NordVal International / NMKL
c/o Danish Technical University
Kemitorvet B201
DK-2800 Kgs Lyngby, Denmark
www.nmkl.org

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**
NordVal No:	025
First approval date:	24 January 2006
Renewal date:	10 June 2019
Valid until:	10 June 2021

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

NordVal International has reviewed the method and the validation studies conducted by BIOTECON Diagnostics and the MQD, Institute for Analytic and Hygiene in Güstrow, Germany. The validations have been carried out according to ISO 16140 and ISO 16140-2. The reference method was EN ISO 11290:1996/Amd 1:2004 technical equivalent to EN ISO 11290-1:2017: Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp- Part 1: Detection method.

The results of the validations document that the alternative method performs equivalent to 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.

The production of the kits are fulfilling the requirements given in ISO 9001.

Date: 9 June 2019

Yours sincerely,

Hilde Skår Norli
Chair of NordVal International

Nina Skall Nielsen
NMKL Secretary General

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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 (Art. No. R 300 23) or the **food**proof® *Listeria monocytogenes* Detection Kit, 5'Nuclease (Art. No. R 302 23).

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 **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 method have been recalculated according to the ISO 16140-2:2016 protocol.

In 2019, additional results from previous validations on different matrices have been included in line with ISO 16140-2.

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METHOD PERFORMANCE CHARACTERISTICS

Selectivity studies

Selectivity studies have been carried out in 2005 and 2011. In total 153 *Listeria monocytogenes* strains have been tested for the specificity. All isolates were positively detected by the alternative method. For the exclusivity 95 strains 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%.

Sensitivity studies:

Meat, fish, milk, eggs, vegetables and environmental samples have been included in the sensitivity studies. The samples have been artificially contaminated by two different serotypes of *Listeria* at 0 = negative control, 1-10 cells per 25 g / area of 100 cm^2 sample and 10-100 cells per 25 g / area of 100 cm^2 sample. The results of the studies carried out in 2005 and 2011 are included in Table 1.

In the study carried out in 2005, a total of 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 acceptability limit for the sensitivity given in ISO 16140-2 is met, and hence the alternative method is regarded as fit for the purpose of the matrices included.

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.

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.

INTERLABORATORY STUDY (2005)

Valid results from six laboratories were obtained. Poultry meat was selected as test matrix. Artificial inoculation was used at 3 levels (negative control, medium 1-10 cells per 25 g and high 10-100 cells per 25 g) each analysed in duplicates. All laboratories detected no positive results in the negative control and all positive at medium and high level. The sensitivity of the alternative method in the interlaboratory study is 100%.

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.

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Table 1: Results of the comparison studies carried out in 2005 and 2011, respectively.

Matrices	PA	NA	PD	ND	FP	Sum	Relative Trueness RT (%)	Sensitivity alternative method SE_{alt} (%)	Sensitivity reference method SE_{ref} (%)	FPR(%)
2005 Study	PA	NA	PD	ND	FP	N	$\frac{(PA+NA)\cdot 100}{N}$	$\frac{(PA+PD)\cdot 100}{PA+PD+ND}$	$\frac{(PA+ND)\cdot 100}{PA+PD+ND}$	$\frac{FP\cdot 100}{NA}$
Meat (minced meat, poultry sausage, heat treated bacon, smoked bacon)	50	27	3	0	3	80	96	100	94	11
Fish (raw coalfish, smoked sprat, shrimps)	42	18	0	0	0	60	100	100	100	0
Milk (firm cheese (Gouda), (blue mould cheese), ice-cream)	34	26	0	0	0	60	100	100	100	0
Eggs (raw eggs, egg salad, salad creme)	35	25	0	0	0	60	100	100	100	0
Vegetable(raw soy bean sprouts, leaf salad, soup vegetable)	34	36	0	0	0	60	100	100	100	0
Environment (metal surface, glass surface, wood surface)	39	21	0	0	0	60	100	100	100	0
TOTAL 2005	234	143	3	0	3	380	99	100	99	2
2011 Study										
Milk (firm cheese (Gouda), (blue mould cheese), ice-cream)	32	25	0	0	0	60	100	100	100	0
Environment (metal surface, glass surface, wood surface)	42	15	0	0	0	60	100	100	100	0
Total 2011	75	40	0	0	0	180	100	100	100	0

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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 and FPR is the false positive rate

Relative AC = The relative accuracy; 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