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NF VALIDATION
Validation of alternative analytical methods
Application in food microbiology

Summary report

EN ISO 16140-2:2016 validation of the GeneDisc® method for the simultaneous detection of *Salmonella spp.* and *Escherichia coli O157:H7*
- Detection of *Salmonella spp* -

Qualitative method

This report consists of 110 pages, including 9 appendices.
Only copies including the totality of this document are authorised.

Competencies of the laboratory are certified by COFRAC accreditation for the analyses marked with the symbol *. 

Version 0
January 16, 2017
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Quality Assurance documents related to this study can be consulted upon request from PALL GENEDISC TECHNOLOGIES.

The technical protocol and the result interpretation were realised according to the EN ISO 16140:2016 and the AFNOR technical rules.

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✓ Studied method: GeneDisc ® Salmonella spp

✓ Validation standard: EN ISO 16140:2016 - Microbiology of the food chain
  - Method validation
    Part 1: Vocabulary
    Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method

✓ Reference method*: EN ISO 6579: Horizontal method for the detection of Salmonella spp

✓ Scope: All human and animal food products

✓ Certification organism: AFNOR Certification (http://nf-validation.org/)

* Analyses performed according to the COFRAC accreditation
1 INTRODUCTION

The GeneDisc method for the simultaneous detection of *Salmonella* spp. and *Escherichia coli* O157:H7 in dairy products, meat products and raw beef meats was validated on the 28th November 2008 (certificate n° GEN 25/05 - 11/08).

Extensions were obtained:

- January 2009 for two versions of GeneDisc Plates with 12 sectors,
- February 2010 for extension to all the food categories,
- March 2011 to extend the validation to the GeneDisc Cycler V3,
- October 2012 for using the GeneDisc Ultra-lyser,
- March 2014 for:
  * 375 g sample size for “Raw beef meats”,
  * Modification of the enrichment volume used for the DNA extraction step for dairy products and raw beef meat (short protocol),
  * Extension to a new format of extraction pack: Extraction Pack Food 2.
- October 2015: to extend to a new provider of raw material

The renewal of the method was obtained in May 2013. Complementary inclusivity tests were then performed to be in agreement with the AFNOR technical rules.

The method was renewed in November 2016.

2 METHOD PROTOCOLS

2.1 Reference method *

The reference method corresponds to the EN ISO 6579 standard: Horizontal method for the detection of *Salmonella* spp (See Appendix 1).

* Analyses performed according to the COFRAC accreditation
2.2 Alternative method

**Principle**

The DNA extraction is based on cell lysis by heat treatment. A specific target of the *Salmonella* genome is then detected by Real-Time PCR using the following GeneDisc:

- GeneDisc *Salmonella* spp., 6 or 12 sectors (GSLMSPP206006 or GSLMSPP2012006),
- GeneDisc pathogenic *E. coli* O157 & *Salmonella* spp., 6 or 12 sectors (GECOSLM206006 or GECOSLM212006),
- GeneDisc STEC and *Salmonella* spp, 6 or 12 sectors (GSTECSL206006 or GSTECSL212006).

**Protocols (see Appendix 2)**

<table>
<thead>
<tr>
<th>Protocol steps</th>
<th>Raw beef meat</th>
<th>Dairy products</th>
<th>All food / Feed samples / Except dairy products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test portion</td>
<td>25 g</td>
<td>375 g</td>
<td>25 g</td>
</tr>
<tr>
<td>Enrichment broth</td>
<td>BPW pre-warmed at 41.5°C ± 1°C</td>
<td>BPW + Acriflavine 10 mg/l</td>
<td>BPW</td>
</tr>
<tr>
<td>Volume of enrichment broth</td>
<td>225 ml</td>
<td>1.5 l</td>
<td>225 ml</td>
</tr>
<tr>
<td>Enrichment conditions</td>
<td>10 h ± 2 h at 41.5°C ± 1°C</td>
<td>10 h - 20 h at 41.5°C ± 1°C</td>
<td>18 h ± 2 h at 37°C ± 1°C</td>
</tr>
<tr>
<td>Sample volume to be processed for the DNA extraction</td>
<td>1 ml 50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>Extraction Pack Food</td>
<td>1 ml 1 or 2 1 or 2</td>
<td>1 ml 1 or 2 1 or 2</td>
<td>1 or 2</td>
</tr>
<tr>
<td>PCR analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confirmation <em>Salmonella</em> spp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Brilliance Salmonella or other appropriate selective agar, purification, latex test</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• RVS, Brilliance Salmonella or other appropriate selective agar, purification, latex test</td>
</tr>
</tbody>
</table>

Confirmatory tests are realised by:

- Direct streaking of 50 µl enrichment broth onto *Brilliance Salmonella*, followed by latex test directly on isolated colonies,
- Subculture in RVS broth (24 h ± 2 h at 41.5°C ± 1°C) and streaking onto *Brilliance Salmonella* and latex test on isolated colonies.

1 Including raw beef meat samples
Reference of the kit insert since the last validation study

The reference of the current kit inserts are listed below:

- GeneDisc Plate *Salmonella* spp. 6 or 12 sectors (GSLMLSPP206006 & GSLMSPP212006): FBIFUGDSSPPENb
- GeneDisc Plate Pathogenic *E. coli* O157 & *Salmonella* spp. 6 or 12 sectors (GECOSLM206006 & GECOSLM212006): FBIFUGDECO157SSPPENb
- GeneDisc Plate STEC & *Salmonella* spp. 6 or 12 sectors (GSTECSL206006 & GSTECSL212006): FBIFUGDSTECSSPPENb
- Extraction Pack Food 1 (PFOOD1100): FBIFUGDEPFOOD1EN
- Extraction Pack Food 2 (PFOOD2096): FBIFUGDPFOODEN

No modification has been made since the last validation study.

