NF VALIDATION
Validation of alternative analytical methods
Application in food microbiology

Summary report
Validation study according to the EN ISO 16140-2:2016

iQ-Check *Listeria* spp.
(certificate number BRD 07/13 - 05/07)
for the detection of *Listeria* spp. in a broad range of food and production environmental samples

Qualitative method

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This report consists of 199 pages, including 10 appendices. Only copies including the totality of this report are authorised. Competencies of the laboratory are certified by COFRAC accreditation for the analyses marked with the symbol◆.

Version 0
November 16, 2023

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Quality Assurance documents related to this study can be consulted upon request from BIO-RAD.

The technical protocol and the result interpretation were carried out according to the EN ISO 16140-2:2016 and the AFNOR technical rules (PR Revision 7).

<table>
<thead>
<tr>
<th>Validation protocols</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFNOR technical rules (PR Revision 7).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reference method*</th>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Alternative method</th>
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<tbody>
<tr>
<td>iQ-Check <em>Listeria</em> spp.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Scope</th>
</tr>
</thead>
<tbody>
<tr>
<td>✖️ Broad range of food</td>
</tr>
<tr>
<td>✖️ Production environmental samples</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Certification organism</th>
</tr>
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</table>

* Analyses performed according to the COFRAC accreditation

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INTRODUCTION

The iQ-Check® *Listeria* spp. method was validated on the 14th May 2007 (certificate number BRD 07/13 - 05/07) for food products and environmental samples. The study was performed by IPL. The following renewals and extensions were performed:

<table>
<thead>
<tr>
<th>Date</th>
<th>Validation</th>
<th>Reference method</th>
<th>Validation standard</th>
<th>Expert laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>▪ Modification of the extraction step with the utilization of a new consumable (Deep Well plate) in addition to the tube format previously validated</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>▪ Use of the CFX Manager Software to handle the CFX96 and MiniOpticon real-time PCR thermal cyclers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>Validation</td>
<td>Reference method</td>
<td>Validation standard</td>
<td>Expert laboratory</td>
</tr>
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<td>--------------</td>
<td>----------------------------------------------------------------------------</td>
<td>------------------</td>
<td>---------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>October 2019</td>
<td>Renewal study for a broad range food claim and production environmental samples</td>
<td>ISO 11290-1 (2017)</td>
<td>ISO 16140-2 (2016)</td>
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</tr>
<tr>
<td></td>
<td>Extension study for the production environmental samples category:</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>▪ For a new enrichment protocol associated with the Easy II lysis protocol.</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>▪ For the optional use of the iQ-Check Free DNA Removal Solution (FDRS) protocol associated with the Easy II lysis protocol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>▪ For the use of a new Application Protocol File (APF). The classical iQ-Check APF (Application Protocol File) corresponds to a 1h50 min PCR run. Some optimization (reduction of the number of cycles + time reduction of some steps) led to release the APF Fast for iQ-Check methods (here <em>Listeria</em> spp) to reduce the PCR run time down to 1h10 min without impact on the performances (sensitivity and specificity).</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April 2021</td>
<td>Extension for the use of the APF fast for all the categories and protocols</td>
<td>/</td>
<td>/</td>
<td>Bio-Rad internal data*</td>
</tr>
<tr>
<td>May 2021</td>
<td>Extension for the use of the iQ-Check Prep v4</td>
<td>/</td>
<td>/</td>
<td>Bio-Rad internal data*</td>
</tr>
<tr>
<td>December 2022</td>
<td>Extension for the use of the CFX Opus Deep Well and the CFX Manager Software IDE 3.1</td>
<td>/</td>
<td>ISO 16140-2 (2016)</td>
<td>Bio-Rad internal data*</td>
</tr>
</tbody>
</table>

* Manufacturer internal data are not included in this report
2 METHOD PROTOCOLS

2.1 Alternative method

The flow diagrams of the alternative method are provided in Appendix 1.

2.1.1 Principle

The iQ-Check *Listeria* spp. kit is tests based on gene amplification and detection by real-time PCR. Ready-to-use PCR reagents contain oligonucleotides (primers and probes) specific for *Listeria* spp., as well as DNA polymerase and nucleotides. Detection and data analysis are optimized for use with a Bio-Rad real-time PCR instrument, such as the CFX96™ Touch standard or Deep Well (DW) or CFX Opus Deep Well (DW) systems.

PCR is a powerful technique used to generate many copies of target DNA. During the PCR reaction, several cycles of heating and cooling allow DNA denaturation, by heat, followed by primers binding to the target region. The DNA polymerase then uses these primers and deoxynucleotide triphosphates (dNTPs) to extend the DNA, creating copies of the target DNA. These copies are called amplicons.

This test allows the detection of *Listeria* spp. in environmental samples and food products previously enriched by culture in *Listeria* Special Broth (LSB or LSB II). It includes the following 4 main steps:

- Enrichment,
- DNA extraction,
- Real-time PCR,
- Data analysis & interpretation.

The FDRS protocol (Free DNA Removal Solution) can be applied to remove free DNA from food and environmental enriched samples prior to PCR analysis. In the context of validation, this protocol only concerns the environmental samples. It is performed by a selected enzyme and its specific buffer under optimized conditions. The iQ-Check lysis buffer associated with thermal lysis inactivates the enzyme, allowing extraction from intact and living cells.

The PCR can be performed using either the classical iQ-Check APF (Application Protocol File) which corresponds to a 1h50 min PCR run or the APF Fast (reduction of the number of cycles + time reduction of some steps) to reduce the PCR run time down to 1h10 min.
2.1.2 Protocols

The different steps are described below:

<table>
<thead>
<tr>
<th>Enrichment step</th>
<th>- Protocols available</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LSB broth for 22 - 24 h ± 1 h at 30°C ± 1°C for all categories. (Protocol 1)</td>
</tr>
<tr>
<td></td>
<td>LSB broth for 24 - 26 h at 30°C ± 1°C for all categories. (Protocol 2)</td>
</tr>
<tr>
<td></td>
<td>LSB broth for 18 - 26 h at 30°C ± 1°C for the production environmental category (Protocols 3 and 4)</td>
</tr>
<tr>
<td></td>
<td>LSB II broth for 18 - 26 h at 37°C ± 1°C for composite foods and environmental samples (Protocol 5)</td>
</tr>
<tr>
<td></td>
<td>LSB II broth for 18 - 26 h at 37°C ± 1°C after pre-warming of the broth for dairy products (Protocol 6)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Optional iQ-Check Free DNA Removal Solution (FDRS)</th>
<th>- Activate the iQ-Check Free DNA Removal Solution (FDRS).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- Pipette 10 µl of activate reagent into the bottom of each well of a 96-Deep Well microplate.</td>
</tr>
<tr>
<td></td>
<td>- Add 100 µl of decanted enriched LSB or LSB II per well. Seal the Deep Well microplate with the X-Pierce sealing film.</td>
</tr>
<tr>
<td></td>
<td>- Incubate in the thermoshaker without shaking for 15 to 30 min at 37°C.</td>
</tr>
<tr>
<td></td>
<td>- Proceed to Easy II DNA extraction protocol using 100 µl of treated enriched sample.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lysis step</th>
<th>- <strong>Standard II protocol (Protocol 1):</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- Centrifugation of 1.5 ml enriched LSB broth at 10000 - 12000 g for 5 min.</td>
</tr>
<tr>
<td></td>
<td>- Discard the supernatant.</td>
</tr>
<tr>
<td></td>
<td>- Resuspend pellet by pipetting the reagent up and down in the tube.</td>
</tr>
<tr>
<td></td>
<td>- Agitation using the &quot;Disruptor Genie&quot; for 3 min ± 1 min.</td>
</tr>
<tr>
<td></td>
<td>- Incubation at 95 - 100°C for 15 - 20 min.</td>
</tr>
<tr>
<td></td>
<td>- Vortex at high speed.</td>
</tr>
<tr>
<td></td>
<td>- Centrifugation at 10000 - 12000 g for 5 min.</td>
</tr>
</tbody>
</table>
- **Easy II protocol (Protocols 2 to 6):**
  - Aliquot 100 µl of lysis reagent (A + F) to tubes of wells of Deep Well plate.
  - Add 100 µl of enriched LSB or LSB II broth.
  - Place tubes in the cell disruptor for 3 min ± 1 min (for tubes only).
  - Incubation at 95 - 100°C for 15 - 20 min in a heat block (tubes)
    or in the plate agitator-incubator (Deep Well plates and tubes)
    under agitation at 1300 rpm.
  - Centrifugation at 10000 - 12000 g for 2 min (tubes only).

**PCR**

- Add 5 µl of lysates supernatant to 45 µl of PCR mix (reagent B +
  reagent C).
- Run PCR.
- In case of inhibition, a 1/10 dilution is applied to the DNA extract.

**Confirmation**

The positive PCR tests are confirmed by:

- Reference method protocol: streaking 10 µl of the enriched LSB broth
  or LSB II onto O&A or Palcam plates incubated for 24 to 48 h at 37°C
  ± 1°C. The typical colonies are confirmed by the tests described in the
  ISO method.

- Alternative method: streaking 100 µl of enriched LSB or LSB II broth
  onto Agar *Listeria* or RAPID’*Listeria* Agar or RAPID’*L.mono* Agar
  incubated for 24 h at 37°C ± 1°C. The only presence of typical
  colonies allows to confirm the positive PCR result.

- By any other method certified by NF Validation based on a principle
  different from that used in the iQ-Check method starting from the same
  enrichment step.

It is possible to store the enrichment broths for 72 h at 5°C ± 3°C before proceeding
 to PCR and confirmatory tests.

The protocols are summarized in **Table 1**.
Table 1 – Summary of the protocols

<table>
<thead>
<tr>
<th>Validation study</th>
<th>Protocol</th>
<th>Scope</th>
<th>Enrichment step</th>
<th>FDRS</th>
<th>Lysis step</th>
<th>APF¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial 2007</td>
<td>Protocol 1</td>
<td>All categories</td>
<td>LSB for 22 – 24 h at 30°C ± 1°C</td>
<td>No</td>
<td>Standard II</td>
<td>Classic Fast</td>
</tr>
<tr>
<td></td>
<td>Protocol 2</td>
<td></td>
<td>LSB for 24 – 26 h at 30°C ± 1°C</td>
<td>No</td>
<td>Easy II</td>
<td>Classic Fast</td>
</tr>
<tr>
<td>Extension 2019</td>
<td>Protocol 3</td>
<td>Environmental samples</td>
<td>LSB for 18 – 26 h at 30°C ± 1°C</td>
<td>No</td>
<td>Easy II</td>
<td>Classic Fast</td>
</tr>
<tr>
<td></td>
<td>Protocol 4</td>
<td></td>
<td>LSB for 18 – 26 h at 30°C ± 1°C</td>
<td>Yes</td>
<td>Easy II</td>
<td>Classic Fast</td>
</tr>
<tr>
<td>Extension 2023</td>
<td>Protocol 5</td>
<td>Composite foods Environmental samples</td>
<td>LSB II for 18 – 26 h at 37°C ± 1°C</td>
<td>Optional</td>
<td>Easy II</td>
<td>Fast</td>
</tr>
<tr>
<td></td>
<td>Protocol 6</td>
<td>Dairy products</td>
<td>Prewarmed LSB II for 18 – 26 h at 37°C ± 1°C</td>
<td>Optional</td>
<td>Easy II</td>
<td>Fast</td>
</tr>
</tbody>
</table>

2.1.3 Restriction

There is no restriction for use.

2.2 Reference method


The flow diagram is given in Appendix 2.

2.3 Study design

The enrichment broths used for the reference method and the alternative method are different; it was thus an unpaired study design for all the protocols tested.

¹ Application Protocol File
## 3 METHOD COMPARISON STUDY

The method comparison study is a study performed by the expert laboratory to compare the alternative method with the reference method.

The study was carried out on a diversity of samples and strains representative of agri-food products. This does not constitute an exhaustive list of the different matrices included in the scope.

For any comment on the alternative method, please contact AFNOR Certification at http://nf-validation.afnor.org/contact-2/.

### 3.1 Initial validation study and extension/renewal studies: results - LSB protocol

The following combinations of protocols were used for interpretation of the data obtained for this study (See Table 2).

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>Protocol used for the categories tested in previous validation studies</th>
<th>Extension Environmental samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Protocol 1 (Categories 1 to 6)</td>
<td>/</td>
</tr>
<tr>
<td>B1</td>
<td>Protocol 1 (Categories 1 to 5)</td>
<td>Protocol 3 – APF Classic</td>
</tr>
<tr>
<td>C1</td>
<td>Protocol 1 (Categories 1 to 5)</td>
<td>Protocol 4 – APF Classic</td>
</tr>
<tr>
<td>D1</td>
<td>Protocol 1 (Categories 1 to 5)</td>
<td>Protocol 3 – APF Fast</td>
</tr>
<tr>
<td>E1</td>
<td>Protocol 1 (Categories 1 to 5)</td>
<td>Protocol 4 – APF Fast</td>
</tr>
<tr>
<td>A2</td>
<td>Protocol 2 (Categories 1 to 6)</td>
<td>/</td>
</tr>
<tr>
<td>B2</td>
<td>Protocol 2 (Categories 1 to 5)</td>
<td>Protocol 3 – APF Classic</td>
</tr>
<tr>
<td>C2</td>
<td>Protocol 2 (Categories 1 to 5)</td>
<td>Protocol 4 – APF Classic</td>
</tr>
<tr>
<td>D2</td>
<td>Protocol 2 (Categories 1 to 5)</td>
<td>Protocol 3 – APF Fast</td>
</tr>
<tr>
<td>E2</td>
<td>Protocol 2 (Categories 1 to 5)</td>
<td>Protocol 4 – APF Fast</td>
</tr>
</tbody>
</table>
3.1.1 Sensitivity study

The sensitivity (SE) is the ability of the method to detect the analyte by either the reference or alternative method.

3.1.1.1 Number and nature of the samples

363 samples were analyzed for the initial validation study in 2007 covering 4 food categories and production environmental samples.

For the extension study run in 2008 (modification of Taq provider and modification of probes chemistry), the following tests were carried on:

- 218 lysates from the initial validation study were tested again by Bio-Rad using both versions of the kit (Standard II lysis protocol).
- 133 additional samples were tested with both versions of the kit by the expert laboratory (IPL).

Note that only the results obtained with the modified mix are provided in the raw data tables for the tests run in 2008 as same results were observed using both kits.

47 samples have been excluded from the previous validation studies as they were highly contaminated or because the contamination was not determined.

For the renewal study, 101 samples were tested with the Standard II protocol (Protocol 1) providing 60 positive and 41 negative results; 127 samples were tested with the Easy II protocol (Protocol 2) providing 64 positive and 63 negative results.

For the extension study which concerns the environmental samples category, 68 samples were tested with and without applying FDRS protocol with both APF (Classic and Fast), providing 38 positive and 30 negative results.

Taking into account all the studies, 550 samples were tested using protocol 1 and 469 using protocol 2. The distribution per tested category and type is given in Tables 3 and 4.
## Table 3 - Number of samples tested per study - Standard II lysis (protocol 1)

<table>
<thead>
<tr>
<th>Category</th>
<th>Protocol</th>
<th>APF</th>
<th>Type</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>a RTE</td>
<td>14</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b RTRH</td>
<td>12</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c Pastries, egg products</td>
<td>13</td>
<td>14</td>
<td>27</td>
</tr>
<tr>
<td>Composite foods</td>
<td>P1</td>
<td>Classic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>39</td>
<td>31</td>
<td>70</td>
</tr>
<tr>
<td>Meat products</td>
<td>P1</td>
<td>Classic</td>
<td>a Raw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b RTE, RTRH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c Delicatessen</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>74</td>
<td>39</td>
<td>113</td>
</tr>
<tr>
<td>Dairy products</td>
<td>P1</td>
<td>Classic</td>
<td>a Raw milk cheeses</td>
<td>19</td>
<td>15</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b Raw milk</td>
<td>15</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c Heat treated dairy products</td>
<td>22</td>
<td>26</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>56</td>
<td>45</td>
<td>101</td>
</tr>
<tr>
<td>Fishery products</td>
<td>P1</td>
<td>Classic</td>
<td>a Raw fish</td>
<td>16</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b Smoked and cured fish</td>
<td>17</td>
<td>17</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c RTE, RTRH</td>
<td>14</td>
<td>13</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>47</td>
<td>46</td>
<td>93</td>
</tr>
<tr>
<td>Vegetables</td>
<td>P1</td>
<td>Classic</td>
<td>a Fresh and frozen vegetables</td>
<td>16</td>
<td>12</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b Pre-cooked, under atmosphere</td>
<td>18</td>
<td>14</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c RTE, RTRH</td>
<td>6</td>
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<td>22</td>
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<td></td>
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<td>Total</td>
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<td>42</td>
<td>82</td>
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<tr>
<td>Environmental Samples</td>
<td>P1</td>
<td>Classic</td>
<td>a Process water</td>
<td>13</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b Sponges, swabs</td>
<td>20</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c Dusts, residues</td>
<td>21</td>
<td>7</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
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<td>37</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>Classic</td>
<td>a Process water</td>
<td>12</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b Sponges, swabs</td>
<td>11</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c Dusts, residues</td>
<td>15</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
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<td>P4</td>
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<td>12</td>
<td>11</td>
<td>23</td>
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<td></td>
<td></td>
<td>b Sponges, swabs</td>
<td>11</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c Dusts, residues</td>
<td>15</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>38</td>
<td>30</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>Fast</td>
<td>a Process water</td>
<td>12</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b Sponges, swabs</td>
<td>11</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c Dusts, residues</td>
<td>15</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>38</td>
<td>30</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>P4</td>
<td>Fast</td>
<td>a Process water</td>
<td>12</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b Sponges, swabs</td>
<td>11</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c Dusts, residues</td>
<td>15</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>38</td>
<td>30</td>
<td>68</td>
</tr>
</tbody>
</table>

A1: Total P1  
310 240 550
B1: Total P1 + P3 Classic  
294 233 527
C1: Total P1 + P4 Classic  
294 233 527
D1: Total P1 + P3 Fast  
294 233 527
E1: Total P1 + P4 Fast  
294 233 527
Table 4 - Number of samples tested per study — Easy II lysis (protocol 2)

<table>
<thead>
<tr>
<th>Category</th>
<th>Protocol</th>
<th>APF</th>
<th>Type</th>
<th>Positifs</th>
<th>Négatifs</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Composite foods</td>
<td>P2</td>
<td>Classic</td>
<td>a RTE</td>
<td>14</td>
<td>9</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b RTRH</td>
<td>12</td>
<td>10</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c Pastries, egg products</td>
<td>12</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>38</td>
<td>30</td>
<td>68</td>
</tr>
<tr>
<td>2 Meat products</td>
<td>P2</td>
<td>Classic</td>
<td>a Raw</td>
<td>25</td>
<td>11</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b RTE, RTRH</td>
<td>11</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c Delicatessen</td>
<td>29</td>
<td>12</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>65</td>
<td>30</td>
<td>95</td>
</tr>
<tr>
<td>3 Dairy products</td>
<td>P2</td>
<td>Classic</td>
<td>a Raw milk cheeses</td>
<td>14</td>
<td>16</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b Raw milk</td>
<td>14</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c Heat treated dairy products</td>
<td>18</td>
<td>21</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>46</td>
<td>41</td>
<td>87</td>
</tr>
<tr>
<td>4 Fishery products</td>
<td>P2</td>
<td>Classic</td>
<td>a Raw fish</td>
<td>11</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b Smoked and cured fish</td>
<td>13</td>
<td>11</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c RTE, RTRH</td>
<td>11</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>35</td>
<td>31</td>
<td>66</td>
</tr>
<tr>
<td>5 Vegetables</td>
<td>P2</td>
<td>Classic</td>
<td>a Fresh and frozen vegetables</td>
<td>13</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b Precooked, under atmosphere</td>
<td>18</td>
<td>13</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c RTE, RTRH</td>
<td>6</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>37</td>
<td>36</td>
<td>73</td>
</tr>
<tr>
<td>6 Environmental Samples</td>
<td>P2</td>
<td>Classic</td>
<td>a Process water</td>
<td>8</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b Sponges, swabs</td>
<td>20</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c Dusts, residues</td>
<td>11</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>39</td>
<td>41</td>
<td>80</td>
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<tr>
<td></td>
<td>P3</td>
<td>Classic</td>
<td>a Process water</td>
<td>12</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b Sponges, swabs</td>
<td>11</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c Dusts, residues</td>
<td>15</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>38</td>
<td>30</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>P4</td>
<td>Classic</td>
<td>a Process water</td>
<td>12</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b Sponges, swabs</td>
<td>11</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c Dusts, residues</td>
<td>15</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>38</td>
<td>30</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>Fast</td>
<td>a Process water</td>
<td>12</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b Sponges, swabs</td>
<td>11</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c Dusts, residues</td>
<td>15</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>38</td>
<td>30</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>P4</td>
<td>Fast</td>
<td>a Process water</td>
<td>12</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b Sponges, swabs</td>
<td>11</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c Dusts, residues</td>
<td>15</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>38</td>
<td>30</td>
<td>68</td>
</tr>
</tbody>
</table>

A2: Total P2 260 209 469
B2: Total P2 + P3 Classic 259 198 457
C2: Total P2 + P4 Classic 259 198 457
D2: Total P2 + P3 Fast 259 198 457
E2: Total P2 + P4 Fast 259 198 457
3.1.1.2 Distribution of the contamination for Listeria spp. detection

The number of samples contaminated per *Listeria* spp., *Listeria* spp. and *Listeria monocytogenes*, and *Listeria monocytogenes* for each extraction protocol is given in Table 5 to Table 7.

**Table 5 - Number of samples contaminated per *Listeria* spp., *Listeria* spp. and *Listeria monocytogenes* – Standard II lysis (Protocol 1)**

<table>
<thead>
<tr>
<th>Category</th>
<th><em>Listeria</em> spp. other than <em>Listeria monocytogenes</em> (A)</th>
<th><em>Listeria monocytogenes</em> (B)</th>
<th><em>Listeria monocytogenes</em> + <em>Listeria</em> spp. other than L. monocytogenes (C)</th>
<th>A+C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>22</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>14</td>
<td>34</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>37</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>31</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>22</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>29</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>All categories</td>
<td><strong>77</strong></td>
<td><strong>155</strong></td>
<td><strong>78</strong></td>
<td><strong>155</strong></td>
</tr>
</tbody>
</table>

**Table 6 - Number of samples contaminated per *Listeria* spp., *Listeria* spp. and *Listeria monocytogenes* – Easy II lysis (Protocol 2)**

<table>
<thead>
<tr>
<th>Category</th>
<th><em>Listeria</em> spp. other than <em>Listeria monocytogenes</em> (A)</th>
<th><em>Listeria monocytogenes</em> (B)</th>
<th><em>Listeria monocytogenes</em> + <em>Listeria</em> spp. other than L. monocytogenes (C)</th>
<th>A+C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>20</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>12</td>
<td>31</td>
<td>53</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>27</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>20</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>18</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>14</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>All categories</td>
<td><strong>74</strong></td>
<td><strong>111</strong></td>
<td><strong>75</strong></td>
<td><strong>149</strong></td>
</tr>
</tbody>
</table>

**Table 7 - Number of samples contaminated per *Listeria* spp., *Listeria* spp. and *Listeria monocytogenes* – Easy II lysis (Protocol 3 or 4)**

<table>
<thead>
<tr>
<th>Category</th>
<th><em>Listeria</em> spp. other than <em>Listeria monocytogenes</em> (A)</th>
<th><em>Listeria monocytogenes</em> (B)</th>
<th><em>Listeria monocytogenes</em> + <em>Listeria</em> spp. other than L. monocytogenes (C)</th>
<th>A+C</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>7</td>
<td>15</td>
<td>15</td>
<td>22</td>
</tr>
</tbody>
</table>
According to the AFNOR technical rules (Revision 6), 15 to 25 samples per category need to be contaminated with *Listeria* spp. alone or associated with *Listeria monocytogenes*. This is the case for each category and each tested protocol.

### 3.1.1.3 Artificial contamination of the samples

Artificial contaminations were done by spiking (See Appendix 3). Strains were injured using different protocols and the injury level was evaluated by comparing enumeration done onto selective media (Palcam) and non-selective media (TSYEAA).

148 samples were artificially contaminated; 138 gave positive result by at least one of the methods.

The repartition of positive samples per inoculation protocol and inoculation level is given in Table 8.

<table>
<thead>
<tr>
<th>Naturally contaminated</th>
<th>Artificially contaminated</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spiking protocol</td>
<td>Seeding protocol</td>
</tr>
<tr>
<td></td>
<td>≤ 5 CFU</td>
<td>3 ≤ x ≤ 10 CFU</td>
</tr>
<tr>
<td>Protocol 1</td>
<td>Samples number</td>
<td>207</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>66,8%</td>
</tr>
<tr>
<td>Protocol 2</td>
<td>Samples number</td>
<td>185</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>71,2%</td>
</tr>
<tr>
<td>Protocol 1 (cat 6)</td>
<td>Samples number</td>
<td>41</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>75,9%</td>
</tr>
<tr>
<td>Protocol 2 (cat 6)</td>
<td>Samples number</td>
<td>37</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>94,9%</td>
</tr>
<tr>
<td>Protocol 3 or 4</td>
<td>Samples number</td>
<td>3</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>7,91%</td>
</tr>
<tr>
<td>Protocol 1 + 3 or 4</td>
<td>Samples number</td>
<td>169</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>57,5%</td>
</tr>
<tr>
<td>Protocol 2 + 3 or 4</td>
<td>Samples number</td>
<td>151</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>58,8%</td>
</tr>
</tbody>
</table>

66.8 % and 71.2 % of the samples were naturally contaminated respectively for the Standard II lysis protocol and the Easy II lysis protocol.

57.5 % and 58.3 % of the samples were naturally contaminated when protocol 3 or 4 (environmental samples category) were respectively combined with protocols 1 and 2 (food categories).
3.1.1.4 Protocol applied during the validation study

> **Enrichment, lysis and PCR protocols**

The minimum incubation time was evaluated for all the study. A summary of the protocols evaluated during the initial validation study and the extensions in 2019 is shown in Table 9.

**Table 9 – Protocols applied**

<table>
<thead>
<tr>
<th>Validation study</th>
<th>Protocol</th>
<th>Scope</th>
<th>Enrichment step</th>
<th>FDRS</th>
<th>Lysis step</th>
<th>APF²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial 2007</td>
<td>Protocol 1</td>
<td>All categories</td>
<td>LSB for 22 h at 30°C ± 1°C</td>
<td>No</td>
<td>Standard II</td>
<td>Classic</td>
</tr>
<tr>
<td></td>
<td>Protocol 2</td>
<td></td>
<td>LSB for 24 h at 30°C ± 1°C</td>
<td>No</td>
<td>Easy II</td>
<td>Classic</td>
</tr>
<tr>
<td>Extension 2019</td>
<td>Protocol 3</td>
<td>Environmental samples</td>
<td>LSB for 18 h at 30°C ± 1°C</td>
<td>No</td>
<td>Easy II</td>
<td>Fast</td>
</tr>
<tr>
<td></td>
<td>Protocol 4</td>
<td></td>
<td>LSB for 18 h at 30°C ± 1°C</td>
<td>Yes*</td>
<td>Easy II</td>
<td>Classic Fast</td>
</tr>
</tbody>
</table>

An incubation time of 15 min in the thermoshaker was applied for the validation study.

> **Confirmation protocols**

During the validation study, the following protocols were tested:

- Streaking 10 µl of enriched LSB broth onto O&A and Palcam plates;
- Streaking 0.1 ml enriched LSB broth onto RAPID’*Listeria* Agar;
- Streaking 0.1 ml enriched LSB broth onto RAPID’*L.mono* Agar;

The presence of typical colonies allows to confirm the positive PCR Tests but during the validation study, the typical colonies were confirmed using the tests described in the reference method (Gram, catalase) and by biochemical galleries (API *Listeria*). The confirmation was run after 22 h incubation time (shortest incubation time applied for the standard II extraction protocol).

