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:	**foodproof® Listeria monocytogenes Detection LyoKit - 5'Nuclease - for Listeria monocytogenes detection in food products and production environmental samples**
NordVal No:	025
First approval date:	24 January 2006
Renewal date:	23 October 2023
Valid until:	02 November 2025

foodproof® Listeria monocytogenes Detection LyoKit - 5'Nuclease

Manufactured and supplied by:

Hygiena Diagnostics GmbH
Hermannswerder 17
D-14473 Potsdam
Germany

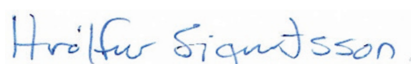
fulfills the requirements of the NordVal validation protocol / ISO 16140-2. The reference method was EN ISO 11290-1:2017: Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Detection method.

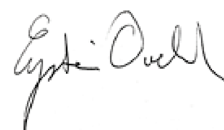
NordVal International has reviewed the method and the validation study conducted by ADRIA Développement, France. The results of the validation document that the alternative method performs equivalently to the reference method for the detection of *Listeria monocytogenes* in food products and production environmental samples. NordVal International has concluded that it has been satisfactorily demonstrated that the method performance characteristic requirements have been fulfilled.

The production of the **food**proof® *Listeria monocytogenes* Detection LyoKit - 5'Nuclease - fulfills the requirements outlined by ISO 9001.

Date: 23. October 2023

Yours sincerely,





Hrólfur Sigurðsson
Chair of NordVal International

Eystein Oveland
NMKL Executive Director

PRINCIPLE OF THE METHOD

The method is based on an enrichment step, DNA extraction and real-time PCR detection using PCR instruments with a FAM, a VIC/Yakima Yellow or HEX detection channel.

The following three enrichment / DNA extraction protocols were evaluated during the validation study. There is a declaration of equivalence between the **food**proof® StarPrep Two Kit and the **food**proof® StarPrep Two 8-Strip Kit:

		Protocol A	Protocol B	Protocol C
Enrichment step		Actero *Listeria* Enrichment Media (ALEM) 22 h ± 2 h 36°C ± 1°C (dilution 1:7)	Half Fraser (ISO) 25 h ± 1 h 30°C ± 1°C (dilution 1:10)	Half Fraser (ISO) 48 h ± 2 h 30°C ± 1°C (dilution 1:10)
Extraction	Kit	**food**proof StarPrep Two 8-Strip Kit	**food**proof StarPrep Two Kit	**food**proof StarPrep Two Kit
	Protocol	Procedure A STANDARD	Procedure A STANDARD	Procedure B RAPID
	Enrichment volume	800 µl	800 µl	200 µl
	PCR	5 µl	5 µl	5 µl
Confirmation		Streaking 10 µl onto O&A The presence of only typical colonies allows confirmation of the positive PCR result.		

Kits for DNA extraction:
- **food**proof® StarPrep Two Kit (order No. S 400 08.1);
- **food**proof® StarPrep Two 8-Strip Kit (order No. S 400 17 L).

Kit for PCR:
- **food**proof® *Listeria monocytogenes* Detection LyoKit
(order No. R 602 23-1, R 602 23-2 or R 602 23-3).

The detection kit provides all reagents required for PCR.

FIELD OF APPLICATION

The **food**proof® *Listeria monocytogenes* LyoKit - 5' Nuclease – is applicable for detection of *Listeria monocytogenes* in food products and production environmental samples.

HISTORY

The **food**proof® *Listeria monocytogenes* Detection Kit, Hybridization Probes and **food**proof® *Listeria monocytogenes* Detection Kit, 5' Nuclease, in combination with **food**proof® ShortPrep II Kit or **food**proof® StarPrep Two Kit was first approved in 2006 and further extended in 2011. The studies were conducted by MQD, Institute for Analytic and Hygiene in Güstrow, Germany.

In 2021, the method was expanded to the use of three protocols and hence completely new validations studies were carried out by ADRIA Développement, France. The results included in this certificate are based on these recent studies.

In 2023, the name of manufacturer and supplier was changed to Hygiena Diagnostics GmbH due to a rebranding.

METHOD PERFORMANCE CHARACTERISTICS

Selectivity studies

The inclusivity and exclusivity testing gave the expected results for the 50 target strains and 30 non-target strains.

Sensitivity studies

The sensitivity is the ability of the method to detect the analyte.