3 VALIDATION STUDIES RESULTS

3.1 Method comparison study


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

3.1.1.1 Number and nature of samples

236 samples were analyzed in 2008, 213 in 2010, 197 in 2014 and 30 in 2016. The number of samples tested per tested category for the studies performed in 2008, 2010, 2014 and 2016 are given in Table 1.
## Table 1 – Repartition per tested category and type

<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><strong>Raw beef meats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>25g (2008)</strong></td>
<td>a Raw</td>
<td>8</td>
<td>27</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>b Frozen</td>
<td>16</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>c Seasoned</td>
<td>8</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>32</td>
<td>34</td>
<td>66</td>
</tr>
<tr>
<td><strong>25g (2014)</strong></td>
<td>a Raw</td>
<td>8</td>
<td>15</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>b Frozen</td>
<td>7</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>c Seasoned</td>
<td>9</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>24</td>
<td>30</td>
<td>54</td>
</tr>
<tr>
<td><strong>25g (2008 + 2014)</strong></td>
<td>a Raw</td>
<td>16</td>
<td>42</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>b Frozen</td>
<td>23</td>
<td>10</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>c Seasoned</td>
<td>17</td>
<td>12</td>
<td>29</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>56</td>
<td>64</td>
<td>120</td>
</tr>
<tr>
<td><strong>Meat Products</strong></td>
<td>a Raw meat (except poultry)</td>
<td>16</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>b Raw poultry meat</td>
<td>13</td>
<td>22</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>c RTE and RTC</td>
<td>11</td>
<td>18</td>
<td>29</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>40</td>
<td>56</td>
<td>96</td>
</tr>
<tr>
<td><strong>Dairy products</strong></td>
<td>a Raw milks</td>
<td>6</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>b Raw milk cheeses</td>
<td>16</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>c Creams and fermented milks</td>
<td>10</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>32</td>
<td>35</td>
<td>67</td>
</tr>
<tr>
<td><strong>Dairy products</strong> (2014)**</td>
<td>a Raw milks</td>
<td>3</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>b Raw milk cheeses</td>
<td>19</td>
<td>18</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>c Creams and fermented milks</td>
<td>10</td>
<td>18</td>
<td>28</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>32</td>
<td>45</td>
<td>77</td>
</tr>
<tr>
<td><strong>Dairy products</strong> (2008 + 2014)**</td>
<td>a Raw milks</td>
<td>9</td>
<td>19</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>b Raw milk cheeses</td>
<td>35</td>
<td>28</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>c Creams and fermented milks</td>
<td>20</td>
<td>33</td>
<td>53</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>64</td>
<td>80</td>
<td>144</td>
</tr>
<tr>
<td><strong>Egg products</strong></td>
<td>a Liquid egg products</td>
<td>8</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>b Egg powders and egg based preparations</td>
<td>9</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>c Desserts and pastries</td>
<td>16</td>
<td>14</td>
<td>30</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>33</td>
<td>40</td>
<td>73</td>
</tr>
<tr>
<td><strong>Seafood and Vegetables</strong></td>
<td>a Raw seafood</td>
<td>9</td>
<td>12</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>b Raw vegetables and spices</td>
<td>8</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>c RTE and RTRH</td>
<td>16</td>
<td>6</td>
<td>22</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>33</td>
<td>31</td>
<td>64</td>
</tr>
<tr>
<td><strong>Feed stuffs</strong></td>
<td>a Raw materials and ingredients</td>
<td>7</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>b Low moisture finished products</td>
<td>10</td>
<td>17</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>c High moisture finished products</td>
<td>15</td>
<td>18</td>
<td>33</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>32</td>
<td>56</td>
<td>88</td>
</tr>
<tr>
<td><strong>Raw beef meats</strong></td>
<td>a Raw</td>
<td>11</td>
<td>13</td>
<td>24</td>
</tr>
<tr>
<td><strong>375g - 10h (2014 + 2016)</strong></td>
<td>b Frozen</td>
<td>10</td>
<td>13</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>c Seasoned</td>
<td>10</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>31</td>
<td>37</td>
<td>68</td>
</tr>
<tr>
<td><strong>Raw beef meats</strong></td>
<td>a Raw</td>
<td>11</td>
<td>13</td>
<td>24</td>
</tr>
<tr>
<td><strong>375g - 20h (2014 + 2016)</strong></td>
<td>b Frozen</td>
<td>10</td>
<td>13</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>c Seasoned</td>
<td>10</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>31</td>
<td>37</td>
<td>68</td>
</tr>
</tbody>
</table>

**All products (Raw beef meat -375g 10h)** 289 364 653

**All products (Raw beef meat -375g 20h)** 289 364 653
Due to changes in the AFNOR technical rules concerning the inoculation, 23 samples have been removed for interpretation; they are listed in Table 2.

### Table 2 - Samples removed

<table>
<thead>
<tr>
<th>Category</th>
<th>Type</th>
<th>Samples number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Raw beef meat</td>
<td>a - Raw</td>
<td>511, 576, 577, 578,</td>
</tr>
<tr>
<td></td>
<td>b - Frozen</td>
<td>649, 650, 651, 642, 648</td>
</tr>
<tr>
<td></td>
<td>c - Seasoned</td>
<td>646</td>
</tr>
<tr>
<td>2 Meat products</td>
<td>b - Raw poultry meat</td>
<td>1115, 1116, 1117</td>
</tr>
<tr>
<td>5 Seafood and vegetables</td>
<td>c - Ready to eat and ready to reheat</td>
<td>68</td>
</tr>
<tr>
<td>7 Raw beef meats (375 g)</td>
<td>a - Raw</td>
<td>655, 656, 657, 659</td>
</tr>
<tr>
<td></td>
<td>b - Frozen</td>
<td>5841, 5842, 5844, 5846</td>
</tr>
<tr>
<td></td>
<td>c - Seasoned</td>
<td>654</td>
</tr>
</tbody>
</table>

### 3.1.1.2 Artificial contamination of samples

The strains were stressed using various injury protocols. The injury efficiency was evaluated by comparing enumeration results onto selective and non-selective agar plates (respectively XLD and TSA). The artificial contaminations are provided in Appendix 3.