² Application Protocol File
For negative PCR samples, the enriched LSB was subcultured in Fraser for 24h ± 2h at 37°C in order to have a total duration of incubation equivalent to the ISO method before streaking onto O&A and Palcam plates. This protocol only concerns the samples tested for this study.

**Enrichment broth storage**

The enriched LSB broths from positive and discordant samples were tested again after storage for 72 h at 5°C ± 3°C. DNA extractions, PCR tests and confirmatory tests were run again.

### 3.1.1.5 Test results

Raw data per category are given in Appendix 4. The results are given in Tables 10 and 11.

#### Table 10 – Interpretation of sample results between the reference and alternative method (based on the confirmed alternative) – Standard II lysis

<table>
<thead>
<tr>
<th>Category</th>
<th>Protocol</th>
<th>APF</th>
<th>PA</th>
<th>NA*</th>
<th>PD</th>
<th>ND**</th>
<th>PPND</th>
<th>PPNA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Composite foods</td>
<td>Protocol 1</td>
<td>Classic</td>
<td>24</td>
<td>25</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>6</td>
<td>70</td>
</tr>
<tr>
<td>2 Meat products</td>
<td>Protocol 1</td>
<td>Classic</td>
<td>59</td>
<td>36</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>113</td>
</tr>
<tr>
<td>3 Dairy products</td>
<td>Protocol 1</td>
<td>Classic</td>
<td>43</td>
<td>44</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>101</td>
</tr>
<tr>
<td>4 Fishery products</td>
<td>Protocol 1</td>
<td>Classic</td>
<td>39</td>
<td>45</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>93</td>
</tr>
<tr>
<td>5 Vegetable products</td>
<td>Protocol 1</td>
<td>Classic</td>
<td>30</td>
<td>39</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>82</td>
</tr>
<tr>
<td>6 Environmental samples</td>
<td>Protocol 1</td>
<td>Classic</td>
<td>47</td>
<td>36</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>Protocol 3</td>
<td>Classic</td>
<td>27</td>
<td>28</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>68</td>
</tr>
<tr>
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<td>45</td>
<td>23</td>
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</tr>
<tr>
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<td>219</td>
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<td>D1: Total P1 + P3 Fast</td>
<td></td>
<td></td>
<td>222</td>
<td>215</td>
<td>45</td>
<td>24</td>
<td>3</td>
<td>18</td>
<td>527</td>
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<tr>
<td>E1: Total P1 + P4 Fast</td>
<td></td>
<td></td>
<td>222</td>
<td>219</td>
<td>45</td>
<td>24</td>
<td>3</td>
<td>14</td>
<td>527</td>
</tr>
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</table>

* PPNA not included  ** PPND not included
Table 11 – Interpretation of sample results between the reference and alternative method (based on the confirmed alternative) – Easy II lysis

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<thead>
<tr>
<th>Category</th>
<th>Protocol</th>
<th>APF</th>
<th>PA</th>
<th>NA*</th>
<th>PD</th>
<th>ND**</th>
<th>PPND</th>
<th>PPNA</th>
<th>Total</th>
</tr>
</thead>
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<td>23</td>
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<td>10</td>
<td>5</td>
<td>0</td>
<td>7</td>
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</tr>
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<td>Protocol 2 Classic</td>
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<td>9</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>95</td>
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</tr>
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<td>Protocol 2 Classic</td>
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</tr>
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<td>Protocol 2 Classic</td>
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</tr>
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<td>0</td>
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</tr>
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<td>6 Environmental samples</td>
<td>Protocol 2 Classic</td>
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<td>4</td>
<td>68</td>
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</tr>
<tr>
<td></td>
<td>Protocol 4 Fast</td>
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<td>30</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>40</td>
<td>22</td>
<td>1</td>
<td>20</td>
<td>457</td>
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<td>C2: Total P2 + P4 Classic</td>
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<td>180</td>
<td>40</td>
<td>23</td>
<td>0</td>
<td>18</td>
<td>457</td>
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<td>D2: Total P2 + P3 Fast</td>
<td></td>
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<td>176</td>
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<td>23</td>
<td>0</td>
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<tr>
<td>E2: Total P2 + P4 Fast</td>
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<td>40</td>
<td>23</td>
<td>0</td>
<td>18</td>
<td>457</td>
<td></td>
</tr>
</tbody>
</table>

* PPNA not included    ** PPND not included

3.1.1.6 Calculation of relative trueness (RT), sensitivity (SE) and false positive ratio (FPR)

The calculations are presented in Table 12 and Table 13. A summary of the results is given in Tables 14 and 15.
### Table 12 – Calculation of the relative trueness (RT), the sensitivity (SE) and the false positive ratio (FPR) - Standard II lysis

<table>
<thead>
<tr>
<th>Category</th>
<th>Protocol</th>
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<th>Type</th>
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<th>NA*</th>
<th>PD</th>
<th>ND**</th>
<th>PPND</th>
<th>PPNA</th>
<th>SEalt</th>
<th>SEref</th>
<th>RT</th>
<th>FPR</th>
</tr>
</thead>
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<td>P1</td>
<td>Classic</td>
<td>a RTE</td>
<td>11</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>100,0%</td>
<td>78,6%</td>
<td>86,4%</td>
<td>25,0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b RTRH</td>
<td>7</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>83,3%</td>
<td>75,0%</td>
<td>76,2%</td>
<td>33,3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c Pastries, egg products</td>
<td>6</td>
<td>12</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>76,9%</td>
<td>69,2%</td>
<td>74,1%</td>
<td>14,3%</td>
</tr>
<tr>
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<td></td>
<td></td>
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<td>25</td>
<td>10</td>
<td>4</td>
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<td>6</td>
<td>87,2%</td>
<td>74,4%</td>
<td>78,6%</td>
<td>22,6%</td>
</tr>
<tr>
<td><strong>Meat products</strong></td>
<td>P1</td>
<td>Classic</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>96,4%</td>
<td>82,1%</td>
<td>86,4%</td>
<td>0,0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b RTE, RTRH</td>
<td>15</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>93,8%</td>
<td>100,0%</td>
<td>96,0%</td>
<td>11,1%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c Delicatessen</td>
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<td>12</td>
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<td>86,7%</td>
<td>10,3%</td>
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<td>15</td>
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<td>1</td>
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<td>76,5%</td>
<td>6,7%</td>
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<td></td>
<td></td>
<td></td>
<td>b Raw milk</td>
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<td>1</td>
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<td>93,3%</td>
<td>89,5%</td>
<td>0,0%</td>
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<tr>
<td></td>
<td></td>
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<td>c Heat treated dairy products</td>
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<td>0</td>
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<td>93,8%</td>
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<td>44</td>
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<td>4</td>
<td>1</td>
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<td>91,1%</td>
<td>85,7%</td>
<td>87,1%</td>
<td>4,4%</td>
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<td>P1</td>
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<td>a Raw fish</td>
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<td>16</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>93,8%</td>
<td>87,5%</td>
<td>90,6%</td>
<td>0,0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b Smoked and cured fish</td>
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<td>17</td>
<td>3</td>
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<td>0</td>
<td>0</td>
<td>100,0%</td>
<td>82,4%</td>
<td>91,2%</td>
<td>0,0%</td>
</tr>
<tr>
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<td>1</td>
<td>1</td>
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<td>92,9%</td>
<td>92,6%</td>
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<tr>
<td><strong>Total</strong></td>
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<td>39</td>
<td>45</td>
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<td>0</td>
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<td>95,7%</td>
<td>87,2%</td>
<td>91,4%</td>
<td>2,2%</td>
</tr>
<tr>
<td><strong>Vegetables</strong></td>
<td>P1</td>
<td>Classic</td>
<td>a Fresh and frozen vegetables</td>
<td>12</td>
<td>11</td>
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<td>2</td>
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<td>87,5%</td>
<td>85,7%</td>
<td>8,3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b Precooked, under atmosphere</td>
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<td>1</td>
<td>0</td>
<td>1</td>
<td>94,4%</td>
<td>88,9%</td>
<td>90,6%</td>
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</tr>
<tr>
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<td>c RTE, RTRH</td>
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<td>15</td>
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<td>1</td>
<td>0</td>
<td>1</td>
<td>83,3%</td>
<td>66,7%</td>
<td>86,4%</td>
<td>6,3%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
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<td>30</td>
<td>39</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>90,0%</td>
<td>85,0%</td>
<td>87,8%</td>
<td>7,1%</td>
</tr>
</tbody>
</table>
### Summary report

**IQ Check** *Listeria* spp.

| Category | Protocol | APF | Type                  | PA | NA* | PD | ND** | PPND | PPNA | Selt | Sref | RT | FPR |
|----------|----------|-----|-----------------------|----|-----|----|------|------|------|------|-----|-----|----|-----|
|          |          |     | a Process water       | 12 | 9   | 0  | 1    | 0    | 0    | 92,3%| 100,0%| 95,7%| 10,0%|
|          |          |     | b Sponges, swabs      | 17 | 20  | 1  | 2    | 0    | 0    | 90,0%| 95,0% | 92,5%| 0,0% |
|          |          |     | c Dusts, residues     | 18 | 7   | 3  | 0    | 0    | 0    | 100,0%| 85,7% | 89,3%| 0,0% |
|          |          |     | Total                 | 47 | 36  | 4  | 3    | 0    | 1    | 94,4%| 92,6% | 92,3%| 2,7% |
|          | P3       | Classic | a Process water      | 9  | 10  | 2  | 1    | 0    | 1    | 91,7%| 83,3% | 87,0%| 9,1% |
|          | P3       | Classic | b Sponges, swabs     | 10 | 9   | 1  | 0    | 0    | 0    | 100,0%| 90,9% | 95,2%| 10,0%|
|          | P3       | Classic | c Dusts, residues    | 8  | 9   | 2  | 4    | 1    | 0    | 66,7%| 86,7% | 70,8%| 11,1%|
|          |          | Total   | a Process water      | 27 | 28  | 5  | 5    | 1    | 2    | 84,2%| 86,8% | 83,8%| 10,0%|
|          | P4       | Classic | a Process water      | 9  | 11  | 2  | 1    | 0    | 1    | 91,7%| 83,3% | 87,0%| 9,1% |
|          | P4       | Classic | b Sponges, swabs     | 10 | 10  | 1  | 0    | 0    | 0    | 100,0%| 90,9% | 95,2%| 10,0%|
|          | P4       | Classic | c Dusts, residues    | 8  | 9   | 2  | 5    | 0    | 2    | 66,7%| 86,7% | 70,8%| 22,2%|
|          |          | Total   | a Process water      | 27 | 30  | 5  | 6    | 0    | 0    | 84,2%| 86,8% | 83,8%| 0,0% |
|          | P3       | Fast    | a Process water      | 9  | 10  | 2  | 1    | 0    | 1    | 91,7%| 83,3% | 87,0%| 9,1% |
|          | P3       | Fast    | b Sponges, swabs     | 10 | 9   | 1  | 0    | 0    | 0    | 100,0%| 90,9% | 95,2%| 0,0% |
|          | P3       | Fast    | c Dusts, residues    | 8  | 9   | 2  | 5    | 0    | 4    | 66,7%| 86,7% | 70,8%| 13,3%|
|          |          | Total   | a Process water      | 27 | 30  | 5  | 6    | 0    | 0    | 84,2%| 86,8% | 83,8%| 0,0% |

**Environmental Samples**

| Category | Protocol | APF | Type                  | PA | NA* | PD | ND** | PPND | PPNA | Selt | Sref | RT | FPR |
|----------|----------|-----|-----------------------|----|-----|----|------|------|------|------|-----|-----|----|-----|
|          |          |     | a Process water       | 12 | 9   | 0  | 1    | 0    | 0    | 92,3%| 100,0%| 95,7%| 10,0%|
|          |          |     | b Sponges, swabs      | 17 | 20  | 1  | 2    | 0    | 0    | 90,0%| 95,0% | 92,5%| 0,0% |
|          |          |     | c Dusts, residues     | 18 | 7   | 3  | 0    | 0    | 0    | 100,0%| 85,7% | 89,3%| 0,0% |
|          |          |     | Total                 | 47 | 36  | 4  | 3    | 0    | 1    | 94,4%| 92,6% | 92,3%| 2,7% |
|          |          |     | a Process water       | 9  | 10  | 2  | 1    | 0    | 1    | 91,7%| 83,3% | 87,0%| 9,1% |
|          |          |     | b Sponges, swabs      | 10 | 9   | 1  | 0    | 0    | 0    | 100,0%| 90,9% | 95,2%| 10,0%|
|          |          |     | c Dusts, residues     | 8  | 9   | 2  | 4    | 1    | 0    | 66,7%| 86,7% | 70,8%| 11,1%|
|          |          |     | Total                 | 27 | 28  | 5  | 5    | 1    | 2    | 84,2%| 86,8% | 83,8%| 10,0%|

**A1: Total P1**

|          |          |     | Total P1              | 242| 225| 44 | 21 | 15 | 92,3%| 85,8% | 87,6%| 7,5% |

**B1: Total P1 + P3 Classic**

|          |          |     | Total P1 + P3 Classic | 222| 217| 45 | 23 | 16 | 90,8%| 84,7% | 86,3%| 8,6% |

**C1: Total P1 + P4 Classic**

|          |          |     | Total P1 + P4 Classic | 222| 219| 45 | 24 | 14 | 90,8%| 84,7% | 86,3%| 7,3% |

**D1: Total P1 + P3 Fast**

|          |          |     | Total P1 + P3 Fast    | 222| 215| 45 | 24 | 18 | 90,8%| 84,7% | 86,3%| 9,0% |

**E1: Total P1 + P4 Fast**

|          |          |     | Total P1 + P4 Fast    | 222| 219| 45 | 24 | 14 | 90,8%| 84,7% | 86,3%| 7,3% |

* PPNA not included  ** PPND not included
Table 13 – Calculation of the relative trueness (RT), the sensitivity (SE) and the false positive ratio (FPR) - Easy II lysis

<table>
<thead>
<tr>
<th>Category</th>
<th>Protocol</th>
<th>Type</th>
<th>PA</th>
<th>NA*</th>
<th>PD</th>
<th>ND**</th>
<th>PPND</th>
<th>PPNA</th>
<th>Se&lt;sub&gt;ref&lt;/sub&gt;</th>
<th>RT</th>
<th>FPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Composite foods</td>
<td>P2</td>
<td>Classic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a RTE</td>
<td>11</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>100.0%</td>
<td>78.6%</td>
<td>87.0%</td>
<td>33.3%</td>
<td></td>
</tr>
<tr>
<td>b RTRH</td>
<td>7</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>83.3%</td>
<td>75.0%</td>
<td>77.3%</td>
<td>10.0%</td>
<td></td>
</tr>
<tr>
<td>c Pastries, egg products</td>
<td>5</td>
<td>8</td>
<td>4</td>
<td>3</td>
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<td>3</td>
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<tr>
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<td>23</td>
<td>23</td>
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<td>7</td>
<td>86.8%</td>
<td>73.7%</td>
<td>77.9%</td>
<td>23.3%</td>
<td></td>
</tr>
<tr>
<td>2 Meat products</td>
<td>P2</td>
<td>Classic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a Raw</td>
<td>20</td>
<td>11</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>96.0%</td>
<td>84.0%</td>
<td>86.1%</td>
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<td></td>
</tr>
<tr>
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<td>10</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>90.9%</td>
<td>100.0%</td>
<td>94.4%</td>
<td>14.3%</td>
<td></td>
</tr>
<tr>
<td>c Delicatessen</td>
<td>21</td>
<td>11</td>
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<td>1</td>
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<td>80.5%</td>
<td>8.3%</td>
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<td>9</td>
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<td>86.2%</td>
<td>85.3%</td>
<td>6.7%</td>
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</tr>
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<td>3 Dairy products</td>
<td>P2</td>
<td>Classic</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>a Raw milk cheeses</td>
<td>9</td>
<td>13</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>85.7%</td>
<td>78.6%</td>
<td>83.3%</td>
<td>18.8%</td>
<td></td>
</tr>
<tr>
<td>b Raw milk</td>
<td>12</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>92.9%</td>
<td>92.9%</td>
<td>88.9%</td>
<td>50.0%</td>
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</tr>
<tr>
<td>c Heat treated dairy products</td>
<td>15</td>
<td>20</td>
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<td>0</td>
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<td>88.5%</td>
<td>14.6%</td>
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<td>4 Fishery products</td>
<td>P2</td>
<td>Classic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a Raw fish</td>
<td>9</td>
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<td>0</td>
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<td>0</td>
<td>100.0%</td>
<td>81.8%</td>
<td>90.5%</td>
<td>0.0%</td>
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</tr>
<tr>
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<td>10</td>
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<td>3</td>
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<td>100.0%</td>
<td>76.9%</td>
<td>87.5%</td>
<td>0.0%</td>
<td></td>
</tr>
<tr>
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</tr>
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<td>P2</td>
<td>Classic</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>a Fresh and frozen vegetables</td>
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<td>9</td>
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<td>1</td>
<td>0</td>
<td>1</td>
<td>92.3%</td>
<td>100.0%</td>
<td>95.7%</td>
<td>10.0%</td>
<td></td>
</tr>
<tr>
<td>b Precooked, under atmosphere</td>
<td>15</td>
<td>13</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>94.4%</td>
<td>88.9%</td>
<td>90.3%</td>
<td>0.0%</td>
<td></td>
</tr>
<tr>
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<td>3</td>
<td>11</td>
<td>2</td>
<td>1</td>
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<td>2</td>
<td>83.3%</td>
<td>66.7%</td>
<td>84.2%</td>
<td>15.4%</td>
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</tr>
<tr>
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<td>33</td>
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<td>3</td>
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<td>3</td>
<td>91.9%</td>
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<td>90.4%</td>
<td>8.3%</td>
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</tr>
<tr>
<td>Category</td>
<td>Protocol</td>
<td>Type</td>
<td>PA</td>
<td>NA*</td>
<td>PD</td>
<td>ND**</td>
<td>PPND</td>
<td>PPNA</td>
<td>Sealt</td>
<td>Seef</td>
<td>RT</td>
</tr>
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<td>------</td>
<td>------</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>a Process water</td>
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<td>11</td>
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<td>87,5%</td>
<td>95,0%</td>
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<td>17</td>
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<td>95,0%</td>
<td>92,5%</td>
</tr>
<tr>
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<td></td>
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<td>10</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>90,9%</td>
<td>100,0%</td>
<td>95,0%</td>
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<tr>
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<td>93,8%</td>
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<tr>
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<td></td>
<td>a Process water</td>
<td>9</td>
<td>10</td>
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<td>0</td>
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<td>83,3%</td>
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<td>9</td>
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<td>0</td>
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<td>100,0%</td>
<td>90,9%</td>
<td>95,2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c Dusts, residues</td>
<td>8</td>
<td>9</td>
<td>2</td>
<td>4</td>
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<td>66,7%</td>
<td>86,7%</td>
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<tr>
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<td>28</td>
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<td>86,8%</td>
<td>83,8%</td>
</tr>
<tr>
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<td></td>
<td>a Process water</td>
<td>9</td>
<td>11</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>91,7%</td>
<td>83,3%</td>
<td>87,0%</td>
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<tr>
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<td>b Sponges, swabs</td>
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<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100,0%</td>
<td>90,9%</td>
<td>95,2%</td>
</tr>
<tr>
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<td></td>
<td>c Dusts, residues</td>
<td>8</td>
<td>9</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>66,7%</td>
<td>86,7%</td>
<td>70,8%</td>
</tr>
<tr>
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<td>Total</td>
<td>27</td>
<td>30</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>84,2%</td>
<td>86,8%</td>
<td>83,8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a Process water</td>
<td>9</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>91,7%</td>
<td>83,3%</td>
<td>87,0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b Sponges, swabs</td>
<td>10</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>100,0%</td>
<td>90,9%</td>
<td>95,2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c Dusts, residues</td>
<td>8</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>66,7%</td>
<td>86,7%</td>
<td>70,8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>27</td>
<td>26</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>4</td>
<td>84,2%</td>
<td>86,8%</td>
<td>83,8%</td>
</tr>
<tr>
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<td></td>
<td>a Process water</td>
<td>9</td>
<td>11</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>91,7%</td>
<td>83,3%</td>
<td>87,0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b Sponges, swabs</td>
<td>10</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100,0%</td>
<td>90,9%</td>
<td>95,2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c Dusts, residues</td>
<td>8</td>
<td>9</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>66,7%</td>
<td>86,7%</td>
<td>70,8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>27</td>
<td>30</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>84,2%</td>
<td>86,8%</td>
<td>83,8%</td>
</tr>
<tr>
<td></td>
<td>A2:Total P2</td>
<td>203</td>
<td>189</td>
<td>37</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>92,3%</td>
<td>85,8%</td>
<td>87,8%</td>
<td>9,6%</td>
</tr>
<tr>
<td></td>
<td>B2:Total P2 + P3 Classic</td>
<td>196</td>
<td>178</td>
<td>40</td>
<td>22</td>
<td>1</td>
<td>18</td>
<td>91,1%</td>
<td>84,6%</td>
<td>86,2%</td>
<td>10,6%</td>
</tr>
<tr>
<td></td>
<td>C2:Total P2 + P4 Classic</td>
<td>196</td>
<td>180</td>
<td>40</td>
<td>23</td>
<td>0</td>
<td>18</td>
<td>91,1%</td>
<td>84,6%</td>
<td>86,2%</td>
<td>9,1%</td>
</tr>
<tr>
<td></td>
<td>D2:Total P2 + P3 Fast</td>
<td>196</td>
<td>176</td>
<td>40</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>91,1%</td>
<td>84,6%</td>
<td>86,2%</td>
<td>11,1%</td>
</tr>
<tr>
<td></td>
<td>E2:Total P2 + P4 Fast</td>
<td>196</td>
<td>180</td>
<td>40</td>
<td>23</td>
<td>0</td>
<td>18</td>
<td>91,1%</td>
<td>84,6%</td>
<td>86,2%</td>
<td>9,1%</td>
</tr>
</tbody>
</table>

* PPNA not included ** PPND not included
Table 14 - Summary of results – Standard II lysis

<table>
<thead>
<tr>
<th></th>
<th>A1</th>
<th>B1</th>
<th>C1</th>
<th>D1</th>
<th>E1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity for the alternative method</td>
<td>$SE_{alt} = \frac{(PA + PD)}{(PA + ND + PD)} \times 100%$</td>
<td>92.3%</td>
<td>90.8%</td>
<td>90.8%</td>
<td>90.8%</td>
</tr>
<tr>
<td>Sensitivity for the reference method</td>
<td>$SE_{ref} = \frac{(PA + ND)}{(PA + ND + PD)} \times 100%$</td>
<td>85.8%</td>
<td>84.7%</td>
<td>54.7%</td>
<td>84.7%</td>
</tr>
<tr>
<td>Relative trueness</td>
<td>$RT = \frac{(PA + NA)}{N} \times 100%$</td>
<td>87.6%</td>
<td>86.3%</td>
<td>86.3%</td>
<td>86.3%</td>
</tr>
<tr>
<td>False positive ratio for the alternative method*</td>
<td>$FPR = \frac{FP}{NA} \times 100%$</td>
<td>7.5%</td>
<td>8.6%</td>
<td>7.3%</td>
<td>9.0%</td>
</tr>
</tbody>
</table>

Table 15 - Summary of results – Easy II lysis

<table>
<thead>
<tr>
<th></th>
<th>A2</th>
<th>B2</th>
<th>C2</th>
<th>D2</th>
<th>E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity for the alternative method</td>
<td>$SE_{alt} = \frac{(PA + PD)}{(PA + ND + PD)} \times 100%$</td>
<td>92.3%</td>
<td>91.1%</td>
<td>91.1%</td>
<td>91.1%</td>
</tr>
<tr>
<td>Sensitivity for the reference method</td>
<td>$SE_{ref} = \frac{(PA + ND)}{(PA + ND + PD)} \times 100%$</td>
<td>85.8%</td>
<td>84.6%</td>
<td>84.6%</td>
<td>84.6%</td>
</tr>
<tr>
<td>Relative trueness</td>
<td>$RT = \frac{(PA + NA)}{N} \times 100%$</td>
<td>87.8%</td>
<td>86.2%</td>
<td>86.2%</td>
<td>86.2%</td>
</tr>
<tr>
<td>False positive ratio for the alternative method*</td>
<td>$FPR = \frac{FP}{NA} \times 100%$</td>
<td>9.6%</td>
<td>10.6%</td>
<td>9.1%</td>
<td>11.1%</td>
</tr>
</tbody>
</table>

With $ND = ND + PPND \quad NA = NA + PPNA$
3.1.1.7 Analysis of discordant results

＞ Negative deviations

The negative deviations are listed in Table 16 for the renewal study and Table 17 for the extension study.

For the renewal study, 24 and 20 negative deviations were respectively observed for the Standard II protocol and the Easy II protocol. For 3 samples (3474, 5340 and 1312), Listeria spp. strains were isolated from the enrichment broth. In these cases, the contamination level was probably just at the limit of detection of the alternative method.

For sample 3474 (raw milk cheese), the PCR was positive using the Easy II protocol after 72 h storage of the LSB broth. For sample 5340 (raw milk cheese), the PCR result was positive using the Standard II protocol after storage.

For the environmental samples tested for the extension, 6 negative deviations were observed with all the protocols (with or without FDRS, APF Classic and Fast). The presence of Listeria spp. was not confirmed in the enrichment broth for these samples. These discordant results were probably linked to the unpaired study design.

None of the samples in negative agreement (NA) for both extraction protocols was confirmed positive with cultural methods.

＞ Positive deviations

They are listed in Table 18 for the renewal and Table 19 for the extension.

For the renewal study, 44 and 37 positive deviations were respectively observed with the Standard II and the Easy II protocols. They concern 16 artificially contaminated samples and 30 naturally contaminated samples. 28 samples were contaminated with Listeria monocytogenes.

For the extension study, 5 positive deviations were observed for the 4 tested protocols; 2 samples were naturally contaminated and 3 artificially contaminated.
Table 16 - Negative deviations (renewal study)

<table>
<thead>
<tr>
<th>Date of analysis</th>
<th>Ref.</th>
<th>Product</th>
<th>Inoculation level (CFU/sample)</th>
<th>ISO 11290-1 result</th>
<th>Alternative method: iQ Check Listeria spp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>After incubation time</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Standard II protocol 2006</td>
</tr>
<tr>
<td>2019 1666</td>
<td>RTRH (puff ham and cheese)</td>
<td>1,6 +</td>
<td>37,12/38,64/40,45</td>
<td>+/+/+</td>
<td>-</td>
</tr>
<tr>
<td>2019 4457</td>
<td>RTRH (quiche lorraine)</td>
<td>2,8 +</td>
<td>&lt;35,00/-</td>
<td>+/+/-</td>
<td>-/+/-</td>
</tr>
<tr>
<td>2006 N10</td>
<td>Pastry</td>
<td>/ +</td>
<td>N/A -</td>
<td>N/A -</td>
<td>N/A -</td>
</tr>
<tr>
<td>2019 1661</td>
<td>Tortilla</td>
<td>3,4 +</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2019 5333</td>
<td>Pastry</td>
<td>2,6 +</td>
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<td>/ +</td>
<td>38,53 +</td>
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<td>-41,12/-</td>
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<td>36,17 +</td>
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<td>3,8 +</td>
<td>47,78/-</td>
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<td>j*</td>
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Table 17 - Negative deviations (extension study)

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<th>ISO 11290-1 result</th>
<th>Alternative method: iQ Check Listeria spp</th>
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Analyses performed according to the COFRAC accreditation
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**Table 18 - Positive deviations (renewal study)**

**Summary report (Version 0)**

iQ Check Listeria spp.
Table 19 - Positive deviations (Extension study)

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<th>Date of analysis</th>
<th>Ref</th>
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<th>Inoculation level (CFU/sample)</th>
<th>ISO 11290-1 result</th>
<th>Alternative method: IQ Check Listeria spp</th>
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<td><strong>APF Classic</strong></td>
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**Note:** PD = Positive deviation
The analyses of discordant results according to the EN ISO 16140-2:2016 is the following (See Table 20 and Table 21):

**Table 20 - Analyses of discordant results - Standard II lysis**

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<th>PD</th>
<th>ND</th>
<th>PPND</th>
<th>N+</th>
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<td>a RTE</td>
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<td>3</td>
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<td>b RTRH</td>
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**C1:** Total P1 + P4 Classic 222 45 24 3 294 -18 6
**D1:** Total P1 + P3 Fast 222 45 24 3 294 -18 6
**E1:** Total P1 + P4 Fast 222 45 24 3 294 -18 6

iQ Check *Listeria* spp.
Table 21 - Analyses of discordant results - Easy II lysis

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<td>B2:Total P2 + P3</td>
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<td>22</td>
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<td>E2:Total P2 + P4</td>
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<td>23</td>
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The observed values for ND - PD meet the acceptability limit for each individual category and for all the combined categories (calculated values \( \leq AL \)) whatever the protocols taken into account for interpretation.

