NordVal International / NMKL
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NordVal International Certificate

Issued for:	**foodproof® *Listeria monocytogenes* Detection Kit, Hybridization Probes and foodproof® *Listeria monocytogenes* Detection Kit, 5' Nuclease, in combination with foodproof® ShortPrep II Kit or foodproof® StarPrep Two Kit**
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Valid until:	10 June 2019

foodproof® *Listeria monocytogenes* Detection Kit, Hybridization Probes and foodproof® *Listeria monocytogenes* Detection Kit, 5' Nuclease, in combination with foodproof® ShortPrep II Kit or foodproof® StarPrep Two Kit

Manufactured and supplied by:
BIOTECON Diagnostics GmbH,
Hermannswerder 17,
14473 Potsdam, Germany.

fulfils the requirements of the NordVal validation protocol. The reference method was EN ISO 11290:1996/Amd 1:2004: Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of *Listeria monocytogenes* -- Part 1: Detection method (Amd 1:2004 Modification of the isolation media and the haemolysis test, and inclusion of precision data).

NordVal International has reviewed the method and the validation studies conducted by the MQD, Institute for Analytic and Hygiene in Güstrow, Germany, studied the enclosures to the application and evaluated the results obtained in the validations. The results document no statistical differences in the performances between alternative methods and the reference method for the detection of *Listeria monocytogenes*. NordVal International has concluded that it has been satisfactorily demonstrated that the requirements for the sensitivity and the agreement between the methods are fulfilled, further that confirmation of obtained positives are not necessary.

Date: 9 June 2017

Yours sincerely

Hilde Skår Norli
Chair of NordVal International

Nina Skall Nielsen
NMKL Secretary General

PRINCIPLE OF THE METHOD

The principle is real-time PCR and detection with specific, fluorescence labelled probes.

After DNA isolation using the **food**proof® ShortPrep II Kit (Art. No. S 400 02) or the bulk version of this kit, the **food**proof® StarPrep Two Kit (Art. No. S 400 08), designed for the rapid preparation of bacterial DNA for direct use in PCR, the real-time detection of *Listeria monocytogenes* DNA is carried out either by using the **food**proof® *Listeria monocytogenes* Detection Kit, Hybridization Probes or the **food**proof® *Listeria monocytogenes* Detection Kit, 5'Nuclease.

For food samples inoculate 25 g. For environmental samples inoculate an area of 100 cm^2. Perform the pre-enrichment according to EN ISO 11290. The detection kit provides all the reagents required for the PCR.

FIELD OF APPLICATION

The **food**proof® *Listeria monocytogenes* Detection Kit, Hybridization Probes and the **food**proof® *Listeria monocytogenes* Detection Kit, 5' Nuclease in combination with **food**proof® ShortPrep II Kit are intended for the detection of *Listeria monocytogenes* DNA isolated from enrichment cultures prepared by various valid methods inoculated with food samples that are potentially contaminated with *Listeria monocytogenes.*

The methods are tested on foods and environmental samples.

HISTORY

The **food**proof® *Listeria monocytogenes* Detection Kit, Hybridization Probes in combination with **food**proof® ShortPrep II Kit was first approved in 2006 based on a comparison study and a collaborative study.

In 2011, the method was extended: A new system was evolved using hydrolysis probes instead of hybridisation probes. The modification, using a new primer, required a new comparison study of the selectivity (inclusivity and exclusivity) and a comparison study of the relative accuracy to measure the degree of correspondence between the results obtained by the **food**proof® *Listeria monocytogenes,* 5' Nuclease Detection Kit and the reference method. In 2011 it also was an extension of the method, inclusion of environmental samples, and hence it was required to include this matrix in the comparison study. However, it was not required to make a full comparison study with five food matrices. As the method procedure was unchanged, NordVal did not require an additional collaborative study.

In 2017, the results obtained for the foodproof® Listeria monocytogenes Detection Kit, Hybridization Probes and foodproof® Listeria monocytogenes Detection Kit, 5' Nuclease, in combination with foodproof® ShortPrep II Kit or foodproof® StarPrep Two Kit method have been recalculated according to the ISO 16140-2:2016 protocol.