270 samples were artificially contaminated or cross contaminated; 245 gave a positive result. The repartition of the positive samples per contamination (natural and artificial) level is provided in table 3.
### Table 3 – Repartition of the positive samples per contamination level

<table>
<thead>
<tr>
<th>Category</th>
<th>Type</th>
<th>Naturally contaminated</th>
<th>Cross contamination</th>
<th>Spiking</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Artifically contaminated ≤5 cfu/sample</td>
<td>Artifically contaminated 5&lt;x&lt;10 cfu/sample</td>
<td>Artifically contaminated 10&lt;x&lt;30 cfu/sample</td>
<td>Total</td>
</tr>
<tr>
<td>Raw beef (2008+2014)</td>
<td>a</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0</td>
<td>2</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0</td>
<td>3</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td>Meat products</td>
<td>a</td>
<td>6</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>1</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>18</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Dairy products (2008+2014)</td>
<td>a</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0</td>
<td>0</td>
<td>33</td>
<td>28</td>
</tr>
<tr>
<td>Egg products</td>
<td>a</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>9</td>
<td>0</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Seafood and vegetables</td>
<td>a</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1</td>
<td>0</td>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td>Feed stuffs</td>
<td>a</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>b</td>
<td>7</td>
<td>0</td>
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<td>c</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>12</td>
<td>0</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>Raw beef 375g</td>
<td>a</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>5</td>
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<tr>
<td></td>
<td>b</td>
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<td>4</td>
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<td>Total</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>40</td>
<td>3</td>
<td>146</td>
<td>78</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>14,0%</td>
<td>1,1%</td>
<td>51,2%</td>
<td>27,4%</td>
</tr>
</tbody>
</table>

Taking into account all the studies, 14.0% of the samples were naturally contaminated.

### 3.1.1.3 Confirmation protocols

For the study run in 2008 on raw beef meats, the positive PCR tests were confirmed by direct streaking onto COMPASS *Salmonella* and Brilliance *Salmonella*, and subculture in RVS broth prior streaking onto the two selective agar plates.
For the studies run in 2008 (Meat and dairy products), 2010, 2014 and 2016, only Brilliance *Salmonella* was used for direct streaking and after subculture in RVS broth.

### 3.1.1.4 Results

Raw data are provided in Appendix 4. The results are given in table 4 per confirmation protocol and with all the confirmatory tests combined.

**Table 4 – Interpretation of sample results between the reference and alternative method (based on the confirmed alternative method result using different confirmation protocols)**

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<th>Category</th>
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<th>PD</th>
<th>ND</th>
<th>PPNA</th>
<th>PPND</th>
<th>Total</th>
</tr>
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<td>1</td>
<td>2</td>
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DP: Positive Deviation (R-/A+)
ND: Negative Deviation (R+/A-)
PP: Positive Presumptive non confirmed samples
PPNA: Positive Presumptive non confirmed samples by alternative method
PPND: Positive Presumptive non confirmed samples by reference method
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<th>PD</th>
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3.1.1.5 Calculation of the relative trueness (RT), the relative sensitivity (SE) and the false positive ratio (FPR)

The calculations were done taking into account all the confirmatory tests. They are presented in table 5.
### Table 5 – Calculation of the relative trueness (RT), the relative sensitivity (SE) and the false positive ratio (FPR)

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<th>Type</th>
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<th>ND</th>
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<td>87,5</td>
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<td>PPND</td>
<td>PPNA</td>
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<td>SE ref %</td>
<td>RT %</td>
<td>FPR %</td>
</tr>
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<td>a Raw</td>
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<td>90,9</td>
<td>95,8</td>
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<tr>
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<td>87,0</td>
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<td>93,5</td>
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<td>352</td>
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<td>12</td>
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<td>95,8</td>
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<td>3,3</td>
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<td>All products (Raw beef meat -375g 20h)</td>
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<td>264</td>
<td>352</td>
<td>12</td>
<td>13</td>
<td>0</td>
<td>12</td>
<td>95,5</td>
<td>95,8</td>
<td>96,2</td>
<td>3,3</td>
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</table>
The following results are observed:

**Table 6**

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<th>Shorter incubation time for raw beef (375 g)</th>
<th>Longer incubation time for raw beef (375 g)</th>
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<td>Sensitivity for the alternative method</td>
<td>$SE_{alt} = \frac{(PA + PD)}{(PA + ND + PD)} \times 100%$</td>
<td>95.5 %</td>
</tr>
<tr>
<td>Sensitivity for the reference method</td>
<td>$SE_{ref} = \frac{(PA + ND +)}{(PA + ND + PD)} \times 100%$</td>
<td>95.8 %</td>
</tr>
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<td>Relative trueness</td>
<td>$RT = \frac{(PA + NA)}{N} \times 100%$</td>
<td>96.2 %</td>
</tr>
<tr>
<td>False positive ratio for the alternative method</td>
<td>$FPR = \frac{(FP)}{NA} \times 100%$</td>
<td>3.3 %</td>
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With $ND = ND + PPND$  $NA = NA + PPNA$

3.1.1.6 Analysis of discordant results

- **Negative deviations**

  13 negative deviations were observed (See table 7). 11 samples were artificially contaminated and 2 naturally contaminated. For 8 samples, the confirmatory tests concluded to the presence of *Salmonella* in the enrichment broth.