### 3.1.1.8 Enrichment broth storage at 5 ± 3 °C for 72 h

The following changes were observed (See Table 22 and Table 23).

#### Table 22 - Enrichment broth storage (Renewal study)

<table>
<thead>
<tr>
<th>Date of analysis</th>
<th>Ref</th>
<th>Product</th>
<th>Alternative method: iQ Check <em>Listeria spp</em></th>
<th>Agreement Standard II lysis</th>
<th>Agreement Easy II lysis</th>
<th>Agreement Standard II lysis 72h</th>
<th>Agreement Easy II lysis 72h</th>
<th>Category</th>
<th>Type</th>
</tr>
</thead>
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<td>2019</td>
<td>4457</td>
<td>RTRH (quiche lorraine)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>PA</td>
<td>PA</td>
<td>1</td>
<td>b</td>
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<td>2006</td>
<td>N3</td>
<td>Frozen meat</td>
<td>PA</td>
<td>PA</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>2019</td>
<td>3471</td>
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<td>PD</td>
<td>NA</td>
<td>PD</td>
<td>PD</td>
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<td>a</td>
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<td>ND</td>
<td>ND</td>
<td>PA</td>
<td>3</td>
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<td>5340</td>
<td>Raw milk cheese</td>
<td>ND</td>
<td>PA</td>
<td>ND</td>
<td>PD</td>
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<td>b</td>
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<tr>
<td>2006</td>
<td>C1</td>
<td>Smoked salmon</td>
<td>NA</td>
<td>NA</td>
<td>PD</td>
<td>PD</td>
<td>4</td>
<td>c</td>
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</tr>
<tr>
<td>2006</td>
<td>N4</td>
<td>Sandwich (tuna)</td>
<td>PA</td>
<td>PA</td>
<td>ND</td>
<td>ND</td>
<td>4</td>
<td>c</td>
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</tr>
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<td>N6</td>
<td>Sandwich (shrimp)</td>
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<td>ND</td>
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#### Table 23 - Enrichment broth storage (Renewal study)

<table>
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<th>Ref</th>
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The analyses of discordant become (See Table 24 and Table 25).
### Table 24 - Analysis of discordant after storage 72 h at 5 ± 3°C -

#### Standard II lysis

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<th>Category</th>
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<th>Type</th>
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<th>PD</th>
<th>ND</th>
<th>PPND</th>
<th>N+</th>
<th>(ND+PPND)-PD</th>
<th>AL</th>
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<td>RTE</td>
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</table>

A2: Total P1: 176 33 19 1 229 -13 6
B2: Total P1 + P3 Classic: 171 37 22 1 231 -14 6
C2: Total P1 + P4 Classic: 171 37 21 2 231 -14 6
D2: Total P1 + P3 Fast: 171 37 22 1 231 -14 6
E2: Total P1 + P4 Fast: 170 37 23 1 231 -13 6
### Table 25 - Analysis of discordant after storage 72 h at 5 ± 3°C - Easy II lysis

<table>
<thead>
<tr>
<th>Category</th>
<th>Protocol</th>
<th>APF</th>
<th>Type</th>
<th>Type</th>
<th>PA</th>
<th>PD</th>
<th>ND</th>
<th>PPND</th>
<th>N+</th>
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<td>RTE</td>
<td>11</td>
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<td>Delicatessen</td>
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**A2**: Total P2

179 35 20 0 234 -15 6

**B2**: Total P2 + P3 Classic

174 38 22 0 234 -16 6

**C2**: Total P2 + P4 Classic

174 38 21 1 234 -16 6

**D2**: Total P2 + P3 Fast

174 38 22 0 234 -16 6

**E2**: Total P2 + P4 Fast

173 38 23 0 234 -15 6

ADRIA 33/199 16 November 2023

Summary report (Version 0)
iQ Check Listeria spp.
The observed values for ND - PD meet the acceptability limit for each individual category and for all the combined categories (calculated values ≤ AL) whatever the protocols taken into account for interpretation.

3.1.1.9 Confirmation

The presence of *Listeria* spp. was confirmed from LSB broth for 293 samples. Typical colonies were observed on RAPID’*Listeria* spp. for 288 samples, and on RAPID’*L.mono* for 265 samples.

For 15 samples tested with protocol 1, 20 samples tested with protocol 2, and 6 samples tested combining protocols 3 and 4, it was impossible to confirm the presence of *Listeria* spp. in the enrichment broth even when proceeding to several subcultures in Fraser broth prior streaking onto Agar *Listeria* and Palcam plates. The samples are listed in Table 26 and Table 27.

Note that when the FDRS protocol was applied prior the Easy II lysis protocol, the number of PPNA samples was reduced from 6 to 0 sample. The percentage of false positive results decreases from 10.0% to 0.0% with the APF Classic and from 13.3 % to 0.0% with the APF Fast when using the FDRS protocol.
Table 26 – Positive presumptive not confirmed samples. Protocols 1 and 2

<table>
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<tr>
<th>Date of analysis</th>
<th>Ref</th>
<th>Product</th>
<th>Alternative method: IQ Check <em>Listeria</em> spp</th>
<th>After incubation time</th>
<th>Final result Standard II lysis</th>
<th>Final result Easy II lysis</th>
<th>Agreement Standard II lysis</th>
<th>Agreement Easy II lysis</th>
<th>Category</th>
<th>Type</th>
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**Summary report** (Version 0)

IQ Check *Listeria* spp.
## Summary report (Version 0)

**IQ Check Listeria spp.**

### Date of analysis

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<th>Date of analysis</th>
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<th>Ct FAM</th>
<th>Result</th>
<th>Ct FAM</th>
<th>Result</th>
<th>Identifications</th>
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<th>Agreement Easy II lysis</th>
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<td>X14</td>
<td>Merguez</td>
<td>36.53</td>
<td>+</td>
<td>38.40</td>
<td>+</td>
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<td>-</td>
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<td>4429</td>
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<td>-</td>
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<td>+/-</td>
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<td>+/-</td>
<td>-</td>
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<td>40.23/</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>NA</td>
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<tr>
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<td>4459</td>
<td>Raw milk</td>
<td>-</td>
<td>-</td>
<td>40.42/-</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>NA</td>
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<tr>
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<td>4461</td>
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<td>-</td>
<td>40.37/</td>
<td>+/-</td>
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<td>Ice-cream</td>
<td>41.61</td>
<td>+</td>
<td>41.50</td>
<td>+</td>
<td>/</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>5446</td>
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<td>40.89/</td>
<td>+/-</td>
<td>38.92</td>
<td>+/-</td>
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<td>2006</td>
<td>Z7</td>
<td>Frozen cauliflower</td>
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<td>2006</td>
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<td>Cucumber</td>
<td>44.13</td>
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<td>36.42</td>
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<tr>
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<td>RTE (Macédoine)</td>
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<td>-</td>
<td>40.41/-</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>NA</td>
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<tr>
<td>2006</td>
<td>CC17</td>
<td>Process water (vegetable industry)</td>
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<td>42.24</td>
<td>+</td>
<td>Ø</td>
<td>-</td>
<td>PPNA</td>
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<tr>
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<td>Residues (dairy environment)</td>
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<td>+/-</td>
<td>/</td>
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### Alternative method: IQ Check Listeria spp

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<th>Ct FAM</th>
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<th>Identifications</th>
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<th>Agreement Easy II lysis</th>
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<tr>
<td>2019</td>
<td>4434</td>
<td>RTE salad</td>
<td>45/41</td>
<td>+/-</td>
<td>41.29/35.56</td>
<td>+/-</td>
<td>36.80</td>
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<td>5Fraser/AL/Palcam</td>
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<td>+/-</td>
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<tr>
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<td>CC17</td>
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<td>+</td>
<td>42.24</td>
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<td>Ø</td>
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<td>/</td>
<td>-</td>
<td>PPNA</td>
<td>PPNA</td>
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</table>
## Table 27 – Positive presumptive not confirmed samples. Protocols 3 or 4

| Date of analysis | Ref | Product | Inoculation level (CFU/sample) | ISO 11290-1 result | Alternative method: iQ Check Listeria spp | 18h at 30°C ± 1°C | Confir-
mation | Final result | Agreement |
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<td>Result</td>
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<td>Rinsing water (poultry environment)</td>
<td>/ -</td>
<td>39,35/40,27/-</td>
<td>+/+/-</td>
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<td>- -</td>
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<td>/ -</td>
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<td>- -</td>
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<td>Wipe (vegetable environment)</td>
<td>2,6</td>
<td>39,38/-/</td>
<td>+/-/-</td>
<td>- -</td>
<td>- -</td>
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<td>Wipe after cleaning process (vegetable environment)</td>
<td>/ -</td>
<td>41,02/-/</td>
<td>+/-/-</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
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<td>Residues (seafood environment)</td>
<td>/ -</td>
<td>41,78/-/</td>
<td>+/-/-</td>
<td>- -</td>
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<td>38,75/-/41,03</td>
<td>+/-/+</td>
<td>- -</td>
<td>- -</td>
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</table>
3.1.1.10 PCR inhibition

1086 DNA extracts were tested for the overall validation studies after enrichment broths incubation time and 598 after enrichment broths storage. The inhibitions observed are listed in Table 28.

The inhibitions concern 17 samples for the Standard II extraction protocol and 12 samples for the Easy II extraction protocol.

Results were obtained when a 1/10 dilution of the lysates was applied except for samples 1665 and 1673 for which the dilution was not necessary. The inhibition represents 2.7%.

Note that no PCR inhibition was observed for the extension study concerning the environment samples category.
### Table 28 – PCR inhibitions

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<th>After storage</th>
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<td>Easy II lysis</td>
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<td>CI</td>
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<tr>
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<td>BB18</td>
<td>Delicatessen</td>
<td>N/A/29.52*</td>
<td>N/A/N/A*</td>
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<td>2006</td>
<td>BB5</td>
<td>Seasoned sausage</td>
<td>N/A/29.99*</td>
<td>N/A/N/A*</td>
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<td>O18</td>
<td>Cheese</td>
<td>N/A/35.56*</td>
<td>N/A/N/A*</td>
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<td>C8</td>
<td>Raw milk</td>
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<td>G17</td>
<td>Salmon</td>
<td>N/A/23.78*</td>
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<td>AA16</td>
<td>Mini beef balls</td>
<td>N/A*</td>
<td>N/A/14.48*</td>
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<td>Residues Hareng</td>
<td>-/ 35.79*</td>
<td>-/ 35.79*</td>
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<td>-/ 35.79*</td>
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<td>Raw milk cheese</td>
<td>-/ 32.18*</td>
<td>-/ 32.18*</td>
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<td>5334</td>
<td>Pastry</td>
<td>-/ 32.22*</td>
<td>-/ 30.89*</td>
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<td>N/A/N/A*</td>
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<td>2019</td>
<td>5334</td>
<td>Pastry</td>
<td>-/ 32.33*</td>
<td>-/ 32.33*</td>
</tr>
<tr>
<td>2019</td>
<td>5341</td>
<td>Raw milk cheese</td>
<td>-/ 32.30*</td>
<td>-/ 32.30*</td>
</tr>
<tr>
<td>2019</td>
<td>5442</td>
<td>Beetroot</td>
<td>32.77/33.12*</td>
<td>32.77/33.12*</td>
</tr>
<tr>
<td>2019</td>
<td>1658</td>
<td>RTE salad (ham)</td>
<td>-/ 32.75*</td>
<td>-/ 32.75*</td>
</tr>
<tr>
<td>2019</td>
<td>3474</td>
<td>Raw milk cheese</td>
<td>-/ 32.98*</td>
<td>-/ 32.98*</td>
</tr>
<tr>
<td>2019</td>
<td>3475</td>
<td>Raw milk cheese</td>
<td>-/ 35.30*</td>
<td>-/ 34.42*</td>
</tr>
<tr>
<td>2019</td>
<td>4432</td>
<td>Raw milk cheese</td>
<td>-/ 32.11*</td>
<td>-/ 32.11*</td>
</tr>
<tr>
<td>2019</td>
<td>5334</td>
<td>Pastry</td>
<td>-/ 24.26*</td>
<td>-/ 24.26*</td>
</tr>
<tr>
<td>2019</td>
<td>3468</td>
<td>Raw milk cheese</td>
<td>-/ 26.18*</td>
<td>-/ 26.18*</td>
</tr>
<tr>
<td>2019</td>
<td>3470</td>
<td>Raw milk cheese</td>
<td>-/32.11*</td>
<td>-/32.11*</td>
</tr>
<tr>
<td>2019</td>
<td>3471</td>
<td>Raw milk cheese</td>
<td>-/ 26.01*</td>
<td>-/ 26.01*</td>
</tr>
</tbody>
</table>
3.1.2 Relative Level of Detection (RLOD)

The relative level of detection is the level of detection at $P = 0.50$ ($LOD_{50}$) of the alternative (proprietary) method divided by the level of detection at $P = 0.50$ ($LOD_{50}$) of the reference method.

3.1.2.1 Experimental design

Seven matrix/strain pairs were analyzed by the reference and the alternative methods. The matrix/strain pairs are listed in Table 29.

**Table 29 - Matrix/strain pairs tested**

<table>
<thead>
<tr>
<th>Category</th>
<th>Matrix</th>
<th>Strain</th>
<th>Origin</th>
<th>Inoculation protocol</th>
<th>Enrichment protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite food</td>
<td>Deli salad</td>
<td><em>L. ivanovii</em> Ad1769</td>
<td>Ewe milk</td>
<td>Seeding 48 h at 3°C ± 2°C</td>
<td></td>
</tr>
<tr>
<td>Meat products</td>
<td>Rillettes</td>
<td><em>L. welshimeri</em> L90</td>
<td>Ground beef</td>
<td>/</td>
<td>LSB 22h (Standard II) and 24 h (Easy II)</td>
</tr>
<tr>
<td>Dairy products</td>
<td>Raw milk</td>
<td><em>L. monocytogenes</em> 1/2b L37</td>
<td>Maroilles</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Seafood products</td>
<td>Smoked salmon</td>
<td><em>L. monocytogenes</em> 1/2a L5</td>
<td>Smoked salmon</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Vegetables</td>
<td>Raw vegetable mix</td>
<td><em>L. seeligeri</em> L140</td>
<td>Salad</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Production environmental samples</td>
<td>Process water</td>
<td><em>L. innocua</em> 1/2c L144</td>
<td>Surface</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Process water (vegetables)</td>
<td><em>L. monocytogenes</em> Ad2503</td>
<td>Vegetable industry</td>
<td>Seeding 48 h at 3°C ± 2°C</td>
<td>LSB 16 h with and without FDRS</td>
</tr>
</tbody>
</table>

The following protocol was applied for the RLOD tested for the renewal and extension study:

- A negative control: 5 samples,
- A low contamination level providing fractional recovery data, with 20 replicates,
- A high contamination level, with 5 replicates.

A total plate count determination on each matrix was performed to estimate the total microbial load on the day of analysis.

3.1.2.2 Calculation

The raw data are given in Appendix 5.
The RLOD calculations for the matrix/strain pairs tested for the initial validation study were performed using the Excel spreadsheet available at [http://standards.iso.org/iso/16140](http://standards.iso.org/iso/16140) - RLOD (clause 5-1-4-2 Calculation and interpretation of RLOD) version 15.08.2015. The RLOD are given in Table 30.

**Table 30 - Presentation of RLOD before and after confirmation of the alternative method results**

<table>
<thead>
<tr>
<th>Name</th>
<th>RLOD</th>
<th>RLODL</th>
<th>RLODU</th>
<th>b=ln(RLOD)</th>
<th>sd(b)</th>
<th>z-Test statistic</th>
<th>p-value</th>
<th>AL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deli salad / <em>Listeria ivanovii</em> Ad1769</td>
<td>1,151</td>
<td>0,519</td>
<td>2,553</td>
<td>0,141</td>
<td>0,398</td>
<td>0,354</td>
<td>0,723</td>
<td></td>
</tr>
<tr>
<td>Rillettes / <em>Listeria welshimeri</em> L90 Protocols 1 &amp; 2</td>
<td>0,737</td>
<td>0,311</td>
<td>1,746</td>
<td>-0,305</td>
<td>0,431</td>
<td>0,707</td>
<td>1,520</td>
<td></td>
</tr>
<tr>
<td>Raw milk / <em>Listeria monocytogenes</em> L37 Protocols 1 &amp; 2</td>
<td>1,263</td>
<td>0,403</td>
<td>3,955</td>
<td>0,234</td>
<td>0,571</td>
<td>0,409</td>
<td>0,682</td>
<td></td>
</tr>
<tr>
<td>Vegetables mix / <em>Listeria seeligeri</em> L140 Protocols 1 &amp; 2</td>
<td>1,355</td>
<td>0,557</td>
<td>3,296</td>
<td>0,304</td>
<td>0,445</td>
<td>0,683</td>
<td>0,495</td>
<td></td>
</tr>
<tr>
<td>Smoked salmon / <em>Listeria monocytogenes</em> L5 Protocols 1 &amp; 2</td>
<td>1,163</td>
<td>0,487</td>
<td>2,781</td>
<td>0,151</td>
<td>0,436</td>
<td>0,347</td>
<td>0,729</td>
<td></td>
</tr>
<tr>
<td>Process water / <em>Listeria innocua</em> L144 Protocols 1 &amp; 2</td>
<td>1,000</td>
<td>0,435</td>
<td>2,298</td>
<td>0,000</td>
<td>0,416</td>
<td>0,000</td>
<td>1,000</td>
<td></td>
</tr>
<tr>
<td>Process water / <em>Listeria monocytogenes</em> Ad2503 Protocol 3 (Classic) &amp; Protocol 4 (Classic &amp; Fast) (A)</td>
<td>1,136</td>
<td>0,505</td>
<td>2,559</td>
<td>0,128</td>
<td>0,406</td>
<td>0,315</td>
<td>0,753</td>
<td></td>
</tr>
<tr>
<td>Process water / <em>Listeria monocytogenes</em> Ad2503 Protocol 3 (Fast) (B)</td>
<td>1,273</td>
<td>0,556</td>
<td>2,915</td>
<td>0,242</td>
<td>0,414</td>
<td>0,584</td>
<td>0,559</td>
<td></td>
</tr>
<tr>
<td>Combined (A)</td>
<td>1,062</td>
<td>0,774</td>
<td>1,458</td>
<td>0,060</td>
<td>0,158</td>
<td>0,380</td>
<td>0,704</td>
<td></td>
</tr>
<tr>
<td>Combined (B)</td>
<td>1,083</td>
<td>0,788</td>
<td>1,487</td>
<td>0,079</td>
<td>0,159</td>
<td>0,499</td>
<td>0,617</td>
<td></td>
</tr>
</tbody>
</table>

The LOD\(_{50\%}\) calculations according to Wilrich & Wilrich POD-LOD calculation program - version 11, 2022.10.12 test are given in Table 31.
### Table 31 - LOD$_{50}$ results

<table>
<thead>
<tr>
<th>Strain/Matrix pair</th>
<th>Level of detection at 50% (CFU / sample size) according to Wilrich &amp; Wilrich$^3$</th>
<th>Reference method</th>
<th>Alternative method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deli salad / Listeria ivanovii Ad1769c</td>
<td>0.6[0.4;1.1]</td>
<td>0.7[0.4;1.2]</td>
<td></td>
</tr>
<tr>
<td>Rillettes / Listeria welshimeri L90</td>
<td>0.4[0.2;0.6]</td>
<td>0.2[0.1;0.4]</td>
<td></td>
</tr>
<tr>
<td>Protocols 1 &amp; 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw milk / Listeria monocytogenes L37</td>
<td>0.6[0.3;1.2]</td>
<td>0.7[0.4;1.3]</td>
<td></td>
</tr>
<tr>
<td>Protocols 1 &amp; 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetables mix / Listeria seeligeri L140</td>
<td>0.5[0.3;0.9]</td>
<td>0.6[0.4;1.4]</td>
<td></td>
</tr>
<tr>
<td>Protocols 1 &amp; 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoked salmon / Listeria monocytogenes L5</td>
<td>0.6[0.3;1.0]</td>
<td>0.7[0.4;1.2]</td>
<td></td>
</tr>
<tr>
<td>Protocols 1 &amp; 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Process water / Listeria innocua L144</td>
<td>0.7[0.4;1.3]</td>
<td>0.6[0.3;1.1]</td>
<td></td>
</tr>
<tr>
<td>Protocols 1 &amp; 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Process water / Listeria monocytogenes Ad2503</td>
<td>0.6[0.4;0.7]</td>
<td>0.8[0.5;1.5]</td>
<td></td>
</tr>
<tr>
<td>Protocol 3 (Classic) &amp; Protocol 4 (Classic &amp; Fast) (A)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Process water / Listeria monocytogenes Ad2503</td>
<td>0.9[0.5;1.47]</td>
<td>0.6[0.5;0.8]</td>
<td></td>
</tr>
<tr>
<td>Protocol 3 (Fast) (B)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined (A)</td>
<td>0.6[0.4;0.7]</td>
<td>0.6[0.5;0.8]</td>
<td></td>
</tr>
<tr>
<td>Combined (B)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The RLOD values (using the confirmed alternative method results) meet the acceptability limit of 2.5 for unpaired studies, for all matrix/strain pairs tested whatever the protocol used.

The LOD$_{50}$ varies from 0.4 to 0.7 CFU/sample size for the reference method and from 0.2 to 0.9 CFU/sample size for the alternative method.

---

3.1.3 Inclusivity and exclusivity

The inclusivity study involves pure target strains to be detected or enumerated by the alternative method. The exclusivity study involves pure non-target strains, which can be potentially cross-reactive, but are not expected to be detected or enumerated by the alternative method.

3.1.3.1 Protocols

**Inclusivity**

84 target strains (50 *Listeria monocytogenes* and 34 *Listeria* spp. different from *Listeria monocytogenes*) were tested in 2006 for the initial validation study. Strains were grown in nutrient broth for 22 h at 30°C. The LSB broth was then inoculated between 10 to 100 cells/225 ml and incubated for 24 h at 30°C. The Easy II extraction protocol was applied before performing the PCR test.

For the extension study performed in 2009, 51 target strains (25 *Listeria monocytogenes* and 26 *Listeria* spp. different from *Listeria monocytogenes*) were tested. Strains were grown in nutrient broth for 24 h at 30°C. The LSB broth was then inoculated between 10 to 100 cells/225 ml and incubated for 24 h at 30°C. The Standard II extraction protocol was applied before performing the PCR test (new kit).

For the extension study performed in 2019, 20 *Listeria monocytogenes* and 30 *Listeria* spp. different from *Listeria monocytogenes* were tested. The strains were grown in BHI for 24 h at 37°C and inoculated between 10 to 100 CFU/225 ml LSB broth. The broths were incubated for 18 h at 30°C before applying the protocol of the alternative method (Easy II lysis protocol with and without FDRS).

**Exclusivity**

For the initial validation study, 32 non-target strains were grown in nutrient broth for 24 h at 30°C, inoculated at a level around $10^5$ cells/ml. The broths were incubated for 24 h at 30°C before proceeding to lysis step (Easy II extraction protocol) and PCR.

For the extension study performed in 2009, 30 non-target strains were grown in nutrient broth for 24 h at 30°C, inoculated at a level around $10^5$ cells/ml. The broths were incubated for 24 h at 30°C before proceeding to lysis step (Standard II extraction protocol) and PCR (new kit).

For the extension study performed in 2019, no additional testing was required.
3.1.3.2 Results

The raw data are given in Appendix 6.

> Inclusivity

The 75 *Listeria monocytogenes* strains and 60 *Listeria* spp. different from *Listeria monocytogenes* strains tested in 2006 and 2009 gave positive PCR tests.

For the extension study (2019), the 50 tested strains gave positive PCR results and characteristic colonies on the selective Agar plates (Agar *Listeria*, Palcam, RAPID’*L.mono* and RAPID’*Listeria* spp); for two *Listeria seeligeri* strains (CIP 100100 and BR18), colonies were observed on selective agar plates only after subculture in Fraser broth.

> Exclusivity

No cross reaction was observed among the 62 non-target strains tested.

3.1.4 Practicability

The alternative method practicability was evaluated according to the AFNOR criteria relative to method comparison study.
Storage conditions, shelf-life and modalities of utilisation after first use

The storage temperature is 2 - 8°C. The shelf-life is given on the package. All the reagents shall be stored at the temperature mentioned on the package.

<table>
<thead>
<tr>
<th>Time to result</th>
<th>Steps</th>
<th>Reference method</th>
<th>Alternative method</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative samples</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sampling, enrichment</td>
<td>Day 0</td>
<td>Day 0</td>
<td></td>
</tr>
<tr>
<td>Subculture in Fraser 1</td>
<td>Day 1</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Lysate</td>
<td>/</td>
<td>Day 1</td>
<td></td>
</tr>
<tr>
<td>PCR</td>
<td>/</td>
<td>Day 1</td>
<td></td>
</tr>
<tr>
<td>Streaking onto plates (O1/P1)</td>
<td>Day 1</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Second streaking (O2/P2)</td>
<td>Day 2</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Reading plates (O1/P1)</td>
<td>Days 2 - 3</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Reading plates (O2/P2)</td>
<td>Days 3 - 4</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td><strong>Presumptive positive or positive results</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subculture of typical colonies</td>
<td>Days 3 - 4</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Streaking onto plates</td>
<td>/</td>
<td>Day 1</td>
<td></td>
</tr>
<tr>
<td>Reading plates</td>
<td>/</td>
<td>Days 2 - 3</td>
<td></td>
</tr>
<tr>
<td>Confirmation test</td>
<td>Days 4 - 5</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Results</td>
<td>Days 5 - 6</td>
<td>Days 8 - 10 *</td>
<td>/</td>
</tr>
</tbody>
</table>

* In the case of rhamnose and xylose tests are carried out in tubes.

Common step with the reference method

There is no common step

The negative results are available in 1 day and the positive results in 2 or 3 days.
3.2 Extension study for the use of LSB II medium

3.2.1 Sensitivity study

3.2.1.1 Number and nature of samples

Combining the three categories tested with LSBII broth, 200 samples were tested providing 102 positive and 98 negative results.

The distribution per tested category and type is given in Table 32.

<table>
<thead>
<tr>
<th>Category</th>
<th>Type</th>
<th>Positive samples</th>
<th>Negative samples</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Composite foods</td>
<td>a  Ready-to-eat</td>
<td>10</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>b  Ready-to-reheat</td>
<td>18</td>
<td>13</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>c  Confectionaries, pastries and egg products</td>
<td>7</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>35</td>
<td>38</td>
<td>73</td>
</tr>
<tr>
<td>2 Production environmental samples</td>
<td>a  Surfaces samples</td>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>b  Dusts and wastes</td>
<td>11</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>c  Process water</td>
<td>12</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>33</td>
<td>27</td>
<td>60</td>
</tr>
<tr>
<td>3 Dairy products</td>
<td>a  Raw milk cheese (cow, ewe, goat)</td>
<td>10</td>
<td>13</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>b  Other raw milk-based products (raw milk, cream, butter, fermented milk)</td>
<td>11</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>c  Pasteurized milk-based products (pasteurized cheese, ice cream, milk)</td>
<td>13</td>
<td>11</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>34</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>All categories</td>
<td></td>
<td>102</td>
<td>98</td>
<td>200</td>
</tr>
</tbody>
</table>

3.2.1.2 Artificial contamination of samples

Naturally contaminated products were preferentially analysed, but artificial contaminations were also carried out using the seeding protocol by direct inoculations of products using a liquid inoculum, followed by storage for 48-72 h at 3°C ± 2°C or with lyophilized strain and storage 2 weeks at ambient temperature or by spiking after heat treatment.