A total of 404 samples were analysed using the three protocols. About 55% of the samples tested were naturally contaminated. 108 samples were artificially contaminated using 44 different strains of *L. monocytogenes*, yielding fractional positive recovery data. The results for the categories analysed for Protocol A, B and C are provided in **Table 1**.

Table 1. The results of the sensitivity study for Protocols A, B and C

Matrices	PA	NA	PD	ND	FP	Sum	RT (%)	SE_{alt} (%)	SE_{ref} (%)	FPR(%)
Protocol A										
composite foods/ready to eat and ready to reheat	24	32	9	6	0	71	78.9	84.6	76.9	0
meat products	23	29	6	9	0	67	77.6	76.3	84.2	0
milk and dairy products	28	30	2	2	0	62	93.5	93.8	93.8	0
vegetables	28	43	3	5	1	80	88.8	83.8	91.9	2.3
seafood and fishery products	21	25	7	8	1	62	74.2	75.7	81.1	4.0
environmental samples	31	23	6	2	0	62	87.1	94.9	84.6	0
TOTAL	**155**	**182**	**33**	**32**	**2**	**404**	**83.4**	**84.7**	**85.1**	**1.1**
Protocol B										
composite foods/ready to eat and ready to reheat	30	40	0	0	1	71	100.0	100.0	100.0	2.4
meat products	32	34	1	0	0	67	98.5	100.0	97.0	0
milk and dairy products	30	30	1	0	1	62	98.4	100.0	96.8	3.2
vegetables	31	45	0	3	1	80	96.3	91.2	100.0	2.2
seafood and fishery products	29	31	0	1	1	62	98.4	96.7	100.0	3.1

Matrices	PA	NA	PD	ND	FP	Sum	RT (%)	SE_{alt} (%)	SE_{ref} (%)	FPR(%)
environmental samples	33	29	0	0	0	62	100.0	100.0	100.0	0
TOTAL	**185**	**209**	**2**	**4**	**4**	**404**	**98.5**	**97.9**	**99.0**	**1.9**
Protocol C										
composite foods/ready to eat and ready to reheat	30	40	0	0	1	71	100.0	100.0	100.0	2.4
meat products	32	32	2	0	1	67	97.0	100.0	94.1	3.0
milk and dairy products	29	31	1	0	1	62	96.8	96.8	96.8	3.2
vegetables	34	44	0	0	1	80	100.0	100.0	100.0	4.3
seafood and fishery products	30	30	2	0	1	62	96.8	100.0	93.8	0
environmental samples	33	28	0	0	0	62	100.0	100.0	100.0	3.4
TOTAL	**185**	**205**	**5**	**0**	**5**	**404**	**98.5**	**99.5**	**97.4**	**2.9**

- 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)
- FP = number of false positive
- RT = the relative trueness; the degree of correspondence between the response obtained by the alternative method and the reference method
- SE = the sensitivity; the ability of the method to detect the analyte
  (SEalt = sensitivity of the alternative method, SE_{ref} = sensitivity of the reference method)
- FPR = False positive rate

The observed values for the deviating results (ND-PD) and (ND+PD) are below the acceptability limits and were hence met for each individual category and for all the combined categories for all three protocols.

Level of Detection (LOD) and Relative Level of Detection (RLOD)

The level of detection (LOD_{50}) is the smallest number of culturable microorganisms that can be detected in the sample in 50% of occasions. The relative level of detection is the ratio of the LOD of the alternative method and the LOD of the reference method. A matrix type of each category was tested on low and high levels using the three protocols.

The LOD_{50} of the alternative method varied from 0.4 to 1.0 CFU/test portion (Protocol A) and from 0.3 to 1.1 CFU/test portion (Protocols B and C) and the LOD_{50} of the reference method varied from 0.3 to 1.1 CFU/test portion.

The RLOD is close to 1.0 and is acceptable according to the criteria (acceptance level of 1.5 for the paired studies (Protocols B and C) and 2.5 for the unpaired study (Protocol A), respectively).

INTERLABORATORY STUDY

16 collaborators were involved in the study. Each analyzed 24 blind coded cheese samples inoculated with *Listeria monocytogenes* following both the reference method and Protocol A of the alternative method, using either the **food**proof® StarPrep Two Kit or the **food**proof® StarPrep Two 8-Strip Kit depending on the material available in their laboratory.