- **Positive deviations**

  12 positive deviations were observed (See table 8). 11 samples were artificially cross contaminated and only one was a naturally contaminated sample.
### Table 7 - Negative deviations

<table>
<thead>
<tr>
<th>Category</th>
<th>Type</th>
<th>Sample No</th>
<th>Product</th>
<th>Artificial contamination</th>
<th>Alternative method</th>
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</tr>
<tr>
<td>1</td>
<td>b</td>
<td>517</td>
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<td>S. Typhimurium A00C060</td>
<td>1.8</td>
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<tr>
<td>1</td>
<td>a</td>
<td>530</td>
<td>Beef meat</td>
<td>S. Bredeney 396</td>
<td>4.2</td>
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<tr>
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<td>a</td>
<td>1250</td>
<td>Lamb meat</td>
<td>S. Hadar 4871</td>
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<tr>
<td>2</td>
<td>c</td>
<td>1258</td>
<td>Ready to reheat chicken</td>
<td>S. Hadar 4871</td>
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<tr>
<td>3</td>
<td>a</td>
<td>1272</td>
<td>Raw milk</td>
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</tr>
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<td>3</td>
<td>b</td>
<td>1061</td>
<td>Raw milk cheese</td>
<td>S. Tennessee A00E006</td>
<td>5.2</td>
</tr>
<tr>
<td>3</td>
<td>c</td>
<td>5675</td>
<td>Cream</td>
<td>S. Ohio Ad1482</td>
<td>4.4</td>
</tr>
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<td>3</td>
<td>b</td>
<td>458</td>
<td>Raw milk cheese</td>
<td>S. Ohio Ad1482</td>
<td>5.6</td>
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<td>5</td>
<td>c</td>
<td>1679</td>
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<td>S. Typhimurium Adria 305</td>
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<td>a</td>
<td>2194</td>
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<td>b</td>
<td>2038</td>
<td>Raw meat for pet</td>
<td>S. Kedougou</td>
<td>&lt; 1.0</td>
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<td>S. Enteritidis Ad2295</td>
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<td>S. Typhimurium A00C060</td>
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### Table 8 - Positive deviations

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<th>Inoculation level</th>
<th>PCR</th>
<th>Confirmation</th>
<th>Final result</th>
<th>Agreement</th>
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<td>542</td>
<td>Ground beef</td>
<td>Cross contamination</td>
<td>/</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>PD</td>
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<td>5294</td>
<td>Frozen ground beef</td>
<td>S. Dublin Ad529</td>
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<td>+ (35.0)</td>
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<td>5296</td>
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<td>998</td>
<td>Turkey meat</td>
<td>/</td>
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<td>S. Manhattan 900</td>
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<td>-/+</td>
<td>+</td>
<td>+</td>
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<td>S. houtenae Ad596</td>
<td>6.8</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>PD</td>
</tr>
<tr>
<td>3</td>
<td>c</td>
<td>5671</td>
<td>Fermented milk</td>
<td>S. Anatum Ad298</td>
<td>8.8</td>
<td>+ (22.2)</td>
<td>+</td>
<td>+</td>
<td>PD</td>
</tr>
<tr>
<td>3</td>
<td>c</td>
<td>5676</td>
<td>Cream</td>
<td>S. Mbandaka Ad1722</td>
<td>1.4</td>
<td>+ (35.8)</td>
<td>+</td>
<td>+</td>
<td>PD</td>
</tr>
<tr>
<td>3</td>
<td>c</td>
<td>5677</td>
<td>Cream</td>
<td>S. Anatum Ad298</td>
<td>8.8</td>
<td>+ (25.2)</td>
<td>+</td>
<td>+</td>
<td>PD</td>
</tr>
<tr>
<td>7</td>
<td>a</td>
<td>6787</td>
<td>Beef meat</td>
<td>S. Bredeney 975</td>
<td>3.4</td>
<td>+ (34.6/35.0)</td>
<td>+</td>
<td>+</td>
<td>PD</td>
</tr>
<tr>
<td>7</td>
<td>b</td>
<td>6710</td>
<td>Frozen ground beef</td>
<td>S. Enteritidis Ad2295</td>
<td>2.6</td>
<td>+ (32.8/34.1)</td>
<td>+</td>
<td>+</td>
<td>PD</td>
</tr>
</tbody>
</table>
The interpretation of discordant results according to the EN ISO 16140-2:2016 is the following (See table 9).

### Table 9 - Analysis of discordant

<table>
<thead>
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<th>Category</th>
<th>Type</th>
<th>Paired study</th>
<th>Unpaired study</th>
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<td></td>
<td></td>
<td>PD</td>
<td>ND</td>
</tr>
<tr>
<td>Raw beef meats 25g (2008)</td>
<td>Raw</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Seasoned</td>
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<td>Total</td>
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<td>2</td>
</tr>
<tr>
<td>Raw beef meats 25g (2014)</td>
<td>Raw</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Seasoned</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Raw beef meats 25g (2008 + 2014)</td>
<td>Raw</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Seasoned</td>
<td>0</td>
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</tr>
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<td></td>
<td>Total</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Meat Products (2008)</td>
<td>Raw</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Seasoned</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Dairy products (2008)</td>
<td>Raw milks</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Raw milk cheeses</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Creams and fermented milks</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
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<td>2</td>
</tr>
<tr>
<td>Dairy products (2014)</td>
<td>Raw milks</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Raw milk cheeses</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Creams and fermented milks</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Dairy products (2008 + 2014)</td>
<td>Raw milks</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Raw milk cheeses</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Creams and fermented milks</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>6</td>
<td>4</td>
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<tr>
<td>Egg products (2010 + 2016)</td>
<td>Liquid egg products</td>
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<td>0</td>
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<tr>
<td></td>
<td>Egg powders and egg based preparations</td>
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<td>0</td>
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<tr>
<td></td>
<td>Desserts and pastries</td>
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<td></td>
<td>Total</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Seafood and Vegetables (2010)</td>
<td>Raw seafood</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>Raw vegetables and spices</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>RTE and RTRH</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Feed stuffs (2010 + 2016)</td>
<td>Raw materials and ingredients</td>
<td>0</td>
<td>1</td>
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<tr>
<td></td>
<td>Low moisture finished products</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>High moisture finished products</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Raw beef meats 375g - 10h (2014 + 2016)</td>
<td>Raw</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Seasoned</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Raw beef meats 375g - 20h (2014 + 2016)</td>
<td>Raw</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Seasoned</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>All products (Raw beef meat - 375g 10h)</td>
<td>12</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>All products (Raw beef meat - 375g 20h)</td>
<td>12</td>
<td>13</td>
<td>0</td>
</tr>
</tbody>
</table>
The observed values \( ((ND + PPND) - PD) \) are below the acceptability limit for each category and for all the categories for unpaired and paired study design. The values for \( (ND + PPND + PD) \) are below the acceptability limit for each category and for all the categories for paired study design.