The artificial contaminations are presented in Appendix 7.

The repartition of the positive samples per inoculation protocol and inoculation level is given in Table 33.
Table 33 - Repartition of positive naturally and artificially contaminated samples per inoculation level

<table>
<thead>
<tr>
<th>Category</th>
<th>Naturally contaminated</th>
<th>Spiking protocol</th>
<th>Seeding protocol</th>
<th>Total positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≤ 5 CFU</td>
<td>3 &lt; x ≤ 5,6 CFU</td>
<td>≤ 3 CFU</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>4</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Number of samples</td>
<td>41</td>
<td>4</td>
<td>0</td>
<td>41</td>
</tr>
<tr>
<td>%</td>
<td>40,2</td>
<td>3,9</td>
<td>0,0</td>
<td>40,2</td>
</tr>
</tbody>
</table>

Combining all the categories, 40.2 % of the samples were naturally contaminated.

The distribution per target analytes is given in Table 34.

Table 34 – Distribution per target analytes

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of samples</th>
<th>Listeria spp (A)</th>
<th>Number of samples</th>
<th>Listeria spp + Listeria monocytogenes (B)</th>
<th>Number of samples</th>
<th>Total (A+B)</th>
<th>Number of samples</th>
<th>Listeria monocytogenes (C)</th>
<th>Number of samples</th>
<th>Total positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of samples</td>
<td>Number of samples</td>
<td></td>
<td>Number of samples</td>
<td></td>
<td>Number of samples</td>
<td></td>
<td>Number of samples</td>
<td></td>
<td>Number of samples</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td>23</td>
<td>12</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td>17</td>
<td>16</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>16</td>
<td>18</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
<td>56</td>
<td>46</td>
<td>102</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The number of samples contaminated with Listeria spp. alone or mixed with Listeria monocytogenes is comprised between 15 and 25 per category as required in the AFNOR Technical rules.
3.2.1.3 Protocols applied during the validation study

**Enrichment, lysis and PCR protocols**

The minimum incubation time was evaluated for all studies.
A summary of the protocols evaluated during the extension in 2023 is shown in the table below.

<table>
<thead>
<tr>
<th>Table 35 — Protocols applied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Validation study</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Extension 2023</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

In case of PCR inhibition, 1:10 dilution of the DNA extract in water was applied.

**Instruments**

Two Real-Time PCR instruments were used: CFX96 Deep Well and CFX Opus Deep Well.

**Confirmation protocols**

The positive PCR tests were confirmed by:

- Streaking 10 µl of the enriched LSB II broth onto O&A (AL) and Palcam plates incubated for 24 to 48 h at 37°C ± 1°C. The typical colonies were confirmed by the tests described in the ISO method (gram and catalase). For the study, API *Listeria* galleries were also used to identify the isolated colonies as this information is required for an AFNOR Validation study.

- Streaking 100 µl of enriched LSB II broth onto Agar *Listeria*, RAPID’*Listeria* Agar or RAPID’*L.mono* Agar incubated for 24 h at 37°C ± 1°C. In this case,

---

4 Application Protocol File
the only presence of typical colonies allows to confirm the positive PCR result.

- An additional protocol was applied to be in agreement with the ISO 16140-2 requirements: subculture of the LSB II in Fraser broth (24 h ± 2 h at 37°C) before streaking onto O&A and PALCAM plates.

> **Enrichment broth storage or 72 h at 5°C ± 3°C**

The enrichment broth (LSB II) from positive and discordant samples were stored for 72 h at 5°C ± 3°C and the alternative method was tested again.

### 3.2.1.4 Test results

Raw data are given in Appendix 8.

The results for all categories are given in Table 36 (CFX 96 DW-without FDRS), Table 37 (CFX 96 DW-with FDRS), Table 38 (CFX Opus DW-without FDRS) and Table 39 (CFX Opus DW-with FDRS).

**Table 36 – Interpretation of sample results between the reference and alternative method (based on the confirmed alternative method) - CFX 96 DW - Without FDRS**

<table>
<thead>
<tr>
<th>Category</th>
<th>Type</th>
<th>PA</th>
<th>NA*</th>
<th>PD</th>
<th>ND**</th>
<th>PPND</th>
<th>PPNA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Composite foods</td>
<td>a Ready-to-eat</td>
<td>6</td>
<td>12</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>b Ready-to-reheat</td>
<td>12</td>
<td>11</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>c Confectionaries, pastries and egg products</td>
<td>6</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td>24</td>
<td>33</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td>73</td>
</tr>
<tr>
<td>2 Production environmental samples</td>
<td>a Surfaces samples</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>b Dusts and wastes</td>
<td>9</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>c Process water</td>
<td>9</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td>28</td>
<td>27</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>3 Dairy products</td>
<td>a Raw milk cheese (cow, ewe, goat)</td>
<td>6</td>
<td>13</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>b Other raw milk-based products (raw milk, cream, butter, fermented milk)</td>
<td>7</td>
<td>9</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>c Pasteurized milk-based products (pasteurized cheese, ice cream, milk)</td>
<td>4</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td>17</td>
<td>31</td>
<td>11</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>67</td>
</tr>
<tr>
<td><strong>All categories</strong></td>
<td></td>
<td>69</td>
<td>91</td>
<td>19</td>
<td>13</td>
<td>0</td>
<td>8</td>
<td>200</td>
</tr>
</tbody>
</table>

* PPNA not included  
** PPND not included
Table 37 – Interpretation of sample results between the reference and alternative method (based on the confirmed alternative method) -

**CFX 96 DW - With FDRS**

<table>
<thead>
<tr>
<th>Category</th>
<th>Type</th>
<th>PA</th>
<th>NA*</th>
<th>PD</th>
<th>ND**</th>
<th>PPND</th>
<th>PPNA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Composite foods</td>
<td>a Ready-to-eat</td>
<td>6</td>
<td>12</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>b Ready-to-reheat</td>
<td>12</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>c Confectionaries, pastries and egg products</td>
<td>6</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>24</td>
<td>37</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>73</td>
</tr>
<tr>
<td>2 Production environmental samples</td>
<td>a Surfaces samples</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>b Dusts and wastes</td>
<td>9</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>c Process water</td>
<td>9</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>28</td>
<td>27</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>3 Dairy products</td>
<td>a Raw milk cheese (cow, ewe, goat)</td>
<td>5</td>
<td>13</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>b Other raw milk-based products (raw milk,</td>
<td>7</td>
<td>9</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>cream, butter, fermented milk)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c Pasteurized milk-based products (pasteurized</td>
<td>3</td>
<td>11</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>cheese, ice cream, milk)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>15</td>
<td>33</td>
<td>11</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>67</td>
</tr>
<tr>
<td>All categories</td>
<td></td>
<td>67</td>
<td>97</td>
<td>19</td>
<td>14</td>
<td>2</td>
<td>1</td>
<td>200</td>
</tr>
</tbody>
</table>

* PPNA not included

**CFX Opus DW- Without FDRS**

<table>
<thead>
<tr>
<th>Category</th>
<th>Type</th>
<th>PA</th>
<th>NA*</th>
<th>PD</th>
<th>ND**</th>
<th>PPND</th>
<th>PPNA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Composite foods</td>
<td>a Ready-to-eat</td>
<td>5</td>
<td>11</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>b Ready-to-reheat</td>
<td>12</td>
<td>12</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>c Confectionaries, pastries and egg products</td>
<td>6</td>
<td>11</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>23</td>
<td>34</td>
<td>5</td>
<td>7</td>
<td>0</td>
<td>4</td>
<td>73</td>
</tr>
<tr>
<td>2 Production environmental samples</td>
<td>a Surfaces samples</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>b Dusts and wastes</td>
<td>9</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>c Process water</td>
<td>9</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>28</td>
<td>27</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>3 Dairy products</td>
<td>a Raw milk cheese (cow, ewe, goat)</td>
<td>6</td>
<td>13</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>b Other raw milk-based products (raw milk,</td>
<td>7</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>cream, butter, fermented milk)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c Pasteurized milk-based products (pasteurized</td>
<td>4</td>
<td>11</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>cheese, ice cream, milk)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>17</td>
<td>32</td>
<td>11</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>67</td>
</tr>
<tr>
<td>All categories</td>
<td></td>
<td>68</td>
<td>93</td>
<td>19</td>
<td>14</td>
<td>0</td>
<td>6</td>
<td>200</td>
</tr>
</tbody>
</table>

* PPNA not included

**PPND not included**
### Table 39 – Interpretation of sample results between the reference and alternative method (based on the confirmed alternative method) - CFX Opus- With FDRS

<table>
<thead>
<tr>
<th>Category</th>
<th>Type</th>
<th>PA</th>
<th>NA*</th>
<th>PD</th>
<th>ND**</th>
<th>PPND</th>
<th>PPNA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Composite foods</td>
<td>a  Ready-to-eat</td>
<td>5</td>
<td>11</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>b  Ready-to-reheat</td>
<td>12</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>c  Confectionaries, pastries and egg</td>
<td>6</td>
<td>13</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>products</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>23</td>
<td>37</td>
<td>5</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>73</td>
</tr>
<tr>
<td>2 Production environmental samples</td>
<td>a  Surfaces samples</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>b  Dusts and wastes</td>
<td>9</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>c  Process water</td>
<td>9</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>28</td>
<td>27</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>3 Dairy products</td>
<td>a  Raw milk cheese (cow, ewe, goat)</td>
<td>6</td>
<td>14</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>b  Other raw milk-based products (raw</td>
<td>7</td>
<td>9</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>milk, cream, butter, fermented milk)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c  Pasteurized milk-based products</td>
<td>3</td>
<td>11</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>(pasteurized cheese, ice cream, milk)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>16</td>
<td>34</td>
<td>10</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>67</td>
</tr>
<tr>
<td>All categories</td>
<td></td>
<td>67</td>
<td>98</td>
<td>18</td>
<td>16</td>
<td>0</td>
<td>1</td>
<td>200</td>
</tr>
</tbody>
</table>

* PPNA not included ** PPND not included

#### 3.2.1.5 Calculation of relative trueness (RT), sensitivity (SE) and false positive ratio (FPR)

The calculations are presented in Table 40 (CFX 96 DW-without FDRS), Table 41 (CFX 96 DW-with FDRS), Table 42 (CFX Opus-without FDRS), Table 43 (CFX Opus-with FDRS).
Table 40 – Calculation of the relative trueness (RT), the sensitivity (SE) and the false positive ratio (FPR) CFX 96 DW- Without FDRS

<table>
<thead>
<tr>
<th>Category</th>
<th>Type</th>
<th>PA*</th>
<th>NA</th>
<th>PD</th>
<th>ND**</th>
<th>PPND</th>
<th>PPNA</th>
<th>SE_{alt} %</th>
<th>SE_{ref} %</th>
<th>RT %</th>
<th>FPR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Composite foods</td>
<td>a  Ready-to-eat</td>
<td>6</td>
<td>12</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>60,0</td>
<td>100,0</td>
<td>81,8</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>b  Ready-to-reheat</td>
<td>12</td>
<td>11</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>88,9</td>
<td>77,8</td>
<td>80,6</td>
<td>18,2</td>
</tr>
<tr>
<td></td>
<td>c  Confectionaries, pastries and egg products</td>
<td>6</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>100,0</td>
<td>85,7</td>
<td>95,0</td>
<td>30,0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>24</td>
<td>33</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td>82,9</td>
<td>85,7</td>
<td>84,9</td>
<td>15,2</td>
</tr>
<tr>
<td>2 Production environmental samples</td>
<td>a  Surfaces samples</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100,0</td>
<td>100,0</td>
<td>100,0</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>b  Dusts and wastes</td>
<td>9</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>90,9</td>
<td>90,9</td>
<td>90,0</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>c  Process water</td>
<td>9</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>100,0</td>
<td>81,8</td>
<td>90,0</td>
<td>12,5</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>28</td>
<td>27</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>96,9</td>
<td>90,6</td>
<td>93,3</td>
<td>3,7</td>
</tr>
<tr>
<td>3 Dairy products</td>
<td>a  Raw milk cheese (cow, ewe, goat)</td>
<td>6</td>
<td>13</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100,0</td>
<td>60,0</td>
<td>82,6</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>b  Other raw milk-based products (raw milk, cream, butter, fermented milk)</td>
<td>7</td>
<td>9</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>81,8</td>
<td>81,8</td>
<td>80,0</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>c  Pasteurized milk-based products (pasteurized cheese, ice cream, milk)</td>
<td>4</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>69,2</td>
<td>61,5</td>
<td>62,5</td>
<td>22,2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>17</td>
<td>31</td>
<td>11</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>82,4</td>
<td>67,6</td>
<td>74,6</td>
<td>6,5</td>
</tr>
<tr>
<td>All categories</td>
<td></td>
<td>69</td>
<td>91</td>
<td>19</td>
<td>13</td>
<td>0</td>
<td>8</td>
<td>87,1</td>
<td>81,2</td>
<td>84,0</td>
<td>8,8</td>
</tr>
</tbody>
</table>
Table 41 – Calculation of the relative trueness (RT), the sensitivity (SE) and the false positive ratio (FPR) CFX 96 DW- With FDRS

<table>
<thead>
<tr>
<th>Category</th>
<th>Type</th>
<th>PA*</th>
<th>NA</th>
<th>PD</th>
<th>ND**</th>
<th>PPND</th>
<th>PPNA</th>
<th>SE alt %</th>
<th>SE ref %</th>
<th>RT %</th>
<th>FPR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Composite foods</td>
<td>a Ready-to-eat</td>
<td>6</td>
<td>12</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>60,0</td>
<td>100,0</td>
<td>81,8</td>
<td>8,3</td>
</tr>
<tr>
<td></td>
<td>b Ready-to-reheat</td>
<td>12</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>88,9</td>
<td>77,8</td>
<td>80,6</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>c Confectionaries, pastries and egg products</td>
<td>6</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>100,0</td>
<td>85,7</td>
<td>95,0</td>
<td>8,3</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td>24</td>
<td>37</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>82,9</td>
<td>85,7</td>
<td>84,9</td>
<td>5,4</td>
</tr>
<tr>
<td>2 Production environmental samples</td>
<td>a Surfaces samples</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100,0</td>
<td>100,0</td>
<td>100,0</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>b Dusts and wastes</td>
<td>9</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>90,9</td>
<td>90,9</td>
<td>90,0</td>
<td>11,1</td>
</tr>
<tr>
<td></td>
<td>c Process water</td>
<td>9</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>91,7</td>
<td>83,3</td>
<td>85,0</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td>28</td>
<td>27</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>93,9</td>
<td>90,9</td>
<td>91,7</td>
<td>3,7</td>
</tr>
<tr>
<td>3 Dairy products</td>
<td>a Raw milk cheese (cow, ewe, goat)</td>
<td>5</td>
<td>13</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>90,0</td>
<td>60,0</td>
<td>78,3</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>b Other raw milk-based products (raw milk, cream, butter, fermented milk)</td>
<td>7</td>
<td>9</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>81,8</td>
<td>81,8</td>
<td>80,0</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>c Pasteurized milk-based products (pasteurized cheese, ice cream, milk)</td>
<td>3</td>
<td>11</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>61,5</td>
<td>61,5</td>
<td>58,3</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td>15</td>
<td>33</td>
<td>11</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>76,5</td>
<td>67,6</td>
<td>71,6</td>
<td>0,0</td>
</tr>
<tr>
<td>All categories</td>
<td></td>
<td>67</td>
<td>97</td>
<td>19</td>
<td>14</td>
<td>2</td>
<td>1</td>
<td>84,3</td>
<td>81,4</td>
<td>82,5</td>
<td>3,1</td>
</tr>
</tbody>
</table>

* PPNA not included ** PPND not included
## Table 42 – Calculation of the relative trueness (RT), the sensitivity (SE) and the false positive ratio (FPR) CFX Opus DW- Without FDRS

<table>
<thead>
<tr>
<th>Category</th>
<th>Type</th>
<th>PA*</th>
<th>NA</th>
<th>PD</th>
<th>ND**</th>
<th>PPND</th>
<th>PPNA</th>
<th>SE alt %</th>
<th>SE ref %</th>
<th>RT %</th>
<th>FPR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Composite foods</td>
<td>a Ready-to-eat</td>
<td>5</td>
<td>11</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>50,0</td>
<td>100,0</td>
<td>77,3</td>
<td>9,1</td>
</tr>
<tr>
<td></td>
<td>b Ready-to-reheat</td>
<td>12</td>
<td>12</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>88,9</td>
<td>77,8</td>
<td>80,6</td>
<td>8,3</td>
</tr>
<tr>
<td></td>
<td>c Confectionaries, pastries and egg products</td>
<td>6</td>
<td>11</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>100,0</td>
<td>85,7</td>
<td>95,0</td>
<td>18,2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>23</td>
<td>34</td>
<td>5</td>
<td>7</td>
<td>0</td>
<td>4</td>
<td>80,0</td>
<td>85,7</td>
<td>83,6</td>
<td>11,8</td>
</tr>
<tr>
<td>2 Production environmental samples</td>
<td>a Surfaces samples</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100,0</td>
<td>100,0</td>
<td>100,0</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>b Dusts and wastes</td>
<td>9</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>90,9</td>
<td>90,9</td>
<td>90,0</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>c Process water</td>
<td>9</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>100,0</td>
<td>81,8</td>
<td>90,0</td>
<td>12,5</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>28</td>
<td>27</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>96,9</td>
<td>90,6</td>
<td>93,3</td>
<td>3,7</td>
</tr>
<tr>
<td>3 Dairy products</td>
<td>a Raw milk cheese (cow, ewe, goat)</td>
<td>6</td>
<td>13</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100,0</td>
<td>60,0</td>
<td>82,6</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>b Other raw milk-based products (raw milk, cream, butter, fermented milk)</td>
<td>7</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>81,8</td>
<td>81,8</td>
<td>80,0</td>
<td>12,5</td>
</tr>
<tr>
<td></td>
<td>c Pasteurized milk-based products (pasteurized cheese, ice cream, milk)</td>
<td>4</td>
<td>11</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>69,2</td>
<td>61,5</td>
<td>62,5</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>17</td>
<td>32</td>
<td>11</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>82,4</td>
<td>67,6</td>
<td>74,6</td>
<td>3,1</td>
</tr>
<tr>
<td>All categories</td>
<td></td>
<td>68</td>
<td>93</td>
<td>19</td>
<td>14</td>
<td>0</td>
<td>6</td>
<td>86,1</td>
<td>81,2</td>
<td>83,5</td>
<td>6,5</td>
</tr>
</tbody>
</table>
Table 43 – Calculation of the relative trueness (RT), the sensitivity (SE) and the false positive ratio (FPR) CFX Opus DW- With FDRS

<table>
<thead>
<tr>
<th>Category</th>
<th>Type</th>
<th>PA*</th>
<th>NA</th>
<th>PD</th>
<th>ND**</th>
<th>PPND</th>
<th>PPNA</th>
<th>SE alt %</th>
<th>SE ref %</th>
<th>RT %</th>
<th>FPR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Composite foods</td>
<td>a  Ready-to-eat</td>
<td>5</td>
<td>11</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>50,0</td>
<td>100,0</td>
<td>77,3</td>
<td>9,1</td>
</tr>
<tr>
<td></td>
<td>b  Ready-to-reheat</td>
<td>12</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>88,9</td>
<td>77,8</td>
<td>80,6</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>c  Confectionaries, pastries and egg products</td>
<td>6</td>
<td>13</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100,0</td>
<td>85,7</td>
<td>95,0</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>23</td>
<td>37</td>
<td>5</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>80,0</td>
<td>85,7</td>
<td>83,6</td>
<td>2,7</td>
</tr>
<tr>
<td>2 Production</td>
<td>a  Surfaces samples</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100,0</td>
<td>100,0</td>
<td>100,0</td>
<td>0,0</td>
</tr>
<tr>
<td>environmental</td>
<td>b  Dusts and wastes</td>
<td>9</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>90,9</td>
<td>90,9</td>
<td>90,0</td>
<td>0,0</td>
</tr>
<tr>
<td>samples</td>
<td>c  Process water</td>
<td>9</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>91,7</td>
<td>83,3</td>
<td>85,0</td>
<td>0,0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>28</td>
<td>27</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>93,9</td>
<td>90,9</td>
<td>91,7</td>
<td>0,0</td>
</tr>
<tr>
<td>3 Dairy products</td>
<td>a  Raw milk cheese (cow, ewe, goat)</td>
<td>6</td>
<td>14</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100,0</td>
<td>66,7</td>
<td>87,0</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>b  Other raw milk-based products (raw milk, cream, butter, fermented milk)</td>
<td>7</td>
<td>9</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>81,8</td>
<td>81,8</td>
<td>80,0</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>c  Pasteurized milk-based products (pasteurized cheese, ice cream, milk)</td>
<td>3</td>
<td>11</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>61,5</td>
<td>61,5</td>
<td>58,3</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>16</td>
<td>34</td>
<td>10</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>78,8</td>
<td>69,7</td>
<td>74,6</td>
<td>0,0</td>
</tr>
<tr>
<td>All categories</td>
<td></td>
<td>67</td>
<td>98</td>
<td>18</td>
<td>16</td>
<td>0</td>
<td>1</td>
<td>84,2</td>
<td>82,2</td>
<td>83,0</td>
<td>1,0</td>
</tr>
</tbody>
</table>

* PPNA not included  ** PPND not included
A summary of the results is given in Table 44.

**Table 44 - Summary of results**

<table>
<thead>
<tr>
<th>All categories</th>
<th>CFX 96 DW w/o FDRS</th>
<th>CFX Opus DW w/o FDRS</th>
<th>CFX 96 DW w FDRS</th>
<th>CFX Opus DW w FDRS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity for the alternative method</strong></td>
<td>87,1</td>
<td>84,3</td>
<td>86,1</td>
<td>84,2</td>
</tr>
<tr>
<td><strong>Sensitivity for the reference method</strong></td>
<td>81,2</td>
<td>81,4</td>
<td>81,2</td>
<td>82,2</td>
</tr>
<tr>
<td><strong>Relative trueness</strong></td>
<td>84,0</td>
<td>82,5</td>
<td>83,5</td>
<td>83,0</td>
</tr>
<tr>
<td><strong>False positive ratio for the alternative method</strong></td>
<td>8,8</td>
<td>3,1</td>
<td>6,5</td>
<td>1,0</td>
</tr>
</tbody>
</table>

\[ SE_{alt} = \frac{(PA + PD)}{(PA + ND + PD)} \times 100\% \]
\[ SE_{ref} = \frac{(PA + ND)}{(PA + ND + PD)} \times 100\% \]
\[ RT = \frac{(PA + NA)}{N} \times 100\% \]
\[ FPR = \frac{(FP)}{NA} \times 100\% \]

With \( ND = ND + PPND \)
\( NA = NA + PPNA \)

3.2.1.6 *Analysis of discordant results*

The negative deviations for all categories are given in Table 45 and the positive deviations in Table 46.
## Table 45 - Negative deviations protocols 5 and 6

<table>
<thead>
<tr>
<th>Sample N°</th>
<th>Product</th>
<th>Strain inoculated</th>
<th>Inoculation level CFU/1 portion</th>
<th>Reference method: ISO 11290-1</th>
<th>PCR result Without FDRS</th>
<th>Agreement Without FDRS</th>
<th>PCR result With FDRS</th>
<th>Agreement With FDRS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CFX96DW</td>
<td>CFX Opus DW</td>
<td>CFX96DW</td>
<td>CFX Opus DW</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CQ</td>
<td>Result</td>
<td>CQ</td>
<td>Result</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3302</td>
<td>RTE (Sandwich tuna and crusty)</td>
<td>/</td>
<td>/</td>
<td>L. monocytogenes, L. innocua</td>
<td>N/A 33.78 -</td>
<td>N/A 33.93 -</td>
<td>N/A 34.23 -</td>
<td>N/A 35.61 -</td>
</tr>
<tr>
<td>3670</td>
<td>RTE (Sandwich ham and cheese)</td>
<td>/</td>
<td>/</td>
<td>L. seeligeri</td>
<td>40.54 36.26 +</td>
<td>39.74 34.07 +</td>
<td>38.72 36.02 +</td>
<td>N/A 36.50 +</td>
</tr>
<tr>
<td>749</td>
<td>Potato fritter with cheese</td>
<td>/</td>
<td>/</td>
<td>L. monocytogenes</td>
<td>N/A 36.82 -</td>
<td>N/A 35.87 -</td>
<td>N/A 36.30 -</td>
<td>N/A 35.96 -</td>
</tr>
<tr>
<td>761</td>
<td>RTE (vegetable and cheese)</td>
<td>/</td>
<td>/</td>
<td>L. monocytogenes</td>
<td>N/A 37.41 -</td>
<td>N/A 35.47 -</td>
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<th>Inoculation level CFU/1 portion</th>
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<th>PCR result With FDRS</th>
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* Analyses performed according to the COFRAC accreditation

ADRIA

Summary report (Version 0)

iQ-Check Listeria spp.

57/199

November 16, 2023
## Table 46 - Positive deviations protocols 5 and 6

<table>
<thead>
<tr>
<th>Sample N°</th>
<th>Product</th>
<th>Strain inoculated</th>
<th>Inoculation level CFU/test portion</th>
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<th>Alternative method: iQ-Check Listeria spp.- LSB II 18 h at 37°C</th>
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<th>Strain inoculated</th>
<th>Inoculation level CFU/test portion</th>
<th>Reference method: ISO 11290-1*</th>
<th>Alternative method: iQ-Check Listeria spp.- with prewarmed LSB II 18 h at 37°C</th>
<th>Agreement</th>
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<th>Type</th>
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Negative deviations

- Protocol 5:

Seven to nine deviations were observed for the categories tested with protocol 5 and concerns 2 artificially contaminated samples and 7 naturally contaminated samples. The presence of *Listeria monocytogenes* was confirmed in the enrichment broth for 1 sample (3670: sandwich,) and *Listeria* spp. strain (not belonging to *L. monocytogenes* species) for 2 samples (3304: sandwich, 3297: Falafel).

- Protocol 6:

Six to eight deviations were observed for the categories tested with protocol 6 and concerns 5 artificially contaminated samples and 3 naturally contaminated samples. The presence of *Listeria innocua* was confirmed in the enrichment broth for 2 samples (1729: Raw cow milk cheese, 3544: Pasteurized ewe milk cheese).

Positive deviations

- Protocol 5:

8 positive deviations were observed for the categories tested with protocol 5. They concern 3 artificially contaminated samples and 5 naturally contaminated samples.

- Protocol 6:

Ten to eleven positive deviations were observed for the categories tested with protocol 6. They concern 8 artificially contaminated samples and 3 naturally contaminated samples.

The analyses of discordant results according to the EN ISO 16140-2:2016 for all the categories is the following (See Tables 47 to 50).

As requested in the ISO 16140-2 (2016), the samples in negative agreement were tested using the same incubation time as the reference method (subculture of LSBII in Fraser broth for 24 h at 37°C before streaking onto selective agar plates); all the results were negative.
Table 47 - Analyses of discordant results

**CFX96 DW- Without FDRS**

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<th>N+</th>
<th>ND</th>
<th>PPND</th>
<th>PD</th>
<th>(ND+PPND)-PD</th>
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Table 48 - Analyses of discordant results-

**CFX96 DW- With FDRS**

<table>
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<th>Category</th>
<th>Type</th>
<th>N+</th>
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<th>PD</th>
<th>(ND+PPND)-PD</th>
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## Table 49 - Analyses of discordant results - 
**CFX Opus DW- Without FDRS**

<table>
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<th>PD</th>
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</table>

The observed values for ND+ PPND - PD meet the acceptability limit for each category as well as for all combined categories (calculated values ≤ AL), for both extraction protocols (with or without the optional FDRS step), and both Real-Time PCR instruments tested (CFX96 Deep Well or CFX Opus Deep Well).