METHOD PERFORMANCE CHARACTERISTICS

Selectivity: Inclusivity/Exclusivity

The study approved in 2006:

All tested 102 *Listeria monocytogenes* strains were positive. All tested non-*Listeria monocytogenes* strains were negative.

The additional study approved in 2011:

Inclusivity: Specificity: 51 isolates from *Listeria monocytogenes* were tested for the specificity of the PCR method. All isolates were positively detected.

Exclusivity: 35 samples with bacteria from taxonomically related species or other food related species were studied. None of the tested isolates gave a false positive result.

The selectivity, i.e. the inclusivity and exclusivity, was 100%.

Relative accuracy, relative sensitivity and relative specificity:

The study approved in 2006:

In total six samples were positive with the alternative method and negative with the reference method. Three of these samples were confirmed as true positives. Differences between the alternative and the reference method were found for meat products and a leaf salad sample. A high amount of background flora of these matrices, especially non-*Listeria monocytogenes* species, might be responsible for the differences between the methods. By identification with the reference method CAMP-test, *L. innocua* – and *L. ivanovii*-types were found. By reanalysing with a *Listeria* Genus specific PCR-system in one of the PCR-positive non-inoculated minced meat samples an approximately 1000 times higher amount of *Listeria* Genus than *Listeria monocytogenes* DNA was found.

The results obtained were:

The relative accuracy: 99.2%

The relative sensitivity: 100%

The relative specificity: 96.0%

As the results are satisfactory after screening, confirmation is not necessary.

The additional study approved in 2011:

Three different sub matrices of milk and three different sub matrices of environmental samples were included in the study. Two strains relevant for each matrix were selected. Three inocoluation levels were used: 0 = negative control, 1-10 cells per 25 g/100cm^2 sample and 10-100 cells per 25 g/100cm^2 sample. For each matrix 60 samples were analysed.

The following results were obtained:

Table 1: Results after screening

Matrix	* PA	NA	ND	PD	Sum	Relative AC %	Relative SE %	Relative SP %	Kappa
Milk	33	25	0	2	60	96,6	100	92.6	0.93
Environmental	42	15	0	3	60	95,0	100	83.3	0.88
Total	75	40	0	5	120	95,8	100	88.8	0.91

* see definitions of the abbreviations below.

Table 2: Results after confirmation

Matrix	* PA	NA	ND	PD	TP	FP	Sum	Relative AC %	Relative SE %	Relative SP %	Kappa
Milk	32	25	0	0	2	0	60	100	100	100	1.00
Environmental	42	15	0	0	3	0	60	100	100	100	1.00
Total	75	40	0	0	5	0	120	100	100	100	1.00

* see definitions of the abbreviations below.

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 PDs that after confirmation proved to be negative.

TP = number of PDs that after confirmation proved to be 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.

The five samples obtained positive with the alternative method (the **food**proof® *Listeria monocytogenes* Detection kit, 5'Nuclease in combination with the **food**proof® Short PrepII Kit) but negative with the ISO method turned out to be positive after confirmation. That means that the reference method had some false negatives, and has a poorer sensitivity than the alternative method.

The degree of agreement between the alternative method and the reference method, kappa, was above 0.80 for all categories and indicate very good agreement between the methods.

The sum of deviations (FN+FP+TP = 0+0+5 =5) is below the acceptability limit for the sensitivity; tabled as 8 for two categories.

According to the obtained results, the method can be used without any confirmation.

Detection Level

The limit of detection is 1-10 cells per 25 g/100 cm^2, which was obtained both with the alternative method and the reference method for all food matrices.

CONCLUSION

The **food**proof® *Listeria monocytogenes* Detection Kit, Hybridization Probes and the **food**proof® *Listeria monocytogenes* Detection Kit, 5' Nuclease in combination with **food**proof® ShortPrep II Kit or **food**proof® StarPrep Two Kit perform equivalent to the reference method.