3.1.1.7 Confirmatory tests

A summary of the differences observed between the different confirmation protocols is presented in table 10.

**Table 10 - Differences observed between the different confirmation protocols**

<table>
<thead>
<tr>
<th>Category</th>
<th>Confirmation protocol</th>
<th>PA</th>
<th>NA</th>
<th>PD</th>
<th>ND</th>
<th>PPNA</th>
<th>PPND</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>All samples (shorter incubation time for raw beef 375g category)</td>
<td>DS Compass Salmonella</td>
<td>25</td>
<td>34</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>DS Brilliance Salmonella</td>
<td>252</td>
<td>352</td>
<td>12</td>
<td>19</td>
<td>12</td>
<td>6</td>
<td>653</td>
</tr>
<tr>
<td></td>
<td>RV5/Compass Salmonella</td>
<td>27</td>
<td>34</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>RV5/Brilliance Salmonella</td>
<td>262</td>
<td>351</td>
<td>14</td>
<td>15</td>
<td>11</td>
<td>0</td>
<td>653</td>
</tr>
<tr>
<td></td>
<td>All confirmatory tests</td>
<td>264</td>
<td>355</td>
<td>12</td>
<td>13</td>
<td>9</td>
<td>0</td>
<td>653</td>
</tr>
<tr>
<td>All samples (longer incubation time or raw beef 375g category)</td>
<td>DS Compass Salmonella</td>
<td>25</td>
<td>34</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>DS Brilliance Salmonella</td>
<td>249</td>
<td>352</td>
<td>12</td>
<td>18</td>
<td>12</td>
<td>10</td>
<td>653</td>
</tr>
<tr>
<td></td>
<td>RV5/Compass Salmonella</td>
<td>27</td>
<td>34</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>RV5/Brilliance Salmonella</td>
<td>262</td>
<td>351</td>
<td>14</td>
<td>14</td>
<td>11</td>
<td>1</td>
<td>653</td>
</tr>
<tr>
<td></td>
<td>All confirmatory tests</td>
<td>264</td>
<td>355</td>
<td>12</td>
<td>13</td>
<td>9</td>
<td>0</td>
<td>653</td>
</tr>
</tbody>
</table>

Few results were available using the COMPASS Salmonella Agar. The best results were observed when proceeding to a subculture in RVS broth prior streaking onto Brilliance Salmonella. The Salmonella strains were recovered in 276 samples using RVS while only 264 were confirmed positive using direct streaking onto Brilliance Salmonella.

3.1.1.8 Enrichment broth storage for 24 h at 2 - 8°C

97 samples were tested again after enrichment broth storage for 24 h at 2 - 8°C in 2010, 2014 and 2016. The following changes were observed (see table 11).

**Table 11**

<table>
<thead>
<tr>
<th>Category</th>
<th>Type</th>
<th>Sample No</th>
<th>Product</th>
<th>Before storage</th>
<th>After storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>b</td>
<td>459</td>
<td>Raw milk cheese</td>
<td>ND</td>
<td>PA</td>
</tr>
<tr>
<td>4</td>
<td>a</td>
<td>1610</td>
<td>Whole liquid egg</td>
<td>PPNA</td>
<td>PD</td>
</tr>
<tr>
<td>5</td>
<td>c</td>
<td>1680</td>
<td>Ready to reheat</td>
<td>PA</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>a</td>
<td>2194</td>
<td>Hemoglobin</td>
<td>ND</td>
<td>PA</td>
</tr>
<tr>
<td>7</td>
<td>a</td>
<td>5712</td>
<td>Beef tartar</td>
<td>PA (20 h)</td>
<td>PPND</td>
</tr>
</tbody>
</table>
According to the EN ISO 16140-2:2016, the analyses of discordant became (See table 11).

### Table 12

<table>
<thead>
<tr>
<th>Category</th>
<th>Type</th>
<th>PD</th>
<th>ND</th>
<th>PPND</th>
<th>(ND+PPND) - PD</th>
<th>AL</th>
<th>(ND+PPND) + PD</th>
<th>AL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw beef meats 25g (2008)</td>
<td>a Raw</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>b Frozen</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c Seasoned</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td></td>
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<td>0</td>
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<td>/</td>
</tr>
<tr>
<td>Raw beef meats 25g (2014)</td>
<td>a Raw</td>
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<td>0</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c Seasoned</td>
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<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>/</td>
</tr>
<tr>
<td>Meat Products (2008)</td>
<td>a Raw</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>b Frozen</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td></td>
<td></td>
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<td></td>
</tr>
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<td></td>
<td>c Seasoned</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td></td>
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<td>0</td>
<td>0</td>
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<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Dairy products (2008)</td>
<td>a Raw milks</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>b Raw milk cheeses</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c Creams and fermented milks</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td></td>
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<td>0</td>
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<td></td>
</tr>
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<td>c Creams and fermented milks</td>
<td>3</td>
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<td>0</td>
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<td>2</td>
<td>0</td>
<td>-1</td>
<td>3</td>
<td></td>
<td>/</td>
</tr>
<tr>
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<td>0</td>
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<td></td>
<td></td>
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</tr>
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<td>0</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c Desserts and pastries</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>0</td>
<td>-1</td>
<td>3</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Seafood and Vegetables (2010)</td>
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<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>b Raw vegetables and spices</td>
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<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c RTE and RTRH</td>
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<td></td>
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<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Feed stuffs (2010 + 2016)</td>
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<td>0</td>
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<td></td>
<td></td>
<td></td>
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</tr>
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<td></td>
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<td></td>
</tr>
<tr>
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<td>c Cooked products</td>
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<td>0</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Raw beef meats 375g - 10h (2014 + 2016)</td>
<td>a Raw</td>
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<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>b Frozen</td>
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<td>2</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c Seasoned</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>/</td>
</tr>
<tr>
<td>Raw beef meats 375g - 20h (2014 + 2016)</td>
<td>a Raw</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>b Frozen</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c Seasoned</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
<td>/</td>
</tr>
<tr>
<td>All products (Raw beef meat -375g 10h)</td>
<td></td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>All products (Raw beef meat -375g 20h)</td>
<td></td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>4</td>
<td>12</td>
</tr>
</tbody>
</table>
3.1.1.9 PCR inhibitions

The following inhibitions were observed (See table 13).