## Table 50 - Analyses of discordant results - 
**CFX Opus DW- With FDRS**

<table>
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<th>PD</th>
<th>(ND+PPND)-PD</th>
<th>AL</th>
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<td>0</td>
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</table>

The observed values for ND+ PPND - PD meet the acceptability limit for each category as well as for all combined categories (calculated values ≤ AL), for both extraction protocols (with or without the optional FDRS step), and both Real-Time PCR instruments tested (CFX96 Deep Well or CFX Opus Deep Well).
3.2.1.7 Enrichment broth storage at 5 ± 3 °C for 72 h

111 enriched samples were tested again after storage of the enriched LSB II broths for 72h at 5°C ± 3°C with both extraction protocol and thermocyclers. The following changes were observed (see Table 51).

### Table 51 – Changes observed after enrichment broths storage

<table>
<thead>
<tr>
<th>Sample N°</th>
<th>Product</th>
<th>iQ-Check Listeria spp.</th>
<th>LSB II 18h at 37°C</th>
<th>LSB II 18h at 37°C + 72 h at 5°C</th>
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<tr>
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<td>With FDRS</td>
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<td>CFX 96 DW</td>
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</tr>
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<td>CFX 96 DW</td>
<td>CFX Opus</td>
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<td>CFX Opus</td>
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<td></td>
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<td>CFX 96 DW</td>
<td>CFX Opus</td>
</tr>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
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<td>RTE (Sandwich ham and cheese)</td>
<td>PA</td>
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<td>3308</td>
<td>Salmon nuggets</td>
<td>PA</td>
<td>PA</td>
<td>ND</td>
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<tr>
<td>733</td>
<td>Rinsing water</td>
<td>PPNA</td>
<td>ND</td>
<td>PPND</td>
</tr>
<tr>
<td>1729</td>
<td>Raw cow milk cheese</td>
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The analyses of discordant results become (See Tables 52 to 55).

### Table 52 - Analysis of discordant results after storage for 72 h at 5 ± 3°C - CFX96 DW- Without FDRS

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<th>Category</th>
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<th>PPND</th>
<th>PD</th>
<th>(ND+PPND)-PD</th>
<th>AL</th>
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All categories: 102, 13, 1, 19, -5, 5
### Table 53 - Analysis of discordant results after storage for 72 h at 5 ± 3°C - CFX96 DW - With FDRS

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### Table 54 - Analysis of discordant results after storage for 72 h at 5 ± 3°C - CFX Opus DW - Without FDRS

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## Table 55 - Analysis of discordant results after storage for 72 h at 5 ± 3°C - CFX Opus DW- With FDRS

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</tr>
<tr>
<td>c</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>-1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>3 Dairy products</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>11</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>13</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>7</td>
<td>0</td>
<td>11</td>
<td>-4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>All categories</td>
<td>102</td>
<td>14</td>
<td>1</td>
<td>19</td>
<td>-4</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

The calculated values for ND+ PPND - PD meet the acceptability limit for each category as well as for all the combined categories (calculated values ≤ AL) for both extraction protocols (with or without the optional FDRS step), and both Real-Time PCR instruments (CFX 96 Deep Well or CFX Opus Deep Well). Thus, the enriched LSB II broths can be kept at 5°C± 3°C for 72 h.

### 3.2.1.8 Confirmation

It was impossible to confirm the presence of *Listeria* spp in the enrichment broth after incubation time for 13 samples even if additional confirmation were carried out (5 Fraser broths, 5 AL and 5 Palcam). The samples concerned as well as the PCR results observed are given in Table 56.

Difference between tested conditions are observed with less unconfirmed positive results when using the FDRS protocol. Some of those unconfirmed positives could be therefore linked to the presence of free DNA in the test sample.

For samples confirmed positive by direct streaking onto selective agar plates, typical *Listeria* spp. colonies were observed for 85 samples on RAPID’L.mono plates, for 85 samples on RAPID’Listeria spp plates and 89 on AL plates.
### Table 56 – Positive PCR results not confirmed by cultural methods - protocols 5 and 6

<table>
<thead>
<tr>
<th>Sample N°</th>
<th>Product</th>
<th>Reference method : ISO 11290-1*</th>
<th>Alternative method: iQ-Check Listeria spp- LSB II 18 h at 37°C</th>
<th>Agreement</th>
<th>Category</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Without FDRS</td>
<td>With FDRS</td>
<td>CFX 96 DW</td>
<td>CFX Opus DW</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FAM Cq</td>
<td>IC Cq</td>
<td>Result</td>
<td>FAM Cq</td>
</tr>
<tr>
<td>110</td>
<td>Mini-involtini with cheese</td>
<td>N/A</td>
<td>34.89</td>
<td>-</td>
<td>44.95</td>
<td>33.75</td>
</tr>
<tr>
<td>749</td>
<td>Potato fritter with cheese</td>
<td>L. monocytogenes</td>
<td>N/A</td>
<td>36.82</td>
<td>-</td>
<td>43.22</td>
</tr>
<tr>
<td>106</td>
<td>RTE (vegetable macedoine)</td>
<td>N/A</td>
<td>33.31</td>
<td>-</td>
<td>32.96</td>
<td>33.05</td>
</tr>
<tr>
<td>3301</td>
<td>RTRH (Tomato, cereals)</td>
<td>N/A</td>
<td>39.10</td>
<td>32.93</td>
<td>+</td>
<td>33.58</td>
</tr>
<tr>
<td>3464</td>
<td>RTRH (Paella)</td>
<td>N/A</td>
<td>39.69</td>
<td>33.08</td>
<td>+</td>
<td>32.97</td>
</tr>
<tr>
<td>114</td>
<td>Pastry</td>
<td>N/A</td>
<td>36.50</td>
<td>33.16</td>
<td>+</td>
<td>37.32</td>
</tr>
<tr>
<td>118</td>
<td>Chocolate mousse</td>
<td>N/A</td>
<td>40.16</td>
<td>35.84</td>
<td>+</td>
<td>32.87</td>
</tr>
<tr>
<td>517</td>
<td>Chocolate mousse</td>
<td>N/A</td>
<td>38.89</td>
<td>33.97</td>
<td>+</td>
<td>38.26</td>
</tr>
<tr>
<td>961</td>
<td>Wastes (Salmon)</td>
<td>L. monocytogenes</td>
<td>N/A</td>
<td>34.89</td>
<td>-</td>
<td>34.30</td>
</tr>
<tr>
<td>733</td>
<td>Rinsing water</td>
<td>L. monocytogenes</td>
<td>29.33</td>
<td>32.25</td>
<td>+</td>
<td>29.24</td>
</tr>
</tbody>
</table>

* Analyses performed according to the COFRAC accreditation

ADRIA
Summary report (Version 0)
iQ-Check Listeria spp.

November 16, 2023

65/199
**Sample N°** | **Product** | **Reference method : ISO 11290-1** | **Alternative method: iQ-Check Listeria spp- LSB II 18 h at 37°C**
---|---|---|---
| | | **Without FDRS** | **With FDRS** | **Agreement** | **Without FDRS** | **With FDRS** |
| **CXF 96** | **CFX Opus** | **CXF 96** | **CFX Opus** | **Confirmation** | **CXF 96** | **CFX Opus** | **CXF 96** | **CFX Opus** |
| **FAM Cq** | **IC Cq** | **Result** | **FAM Cq** | **IC Cq** | **Result** | **FAM Cq** | **IC Cq** | **Result** | **FAM Cq** | **IC Cq** | **Result** | **FAM Cq** | **IC Cq** | **Result** |
| 1724 | Raw ewe milk | - | N/A | 32.65 | - | 39.24 | N/A | N/A | 32.55 | + / - / - | N/A | 32.55 | - | N/A | 32.55 | - | NA | PPNA | NA | NA | 3 | b |
| 2754 | Ice cream | - | 40.38 | 33.1 | 32.82 | N/A | N/A | 33.36 | N/A | 33.6 | - | PPNA | NA | NA | NA | 3 | c |
| 3077 | Half-skimmed milk powder | - | 40.07 | 32.26 | 32.09 | - | N/A | 32.83 | - | N/A | 32.68 | - | PPNA | NA | NA | NA | 3 | c |

* Analyses performed according to the COFRAC accreditation

ADRIA
Summary report (Version 0)
iQ-Check Listeria spp.
3.2.1.9 PCR inhibition

400 DNA extracts prepared with and without applying the FDRS protocol were tested after incubation time and 222 after enrichment broths storage with both thermocyclers (CFX96 Deep Well and CFX Opus Deep Well), only 13 PCR inhibitions (2.1%) were observed and concerns 5 samples (see Table 57). A 1/10 dilution in sterile water allowed to obtain a result.
### Table 57 - Samples with PCR inhibitions – protocols 5 and 6

<table>
<thead>
<tr>
<th>Sample N°</th>
<th>Product</th>
<th>Incubation time</th>
<th>Without FDRS</th>
<th>With DFRS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CFX96 DW</td>
<td>CFX Opus DW</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FAM Cq</td>
<td>IC Cq</td>
</tr>
<tr>
<td>118</td>
<td>Chocolate mousse</td>
<td>18h</td>
<td>40.16</td>
<td>35.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18h + 72 h</td>
<td>N/A</td>
<td>32.61</td>
</tr>
<tr>
<td>725</td>
<td>Wastes (Meat)</td>
<td>18h</td>
<td>N/A</td>
<td>34.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N/A</td>
<td>32.6</td>
</tr>
<tr>
<td>2747</td>
<td>Raw goat milk cheese</td>
<td>18h (pre-warmed)</td>
<td>N/A</td>
<td>N/A*</td>
</tr>
<tr>
<td>2750</td>
<td>Raw ewe milk cheese</td>
<td>18h (pre-warmed)</td>
<td>N/A</td>
<td>32.6</td>
</tr>
<tr>
<td>2752</td>
<td>Raw goat milk cheese</td>
<td>18h (pre-warmed) + 72h</td>
<td>34.97</td>
<td>32.6</td>
</tr>
</tbody>
</table>

*: Testing after 1/10 dilution of the extract
ac: atypical curve
3.2.2 Relative level of detection

The relative level of detection is the level of detection at $P = 0.50$ (LOD$_{50}$) of the alternative (proprietary) method divided by the level of detection at $P = 0.50$ (LOD$_{50}$) of the reference method.

The RLOD is defined as the ratio of the alternative and reference methods:

$$RLOD = \frac{LOD_{\text{Alt.}}}{LOD_{\text{Ref.}}}$$

The relative detection level is the smallest number of culturable micro-organisms that can be detected in the sample in 50% of occasions by the alternative and reference methods.

3.2.2.1 Experimental design

Two matrix/strain pairs were tested for the extension study using the following protocol:

- A negative control: 5 samples,
- A low contamination level providing fractional recovery data, with 20 replicates,
- A high contamination level, with 5 replicates.

A total plate count was performed to estimate the total microbial load on the day of analysis.

The extraction was performed using the Easy II protocol with and without the FDRS step. Two PCR instruments were tested: CFX 96 Deep Well and CFX Opus Deep Well.

The matrix/strain pairs tested are listed in Table 58.

Table 58 - Defined (matrix/strain) pairs for the RLOD determination

<table>
<thead>
<tr>
<th>Category</th>
<th>Matrix</th>
<th>Strain</th>
<th>Origin</th>
<th>Inoculation and storage condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Composite food</td>
<td>Deli salad: piémontaise</td>
<td><em>L. innocua</em> Ad3269</td>
<td>Vegetables mix</td>
<td>48-72 h at 3°C ± 2°C</td>
</tr>
<tr>
<td>2 Production environmental samples</td>
<td>Process water</td>
<td><em>Listeria monocytogenes</em> Ad2503</td>
<td>Environment from vegetables production site</td>
<td>48-72 h at 3°C ± 2°C</td>
</tr>
<tr>
<td>3 Dairy products</td>
<td>Raw ewe milk cheese</td>
<td><em>L. ivanovii</em> Ad1737</td>
<td>Raw milk cheese</td>
<td>48-72 h at 3°C ± 2°C</td>
</tr>
</tbody>
</table>
3.2.2.2 Calculation and interpretation of the RLOD

The raw data are given in Appendix 9.

The RLOD calculations were performed using the Excel spreadsheet available at [http://standards.iso.org/iso/16140](http://standards.iso.org/iso/16140) - RLOD (clause 5-1-4-2 Calculation and interpretation of RLOD) version 15.08.2015. The RLOD are given in Tables 59 and 60.

**Table 59 – Presentation of RLOD before and after confirmation of the alternative method results - CFX96 DW and Opus DW- Without FDRS**

<table>
<thead>
<tr>
<th>Category</th>
<th>Matrix / strain pair</th>
<th>RLOD</th>
<th>RLODL</th>
<th>RLODU</th>
<th>b=ln (RLOD)</th>
<th>sd(b)</th>
<th>z-Test statistic</th>
<th>p-value</th>
<th>AL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Composite food</td>
<td>Piemontaise L. innocua Ad3269</td>
<td>1,151</td>
<td>0,519</td>
<td>2,553</td>
<td>0,141</td>
<td>0,398</td>
<td>0,354</td>
<td>0,723</td>
<td></td>
</tr>
<tr>
<td>2 Production environmental samples</td>
<td>Process water L. mono Ad2503</td>
<td>2,175</td>
<td>0,968</td>
<td>4,890</td>
<td>0,777</td>
<td>0,405</td>
<td>1,918</td>
<td>0,055</td>
<td>2,5</td>
</tr>
<tr>
<td>3 Dairy products</td>
<td>Raw ewe milk cheese L. ivanovii Ad1737</td>
<td>1,000</td>
<td>0,491</td>
<td>2,037</td>
<td>0,000</td>
<td>0,356</td>
<td>0,000</td>
<td>1,000</td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td></td>
<td>1,321</td>
<td>0,864</td>
<td>2,018</td>
<td>0,212</td>
<td>1,312</td>
<td>0,189</td>
<td>/</td>
<td></td>
</tr>
</tbody>
</table>

**Table 60 – Presentation of RLOD before and after confirmation of the alternative method results - CFX96 DW and Opus DW- With FDRS**

<table>
<thead>
<tr>
<th>Category</th>
<th>Matrix / strain pair</th>
<th>RLOD</th>
<th>RLODL</th>
<th>RLODU</th>
<th>b=ln (RLOD)</th>
<th>sd(b)</th>
<th>z-Test statistic</th>
<th>p-value</th>
<th>AL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Composite food</td>
<td>Piemontaise L. innocua Ad3269</td>
<td>1,151</td>
<td>0,519</td>
<td>2,553</td>
<td>0,141</td>
<td>0,398</td>
<td>0,354</td>
<td>0,723</td>
<td></td>
</tr>
<tr>
<td>2 Production environmental samples</td>
<td>Process water L. mono Ad2503</td>
<td>2,175</td>
<td>0,968</td>
<td>4,890</td>
<td>0,777</td>
<td>0,405</td>
<td>1,918</td>
<td>0,055</td>
<td>2,5</td>
</tr>
<tr>
<td>3 Dairy products</td>
<td>Raw ewe milk cheese L. ivanovii Ad1737</td>
<td>0,843</td>
<td>0,409</td>
<td>1,740</td>
<td>-0,171</td>
<td>0,362</td>
<td>0,471</td>
<td>1,363</td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td></td>
<td>1,252</td>
<td>0,817</td>
<td>1,921</td>
<td>0,225</td>
<td>0,214</td>
<td>1,052</td>
<td>0,293</td>
<td>/</td>
</tr>
</tbody>
</table>

The LOD\(_{50\%}\) calculations according to Wilrich & Wilrich POD-LOD calculation program - version 11, 2022-10-12 test are given in Table 61.
### Table 61 – LOD$_{50}$ results

<table>
<thead>
<tr>
<th>Category</th>
<th>Food item</th>
<th>Strain</th>
<th>Level of detection at 50% (CFU / sample size) according to Wilrich &amp; Wilrich$^5$</th>
<th>Reference method</th>
<th>Alternative method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CFX96 DW</td>
<td>CFX Opus DW</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Without FDRS</td>
<td>With FDRS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Without FDRS</td>
<td>With FDRS</td>
</tr>
<tr>
<td>1 Composite foods</td>
<td>Piemontaise</td>
<td><em>Listeria innocua</em></td>
<td>0.5 [0.3-0.9]</td>
<td>0.5 [0.3-1.0]</td>
<td>0.5 [0.3-1.0]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ad3269</td>
<td></td>
<td>0.5 [0.3-1.0]</td>
<td>0.5 [0.3-1.0]</td>
</tr>
<tr>
<td>2 Production</td>
<td>Process water</td>
<td><em>Listeria monocytogenes</em></td>
<td>0.5 [0.3-0.8]</td>
<td>1.1 [0.6-2.0]</td>
<td>1.1 [0.6-2.0]</td>
</tr>
<tr>
<td>environmental samples</td>
<td></td>
<td>Ad2503</td>
<td></td>
<td>1.1 [0.6-2.0]</td>
<td>1.1 [0.6-2.0]</td>
</tr>
<tr>
<td>3 Dairy products</td>
<td>Raw ewe milk cheese</td>
<td><em>Listeria ivanovii</em></td>
<td>1.1 [0.7-1.9]</td>
<td>1.0 [0.6-1.6]</td>
<td>1.1 [0.7-1.9]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ad1737</td>
<td></td>
<td>1.0 [0.6-1.6]</td>
<td>1.0 [0.6-1.6]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.7 [0.5-0.9]</td>
<td>0.9 [0.7-1.2]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.8 [0.6-1.1]</td>
<td>0.9 [0.7-1.2]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.8 [0.6-1.1]</td>
<td>0.8 [0.6-1.1]</td>
</tr>
</tbody>
</table>

The RLOD values (using the confirmed alternative method results) meet the acceptability limit of 2.5 for unpaired studies, for all matrix/strain pairs tested.

The combined LOD$_{50}$ is 0.7 CFU/test portion for the reference method and from 0.8 to 0.9 CFU/test portion for the alternative method depending on the protocol tested (without or with FDRS) and thermocycler used (CFX96 Deep Well or CFX Opus Deep Well).

---

4 INTER-LABORATORY STUDY

The aim of the inter-Laboratory study is to determine the variability of the results obtained in different laboratories using identical samples and to compare these results with those obtained in the methods comparison study.

The inter-laboratory study was carried out in April 2007. Pasteurized milk was contaminated with *Listeria innocua* (L4) isolated from a raw milk cheese. The results were interpreted according to the ISO 16140-2:2016.

4.1 Study organisation

The study was carried out on pasteurized milk. The samples were inoculated with *Listeria innocua* L64 isolated from raw milk cheese. 13 collaborators were involved in the study.

Samples were inoculated individually. Each collaborator received:

- 24 samples for analysis with the reference method
- 24 samples for analysis with the alternative method using the Easy II extraction protocol
- 1 sample for aerobic mesophilic microflora enumeration.

4.2 Experimental parameters controls

4.2.1 Strain stability and background microflora stability

Strain stability was checked by inoculating the matrix at 3 CFU/25 ml and 30 CFU/25 ml. Enumerations were performed for the high contamination level and detection analyses were performed for the low contamination level after 24 h and 48 h storage at 5 ± 3°C. The results are given in Table 62.

<table>
<thead>
<tr>
<th>Day</th>
<th>Reference method (detection)</th>
<th>Alternative method (detection)</th>
<th>Enumeration CFU/25 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>-</td>
<td>-</td>
<td>34</td>
</tr>
<tr>
<td>Day 1</td>
<td>+</td>
<td>+</td>
<td>25</td>
</tr>
<tr>
<td>Day 2</td>
<td>+</td>
<td>+</td>
<td>33</td>
</tr>
</tbody>
</table>

No evolution was observed during storage at 5°C ± 3°C.
4.2.2 Contamination levels

The contamination levels and the sample codification were the following (see Table 63).

Table 63 - Contamination levels

<table>
<thead>
<tr>
<th>Level</th>
<th>Samples</th>
<th>Theoretical target level (b/25 ml)</th>
<th>True level (b/25 ml sample)</th>
<th>Low limit / 25 ml sample</th>
<th>High limit / 25 ml sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1 - 2 - 3 - 10 - 11 - 12 - 19 - 20</td>
<td>0</td>
<td>0</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>1</td>
<td>4 - 5 - 6 - 13 - 14 - 15 - 21 - 22</td>
<td>3</td>
<td>3.7</td>
<td>0.8</td>
<td>10.7</td>
</tr>
<tr>
<td>2</td>
<td>7 - 8 - 9 - 16 - 17 - 18 - 23 - 24</td>
<td>30</td>
<td>34.0</td>
<td>24.0</td>
<td>47.0</td>
</tr>
</tbody>
</table>

4.2.3 Logistic conditions

Temperature conditions are given in Table 64.

Table 64 - Sample temperatures at receipt

<table>
<thead>
<tr>
<th>Collaborators</th>
<th>Temperature measured by the probe (°C)</th>
<th>Temperature measured at receipt (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.1</td>
<td>5.8</td>
</tr>
<tr>
<td>B</td>
<td>12.1</td>
<td>0.3</td>
</tr>
<tr>
<td>C</td>
<td>4.6</td>
<td>5.7</td>
</tr>
<tr>
<td>D</td>
<td>3.7</td>
<td>6.6</td>
</tr>
<tr>
<td>E</td>
<td>4.7</td>
<td>Not communicated</td>
</tr>
<tr>
<td>F</td>
<td>4.2</td>
<td>6.0</td>
</tr>
<tr>
<td>G</td>
<td>3.6</td>
<td>15.5</td>
</tr>
<tr>
<td>H</td>
<td>3.3</td>
<td>Not communicated</td>
</tr>
<tr>
<td>I</td>
<td>4.2</td>
<td>5.5</td>
</tr>
<tr>
<td>J</td>
<td>2.1</td>
<td>7.4</td>
</tr>
<tr>
<td>K</td>
<td>4.2</td>
<td>5.5</td>
</tr>
<tr>
<td>L</td>
<td>11.2</td>
<td>15.0</td>
</tr>
<tr>
<td>M</td>
<td>3.3</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Labs B and L received their samples at Day 2. The temperatures at receipt were above 8°C. These Labs performed the analyses but their results were not taken into account for interpretation.
4.3 Results analysis

The raw data are given in Appendix 10.

4.3.1 Expert laboratory results

The results obtained by the expert laboratory are given in Table 65.

Table 65 – Results obtained by the expert Lab.

<table>
<thead>
<tr>
<th>Level</th>
<th>Reference method</th>
<th>Alternative method</th>
</tr>
</thead>
<tbody>
<tr>
<td>L0</td>
<td>0/8</td>
<td>0/8</td>
</tr>
<tr>
<td>L1</td>
<td>7/8</td>
<td>7/8</td>
</tr>
<tr>
<td>L2</td>
<td>8/8</td>
<td>8/8</td>
</tr>
</tbody>
</table>

4.3.2 Results observed by the collaborative laboratories

- **Aerobic mesophilic flora enumeration**

Depending on the Lab results, the enumeration levels varied from < 1 CFU/ml to 31 CFU/ml.

- **Listeria spp. detection**

13 collaborators participated to the study. The results obtained are provided in Table 66 (reference method) and Table 67 (alternative method).
Table 66 - Positive results by the reference method
(ALL the collaborators)

<table>
<thead>
<tr>
<th>Collaborators</th>
<th>Contamination level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L0</td>
</tr>
<tr>
<td>A</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>0</td>
</tr>
<tr>
<td>H</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
</tr>
<tr>
<td>J</td>
<td>0</td>
</tr>
<tr>
<td>K</td>
<td>0</td>
</tr>
<tr>
<td>L</td>
<td>0</td>
</tr>
<tr>
<td>M</td>
<td>0</td>
</tr>
</tbody>
</table>

Total: \( P_0 = 0 \) \( P_1 = 100 \) \( P_2 = 104 \)

Table 67 - Positive results (before and after confirmation)
by the alternative method (ALL the collaborators)

<table>
<thead>
<tr>
<th>Collaborators</th>
<th>Contamination level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L0</td>
</tr>
<tr>
<td></td>
<td>PCR result</td>
</tr>
<tr>
<td>A</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>0</td>
</tr>
<tr>
<td>H</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
</tr>
<tr>
<td>J</td>
<td>6</td>
</tr>
<tr>
<td>K</td>
<td>0</td>
</tr>
<tr>
<td>L</td>
<td>0</td>
</tr>
<tr>
<td>M</td>
<td>0</td>
</tr>
</tbody>
</table>

Total: \( P_0 = 9 \) \( C_0 = 0 \) \( CP_0 = 0 \) \( P_1 = 101 \) \( C_1 = 101 \) \( CP_1 = 101 \) \( P_2 = 104 \) \( C_2 = 104 \) \( CP_2 = 104 \)

According to the AFNOR technical rules, it is possible to include the results from a collaborator with maximum one cross contamination at Level 0. For this study, this rule was applied and the results from Lab J were excluded for interpretation (6 unspiked samples with positive PCR results not confirmed). Lab B also obtained
2 unspiked samples with positive PCR results not confirmed by cultural method but this Lab is also excluded for samples receipt at 12.1°C.

4.3.3 Results of the collaborators retained for interpretation

The results obtained with the 10 labs kept for interpretation are presented in Table 68 (reference method) and Table 69 (alternative method).

Table 68 - Positive results by the reference method
(Without Labs B, J and L)

<table>
<thead>
<tr>
<th>Collaborators</th>
<th>Contamination level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L0</td>
</tr>
<tr>
<td>A</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>0</td>
</tr>
<tr>
<td>H</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
</tr>
<tr>
<td>K</td>
<td>0</td>
</tr>
<tr>
<td>M</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>P₀ = 0</strong></td>
</tr>
</tbody>
</table>

Table 69 - Positive results (before and after confirmation)
by the alternative method (Without Labs B, J and L)

<table>
<thead>
<tr>
<th>Collaborators</th>
<th>Contamination level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L0</td>
</tr>
<tr>
<td></td>
<td>PCR result</td>
</tr>
<tr>
<td>A</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>0</td>
</tr>
<tr>
<td>H</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
</tr>
<tr>
<td>K</td>
<td>0</td>
</tr>
<tr>
<td>M</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>P₀ = 1</strong></td>
</tr>
</tbody>
</table>
4.4 Calculation and interpretation

4.4.1 Calculation of the specificity percentage (SP)

The percentage specificities (SP) of the reference method and of the alternative method, using the data after confirmation, based on the results of level L0 are the following (See Table 70).

**Table 70 - Percentage specificity**

<table>
<thead>
<tr>
<th>Specificity for the reference method</th>
<th>( SP_{ref} = \left( 1 - \frac{P_0}{N} \right) \times 100 % = 100 % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity for the alternative method</td>
<td>( SP_{alt} = \left( 1 - \frac{CP_0}{N} \right) \times 100 % = 100 % )</td>
</tr>
</tbody>
</table>

\( N \): number of all L0 tests  
\( P_0 \): total number of false-positive results obtained with the blank samples before confirmation  
\( CP_0 \): total number of false-positive results obtained with the blank samples

4.4.2 Calculation of the sensitivity (SE_{alt}), the sensitivity for the reference method (SE_{ref}), the relative trueness (RT) and the false positive ratio for the alternative method (FPR)

Fractional positive results were obtained for the low inoculation level (L1). This inoculation level was retained for calculation.

A summary of the results of the collaborators retained for interpretation, and obtained with the reference and the alternative methods for Level 1 is provided in Table 71.

**Table 71 - Summary of the obtained results with the reference method and the alternative method for Level 1**

<table>
<thead>
<tr>
<th>Level</th>
<th>Response</th>
<th>Reference method positive (R+)</th>
<th>Reference method negative (R-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alternative method positive (A+)</td>
<td>Positive agreement (A+/R+)  ( PA = 78 )</td>
<td>Positive deviation (R-/A+)  ( PD = 1 )</td>
</tr>
<tr>
<td></td>
<td>Alternative method negative (A-)</td>
<td>Negative deviation (A-/R+)  ( ND = 1 ) (PPND = 0)</td>
<td>Negative agreement (A-/R-)  ( NA = 0 ) (PPNA = 0)</td>
</tr>
</tbody>
</table>
Based on the data summarized in Table 71, the values of sensitivity of the alternative and reference methods, as well as the relative trueness and false positive ratio for the alternative method taking account the confirmations, are the following (See Table 72).