The targeted inoculation levels were 0 CFU/25g, 1.5 CFU/25g and 6 CFU/25g.

Results from five collaborators were excluded for interpretation as either the analyses were carried out too late or due to the fact that a high number of positive PCR results were obtained for uninoculated samples, leaving valid results from 11 collaborators.

The positive results obtained by the reference and the alternative method are provided in **Table 2** and **Table 3**, respectively.

Table 2. Positive results by the reference method

Laboratories	Contamination level		
	L_0	L_1	L_2
D	0/8[a]	4/8[b]	8/8[c]
E	0/8	6/8	8/8
F	0/8	7/8	8/8
G	0/8	7/8	8/8
H	0/8	6/8	8/8
I	0/8	7/8	8/8
K	0/8	7/8	8/8
M	0/8	6/8	8/8
N	0/8	8/8	8/8
O	0/8	7/8	8/8
P	0/8	4/8	8/8
Total	P_0**=0/8**	P_1**=69/88**	P_2**=88/88**

L_0 = negative control
L_1 = 1.5 CFU/25g
L_2 = 6.0 CFU/25g
a Number of positive reference method results at level 0
b Number of positive reference method results at level 1
c Number of positive reference method results at level 2

Table 3. Positive results (before and after confirmation) by the alternative method

Laboratories	Contamination level					
	L_0		L_1		L_2	
	Screening	Confirmed	Screening	Confirmed	Screening	Confirmed
D	0/8[a]	0/8[b]	6/8[c]	6/8[d]	8/8[e]	8/8[f]
E	0/8	0/8	6/8	6/8	8/8	8/8
F	1/8	0/8	8/8	8/8	8/8	8/8
G	0/8	0/8	6/8	6/8	8/8	8/8
H	0/8	0/8	6/8	6/8	8/8	8/8
I	0/8	0/8	6/8	6/8	8/8	8/8
K	0/8	0/8	6/8	6/8	8/8	8/8
M	0/8	0/8	5/8	5/8	8/8	8/8

Laboratories	Contamination level					
	L_0		L_1		L_2	
	Screening	Confirmed	Screening	Confirmed	Screening	Confirmed
N	0/8	0/8	8/8	8/8	8/8	8/8
O	0/8	0/8	7/8	7/8	8/8	8/8
P	1/8	0/8	7/8	6/8	8/8	8/8
Total	P_0=2/88	CP_0=0/88	P_1=71/88	CP_1=70/88	P_2=88/88	CP_2=88/88

L_0 = negative control
L_1 = 1.5 CFU/25g
L_2 = 6.0 CFU/25g
a Number of positive alternative method results at level 0
b Number of confirmed positive alternative method results at level 0
c Number of positive alternative method results at level 1
d Number of confirmed positive alternative method results at level 1
e Number of positive alternative method results at level 2
f Number of confirmed positive alternative method results at level 2

The specificity (%) of the reference method and the alternative method:

Specificity for the reference method $SP_{ref} = \left[1 - \frac{P_0}{N_-}\right] \cdot 100\% = 100\%$

Specificity for the alternative method $SP_{alt} = \left[1 - \frac{CP_0}{N_-}\right] \cdot 100\% = 100\%$

Where:

N_- is the total number of all L_0 tests
P_0 is the number of false positive obtained by the reference method
CP_0 is the number of false positive obtained by the alternative method

The relative trueness, the sensitivity of the alternative method and the reference method and the false positive rate of level 1 and 2 are provided in **Table 4**.

Table 4. Results of the Interlaboratory study

Level	PA*	NA	PD	ND	FP	Sum	RT (%)	SE_{alt} (%)	SE_{ref} (%)	FPR(%)
L_1 = 1.5 CFU/25g	55	3	15	14	1	88	67.0	83.3	82.1	25.0
L_2 = 6.0 CFU/25g	88	0	0	0	0	88	100.0	100.0	100.0	0

* See abbreviations under Table 1

As the positive and the negative deviations are in the same magnitude, resulting in comparable sensitivity rates, it can be interpreted that the methods perform equivalently. When calculating the acceptability limit, AL, according to ISO 16140-2, it demonstrates that the acceptance criteria have been met.

CONCLUSION

The studies have shown that the alternative method fulfills the requirements of the NordVal International Protocol No. 1 / ISO 16140-2 and guarantees equivalent results to the reference method.