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.


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 Nortli
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 foodproof® ShortPrep II Kit (Art. No. S 400 02) or the bulk version of this kit, the foodproof® 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 foodproof® Listeria monocytogenes Detection Kit, Hybridization Probes or the foodproof® Listeria monocytogenes Detection Kit, 5'Nuclease.

For food samples inoculate 25 g. For environmental samples inoculate an area of 100 cm². Perform the pre-enrichment according to EN ISO 11290. The detection kit provides all the reagents required for the PCR.

FIELD OF APPLICATION

The foodproof® Listeria monocytogenes Detection Kit, Hybridization Probes and the foodproof® Listeria monocytogenes Detection Kit, 5’ Nuclease in combination with foodproof® 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 foodproof® Listeria monocytogenes Detection Kit, Hybridization Probes in combination with foodproof® 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 foodproof® 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 inoculation levels were used: 0 = negative control, 1-10 cells per 25 g/100cm² sample and 10-100 cells per 25 g/100cm² sample. For each matrix 60 samples were analysed. The following results were obtained:

### Table 1: Results after screening

<table>
<thead>
<tr>
<th>Matrix</th>
<th>PA</th>
<th>NA</th>
<th>N</th>
<th>D</th>
<th>P</th>
<th>D</th>
<th>Sum</th>
<th>Relative AC %</th>
<th>Relative SE %</th>
<th>Relative SP %</th>
<th>Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>33</td>
<td>25</td>
<td>0</td>
<td>2</td>
<td>60</td>
<td></td>
<td></td>
<td>96,6</td>
<td>100</td>
<td>92,6</td>
<td>0,93</td>
</tr>
<tr>
<td>Environmental</td>
<td>42</td>
<td>15</td>
<td>0</td>
<td>3</td>
<td>60</td>
<td></td>
<td></td>
<td>95,0</td>
<td>100</td>
<td>83,3</td>
<td>0,88</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>40</td>
<td>0</td>
<td>5</td>
<td>120</td>
<td></td>
<td></td>
<td>95,8</td>
<td>100</td>
<td>88,8</td>
<td>0,91</td>
</tr>
</tbody>
</table>

* see definitions of the abbreviations below.

### Table 2: Results after confirmation

<table>
<thead>
<tr>
<th>Matrix</th>
<th>PA</th>
<th>NA</th>
<th>N</th>
<th>D</th>
<th>P</th>
<th>D</th>
<th>TP</th>
<th>FP</th>
<th>Sum</th>
<th>Relative AC %</th>
<th>Relative SE %</th>
<th>Relative SP %</th>
<th>Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>32</td>
<td>25</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>60</td>
<td></td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>1,00</td>
<td></td>
</tr>
<tr>
<td>Environmental</td>
<td>42</td>
<td>15</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>60</td>
<td></td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>1,00</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>40</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>120</td>
<td></td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>1,00</td>
<td></td>
</tr>
</tbody>
</table>

* 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 foodproof® Listeria
monocytogenes Detection kit, 5’Nuclease in combination with the foodproof® Short PreplII
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 cm², which was obtained both with the
alternative method and the reference method for all food matrices.

CONCLUSION
The foodproof® Listeria monocytogenes Detection Kit, Hybridization Probes and the
foodproof® Listeria monocytogenes Detection Kit, 5’ Nuclease in combination with
foodproof® ShortPrep II Kit or foodproof® StarPrep Two Kit perform equivalent to the
reference method.