Table 72 - Sensitivity, relative trueness and false positive ratio percentages

| Level 1 |
|------------------|------------------|
| Sensitivity for the alternative method: | \[ SE_{alt} = \frac{(PA+PD)}{(PA+PD+ND)} \times 100\% = 98.8\% \] |
| Sensitivity for the reference method: | \[ SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)} \times 100\% = 98.8\% \] |
| Relative trueness | \[ RT = \frac{(PA+NA)}{N} \times 100\% = 97.5\% \] |
| False positive ratio for the alternative method | \[ FPR = \frac{FP}{NA} \times 100\% = / \] |

4.4.3 Interpretation of data

One negative deviation was observed for Lab K (sample 13); no typical colony was observed on RAPID’Listeria spp. plates for this sample.

For an unpaired study design, the difference between (ND – PD) is calculated for the level(s) where fractional recovery is obtained (L1). The observed value found for (ND – PD) shall not be higher than the AL. The AL is defined as \([(ND – PD)_{\text{max}}]\) and calculated per level where fractional recovery is obtained as described below using the following three parameters:

\[(p^+)_{\text{ref}} = \frac{P_x}{N_x}\]

where

\[P_x = \text{number of samples with a positive result obtained with the reference method at level L1 for all the collaborators}\]

\[N_x = \text{number of samples tested at level L1 with the reference method by all the collaborators}\]

\[(p^+)_{\text{alt}} = \frac{CP_x}{N_x}\]

where

\[CP_x = \text{number of samples with a confirmed positive result obtained with the alternative method at level L1 for all the collaborators}\]

\[N_x = \text{number of samples tested at level L1 with the alternative method by all the collaborators}\]
\[(ND-PD)_{\text{max}} = \sqrt[3]{N_x \times \left( (p^+)_{\text{ref}} + (p^+)_{\text{alt}} - 2 \left( (p^+)_{\text{ref}} \times (p^+)_{\text{alt}} \right) \right)}\]

where

\[N_x = \text{number of samples tested for level } L_1 \text{ with the reference method by all the collaborators.}\]

In this study, fractional recovery was observed at Level 1. The calculations are the following for 10 labs, according to the EN ISO 16140-2:2016:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N_x)</td>
<td>88</td>
</tr>
<tr>
<td>((p^+)_{\text{ref}})</td>
<td>1.0</td>
</tr>
<tr>
<td>((p^+)_{\text{alt}})</td>
<td>1.0</td>
</tr>
<tr>
<td>(AL = (ND - PD)_{\text{max}})</td>
<td>2.43</td>
</tr>
<tr>
<td>(ND - PD)</td>
<td>0</td>
</tr>
</tbody>
</table>

**Conclusion**

\[ND - PD < AL\]

The EN ISO 16140-2:2016 requirements are fulfilled as \((ND - PD)\) meet the \(AL\).

There is indeed no difference between the sensitivity of the compared methods, and the alternative method complies with the reproducibility conditions.

### 4.4.4 Evaluation of the LOD \(_{50\%}\), LOD \(_{95\%}\) and RLOD between laboratories

The LOD \(_{50\%}\), the LOD \(_{95\%}\) and the RLOD was calculated using the EN ISO 16140-2:2016 Excel spreadsheet available at [http://standards.iso.org/iso/16140](http://standards.iso.org/iso/16140) - RLOD (clause 5-1-4-2 Calculation and interpretation of RLOD) version 28.06.2017. The results are given only for information (see Table 73).

**Table 73 - LOD \(_{50\%}\), LOD \(_{95\%}\) and RLOD**

<table>
<thead>
<tr>
<th>Method</th>
<th>LOD (_{50%})</th>
<th>LOD (_{95%})</th>
<th>RLOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>0.59 [0.37;0.92]</td>
<td>2.53 [1.61;3.98]</td>
<td>1.00 [0.59;1.69]</td>
</tr>
<tr>
<td>Alternative</td>
<td>0.59 [0.37;0.92]</td>
<td>2.53 [1.61;3.98]</td>
<td></td>
</tr>
</tbody>
</table>
CONCLUSION

The *method comparison study conclusions* are:

*For the initial validation study and extension/renewal studies - LSB protocol*

- In the sensitivity study, 6 categories were tested: 5 food categories and the environmental samples. The ND - PD meet the acceptability limits (AL) for each of the individual category, and as well for the 6 tested categories whatever the combination of protocols tested.

- The number of positive presumptive non-confirmed samples (PPNA) was significantly reduced by applying the FDRS protocol prior the Easy II lysis extraction protocol for the environmental samples.

- The Relative Levels of Detection (RLOD) are all below the AL fixed at 2.5 for the unpaired data study whatever the matrix/strain pairs.

- The inclusivity and exclusivity testing gave the expected results for the 51 target strains and the 32 non-target strains tested in 2005 and 2006 with the Standard II and the Easy II protocols as well as for the 50 non target strains tested for the extension study performed in 2019 (LSB 22h ± 4h at 37°C, with or without FDRS).

- It is possible to store the enrichment broth for 72 h at 5 ± 3°C.

- The alternative method allows a one-day screening of the negative samples.

- The alternative method fulfils all the EN ISO 16140-2:2016 and AFNOR technical rules (PR revision 7).

*For the extension study for the use of LSB II medium (2023)*

- The extension study scheme corresponds to an UNPAIRED study design as the alternative and reference methods have different enrichment procedure.

- In the sensitivity study, 3 categories were tested: two food category and the production environmental samples. The calculated values for ND+ PPND - PD meet the acceptability limits (AL) for each of the individual category, and as well for the 3 combined categories whatever the protocols tested (without or with FDRS) and thermocycler used (CFX96 Deep Well or CFX Opus Deep Well).
The number of positive presumptive non-confirmed samples (PPNA and PPND) was significantly reduced by applying the FDRS protocol.

It is possible to store the LSB II enriched samples for 72 h at 5°C ± 3°C before proceeding to PCR test and confirmation.

The Relative Levels of Detection (RLOD) are all below the AL fixed at 2.5 for the unpaired data study whatever the matrix/strain pairs tested.

The inter-laboratory study conclusions are:

- The data and interpretations comply with the EN ISO 16140-2:2016 requirements. The iQ-Check Listeria spp. method is considered equivalent to the ISO standard.

Quimper, 16 November 2023

Florian QUERO
Technical Study Manager
Validation of Alternative methods

Maryse RANNOU
Project Manager
Validation of Alternative methods

I hereby attest to the validation of the results of the analyses carried out under the COFRAC accreditation. I hereby attest to the validation of the verification of the conformity of the report (opinion and interpretation).
Appendix 1 – Flow diagrams of the alternative method: iQ-Check® *Listeria* spp.

**Enrichment in LSB broth**

25 g + 225 ml LSB broth at room temperature

- 1 swab + 10 ml LSB broth\(^6\)
- 1 sponge + 100 ml LSB broth
- 1 wipe + 225 ml LSB broth

**Standard II lysis protocol**

- 23 h ± 1 h at 30°C\(^7\)
- Aliquot 1.5 ml in an Eppendorf tube
- Centrifugation for 5 min at 10 000 - 12 000 g
- Discard supernatant
- Add 250 µl lysis reagent with lysis beads
- Mix by pipeting up and down
- Agitation for 3 min ± 1 min with the “Disruptor Genie”
- Heat treatment for 15 - 20 min at 95 - 100°C
- Vortex at high speed
- Centrifugation for 5 min at 10 000 g

**Easy II lysis protocol**

- 25 h ± 1 h at 30°C\(^4\)
- Add 100 µl lysis reagent with lysis beads in a tube or a Deep Well plate
- Add 100 µl enriched LSB broth
- Agitation for 3 min ± 1 min with the cell disruptor (for tubes only)
- Incubation at 95 - 100°C for 15 - 20 min in a heat block (tubes) or in the plate agitator-incubator (plates and tubes) under agitation at 1300 rpm
- Centrifugation for 5 min at 10 000 g

**FDRS protocol** (production environmental samples)

- Transfer G1 to the activation buffer (G2). Mix by inverting the tube
- Pipette 10 µl of activate reagent into the bottom of each well of a 96-Deep Well microplate
- Add 100 µl of decanted enriched LSB per well
- Seal the Deep Well microplate with the X-Pierce sealing film
- Incubate in the thermoshaker without shaking for 15 to 30 min at 37°C
- Transfer 5 µl of supernatant in a PCR tube containing 45 µl or mix PCR reagents

\(^6\) For sampling after cleaning process pre-moisten
- 1 swab + 1 ml broth universal neutralizing (+ 9 ml LSB)
- 1 sponge + 10 ml broth universal neutralizing (+ 90 ml LSB)
- 1 wipe + BPW + 10 % neutralizing agent (+ 225 ml LSB)

\(^7\) *During the validation study a subculture in Fraser broth for 24 h at 37°C was performed before streaking onto O&A and Palcam plates for negative PCR samples*
PCR using the CFX96 or the CFX96 Deep Well thermocycler (2 APF tested)

 Confirmation of positive PCR results

 Steaking
 0.1 ml onto RAPID’Listeria Agar
 Or 0.1 ml onto RAPID’L mono Agar
 Or 10µL onto AL or Palcam

 Incubation for 24 - 48 h at 37°C ± 1°C

 Presence of typical colonies allows to confirm the positive PCR results

---

During the validation study, the typical colonies were confirmed using the tests described in the reference method (Catalase, Gram) and 1 or 2 colonies were identified using mini-biochemical galleries.
Enrichment in LSB II broth

For food products:
25 g + 225 ml LSB II broth at room temperature (composite food)
or prewarmed (Dairy products)

For environmental samples:
25 g + 225 ml LSB II broth at room temperature
1 swab + 10 ml LSB II broth or rewarmed (Dairy products)
1 sponge + 100 ml LSB II broth at room temperature
1 wipe + 225 ml LSB II broth at room temperature

Incubation 18 h - 26 h at 37°C

Possibility to store the enriched samples for 72 h at 5°C±

FDRS protocol (optional)
Rehydrate Reagent G1 in 1 ml distilled water for 5 - 10 min at room temperature

Transfer G1 to the activation buffer (G2). Mix by inverting the tube

Pipe 10 µl of activate reagent into the bottom of each well of a 96-Deep Well microplate

Add 100 µl of decanted enriched LSB II per well

Seal the Deep Well microplate with the X-Pierce sealing film

Incubate in the thermoshaker without shaking for 15 to 30 min at 37°C

Transfer 5 µl of supernatant in a PCR tube containing 45 µl of mix PCR reagents

PCR using the CFX96 Deep Well or the CFX OPUS Deep Well thermocycler (APF Fast tested)

Confirmation of positive PCR results

Easy II protocol:
Add 100 µl lysis reagent with lysis beads in a tube or a Deep Well plate

Add 100 µL of decanted enriched LSB II or 100 µL FDRS treated sample

Agitation for 3 min ± 1 min with the cell disruptor (for tubes only)

Incubation at 95 - 100°C for 15 - 20 min in a heat block (tubes) or in the plate agitator-incubator (plates and tubes) under agitation at 1300 rpm

Centrifugation for 5 min at 10 000 g

Transfer 0.1 ml onto Agar Listeria, RAPID'Listeria Agar, and RAPID'L.mono Agar

Incubation 24 h at 37°C ± 1°C

For sampling after cleaning process pre-moisten
- 1 swab + 1 ml broth universal neutralizing (+ 9 ml LSB)
- 1 sponge + 10 ml broth universal neutralizing (+ 90 ml LSB)
- 1 wipe + BPW + 10 % neutralizing agent (+ 225 ml LSB)
Reference method protocol:
10 µl onto O&A (AL) and Palcam
Incubation for 24 - 48 h at 37°C ± 1°C

Presence of typical colonies allows to confirm the positive PCR results
for the alternative confirmation protocol
For the reference method protocol, the typical colonies are confirmed
using the tests described in the ISO method*

* During the validation the colonies were also tested by biochemical gallery for identification

Test portion (25 g or 25 ml) + 225 ml Half Fraser broth
- 1 swab + 10 ml Half Fraser broth\(^\text{10}\)
- 1 sponge + 100 ml Half Fraser broth
- 1 wipe + 225 ml Half Fraser broth

\[\downarrow\]

Primary enrichment medium (Half Fraser broth)

\[\downarrow\]

Incubation at 30°C ± 24 h to 26 h

0.1 ml of culture in 10 ml of secondary enrichment medium (Fraser broth)

\[\downarrow\]

Incubation at 37°C ± 1°C for 24 h ± 2 h

Plating out on Agar *Listeria* according to Ottaviani and Agosti and second selective medium

\[\downarrow\]

Incubation of Agar *Listeria* according to Ottaviani and Agosti for 24 h ± 2 h and an additional 24 h ± 2 h at 37°C ± 1°C

Incubation of second selective medium according to the chosen medium as specified by the manufacturer

\[\downarrow\]

Confirmation

<table>
<thead>
<tr>
<th>Target</th>
<th>Gram</th>
<th>Catalase</th>
<th>Beta hemolysis</th>
<th>CAMP test</th>
<th>Carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Listeria</em> spp</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{10}\) For sampling after cleaning process pre-moisten
- 1 swab + 1 ml broth universal neutralizing (+ 9 ml Half-Fraser)
- 1 sponge + 10 ml broth universal neutralizing (+ 90 ml Half-Fraser)
- 1 wipe + BPW + 10 % neutralizing agent (+ 225 ml Half-Fraser)
Appendix 3 – Artificial contamination of the samples (initial validation study, IPL, ISHA and extension/renewal, ADRIA)

<table>
<thead>
<tr>
<th>Code</th>
<th>Nom</th>
<th>Strain</th>
<th>Artificial contamination</th>
<th>Injury applied</th>
<th>Injury evaluation</th>
<th>CFU/sample</th>
<th>Final result</th>
</tr>
</thead>
<tbody>
<tr>
<td>O23</td>
<td>Goat cheese</td>
<td>L7</td>
<td>Listeria monocytogenes ½ a</td>
<td>Cheese rind</td>
<td>45 minutes at 50°C, 30 minutes at -80°C</td>
<td>0,4</td>
<td>5,4</td>
</tr>
<tr>
<td>O27</td>
<td>Raw milk</td>
<td>L7</td>
<td>Listeria monocytogenes ½ a</td>
<td>Cheese rind</td>
<td>45 minutes at 50°C, 30 minutes at -80°C</td>
<td>0,4</td>
<td>10,8</td>
</tr>
<tr>
<td>O28</td>
<td>Raw milk</td>
<td>L7</td>
<td>Listeria monocytogenes ½ a</td>
<td>Cheese rind</td>
<td>45 minutes at 50°C, 30 minutes at -80°C</td>
<td>0,4</td>
<td>13,5</td>
</tr>
<tr>
<td>P9</td>
<td>Vegetables mix</td>
<td>L47</td>
<td>Listeria monocytogenes ½ a</td>
<td>Roasted apples</td>
<td>45 minutes at 50°C, 30 minutes at -80°C</td>
<td>2,1</td>
<td>16,0</td>
</tr>
<tr>
<td>P10</td>
<td>Cali flower and</td>
<td>L47</td>
<td>Listeria monocytogenes ½ a</td>
<td>Roasted apples</td>
<td>45 minutes at 50°C, 30 minutes at -80°C</td>
<td>2,1</td>
<td>16,0</td>
</tr>
<tr>
<td>P20</td>
<td>Pasteurized milk</td>
<td>L37</td>
<td>Listeria monocytogenes ½ b</td>
<td>Raw milk cheese</td>
<td>45 minutes at 50°C, 30 minutes at -80°C</td>
<td>0,5</td>
<td>6,0</td>
</tr>
<tr>
<td>P21</td>
<td>Goat cheese</td>
<td>L37</td>
<td>Listeria monocytogenes ½ b</td>
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## Validation study (IPL)

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<th>Injury evaluation</th>
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<th>Final result</th>
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<tr>
<td>2019</td>
<td>1658</td>
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<td>RTE salad (ham)</td>
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<td>L. monocytogenes Ad669 + L. innocua Ad671</td>
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<td>RTRH Quiche Lorraine</td>
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<td>Pastry</td>
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<td>Pastry</td>
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<td>RTRH Quiche Lorraine</td>
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<td>2-0-1-3-1, 1,4</td>
<td>+ + 1 b</td>
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<td>Couscous poulet merguez</td>
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<td>2-0-1-3-1, 1,4</td>
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**Summary report** (Version 0)

IQ Check *Listeria* spp.
## Summary report (Version 0)

I.Q. Check *Listeria spp.*

### Artificial contaminations

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<th>Ref</th>
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<th>Product</th>
<th>Strain</th>
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<td>L. innocua Ad1277</td>
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<td>Pastry</td>
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<td>Rillette</td>
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<td>Pastry</td>
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<td>Omelette</td>
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<td>Rocamadour au lait cru</td>
<td>Raw milk cheese</td>
<td>L. monocytogenes Ad252 + L. welshimeri Ad1667</td>
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### Renewal study (ADRIA Développement, 2019)

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**Type**

- a: Artificial contaminations
- c: Global result

**Inoculation level (CFU/sample)**

- Enumeration
- Mean

**Summary report** (Version 0)

IQ Check *Listeria* spp.
## Artificial contaminations

<table>
<thead>
<tr>
<th>Date of analysis</th>
<th>Ref</th>
<th>Product (French name)</th>
<th>Product</th>
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<th>Origin</th>
<th>Injury protocol</th>
<th>Inoculation level (CFU/sample)</th>
<th>Global result</th>
<th>Type</th>
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<td>Environment</td>
<td>Seeding 48h 3°C±2°C</td>
<td>2-2-4-1-1</td>
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<td></td>
<td>Eau rinçage (thon)</td>
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<td>Environment + Environment</td>
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<td>1.0+1,6</td>
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## Renewal study (ADRIA Développement, 2019)

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<td>Environment +Environment Seeding 48h 3°C±2°C</td>
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<td>Chiffonnette après nettoyage plan de travail (environnement laitier)</td>
<td>Wipe (dairy environment)</td>
<td>L.monocytogenes Ad2600</td>
<td>Environment Seeding 48h 3°C±2°C</td>
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<td>Chiffonnette cutter (environnement végétaux)</td>
<td>Wipe (vegetable environment)</td>
<td>L.monocytogenes Ad2600</td>
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<td>2,6  - b</td>
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</table>
Appendix 4 - Sensitivity study: raw data (initial validation study, IPL, ISHA and extension/renewal, ADRIA)

### IPL - Legend

**Total bacteria growth**
- Ø : no growth
- L = low
- M = medium
- H = high

**Distribution of flora**
- A = pure culture of suspicious colonies
- B = mix with a majority of suspicious colonies
- C = mix with a minority of suspicious colonies
- D = mix with rare suspicious colonies
- E : no suspicious colony

(x) : x typical colonies of *Listeria* if x ≤ 5
(a) : Presence of 2 types of colonies (L. *monocytogenes* and L. *spp*)

### Results

pos : positive  
neg : negative

### ADRIA – Legend

**Bold typing : artificially inoculated samples**

**Listeria detection results:**
- H-: characteristic *Listeria* colonies without halo
- H+: characteristic *Listeria* colonies with halo
- -: no typical colonies but presence of background microflora
- st: plate without any colony
- i: PCR inhibition
- *: Lysate d1/10
- PA: positive agreement
- NA: negative agreement
- ND: negative deviation
- PD: positive deviation
- PPNA: positive presumptive negative agreement
- PPND : positive presumptive negative deviation
- NC: non-characteristic colony on TSYEA
- d: doubtful colony
- F1: Fraser 1
- AL Agar *Listeria*
- Pal Palcam
- RLM RAPID’L. *mono*
- RLSP RAPID’Listeria spp.

Analyses performed according to the COFRAC accreditation (ADRIA)

Samples analysed in 2006

Samples analysed in 2008
<table>
<thead>
<tr>
<th>Date of analysis</th>
<th>Ref</th>
<th>Product (French name)</th>
<th>Product</th>
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<th>Confirmation</th>
<th>Final result</th>
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<td>Q17</td>
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<td>Salad</td>
<td>Ø</td>
<td>Ø</td>
<td>Ø</td>
<td>-</td>
<td>-</td>
<td>NA NA 1 a</td>
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<td>2008 A6</td>
<td>A6</td>
<td>Sandwich crabe</td>
<td>RTE salad</td>
<td>+LA +AX   +MA +HMA +L.monocytogenes</td>
<td>+ 17.53</td>
<td>+ 15.01</td>
<td>+</td>
<td>+A MA +MA</td>
<td>L.monocytogenes</td>
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<td>2008 B18</td>
<td>B18</td>
<td>Tabouleh</td>
<td>Tabouleh</td>
<td>-LE -LE  -LE -LE</td>
<td>- HA -</td>
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<td>165</td>
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<td>RTE salad (ham)</td>
<td>- - H-d +j</td>
<td>L.welshimeri</td>
<td>+</td>
<td>20.02</td>
<td>+25.34</td>
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<td>1659</td>
<td>Trio cho juambon et comté</td>
<td>RTE salad (ham cabbage and cheese)</td>
<td>st st - -</td>
<td>/</td>
<td>-</td>
<td>20.87</td>
<td>+25.69</td>
<td>+ H+ + /</td>
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<td>+ + L.seeigleri</td>
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<td>st st st st</td>
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<td>RTE salad (grapefruit)</td>
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<td>+32.66</td>
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<td>+</td>
<td>+</td>
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## COMPOSITE FOODS

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<td>Spaghettis nature</td>
<td>Pasta</td>
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<td>Quiche lorraine</td>
<td>RTRH (Paella)</td>
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<td>Quiche lorraine</td>
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### Analyzing Results

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### Summary Report

- **ADRIA**: 100/199
- **IQ Check Listeria spp.**: 16 November 2023

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**Alternative method: iQ Check Listeria spp.**

**After incubation time**

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## MEAT PRODUCTS

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### Summary report (Version 0)

iQ Check Listeria spp.
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## MEAT PRODUCTS

**Reference method NF EN ISO 11290-1**

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## DAIRY PRODUCTS

### Alterantive method: iQ-Check Listeria spp

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## DAIRY PRODUCTS

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### Summary report (Version 0)

iQ Check Listeria spp.
## DAIRY PRODUCTS

### Alternative method: IQ-Check Listeria spp

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<td>Napa cabbage</td>
<td>Ø Ø Ø Ø Ø Ø Ø Ø / /</td>
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<td>Poêlée de légumes</td>
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<td>18.52 + 16.22 + 20.93 +</td>
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<td>+MB</td>
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## ENVIRONMENTAL SAMPLES

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**Summary report** (Version 0)

iQ Check Listeria spp.
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**Note:**
- Additional columns and rows may be present in the table, providing more detailed information about the environmental samples and the results of the laboratory analysis.
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## ENVIRONMENTAL SAMPLE (Extension study, 2019 – ADRIA Développement)

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* Analyses performed according to the COFRAC accreditation

ADRIA

Summary report (Version 0)

iQ Check Listeria spp.

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16 November 2023
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Summary report (Version 0)
iQ Check Listeria spp.
16 November 2023
## ENVIRONMENTAL SAMPLE (Extension study, 2019 – ADRIA Développement)

### Reference method NF EN ISO 11290-1*

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<td>H+</td>
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<td>+</td>
<td>H+</td>
<td>*</td>
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<td>H+</td>
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<td>RTE sandwich (tuna)</td>
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<td>L.innocua</td>
<td>-/ 24,26/</td>
<td>-/+/-</td>
<td>26,5</td>
<td>+</td>
<td>L.monocytogenes/ L.aes舆geri</td>
<td>+</td>
<td>+</td>
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<td>Eclair chocolat</td>
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<td>-</td>
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<td>l</td>
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<td>36,85/-36,80/-35,51</td>
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<td>(Fraser/ AL/Pakam)</td>
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<td>-</td>
<td>H+d</td>
<td>-</td>
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**Note:** The results indicate the presence or absence of Listeria monocytogenes. Positive results are marked with a '+'.
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<tr>
<th>Date of analysis</th>
<th>Product (French name)</th>
<th>Reference method NF EN ISO 11290-1</th>
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<th>MEAT PRODUCTS</th>
<th>Alternative method: iQ-Check Listeria spp.</th>
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<th>Agreement</th>
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<td>L. monocytogenes</td>
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<td>Viande de boeuf hachée</td>
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<td>2006 K5</td>
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<td>L. innocua</td>
<td>+ +</td>
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<td>+ +</td>
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<td>2006 O14</td>
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<td>2006 O7</td>
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<tr>
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<td>Braits</td>
<td>Sausages</td>
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<td>+ +</td>
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<td>2006 A2 2</td>
<td>Mini boulettes de boeuf</td>
<td>Mini beef balls</td>
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<td>+ +</td>
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<tr>
<td>2005 AAT 0</td>
<td>Merguez hallal</td>
<td>Merguez</td>
<td>-ME +ME +MA +MA</td>
<td>L. welshimeri</td>
<td>+ +</td>
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**Note:** After enrichment broth storage for 72h at 5°C ± 3°C.
## MEAT PRODUCTS

<table>
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<tr>
<th>Date of analysis</th>
<th>Product (French name)</th>
<th>Product</th>
<th>Reference method NF EN ISO 11290-1</th>
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<td>+LA +MB +MA L.monocytogenes</td>
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<td>Chipolatas</td>
<td>Sausages</td>
<td>+LA +LA +MB +MA L.monocytogenes</td>
<td>+ + + + L.welshimeri</td>
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<td>Saucisse biologique</td>
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<td>-LE -LE +HA +LA L.innocua</td>
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<tr>
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<td>Jambon fumé</td>
<td>Smokeham</td>
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<td>Merguez</td>
<td>-LE -LE Ø -LE</td>
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<td>Bacon</td>
<td>Ø Ø</td>
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**ADRIA**