**Table 13 - PCR inhibitions**

<table>
<thead>
<tr>
<th>Sample No</th>
<th>Product</th>
<th>PCR result</th>
</tr>
</thead>
<tbody>
<tr>
<td>947</td>
<td>Poultry meat</td>
<td>NE / - /+ /+</td>
</tr>
<tr>
<td>1992</td>
<td>Liquid egg product</td>
<td>i/- /-</td>
</tr>
<tr>
<td>1993</td>
<td>Liquid egg product</td>
<td>i/- /-</td>
</tr>
<tr>
<td>1994</td>
<td>Liquid egg product</td>
<td>i/- /-</td>
</tr>
<tr>
<td>2188</td>
<td>Raw material feed stuff</td>
<td>i/- /-</td>
</tr>
<tr>
<td>10</td>
<td>Liquid egg product</td>
<td>i/- /-</td>
</tr>
<tr>
<td>12</td>
<td>Liquid egg product</td>
<td>i/- /-</td>
</tr>
<tr>
<td>25</td>
<td>Raw material pellets</td>
<td>i/- /-</td>
</tr>
<tr>
<td>73</td>
<td>Minerals for bovines</td>
<td>i/+ (24 h storage)</td>
</tr>
<tr>
<td>5286</td>
<td>Puff</td>
<td>i/+ (36.8) * (24 h storage)</td>
</tr>
<tr>
<td>5287</td>
<td>Beef meat</td>
<td>i/+ (39.7) * (24 h storage)</td>
</tr>
<tr>
<td>509</td>
<td>Frozen ground beef</td>
<td>i/- *</td>
</tr>
<tr>
<td>398</td>
<td>Carpaccio</td>
<td>i/- *</td>
</tr>
<tr>
<td>400</td>
<td>Seasoned beef trim</td>
<td>i/- *</td>
</tr>
<tr>
<td>507</td>
<td>Carpaccio</td>
<td>i/- *</td>
</tr>
<tr>
<td>464</td>
<td>Raw milk cheese</td>
<td>i/- *</td>
</tr>
</tbody>
</table>

* 1/10 dilution

16 PCR inhibitions were observed among 914 PCR tests, representing 1.6 % of the tests.

Among the 16 PCR inhibitions, 5 of them were linked to liquid egg products.
3.1.2 **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:

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

3.1.2.1 **Experimental design**

Six matrix/strain pairs were tested using the following protocol:

- 6 non spiked samples,
- 6 samples inoculated at a level required to get 0 to 50 % positive samples,
- 6 samples inoculated at a level required to get 50 to 75 % positive samples,
- 6 samples inoculated at a level required to get 75 to 100 % positive samples.

The samples were analyzed by the reference and the alternative methods, and the background flora was enumerated.

The following matrix/strains were tested:

- Raw beef meat, inoculated by *Salmonella* Infantis 128
- Raw milk inoculated by *Salmonella* Typhimurium 305,
- Sausage meat inoculated by *Salmonella* Virchow 14,
- Egg product inoculated by *Salmonella* Enteritidis 657,
- Raw spinaches inoculated by *Salmonella* Virchow F276,
- Feed stuff inoculated by *Salmonella* Agona A00VO38.

3.1.2.2 **Calculation and interpretation of the RLOD**

The raw data are given in Appendix 5.

The RLOD calculations were performed using Excel spreadsheet of the international standard (ISO 16140), as described in the ISO 16140-2 standard (http://standards.iso.org/iso/16140) - RLOD (clause 5-1-4-2).
Calculation and interpretation of RLOD) ver 06-07-2015. The RLOD are given table 14.

**Table 14 – 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>
</tr>
</thead>
<tbody>
<tr>
<td>Raw beef meat / S.Infantis 128</td>
<td>1.580</td>
<td>0.655</td>
<td>3.809</td>
<td>0.457</td>
<td>0.440</td>
<td>1.039</td>
<td>0.299</td>
</tr>
<tr>
<td>Sausage meat / S.Virchow 647</td>
<td>1.122</td>
<td>0.537</td>
<td>2.343</td>
<td>0.115</td>
<td>0.368</td>
<td>0.311</td>
<td>0.756</td>
</tr>
<tr>
<td>Raw milk / S.typhimurium 305</td>
<td>2.231</td>
<td>0.841</td>
<td>5.916</td>
<td>0.802</td>
<td>0.488</td>
<td>1.645</td>
<td>0.100</td>
</tr>
<tr>
<td>Egg product / S.enteritidis 657</td>
<td>1.000</td>
<td>0.456</td>
<td>2.195</td>
<td>0.000</td>
<td>0.393</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Raw spinach / S.Virchow F276</td>
<td>1.000</td>
<td>0.466</td>
<td>2.146</td>
<td>0.000</td>
<td>0.382</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Feed stuff / S.Agona A00V038</td>
<td>1.000</td>
<td>0.456</td>
<td>2.194</td>
<td>0.000</td>
<td>0.393</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Seasoned beef meat / S.infantis 128</td>
<td>1.166</td>
<td>0.499</td>
<td>2.725</td>
<td>0.154</td>
<td>0.424</td>
<td>0.362</td>
<td>0.717</td>
</tr>
<tr>
<td>Ground beef (375g) / S.Typhimurium A00C060</td>
<td>0.797</td>
<td>0.271</td>
<td>2.341</td>
<td>-0.227</td>
<td>0.539</td>
<td>0.421</td>
<td>1.327</td>
</tr>
<tr>
<td>Raw milk cheese / S.Mbandaka Ad1722</td>
<td>0.139</td>
<td>0.028</td>
<td>0.693</td>
<td>-1.975</td>
<td>0.804</td>
<td>2.456</td>
<td>1.986</td>
</tr>
<tr>
<td><strong>Combined</strong></td>
<td>0.987</td>
<td>0.760</td>
<td>1.281</td>
<td>-0.014</td>
<td>0.131</td>
<td>0.104</td>
<td>1.083</td>
</tr>
</tbody>
</table>

