboratories analysed 8 replicates for each level using both the alternative method using Protocol 1 and the reference method. The results are given in **Table 7**.

Table 7. Results of the Interlaboratory study

Level	PA	NA	ND	PD	N	PPND	PPNA	SE_{alt} (%)	SE_{ref} (%)	RT (%)	FPR (%)	Kappa
Level 0	1	111	0	0	112	0	5	99.1	100	99.1	0	1.0
Level 1	110	1	1	0	112	1	0					
Level 2	112	0	0	0	112	0	0					

Explanations to the abbreviations are given under Table 1.

The results of the interlaboratory study are satisfactory.

CONCLUSION

The comparison study and the interlaboratory study showed that iQ-Check® *Listeria monocytogenes* II performs equivalent to the reference method for a broad range of foods and for environmental samples.