Summary report (Version 0)

iQ-Check Listeria spp.
<table>
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<tr>
<th>Date of analysis</th>
<th>Product (French name)</th>
<th>Product</th>
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<td>&lt;26.01*</td>
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<td>/</td>
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<td>24.08 +</td>
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<td>-34.42* +</td>
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<td>31.05 +</td>
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<td>20.05 +</td>
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<td>2015 5344</td>
<td>Luneburger</td>
<td>Raw milk cheese</td>
<td>Ø +MA +LA + H+ + L.monocytogenes</td>
<td></td>
<td>19.38 +</td>
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<td>2019 3419</td>
<td>Lait cru</td>
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<td>H+ + H+ + L.monocytogenes</td>
<td></td>
<td>17.93 +</td>
<td>20.51 +</td>
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<td>H+ + H+ + L.monocytogenes</td>
<td></td>
<td>21.55 +</td>
<td>19.91 +</td>
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<td>Lait cru</td>
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<td>H+ - H+ - L.innocua</td>
<td></td>
<td>14.64 +</td>
<td>15.69 +</td>
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<td>Raw milk cheese</td>
<td>H+ + H+ + L.monocytogenes</td>
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<td>2014 4461</td>
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<td>Raw milk cheese</td>
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<td>17.34 +</td>
<td>19.96 +</td>
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<td>Munster</td>
<td>Cheese</td>
<td>+MA +MA + H+ + L.monocytogenes</td>
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<td>Cheese</td>
<td>Ø +LA +Ø +LA + H+ + L.monocytogenes</td>
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<td>+LA +LA +HB +MB</td>
<td>L monocytogenes + L innocua</td>
<td>+</td>
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<td>+</td>
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<td>+LA +LB +MB +LB</td>
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<td>L monocytogenes + L innocua</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>L monocytogenes + L innocua</td>
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<tr>
<td>2006 N18</td>
<td>Cœur de Neufchâtel Cheese</td>
<td>+LE +LE +LE +LE</td>
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<td>+</td>
<td>+</td>
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<td>2006 AAT 9</td>
<td>Glace vanille-chocolat Ice-cream</td>
<td>+ME +ME +ME +ME</td>
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<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>2019 5342</td>
<td>Camembert au lait pasteurisé Pasteurised cheese</td>
<td>H-</td>
<td>H-</td>
<td>H-</td>
<td>L innocua</td>
<td>+</td>
<td>17,58</td>
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<td>2019 5343</td>
<td>Cantal au lait pasteurisé Pasteurised cheese</td>
<td>H-</td>
<td>H-</td>
<td>H-</td>
<td>L welshimeri</td>
<td>+</td>
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<td>After enrichment broth storage for 72h at 5°C ± 3°C</td>
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<td>Easy II lyss PCR result</td>
<td>Standard II lyss LSB for 22h at 30°C ± 5°C</td>
<td>Easy II lyss LSB for 24h at 5°C ± 3°C</td>
<td>Easy II lyss APF Classic</td>
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<td>Salmon</td>
<td>Salmo salar</td>
<td>AL  + AL  + AL  + AL  + AL</td>
<td>AL  + AL  + AL  + AL  + AL</td>
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<td>L.monocytogenes</td>
<td>PA</td>
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<td>Salmon</td>
<td>Salmo salar</td>
<td>AL  + AL  + AL  + AL  + AL</td>
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<td>Salmo salar</td>
<td>AL  + AL  + AL  + AL  + AL</td>
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<tr>
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<td>Salmon</td>
<td>Salmo salar</td>
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<td>L.monocytogenes</td>
<td>L.monocytogenes</td>
<td>PA</td>
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<tr>
<td>2006 N8</td>
<td>Cocktail de fruits de mer surgelé</td>
<td>Frozen seafood cocktail</td>
<td>* + * + * + * + *</td>
<td>+</td>
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<td>L.monocytogenes</td>
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<td>Frozen seafood cocktail</td>
<td>* + * + * + * + *</td>
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<td>L.monocytogenes</td>
<td>L.monocytogenes</td>
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<td>2006 W3</td>
<td>Nodi de poisson</td>
<td>Koi fish</td>
<td>* + * + * + * + *</td>
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<td>+</td>
<td>L.monocytogenes</td>
<td>L.monocytogenes</td>
<td>PA</td>
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<tr>
<td>2006 M20</td>
<td>Lutines de lieu noir</td>
<td>Fish</td>
<td>* + * + * + * + *</td>
<td>+</td>
<td>+</td>
<td>L.monocytogenes</td>
<td>L.monocytogenes</td>
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<tr>
<td>2006 M22</td>
<td>Crevettes</td>
<td>Shrimps</td>
<td>* + * + * + * + *</td>
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<td>+</td>
<td>L.monocytogenes</td>
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<tr>
<td>2006 N21</td>
<td>Langoustines</td>
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<td>* + * + * + * + *</td>
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<td>Crevettes entières</td>
<td>Shrimps</td>
<td>* + * + * + * + *</td>
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<td>Filet de perche du Nil</td>
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<td>* + * + * + * + *</td>
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<td>* + * + * + * + *</td>
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<td>Huitres marines</td>
<td>Marinated herring</td>
<td>* + * + * + * + *</td>
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<td>L.monocytogenes</td>
<td>L.monocytogenes</td>
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<td>Smoked fish</td>
<td>* + * + * + * + *</td>
<td>+</td>
<td>+</td>
<td>L.monocytogenes</td>
<td>L.monocytogenes</td>
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<tr>
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<td>L.monocytogenes</td>
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<tr>
<td>2006 C1</td>
<td>Saumon fumé</td>
<td>Smoked salmon</td>
<td>* + * + * + * + *</td>
<td>+</td>
<td>+</td>
<td>L.monocytogenes</td>
<td>L.monocytogenes</td>
<td>PA</td>
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<tr>
<td>2006 C3</td>
<td>Lardons de saumon fumé</td>
<td>Smoked gravlax salmon</td>
<td>* + * + * + * + *</td>
<td>+</td>
<td>+</td>
<td>L.monocytogenes</td>
<td>L.monocytogenes</td>
<td>PA</td>
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<tr>
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<td>Sprats fumés</td>
<td>Smoked fish</td>
<td>* + * + * + * + *</td>
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<td>+</td>
<td>L.monocytogenes</td>
<td>L.monocytogenes</td>
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<tr>
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<td>Saumon fumé d’Ecosse</td>
<td>Smoked tuna</td>
<td>* + * + * + * + *</td>
<td>+</td>
<td>+</td>
<td>L.monocytogenes</td>
<td>L.monocytogenes</td>
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<td>Smoked fish</td>
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<td>L.monocytogenes</td>
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<tr>
<td>2006 S19</td>
<td>Paniers au saumon fumé</td>
<td>Smoked salmon</td>
<td>* + * + * + * + *</td>
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<td>+</td>
<td>L.monocytogenes</td>
<td>L.monocytogenes</td>
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<tr>
<td>2006 M15</td>
<td>Crevettes cuites</td>
<td>Cooked shrimps</td>
<td>* + * + * + * + *</td>
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<td>+</td>
<td>L.monocytogenes</td>
<td>L.monocytogenes</td>
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<tr>
<td>2006 C7</td>
<td>Tarama</td>
<td>Tarama</td>
<td>* + * + * + * + *</td>
<td>+</td>
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<td>L.monocytogenes</td>
<td>L.monocytogenes</td>
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<td>Terrine langoustines</td>
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<td>L.monocytogenes</td>
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<td>2006 K1</td>
<td>Accras de morue</td>
<td>RTRH (cod fritters)</td>
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<td>L.monocytogenes</td>
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<td>PA</td>
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<td>2006 M14</td>
<td>Filet de poisson à la provençale</td>
<td>Cooked fish fillet</td>
<td>* + * + * + * + *</td>
<td>+</td>
<td>+</td>
<td>L.monocytogenes</td>
<td>L.monocytogenes</td>
<td>PA</td>
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<td>Herring</td>
<td>* + * + * + * + *</td>
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<td>+</td>
<td>L.monocytogenes</td>
<td>L.monocytogenes</td>
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<td>2006 N4</td>
<td>Faluche au thon</td>
<td>Sandwich (tuna)</td>
<td>* + * + * + * + *</td>
<td>+</td>
<td>+</td>
<td>L.monocytogenes</td>
<td>L.monocytogenes</td>
<td>PA</td>
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<td>2006 N5</td>
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<td>Sandwich (shrimp)</td>
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<td>+</td>
<td>L.monocytogenes</td>
<td>L.monocytogenes</td>
<td>PA</td>
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<td>Faluche aux crevettes</td>
<td>Sandwich (shrimp)</td>
<td>* + * + * + * + *</td>
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<td>+</td>
<td>L.monocytogenes</td>
<td>L.monocytogenes</td>
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<td>2006 N7</td>
<td>Brochettes de saumon frais</td>
<td>Cooked salmon</td>
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## Summary report (Version 0)

### iQ Check Listeria spp.

#### VEGETABLES

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**Reference method**

NF EN ISO 11290-1

**Alternative method:** iQ-Check Listeria spp.

**After enrichment broth storage for 72h at 5°C ± 3°C**

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<th>Agreement</th>
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### Notes

- For identification, the Fraser and APF methods were used for the first step, followed by the Easy II lysis method for the second step.
- The identification process was followed by PCR analysis using the Easy II lysis method for both Fraser and APF.
- The results were compared with the reference method to determine the agreement.

### References

- Fraser 1/2
- Easy II lysis
- APF Classic
- Standard II lysis
- Final result

### Identification

- **Standard II lysis PCR result (2006, 2019):**
  - Fraser 1/2
  - Easy II lysis

- **Alternative method:** iQ-Check Listeria spp.

### Agreement

- PPNA PPNA 5 c
- NA NA 5 c
- NA NA 5 c
- PPNA PPNA 5 c
- NA NA 5 c

### Date

16 November 2023
### ENVIRONMENTAL SAMPLES

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## ENVIRONMENTAL SAMPLES

### Product

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<th>Easy lysis</th>
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**Legend:**
- **Fraser 1/2**: Fraser PCR result
- **Fraser**: Fraser lysis
- **Identifications**: Identifications
- **Result**: Result
- **Easy II lysis**: Easy II lysis
- **Final result**: Final result
- **Agreement**: Agreement

**Notes:**
- **NF EN ISO 11290-1**: Standard II lysis for 24h at 30°C ± 3°C
- **APF Classic**: APF Classic
- **Ct FAM**: Ct FAM
- **L. monocytogenes**: Listeria monocytogenes
- **L. innocua**: Listeria innocua

**References:**
- L. monocytogenes identification
- L. innocua identification
- L. welshimeri identification

**PCR conditions:**
- 30°C + 72h at 5°C
- 30°C + 72h at 5°C ± 3°C
- 30°C + 72h at ± 3°C
- 72h
- 72h ± 3°C

**Agreement methods:**
- Classic
- Easy
- Standard

**Table notes:**
- **+**: Presence
- **-**: Absence
- **PA**: Positive agreement
- **PA**: Positive agreement
- **PA**: Positive agreement
- **PA**: Positive agreement
- **PA**: Positive agreement
- **PA**: Positive agreement
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- **PA**: Positive agreement

**Summary report (Version 0)**

iQ Check Listeria spp.

**Date:** 16 November 2023

**ADRIA**

**Ref:** 135/199
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* Analyses performed according to the COFRAC accreditation
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Summary report (Version 0)
iQ Check Listeria spp.
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## ENVIRONMENTAL SAMPLES (Extension study, 2019 – ADRIA Développement)

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### Summary report (Version 0)

**iQ Check Listeria spp.**

#### 5.3.1 Extension study, 2019 (ADRIA)

**Appendix 5 - Relative level of detection: raw data in French (initial validation study, ISHA – Renewal study, ADRIA)**

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*Analyses performed according to the COFRAC accreditation*
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**Rillettes inoculated with Listeria welshimeri L90**

6,000 CFU/g et *2400 CFU/g

**Summary report (Version 0)**

IQ Check *Listeria spp.*

ADRIA

140/199

16 November 2023
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Result criteria:
- Positive (+): Presence of growth or lysis
- Negative (-): Absence of growth or lysis
- No data (N/A): No growth or lysis observed

Conclusions:
- Positive result: Presence of growth or lysis
- Negative result: Absence of growth or lysis
- No conclusion: Insufficient data for conclusion

**ADRIA**

**Summary report (Version 0)**

IQ Check *Listeria spp.*
### Summary report (Version 0)

**iQ Check Listeria spp.**

#### Smoked salmon inoculated with *Listeria monocytogenes* 1/2 L5

2 000 CFU/g

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**Conclusion**

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**Raw vegetable mix**
inoculated with *Listeria seeligeri* L140
12 000 000 CFU/g

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### Sample N° 4671

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* Analyses performed according to the COFRAC accreditation

**ADRIA**

**Summary report** (Version 0)

iQ Check Listeria spp.
### Appendix 6 - Inclusivity / exclusivity: raw data
(initial validation and extension study)

#### INCLUSIVITY (initial validation study, IPL)

<table>
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<tr>
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## INCLUSIVITY (initial validation study, IPL)

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### INCLUSIVITY (Extension study, IPL 2009)

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## INCLUSIVITY (Extension study, IPL 2009)

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### INCLUSIVITY (Extension study, ADRIA Développement – 2019)

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## INCLUSIVITY (Extension study, ADRIA Développement – 2019)

<table>
<thead>
<tr>
<th>N°</th>
<th>Strain</th>
<th>Species</th>
<th>Reference</th>
<th>Inoculation level in 225ml LSB broth</th>
<th>Origin</th>
<th>LSB for 18 h at 30°C - FDRS Easy II Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>CT FAM</td>
<td>CT IC</td>
<td>Result</td>
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<tr>
<td>27</td>
<td><em>Listeria</em> innocua *</td>
<td>Ad 663</td>
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<td>23</td>
<td>31,14</td>
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<tr>
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<td><em>Listeria</em> innocua *</td>
<td>Ad 671</td>
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<tr>
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<td>Soft cheese (Pont L’Evêque)</td>
<td>30</td>
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<td>33</td>
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<tr>
<td>34</td>
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<td>34,83 / 35,66*</td>
<td>32,43 / 33,10*</td>
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<td>-36,06*</td>
<td>33,03 / 32,37*</td>
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### INCLUSIVITY (Extension study, ADRIA Développement – 2019)

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<thead>
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<th>N°</th>
<th>Strain</th>
<th>Species</th>
<th>Reference</th>
<th>Origin</th>
<th>LSB for 18 h at 30°C - FDRS Easy II Protocol</th>
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<tbody>
<tr>
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## EXCLUSIVITY (initial validation study, IPL)

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<th>Origin</th>
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<td>Poultry tabbouleh</td>
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<tr>
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### Appendix 7 - Artificial contamination of samples - Enrichment in LSB II broth (Extension study, 2023)

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<th>Product (French name)</th>
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<th>Injury protocol</th>
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<td>Oriental tabbouleh</td>
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<td>Seafood salad / Goat cheese and spinach puff pastry</td>
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<td>Listeria monocytogenes Ad1193 Listeria innocua Ad344</td>
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<td>Fromage au lait cru de chèvre Picondon</td>
<td>Raw goat milk cheese</td>
<td>L. monocytogenes 909 L. welshimeri Ad1587</td>
<td>Raw milk cheese</td>
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<td>0-1-1-0-2/1-0-1-0-3</td>
<td>0.8</td>
<td>0.8</td>
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</tr>
<tr>
<td>2023</td>
<td>3937</td>
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<td>2-0-0-0-0</td>
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<td>0-0-2-0-1</td>
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<td>L. monocytogenes A00L097 L. seeligeri Ad1780</td>
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<td>Raw milk</td>
<td>Seeding storage 48h at 3°C</td>
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<td>Product</td>
<td>Strain</td>
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<td>Crème glacée vanille</td>
<td>Ice cream</td>
<td>L. seeligeri Ad1782</td>
<td>Raw milk</td>
<td>Seeding storage 2 weeks at -20°C</td>
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<td></td>
<td>2,4</td>
<td>+</td>
</tr>
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<td>3261</td>
<td>Crème glacée vanille</td>
<td>Ice cream</td>
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<td>Milk</td>
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<td></td>
<td>1,4</td>
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</tr>
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<td>Crème glacée à la vanille</td>
<td>Ice cream</td>
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<td>Milk</td>
<td>Seeding storage 2 weeks at -20°C</td>
<td></td>
<td></td>
<td>1,4</td>
<td>-</td>
</tr>
<tr>
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<td>3543</td>
<td>Brique pur brebis</td>
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<td>Cheese</td>
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<td>0,5</td>
<td>5</td>
<td>+</td>
<td>+</td>
</tr>
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<td>3544</td>
<td>Brique pur brebis</td>
<td>Pasteurized ewe milk cheese</td>
<td>L. innocua Ad661</td>
<td>Cheese</td>
<td>Spiking 10 min 60°C</td>
<td>1,1</td>
<td>5</td>
<td>+</td>
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<td>Tomme des Pyrénées</td>
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<td>Cheese</td>
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<td>5</td>
<td>+</td>
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<td>Tomme des Pyrénées</td>
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<td>L. innocua Ad661</td>
<td>Cheese</td>
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<td>1,1</td>
<td>5</td>
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Appendix 8 - Sensitivity study: raw results - Enrichment in LSB II broth
(Extension study, 2023)

**Bold typing : artificially inoculated samples**

**Listeria detection results:**

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<thead>
<tr>
<th>DW</th>
<th>Deep Well</th>
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<tbody>
<tr>
<td>H+</td>
<td>characteristic Listeria colonies with halo</td>
</tr>
<tr>
<td>H-</td>
<td>characteristic Listeria colonies without halo</td>
</tr>
<tr>
<td>-</td>
<td>no typical colonies but presence of background microflora</td>
</tr>
<tr>
<td>st</td>
<td>plate without any colony</td>
</tr>
<tr>
<td>PA</td>
<td>positive agreement</td>
</tr>
<tr>
<td>NA</td>
<td>negative agreement</td>
</tr>
<tr>
<td>ND</td>
<td>negative deviation</td>
</tr>
<tr>
<td>PD</td>
<td>positive deviation</td>
</tr>
<tr>
<td>PPNA</td>
<td>positive presumptive negative agreement</td>
</tr>
<tr>
<td>PPND</td>
<td>positive presumptive negative deviation</td>
</tr>
<tr>
<td>NC</td>
<td>non-characteristic colony on TSYEA</td>
</tr>
<tr>
<td>d</td>
<td>doubtful colony</td>
</tr>
<tr>
<td>*</td>
<td>result after enrichment broth dilution at 1/10</td>
</tr>
<tr>
<td>NI</td>
<td>no identification</td>
</tr>
<tr>
<td>ni</td>
<td>not isolated colony</td>
</tr>
<tr>
<td>L.mono</td>
<td><em>Listeria monocytogenes</em></td>
</tr>
<tr>
<td>L.inno</td>
<td><em>Listeria innocua</em></td>
</tr>
<tr>
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</tr>
<tr>
<td>L.welsh</td>
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</tr>
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<td>L.ivan</td>
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</tr>
<tr>
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<td>K's code</td>
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<tr>
<td>1:10 with Half Fraser - 24h at 30°C</td>
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<tr>
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<tr>
<td>2022</td>
<td>3302</td>
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*Analyses performed according to the COFRAC accreditation*
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<th>COMPOsite FOODS</th>
<th>I.M.</th>
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<th>Alternative method: IQ-Check Listeria spp.</th>
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<td>-</td>
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<td>st</td>
<td>st</td>
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<td>-</td>
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<td>st</td>
<td>st</td>
<td>/</td>
<td>-</td>
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<td>-</td>
<td>N/A 33.13</td>
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<tr>
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<td>st</td>
<td>st</td>
<td>st</td>
<td>/</td>
<td>-</td>
<td>N/A 33.13</td>
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<tr>
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<td>st</td>
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<td>/</td>
<td>-</td>
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<td>st</td>
<td>st</td>
<td>/</td>
<td>-</td>
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<td>/</td>
<td>-</td>
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<td>st</td>
<td>st</td>
<td>/</td>
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## COMPOSITE FOODS

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<td>CFX Opus Deep Well</td>
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<td><strong>Confirmation</strong></td>
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<td><strong>OQA</strong></td>
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**Legend:**
- **PD:** Positive Detect
- **ND:** Not Detect
- **PP:** Positive Predict
- **PD:** Positive Predict

**Additional Information:**
- **Alternative method:** iQ-Check Listeria spp.
- **Type:**
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<td>-</td>
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**COMPOSITE FOODS**

Alternative method: IQ-Check Listeria spp.

**PCR**

1:1 with LSB II Broth -18h at 37°C

**Easy II lysis protocol - Without FURS**

APF Fast

**Easy II lysis protocol - With FURS**

APF Fast

**Confirmation**

Identification

**Result**

**Final result**

**Agreement RefAlt**

**Date of analysis**

**Product (French name)**

**Identification**

**Result**

**Final result**

**Agreement RefAlt**

**Type**

**Summary report (Version 0)**

IQ Check Listeria spp.

ADRIA

164/199

16 November 2023
| Date of analysis | K's number | Product (French name) | Product | Date produced | IQ Check | ADRIA | 1:10 with Half Fraser - 24h at 30°C | 1:10 with Fraser - 36h at 37°C | POC | PCR | Confirmation | Final result | Agreement Ref Alt |
|------------------|------------|-----------------------|---------|---------------|-----------|--------|-----------------------------------|--------------------------------|-----|----------------|--------------|------------------|
| 2023 014         | 114        | Coupe froissé         | Pastry  | -             | -         | H-d    | -                                | -                             | -   | -              | -            | -                |
| 2023 015         | 115        | Crème angraise        | Custard | st            | -         | st     | st                               | /                             | -   | -              | -            | -                |
| 2023 016         | 116        | Mousse citron         | Lemon mousse | st  | st           | st     | st                               | /                             | -   | -              | -            | -                |
| 2023 017         | 117        | Feuilletine           | Custard based dessert | st | st           | st     | st                               | /                             | -   | -              | -            | -                |
| 2023 018         | 118        | Mousse chocolat noir  | Chocolate mousse | st | st           | st     | st                               | /                             | -   | -              | -            | -                |
| 2023 019         | 119        | Gâteau fourré frutti  | Pastry  | st            | st         | st     | st                               | /                             | -   | -              | -            | -                |
| 2023 020         | 120        | Choux chantilly       | Pastry  | -             | -          | -      | -                                | -                             | -   | -              | -            | -                |
| 2023 021         | 121        | Tartelette citron     | Lemon tartlet | st | st           | st     | st                               | /                             | -   | -              | -            | -                |
| 2023 022         | 122        | Mousse au chocolat à l'ancienne | Chocolate mousse | - | -           | -      | -                                | /                             | -   | -              | -            | -                |
| 2023 023         | 123        | Pâte feuilletée       | Raw dough | -            | -         | -      | -                                | /                             | -   | -              | -            | -                |
## PRODUCTION ENVIRONMENTAL SAMPLES

**Alternative method:** IQ-Check *Listeria* spp.

### Analyses performed according to the COFRAC accreditation

**Date of analysis:** 16 November 2023

**Reference method:** ISO 11290-1*

**Type:** 

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*Analyses performed according to the COFRAC accreditation

ADRIA 166/199

Summary report (Version 0)

IQ Check *Listeria* spp.
## Summary report (Version 0)

### IQ Check Listeria spp.

**Date of analysis:**
- 09/16/2023 (9/6/2023)
- 08/23/2023 (8/23/2023)
- 08/22/2023 (8/22/2023)
- 07/58/2023 (7/58/2023)
- 07/57/2023 (7/57/2023)
- 07/24/2023 (7/24/2023)
- 07/23/2023 (7/23/2023)

### Production environmental samples

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<td>07/23</td>
<td>Chiffonnets environnement porc (production glace)</td>
<td>Wipe before cleaning process</td>
<td>H+ (4) + H+ + L.mono +</td>
<td>N/A 32.73 - N/A 32.86 + 38.17 32.72 + 36.60 32.68 + + spp + H- H- + + L.seef +</td>
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<td>Chiffonnets environnement laitier après nettoyage</td>
<td>Wipe after cleaning process</td>
<td>st st st st / -</td>
<td>N/A 32.68 - N/A 33.00 - N/A 33.03 - N/A 32.73 - st st st st / - - - - -</td>
<td>NA NA NA NA 2 a</td>
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### PRODUCTION ENVIRONMENTAL SAMPLES

**Reference method:** IQ-Check Listeria spp.

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<th>Date of analysis</th>
<th>Product (french name)</th>
<th>Half Fraser</th>
<th>Frater</th>
<th>OQA</th>
<th>PALCAM</th>
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<th>Identification</th>
<th>Result</th>
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<td>H+</td>
<td>H+</td>
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<td>st</td>
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<td>H+</td>
<td>L.mono + 25.77 = N/A + 25.42 = N/A + 28.20 = N/A + 27.34 = 34.32 + H+H+H+H+L.mono Livre</td>
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<td>H+</td>
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<td>Wastes (Meat)</td>
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<td>H+</td>
<td>L.mono + 27.48 = 34.51 + 27.48 = 32.38 + 27.94 = 33.26 + 28.04 = 31.93 + H+H+H+L.mono L.seef</td>
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<td>Wastes (Salmon)</td>
<td>H+</td>
<td>H+</td>
<td>L.mono + 28.75 = 33.07 + 28.84 = 31.91 + 29.20 = 32.37 + 29.00 = 31.57 + + H+H+H+L.mono</td>
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<td>Wastes (Fish)</td>
<td>H+</td>
<td>H+</td>
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<td>-</td>
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<td>st</td>
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<td>-</td>
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<td>Wastes (Meat)</td>
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<td>H+H+</td>
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<td>Reference method: ISO 11290-1*</td>
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<td>Process water</td>
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<td>Rinsing water</td>
<td>st st st st / - N/A 33.13 - N/A 32.95 - N/A 32.78 - N/A 32.72 - st st st st st / -</td>
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<td>Process water</td>
<td>H- + H- + L.inno + 29.94 32.28 + 30.05 31.81 + 29.69 32.07 + 29.93 31.60 + *spp + H- H- + L.inno - + + + + PA PA PA PA 2 c</td>
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<td>Rinsing water</td>
<td>st st st st / - N/A 32.80 - N/A 32.56 - N/A 32.69 - N/A 32.84 - st st st st st / -</td>
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<td>Wastes (Meat)</td>
<td>st st st st / - N/A 33.29 - N/A 32.80 - N/A 33.28 - N/A 32.69 - st st st st st / -</td>
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<td>Process water</td>
<td>H+ + H+ + L.mono + 29.94 33.00 + 30.20 31.92 + 27.58 32.02 + 27.43 31.44 + + + H+ H+ + L.mono + + + + + PA PA PA PA 2 c</td>
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<td>Process water</td>
<td>st st st st / - 29.93 33.58 + 29.90 32.11 + 29.64 32.56 + 29.48 31.77 + + + H+ H+ + L.mono + + + + + PD PD PD PD 2 c</td>
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</table>
## PRODUCTION ENVIRONMENTAL SAMPLES

**Reference method:** ISO 11290-1*

### 1:10 with Half Fraser - 24h at 30°C

| Date of analysis | Product (french name) | Product (english name) | Q&A | PALCAM | Q&A | PALCAM | Identification | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Final result | Agreement Ref/Alt |
|------------------|-----------------------|------------------------|-----|--------|-----|--------|----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----------------|------------------|
| 2023 733         | Eau de rinçage production galette végétale + algues | Process water | H+ | H+ | + | L.mono | 29.33 | 32.25 | ± | 29.24 | 31.69 | + | N/A | 32.49 | - | N/A | 32.71 | - | st | st | st | st | / | - | - | - | - | - | PP | NA | PPNA | NO | ND | 2 | c |
| 2023 734         | Eau de process production de steak végétal | Process water | H+ | H+ | + | L.mono | 26.88 | 32.73 | ± | 26.75 | 31.20 | + | 27.25 | 33.67 | + | 27.11 | 31.13 | + | + | + | H+ | H+ | + | L.mono | L.mono | + | + | + | + | PA | PA | PA | PA | 2 | c |
| 2023 756         | Eau de process environnement laitier (fin de lavage lait) | Process water | st | st | - | st | / | - | N/A | 32.79 | - | N/A | 32.65 | - | N/A | 32.79 | - | N/A | 32.57 | - | st | st | st | st | / | - | - | - | - | NA | NA | NA | NA | 2 | c |
| 2023 828         | Eau de process environnement laitier | Process water | H+ | H+ | + | L.mono | 31.25 | 32.54 | ± | 30.75 | 32.32 | + | 31.55 | 32.13 | + | 31.07 | 32.33 | + | + | + | H+ | H+ | + | L.mono | L.mono | + | + | + | + | PA | PA | PA | PA | 2 | c |
| 2023 829         | Eau de process environnement végétal | Process water | H+ | H+ | + | L.mono | 32.12 | 32.03 | ± | 31.72 | 32.12 | + | 32.37 | 32.18 | + | 31.48 | 32.18 | + | + | + | H- | H- | + | L.seel | L.seel | + | + | + | + | PA | PA | PA | PA | 2 | c |
| 2023 830         | Eau de rinçage environnement carné | Process water | H+| H+| + | L.mono L.welsh | 33.21 | 32.14 | ± | 32.57 | 32.23 | + | 34.64 | 32.80 | + | 34.30 | 32.62 | + | + | + | H+ | H+ | + | L.mono L.welsh | L.mono L.welsh | + | + | + | + | PA | PA | PA | PA | 2 | c |
| 2023 964         | Eau de process lait | Process water | H+ | H+ | + | L.mono | 31.61 | 34.39 | + | 31.83 | 33.44 | + | 31.16 | 32.72 | + | 31.22 | 32.69 | + | + | + | H+ | H+ | + | L.mono | L.mono | + | + | + | + | PA | PA | PA | PA | 2 | c |
| 2023 965         | Eau de process environnement carné | Process water | st | st | st | / | - | N/A | 33.15 | - | N/A | 32.79 | - | N/A | 32.89 | - | N/A | 33.35 | - | st | st | st | st | / | - | - | - | - | NA | NA | NA | NA | 2 | c |
| 2023 967         | Eau de process lait | Process water | st | st | st | / | - | N/A | 33.21 | - | N/A | 32.91 | - | N/A | 33.19 | - | N/A | 32.71 | - | st | st | st | st | / | - | - | - | - | NA | NA | NA | NA | 2 | c |
| 2023 968         | Eau de process dinde surgelé | Process water | st | st | st | / | - | N/A | 33.08 | - | N/A | 32.78 | - | N/A | 33.07 | - | N/A | 33.05 | - | st | st | st | st | / | - | - | - | - | NA | NA | NA | NA | 2 | c