The RLOD are below the AL fixed at 2.5 for unpaired studies and 1.5 for paired studies for the nine tested matrix/strain pairs.

**3.1.3  Inclusivity / exclusivity**

Inclusivity is the ability of the alternative method to detect the target analyte from a wide range of strains. Exclusivity is the lack of interference from a relevant range of non-target strains of the alternative method.

**3.1.3.1 Test protocols**

☐ **Inclusivity**

Strains were grown in BHI broth and inoculated in BPW (10 to 100 cells / 225 ml). The two protocols of the alternative method protocol were tested: short protocol dedicated to raw beef meats (BPW for 8 h at 41.5°C) and protocol for dairy products (BPW + Acriflavine for 16 h at 37°C).

☐ **Exclusivity**

Strains were grown in BHI broth and inoculated in BPW (10⁵ cells/ml). The alternative method protocol was then performed.
3.1.3.2 Results

Data are provided in Appendix 6.

☐ Inclusivity

Among the tested 50 strains, one strain (*Salmonella Wayne Ad 502*) gave a negative PCR result with the short protocol. They all gave a positive PCR test when the “Dairy products” protocol was applied (BPW + Acriflavine 16 h at 37°C).

For the renewal study carried on 2012, complementary tests were done on 9 *Salmonella* strains. The two enrichment protocols were tested. All the strains gave a positive PCR test. The results are provided Appendix 7.

42 additional strains were tested in 2016, including *Salmonella Wayne Ad502* (See Appendix 8). They all gave positive PCR results with the short protocol. One strain (*Salmonella Arbotusovis Ad2320*) gave a negative PCR result with the Dairy products protocol; this strain gave a positive PCR result when milk was added to the enrichment broth.

☐ Exclusivity

No cross reaction was observed on the 30 tested strains.

The alternative method is specific and selective.
3.2 Practicability

The alternative method practicability was evaluated according to the AFNOR criteria relative to method comparison study.

<table>
<thead>
<tr>
<th>Storage conditions and shelf-life</th>
<th>Store the extraction Pack Food 2 at room temperature (15 - 30°C) Store the GeneDisc plate Salmonella spp. at 5°C ± 3°C Expiration date: see data marked on the pack</th>
</tr>
</thead>
<tbody>
<tr>
<td>Workflow (in minutes) for 24 samples</td>
<td>Negative samples</td>
</tr>
<tr>
<td>Steps</td>
<td>Reference method</td>
</tr>
<tr>
<td>25 g</td>
<td>375 g</td>
</tr>
<tr>
<td>Sampling</td>
<td>85</td>
</tr>
<tr>
<td>Sub-culture in RVS and MKTTn</td>
<td>30</td>
</tr>
<tr>
<td>Transfer enriched sample</td>
<td>/</td>
</tr>
<tr>
<td>Extraction</td>
<td>/</td>
</tr>
<tr>
<td>PCR</td>
<td>/</td>
</tr>
<tr>
<td>Streaking onto selective agar plates</td>
<td>60</td>
</tr>
<tr>
<td>Reading selective agar plates</td>
<td>30</td>
</tr>
<tr>
<td>Total for negative samples analyses</td>
<td>205</td>
</tr>
<tr>
<td>Total/negative sample</td>
<td>8.5</td>
</tr>
</tbody>
</table>

Presumptive samples or positive samples

| Steps | Reference method | Alternative method |
| 25 g | 375 g | Direct streaking | Sub-culture RVS |
| 25 g | 375 g | 25 g | 375 g |
| Direct streaking | / | / | 12 | 12 |
| Sub-culture in RVS | / | / | / | / | 15 | 15 |
| Streaking onto selective agar plates | / | / | / | / | 12 | 12 |
| Reading selective agar plates | / | / | 10 | 10 | 10 | 10 |
| Latex test | / | / | 10 | 10 | 10 | 10 |
| Confirmatory tests | 120 | 120 | / | / | / | / |
| Total for positive samples | 325 | 420 | 167 | 182 | 267 | 282 |
| Total/positive sample | 13.5 | 17.1 | 7.0 | 7.6 | 11.1 | 11.8 |
### Time to result

<table>
<thead>
<tr>
<th>Steps</th>
<th>Reference method</th>
<th>Alternative method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beef (25 g)</td>
<td>Beef (375 g)</td>
</tr>
<tr>
<td>Sampling / pre-enrichment</td>
<td>Day 0</td>
<td>Day 0</td>
</tr>
<tr>
<td>Sub-culture (RVS and MKTTn)</td>
<td></td>
<td>/</td>
</tr>
<tr>
<td>Extraction and PCR</td>
<td>/</td>
<td>Day 0 / Day 1</td>
</tr>
<tr>
<td>Streaking onto selective agar plates</td>
<td>Day 2</td>
<td>/</td>
</tr>
<tr>
<td>Reading plates</td>
<td>Day 3</td>
<td>/</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Steps</th>
<th>Reference method</th>
<th>Alternative method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beef (25 g)</td>
<td>Beef (375 g)</td>
</tr>
<tr>
<td></td>
<td>Direct streaking</td>
<td>Sub-culture RVS</td>
</tr>
<tr>
<td>Direct streaking</td>
<td>/</td>
<td>Day 0</td>
</tr>
<tr>
<td>Sub-culture in RVS</td>
<td>/</td>
<td>Day 0</td>
</tr>
<tr>
<td>Streaking onto selective agar plates</td>
<td>/</td>
<td>Day 1</td>
</tr>
<tr>
<td>Reading plates</td>
<td>/</td>
<td>Day 1</td>
</tr>
<tr>
<td>Latex test</td>
<td>/</td>
<td>Day 1</td>
</tr>
</tbody>
</table>