**Note:** PD indicates presence of DNA degradation products. CQ indicates cycle quantification.
<table>
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<th>N° Sample</th>
<th>Product (French name)</th>
<th>Reference method: ISO 11290-1</th>
<th>Alternative method: iQ-Check Listeria spp. 1:10 with prewarmed LSBII Broth -18h at 37°C</th>
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<td>2022 1728</td>
<td>Fromage au lait cru de vache pâte cuite</td>
<td>Raw cow milk cheese</td>
<td>H+ + H+ + L mono +</td>
<td>34,13 32,78 34,28 32,94 + 32,41 32,75 + 33 32,3 + + + H+ H+ + L mono / + + + + + + PA PA PA PA 3 a</td>
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<td>Fromage au lait cru de vache Camembert</td>
<td>Raw cow milk cheese</td>
<td>H+ + H+ + L inno +</td>
<td>38,03 32,37 37,49 34,16 + N/A N/A 32,85 32,24 32,61 + 39,07 33,25 + + + H+ H+ + L innocua / + + + + + + PA PA ND PA 3 a</td>
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<td>Fromage au lait cru de chèvre Picodon</td>
<td>Raw goat milk cheese</td>
<td>- - H+ + L weisb +</td>
<td>36,13 32,55 38,92 33,89 + 36,9 32,62 + 37,33 33,18 + + + H+ H+ + L mono / + + + + + + PA PA PA PA 3 a</td>
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<td>Raw cow milk cheese</td>
<td>- - st -</td>
<td>- N/A 38,02 - N/A 42,06 - N/A 37,89 - N/A 37,93 - - - - - - - - - - - - - - NA NA NA NA 3 a</td>
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<td>Raw cow milk cheese</td>
<td>st st st -</td>
<td>29,76 33,8 + 29,93 32,66 + 29,82 36,28 + 29,71 36,22 + + + H+ H+ + L mono / + + + + + + PD PD PD PD 3 a</td>
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<td>Brie de Meaux au lait cru de vache</td>
<td>Raw cow milk cheese</td>
<td>st st st st</td>
<td>29,93 39,73 + 29,12 N/A + 28,86 N/A + 28,5 N/A + + + H+ H+ + L inno / + + + + + + PD PD PD PD 3 a</td>
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<tr>
<td>2022 2744</td>
<td>Munster au lait cru de vache</td>
<td>Raw cow milk cheese</td>
<td>H+ + H+ + L inno +</td>
<td>20,62 N/A + 20,88 N/A + 19,43 N/A + 19,49 N/A + + + H+ H+ + L inno / + + + + + + PA PA PA PA 3 a</td>
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<td>Raw cow milk cheese</td>
<td>st st st st</td>
<td>- N/A 33,93 - N/A 33,63 - N/A 33,82 - N/A 33,46 - - - - - - - - - - - - - - NA NA NA NA 3 a</td>
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<td>Raw goat milk cheese</td>
<td>st st st st</td>
<td>- N/A 33,18 - N/A 32,98 - N/A 33,32 - N/A 32,94 - - - - - - - - - - - - - - NA NA NA NA 3 a</td>
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<td>Raw goat milk cheese</td>
<td>st st st st</td>
<td>- N/A N/A* 32,56* - N/A N/A* 32,55* - N/A N/A* 32,41* - N/A N/A* 32,41* - - - - - - - - - - - - - - NA NA NA NA 3 a</td>
</tr>
<tr>
<td>2022 2748</td>
<td>Mâples au lait cru de vache</td>
<td>Raw goat milk cheese</td>
<td>st st st st</td>
<td>- N/A 34,92 - N/A 34,94 - N/A 35,25 - N/A 35,37 - - - - - - - - - - - - - - NA NA NA NA 3 a</td>
</tr>
<tr>
<td>2022 2749</td>
<td>Rives des garrigues au lait cru de chèvre</td>
<td>Raw goat milk cheese</td>
<td>st st st st</td>
<td>- N/A 32,96 - N/A 33,07 - N/A 32,89 - N/A 33,06 - - - - - - - - - - - - - - NA NA NA NA 3 a</td>
</tr>
<tr>
<td>2022 2750</td>
<td>Saint Nicolas de la Galmourie au lait cru de brebis</td>
<td>Raw ewes milk cheese</td>
<td>st st st st</td>
<td>- N/A 32,6 - N/A 32,73 - N/A 34,33 * ac N/A N/A* 32,49 ac 32,85 32,811* - - - - - - - - - - - - - - NA NA NA NA 3 a</td>
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<tr>
<td>2022 2751</td>
<td>Brie de Meaux au lait cru de vache</td>
<td>Raw cow milk cheese</td>
<td>st st st st</td>
<td>- N/A 35,76 - N/A 35,4 - N/A 36,63 - N/A 36,77 - - - - - - - - - - - - - - NA NA NA NA 3 a</td>
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<tr>
<td>2022 2752</td>
<td>Fromage au lait cru de chèvre 158</td>
<td>Raw goat milk cheese</td>
<td>H+ + H+ + L mono +</td>
<td>36,13 32,69 + 36,95 32,46 + 39,78 32,61 + 38,54 32,64 + - / (x5) + d m / + / (x5) + / (x5) + / (x5) + / (x5) + / (x5) + / (x5) + L mono / + + + + + + PA PA PA PA 3 a</td>
</tr>
</tbody>
</table>

* Analyses performed according to the COFRAC accreditation

ADRIA

Summary report (Version 0)
iQ Check Listeria spp.

171/199

16 November 2023
# DAIRY PRODUCTS

## Easy Lysis Protocol

### Without FDRS

**APP Fast**

### With FDRS

**APP Fast**

## Confirmation

### Reference method:
ISO 11290-1

### Alternative method: iQ-Check Listeria spp.

### 1:10 with prewarmed LSBII Broth -18h at 37°C

### Easy II lysis protocol - Without FDRS

<table>
<thead>
<tr>
<th>Product</th>
<th>Result</th>
<th>Subculture</th>
<th>Final result</th>
<th>Agreement Ref/Alt</th>
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</thead>
<tbody>
<tr>
<td>CFX96 DW</td>
<td>CFX Opus DW</td>
<td>CFX96 DW</td>
<td>CFX Opus DW</td>
<td>w/o FDRS</td>
</tr>
</tbody>
</table>

### Easy II lysis protocol - With FDRS

**APP Fast**

### Confirmation

### Subculture in Fraser broth 24h at 37°C (Negative samples)

### Result | Final result confirmation | Listeria |
<table>
<thead>
<tr>
<th></th>
<th></th>
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<tbody>
<tr>
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### Type

<table>
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<tr>
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<th>Agreement Ref/Alt</th>
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<td></td>
<td>PA PA PA 3 a</td>
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</tbody>
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- **CFX96**
- **CFX Opus**
- **Easy II Lysis protocol**

### Summary report

**ADRIA** 172/199

**iQ Check Listeria spp.**

### 16 November 2023
### Reference method:
**ISO 11290-1**

#### Easy II lysis protocol - Without FDRS
**APF Fast**

#### Easy II lysis protocol - With FDRS
**APF Fast**

<table>
<thead>
<tr>
<th>Year of analysis</th>
<th>Product (French name)</th>
<th>Product</th>
<th>Confirmation</th>
<th>Subculture in Fraser broth 24h at 37°C (Negative samples)</th>
<th>Reference method: <strong>iQ-Check Listeria spp.</strong></th>
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</thead>
<tbody>
<tr>
<td>2023 1727</td>
<td>Lait cru de chèvre</td>
<td>Raw goat milk</td>
<td>H+ + H+ + L mono</td>
<td>+ 32.43 32.27 + 33 32.31 + 32.38 32.22 + 32.5 32.44 + + + H+ H+ + L. mono /</td>
<td><strong>iQ-Check</strong></td>
</tr>
<tr>
<td>2023 2821</td>
<td>Crème de fermeière</td>
<td>Raw cream</td>
<td>st st st st</td>
<td>- N/A 33.22 - N/A 33.4 - N/A 33.45 - N/A 33.24 - - - - -</td>
<td><strong>CFX Opus</strong></td>
</tr>
<tr>
<td>2023 2822</td>
<td>Crème crue fermière</td>
<td>Raw cream</td>
<td>st st st st</td>
<td>- N/A 33.56 - N/A 33.11 - N/A 33.64 - N/A 33.41 - - - - -</td>
<td><strong>CFX Opus</strong></td>
</tr>
<tr>
<td>2023 2823</td>
<td>Fromage blanc au lait cru de vache</td>
<td>Raw cottage milk cheese</td>
<td>st st st st</td>
<td>- N/A 35.39 - N/A 34.56 - N/A 36.15 - N/A 36.23 - - - - -</td>
<td><strong>CFX Opus</strong></td>
</tr>
<tr>
<td>2023 2824</td>
<td>Faisselle au lait cru</td>
<td>Raw fermented milk</td>
<td>- - st st</td>
<td>- N/A 33.21 - N/A 33.04 - N/A 33.02 - N/A 32.98 - - - - -</td>
<td><strong>CFX Opus</strong></td>
</tr>
<tr>
<td>2023 2932</td>
<td>Lait cru de vache</td>
<td>Raw cow milk</td>
<td>H+ + H+ + L inno +</td>
<td>N/A 33 - N/A 32.79 - N/A 33.39 - N/A 32.71 - - - - -</td>
<td><strong>CFX Opus</strong></td>
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<tr>
<td>2023 2934</td>
<td>Lait cru de vache</td>
<td>Raw cow milk</td>
<td>H+ + H+ + L. mono +</td>
<td>N/A 32.96 - N/A 33.01 - N/A 32.64 - N/A 33.02 - - - - -</td>
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<tr>
<td>2023 3536</td>
<td>Lait de vache</td>
<td>Fermented milk</td>
<td>st st st st</td>
<td>- N/A 33.05 - N/A 32.98 - N/A 33.1 - N/A 32.83 - st st st -</td>
<td><strong>CFX Opus</strong></td>
</tr>
<tr>
<td>2023 3537</td>
<td>Lait de vache</td>
<td>Fermented milk</td>
<td>- - st -</td>
<td>- N/A 34.29 - N/A 34.35 - N/A 33.22 - N/A 32.81 - - - - -</td>
<td><strong>CFX Opus</strong></td>
</tr>
<tr>
<td>2023 3761</td>
<td>Beurre de baratte non pasteurisé</td>
<td>Raw milk butter</td>
<td>- - - -</td>
<td>- 30.59 33.01 + 30.11 32.35 + 30.56 32.96 + 30.24 32.79 + + + H+ / H+</td>
<td><strong>CFX Opus</strong></td>
</tr>
<tr>
<td>2023 3765</td>
<td>Faisselle au lait cru</td>
<td>Raw fermented milk</td>
<td>- st - -</td>
<td>- N/A 32.82 - N/A 33.1 - N/A 34.05 - N/A 33.53 - st - - - - -</td>
<td><strong>CFX Opus</strong></td>
</tr>
<tr>
<td>2023 3768</td>
<td>Faisselle au lait cru</td>
<td>Raw fermented milk</td>
<td>st st st st</td>
<td>- N/A 33.26 - N/A 32.94 - N/A 33.3 - N/A 32.96 - - st - - - -</td>
<td><strong>CFX Opus</strong></td>
</tr>
<tr>
<td>2023 2754</td>
<td>Crème glacée à la vanille &quot;cookie&quot;</td>
<td>Ice cream</td>
<td>- - - -</td>
<td>- 40.36 34.86 35.37 + 33.1 32.58 32.42 / N/A 32.82 - N/A 33.36 - N/A 33.6 - - - - -</td>
<td><strong>CFX Opus</strong></td>
</tr>
<tr>
<td>2023 2755</td>
<td>Crème glacée à la vanille &quot;caramel&quot;</td>
<td>Ice cream</td>
<td>st st st -</td>
<td>- N/A 32.7 - N/A 32.58 - N/A 32.7 - N/A 32.93 - - - - -</td>
<td><strong>CFX Opus</strong></td>
</tr>
<tr>
<td>2023 2825</td>
<td>Lait de vache 1/2 écrémé pasteurisé</td>
<td>Pasteurized cow milk</td>
<td>H+ + H+ + L. mono +</td>
<td>N/A 32.73 - N/A 32.5 - N/A 32.95 - N/A 32.71 - - - - -</td>
<td><strong>CFX Opus</strong></td>
</tr>
<tr>
<td>2023 2826</td>
<td>Lait de vache 1/2 écrémé pasteurisé</td>
<td>Pasteurized cow milk</td>
<td>H+ + H+ + L. mono +</td>
<td>N/A 33 - N/A 32.92 - N/A 32.86 - N/A 32.55 - - - - -</td>
<td><strong>CFX Opus</strong></td>
</tr>
<tr>
<td>2023 2827</td>
<td>Lait de vache 1/2 écrémé pasteurisé mangue / fruit de la passion</td>
<td>Pasteurized cow milk flavoured</td>
<td>H+ + H+ + L. mono +</td>
<td>27.59 33.29 + 27.54 31.51 + 27.62 32.75 + 27.66 31.82 + - - H+ H+ + L. see/</td>
<td><strong>CFX Opus</strong></td>
</tr>
</tbody>
</table>

**Note:** Each cell contains the method details, including sample type, reaction result, and confirmation status. The table includes a variety of dairy products tested for Listeria spp. using different lysis and detection protocols.

**Summary report (Version 0)**

IQ Check Listeria spp.

**ADRIA**

**16 November 2023**

**173/199**
## DAIRY PRODUCTS

### Summary report (Version 0)

**IQ Check Listeria spp.**

<table>
<thead>
<tr>
<th>Year of analysis</th>
<th>Product (French name)</th>
<th>Sample N°</th>
<th>Country</th>
<th>Type</th>
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<tbody>
<tr>
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<td>Fromage au lait de brebis pasteurisé</td>
<td>3545</td>
<td>3544</td>
<td>3263</td>
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<tr>
<td>2023 2830</td>
<td>Fromage au lait de chèvre pasteurisé</td>
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<td>3260</td>
<td>3078</td>
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<tr>
<td>2022 2832</td>
<td>Lait frais de chèvre pasteurisé</td>
<td>3075</td>
<td>3072</td>
<td>2835</td>
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<tr>
<td>2022 2833</td>
<td>Fromage au lait demi-écroué</td>
<td>3071</td>
<td>3072</td>
<td>3263</td>
</tr>
<tr>
<td>2022 2834</td>
<td>Poudre de lait demi-écroué</td>
<td>3072</td>
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<tr>
<td>2022 2850</td>
<td>Poudre de lait demi-écroué</td>
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<tr>
<td>2022 2851</td>
<td>Poudre de lait demi-écroué</td>
<td>3074</td>
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<td>2022 2852</td>
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<td>3075</td>
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<td>2022 2853</td>
<td>Poudre de lait demi-écroué</td>
<td>3077</td>
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<td>Crème glace vanille</td>
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<tr>
<td>2022 2861</td>
<td>Crème glace vanille</td>
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<td>3261</td>
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<tr>
<td>2022 2862</td>
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<td>3263</td>
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<tr>
<td>2022 2863</td>
<td>Crème glace vanille</td>
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<td>3263</td>
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<tr>
<td>2022 2864</td>
<td>Brique pur brebis</td>
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<tr>
<td>2022 2865</td>
<td>Brique pur brebis</td>
<td>3544</td>
<td>3544</td>
<td>3545</td>
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<tr>
<td>2022 2866</td>
<td>Tomme des Pyrénées</td>
<td>3545</td>
<td>3545</td>
<td>3546</td>
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### Reference method: ISO 11290-1+ 1:10 with prewarmed LSBII Broth -1h8 at 37°C

### Easy II lysis protocol - Without FDRS

**APF Fast**

<table>
<thead>
<tr>
<th>Type</th>
<th>CFX96 DW</th>
<th>CFX Opus DW</th>
<th>CFX96 DW</th>
<th>CFX Opus DW</th>
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<tbody>
<tr>
<td>OIA</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>PACAM</td>
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<tr>
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### Confirmation

**Easy II lysis protocol - With FDRS**

**APF Fast**

<table>
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<th>CFX96 DW</th>
<th>CFX Opus DW</th>
<th>CFX96 DW</th>
<th>CFX Opus DW</th>
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<tbody>
<tr>
<td>w/o FDRS</td>
<td>-</td>
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<tr>
<td>w FDRS</td>
<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>w/o FDRS</td>
<td>-</td>
<td>-</td>
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### Alternative method: IQ-Check Listeria spp.

**CFX Opus**

<table>
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<tr>
<th>Result</th>
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<th>CFX Opus</th>
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<tr>
<td>IW+</td>
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<td>PALCAM</td>
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</table>

### Type

- **Easy II lysis protocol - Without FDRS**
- **Easy II lysis protocol - With FDRS**
- **Confirmation**
- **Reference method: ISO 11290-1+ 1:10 with prewarmed LSBII Broth -1h8 at 37°C**
- **Alternative method: IQ-Check Listeria spp.**

### Notes

- *Easy II lysis protocol - Without FDRS* and *Easy II lysis protocol - With FDRS* results are provided.
- *Confirmation* results are also included for verification.

**Biological Radience**

16 November 2023
### Summary report (Version 0)

**iQ Check Listeria spp.**

<table>
<thead>
<tr>
<th>Year of analysis</th>
<th>N° Sample</th>
<th>Product (French name)</th>
<th>Product (English name)</th>
<th>Reference method: ISO 11290-1+</th>
<th>Alternative method: iQ-Check Listeria spp.</th>
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<tr>
<td></td>
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<td>1:10 with prewarmed LSBII Broth - 18h at 37°C</td>
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<td>Easy II lysis protocol - Without FDRS APF Fast</td>
<td>Easy II lysis protocol - With FDRS APF Fast</td>
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<td>confirmation</td>
<td>Subculture in Fraser broth 24h at 37°C (Negative samples)</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>Final result</td>
<td>Agreement Ref/Alt</td>
</tr>
</tbody>
</table>

<p>|                  | Half Fraser | Fraser | Identification | CFX96 DW | CFX Opus DW | CFX96 DW | CFX Opus DW | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result | Result |
|------------------|-------------|--------|----------------|----------|-------------|----------|-------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
|                  |             |        |                |          |             |          |             |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| 2023             | 3546        | Tomme des Pyrénées | Pasteurized cow cheese |           |             |          |             |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |</p>
<table>
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<tr>
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<th>Product (French name)</th>
<th>Reference method: ISO 11290-1</th>
<th>Alternative method: iQ Check Listeria spp.</th>
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<tr>
<td></td>
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<td>Easy II lysis protocol - Without FDRS</td>
<td>Easy II lysis protocol - With FDRS</td>
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<td>w FDRS</td>
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<tr>
<td></td>
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<td>CFX96 Deep Well</td>
<td>CFX Opus Deep Well</td>
<td>CFX96 Deep Well</td>
<td>CFX OPUS Deep Well</td>
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<td></td>
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<td></td>
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<td>I.C. Cq</td>
<td>Result</td>
<td>FAM Cq</td>
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<td>2022</td>
<td>Sandwich viennois</td>
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<td>2022</td>
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<td>Piémontaise au jambon</td>
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<td>N/A</td>
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<tr>
<td>2022</td>
<td>Taboulé à l'orientale</td>
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<td>N/A</td>
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<td>32.96</td>
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<td>N/A</td>
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<td>N/A</td>
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<td>32.58</td>
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<td>N/A</td>
<td>32.65</td>
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<tr>
<td>1:10 with Half Fraser - 24h at 30°C</td>
<td>RTRH (Puff ham, cheese)</td>
<td>Paniers tariflette</td>
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<td>2:10 with Half Fraser - 24h at 30°C</td>
<td>RTH (Puff chicken, mushroom)</td>
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<td>RTRH(Paella)</td>
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<td>4:10 with Half Fraser - 24h at 30°C</td>
<td>RTRH (vegetables, soya)</td>
<td>Galette soja-légumes</td>
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<td>5:10 with Half Fraser - 24h at 30°C</td>
<td>RTH (soya, tomato, basilic)</td>
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<td>Galette de soja à la provençale</td>
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<td>8:10 with Half Fraser - 24h at 30°C</td>
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<td>Puff pastry</td>
<td>Pailet choux-feuurs brocolis</td>
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<td>13:10 with Half Fraser - 24h at 30°C</td>
<td>Raw dough</td>
<td>Pâte à gueule</td>
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<td>14:10 with Half Fraser - 24h at 30°C</td>
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<td>Charlotte framboise</td>
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<td>Custard based dessert</td>
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<td>Confectionary (chocolate)</td>
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<td>20:10 with Half Fraser - 24h at 30°C</td>
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<td>Coupe profiterole</td>
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<td>Mousse au chocolat à l'ancienne</td>
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## PRODUCTION ENVIRONMENTAL SAMPLES

**Alternative method:** IQ-Check Listeria spp.

**1:10 with LSBill Broth - 18h at 37°C + 72h at 4°C**

### PCR

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<tr>
<th>Date of analysis</th>
<th>N° Sample</th>
<th>Product (French name)</th>
<th>Result</th>
<th>Product (French name)</th>
<th>I.C. Cq</th>
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<th>FAM Cq</th>
<th>I.C. Cq</th>
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**16 November 2023**

**Summary report (Version 0)**

IQ Check Listeria spp.
<table>
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<th>Date of analysis</th>
<th>N° Sample</th>
<th>Product (French name)</th>
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### PRODUCTION ENVIRONMENTAL SAMPLES

**Reference method:** ISO 11290-1

**Alternative method:** iQ-Check Listeria spp.

#### 1:10 with LSBill Broth - 18h at 37°C + 72h at 4°C

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<th>Product</th>
<th>1:10 with Half Fraser - 24h at 30°C</th>
<th>PCR</th>
<th>Confirmation</th>
<th>Final result</th>
<th>Agreement Ref/Alt</th>
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<td>26.82 30.47 + 26.71 29.94 + 26.77 30.69</td>
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<td>PA PA PA PA</td>
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<td>29.39 31.09 + 29.09 31.17 + 28.46 30.79 + 28.53 30.55</td>
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<td>PA PA PA PA</td>
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<td>+</td>
<td>27.64 33.98 + 28.03 33.96</td>
<td>CFX Opus DW CFX Opus DW CFX Opus DW CFX Opus DW</td>
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<td>AL</td>
<td>PA PA PA PA</td>
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- **Result:**
  - FAM Cq
  - I.C. Cq
  - RAPID' L_monol
  - AL
- **Final result:**
  - CFX96 Deep Well
  - CFX Opus Deep Well
- **Confirmation:**
  - w/o FDRS
  - w FDRS
- **Agreement Ref/Alt:**
  - PA
  - PD
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## DAIRY PRODUCTS

### Summary report

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### Reference method:
ISO 11290-1

### Alternative method: IQ-Check Listeria spp.

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<th>Easy II lysis protocol - WITHOUT FDRS</th>
<th>CFX Opus DW</th>
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### Result:

- +: Positive
- -: Negative
- N/A: Not Available
## Appendix 9 - Relative level of detection: raw results - Enrichment in LSB II broth (Extension study, 2023)

### Reference method: ISO 11290-1

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<th>Sample No</th>
<th>Inoculation level (cfu/test portion)</th>
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<th>Fraser</th>
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### PCR results

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#### Confirmations

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<th>RAPID'1 L (100 µL)</th>
<th>RAPID'2 L (100 µL)</th>
<th>AL (100 µL)</th>
<th>AL (10 µL)</th>
<th>Palcam (10 µL)</th>
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### Final result L.spp

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<th>Number positive samples/Total</th>
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### Alternative method : iQ-Check Listeria spp

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<td>Result L.spp</td>
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### Number positive samples/Total

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### Notes:

- **O&A** = Oropharyngeal Antigen
- **Palcam** = Palcam Antigen
- **APF** = Amplification Protocol Fast
- **CFX** = CFX Opus
- **CFX96** = CFX96
- **Cq** = Cycle Threshold
- **L.spp** = Listeria spp
- **RAPID'1** = Rapid Detection Method 1
- **RAPID'2** = Rapid Detection Method 2
- **AL** = Amplification Limit
- **FDRS** = Fast Detection Rapid System

*Analyses performed according to the COFRAC accreditation*
## Matrix: Process water

Strain: *Listeria monocytogenes* AD2503

Seeding 48h at 3±2°C

Aerobic mesophilic flora: 1.4107 CFU/mL

### PCR results

#### Alternative method: IQ-Check *Listeria* spp

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*Analyses performed according to the COFRAC accreditation*

**Summary report (Version 0)**

iQ Check *Listeria* spp.
**iQ Check Listeria spp.**

### ALTERNATIVE METHOD : iQ-Check Listeria spp.

1:10 with pre-warmed LSB II Broth -18h at 37°C

### PCR iQ-Check Listeria spp.

**Easy II lysis protocol - Without FDRS**  
**Easy II lysis protocol - With FDRS**  
**Confirmations**

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### CFX 96

- **FAM Co**: t.C. Co  
- **Result L.app**: RapidL mono (100µL)

### CFX Opus

- **RapidL Lapp**: 
- **AL (15uL)**: 
- **Palcam (10 uL)**: 
- **CFX 96**: 
- **CFX Opus**: 

### Confiramations

**APF Fast**  
**I.C.**: 
**Cq Result**: 
**L.spp**: 

### Summary report

- **Matrix**: Raw milk cheese (Roquefort)  
- **Strain**: L. ivanovich Ad1737  
- **Seeding 48h at 32°C**: Aerobic mesophilic flora: 6.6.10^10 UFC/mL

---

* Analyses performed according to the COFRAC accreditation

ADRIA 185/199  
16 November 2023
### Appendix 10 – Inter-laboratory study: raw data (initial validation study)

**Laboratoire A**

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**Comparison / expected results**

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2. **Fraser**
3. **Confirmation**
4. **Result**
5. **Alternative method: iQ-Check Listeria spp.**
6. **Comparison / expected results**

**Aerobic mesophilic flora (CFU/ml): 2**

**Summary report (Version 0)**

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Aerobic mesophilic flora (CFU/ml)：<1

ADRIA 187/199  
Summary report (Version 0)  
iQ Check Listeria spp.  
16 November 2023
### Laboratory C

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Aerobic mesophilic flora (CFU/ml) : <10

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**Summary report** (Version 0)
iQ Check *Listeria* spp.
## Summary report (Version 0)

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### Aerobic mesophilic flora (CFU/ml) :

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ADRIA

Summary report (Version 0)

iQ Check *Listeria* spp.

189/199

16 November 2023
### Laboratory E

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#### Alternative method: iQ-Check Listeria spp.

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**Aerobic mesophilic flora (CFU/ml) : <1**

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**Summary report (Version 0)**

iQ Check *Listeria* spp.
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### Aerobic mesophilic flora (CFU/ml) : NC

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**Summary report** (Version 0)
iQ Check *Listeria* spp.

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ADRIA

191/199

16 November 2023
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Aerobic mesophilic flora (CFU/ml): 2
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**Comparison / expected results**

**Laboratory**

Reference method: NF EN ISO 11290-1

Alternative method: iQ-Check Listeria spp.

**Aerobic mesophilic flora (CFU/ml): NC**
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**Aerobic mesophilic flora (CFU/ml):** 31

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**Summary report** (Version 0)
iQ Check *Listeria* spp.
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| 16        | +          | +      | +            | +                   | Listeria spp. | +      | =    | N/A    | 20,60                            | +                             | +                              |
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| 24        | +          | +      | +            | +                   | Listeria spp. | +      | =    | N/A    | 19,70                            | +                             | +                              |

### Aerobic mesophilic flora (CFU/ml) : 20

**Summary report** (Version 0)
iQ Check *Listeria* spp.
**Summary report** (Version 0)

iQ Check *Listeria* spp.

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**Aerobic mesophilic flora (CFU/ml)**: 5
**Laboratory M**

**Reference method: NF EN ISO 11290-1**

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**Comparison / expected results**

- **Fraser 1/2**
- **Fraser**
- **Confirmation**
- **Result**
- **Ct Cint**
- **Ct FAM**
- **Test result**
- **Confirmation RLspp**
- **Result**

**Alternative method: iQ-Check Listeria spp.**

- **Confirmation RLspp**
- **Result**

---

**Summary report (Version 0)**

iQ Check *Listeria* spp.

**ADRIA**

198/199

16 November 2023

**Aerobic mesophilic flora (CFU/ml): 30**
## Expert laboratory

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