### Presumptive positive or positive results

| Steps                                      | Reference method | Alternative method |
|                                            | Beef (25 g)      | Beef (375 g)       | All products (18 h incubation time) |
|                                            | Direct streaking | Sub-culture RVS    | Brilliance Salmonella | RVS / Brilliance Salmonella | Direct streaking | Sub-culture RVS |
| Confirmatory tests                         | Day 4 to Day 6   | /                  | /                    | /                        | /                | /                |

Negative results are obtained the day of analysis for beef, and within one day for the protocol with 18 h incubation time, while three days are required with the reference method.

Positive results are obtained in one or three days with the alternative method and in four to six days with the reference method.

**Common step with the reference method**: No common step
3.3 Inter-laboratory study (initial validation study – 2008)

3.3.1 Study organisation

Samples were sent to 12 laboratories. The study was done with ground beef samples contaminated by *Salmonella* Typhimurium A00C060. Samples were inoculated and sent on Monday 29th September 2008. The analyses were started on Wednesday 1st October 2008.

The targeted inoculation levels were:

- 0 CFU/25 g,
- 1 – 10 CFU/25 g,
- 5 – 50 CFU/25 g.

Blinded samples were placed in isothermal boxes, which contained cooling blocks, and express-shipped to the different laboratories.

A temperature control flask containing a sensor was added to the package in order to register the temperature profile during the transport, the package delivery and storage until analyses.

The samples were shipped in express (24 h maximum), in isotherm packages. The temperature conditions had to stay lower or equal to 8.4°C during transport, and between 0°C – 8.4°C in the labs.

The *Salmonella* detection was performed by the standard and the alternative method.

3.3.2 Experimental parameters control

3.3.2.1 Sample stability

![Before inoculation]

In order to detect *Salmonella* spp., the EN ISO 6579 method was performed on five ground beef portions (25 g) before the inoculation. All the results were negative.
Sample stability

Sample stability was checked by inoculating the matrix at 100 CFU/g. Enumerations were performed for the high contamination level and detection analyses were performed for the low contamination level. *Triplicate* samples were analysed, and the results were the following:

**Table 15 - *Salmonella* spp. stability in the matrix**

<table>
<thead>
<tr>
<th>Day</th>
<th>CFU/25 g (XLD)</th>
<th>Detection / 25 g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample 1</td>
<td>Sample 2</td>
</tr>
<tr>
<td>Day 0</td>
<td>105</td>
<td>75</td>
</tr>
<tr>
<td>Day 1</td>
<td>85</td>
<td>75</td>
</tr>
<tr>
<td>Day 2</td>
<td>70</td>
<td>80</td>
</tr>
</tbody>
</table>

No evolution was observed during storage at 4°C.

Contamination levels

The contamination levels and the confidence intervals were:

**Table 16**

<table>
<thead>
<tr>
<th>Level</th>
<th>Samples</th>
<th>Theoretical target level (b/25 g)</th>
<th>True level (b/25 g sample)</th>
<th>Low limit / 25 g sample</th>
<th>High limit / 25 g sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 0</td>
<td>1 - 4 - 8 - 10 - 13 - 17 - 22 - 23</td>
<td>0</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Low level</td>
<td>3 - 7 - 11 - 12 - 14 - 16 - 20 - 24</td>
<td>5</td>
<td>4</td>
<td>3.5</td>
<td>4.7</td>
</tr>
<tr>
<td>High level</td>
<td>2 - 5 - 6 - 9 - 15 - 18 - 19 - 21</td>
<td>25</td>
<td>25</td>
<td>22</td>
<td>29</td>
</tr>
</tbody>
</table>
3.3.3 Logistic conditions

Temperature conditions are given in table 17.

Table 17 - Sample temperatures at receipt

<table>
<thead>
<tr>
<th>Laboratories</th>
<th>Temperature measured by the probe (°C)</th>
<th>Temperature measured at receipt (°C)</th>
<th>Date of analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Not measured</td>
<td>3.2</td>
<td>Day 1</td>
</tr>
<tr>
<td>B</td>
<td>3.0</td>
<td>4.9</td>
<td>Day 1</td>
</tr>
<tr>
<td>C</td>
<td>Probe failed</td>
<td>4.2</td>
<td>Day 1</td>
</tr>
<tr>
<td>D</td>
<td>3.0</td>
<td>3.5</td>
<td>Day 1</td>
</tr>
<tr>
<td>E</td>
<td>2.5</td>
<td>3.6</td>
<td>Day 1</td>
</tr>
<tr>
<td>G</td>
<td>2.5</td>
<td>4.5</td>
<td>Day 1</td>
</tr>
<tr>
<td>H</td>
<td>2.5</td>
<td>5.6</td>
<td>Day 1</td>
</tr>
<tr>
<td>J</td>
<td>3.0</td>
<td>5.0</td>
<td>Day 1</td>
</tr>
<tr>
<td>K</td>
<td>4.0</td>
<td>3.8</td>
<td>Day 1</td>
</tr>
<tr>
<td>L</td>
<td>3.5</td>
<td>2.4</td>
<td>Day 1</td>
</tr>
<tr>
<td>M</td>
<td>3.0</td>
<td>4.3</td>
<td>Day 1</td>
</tr>
<tr>
<td>N</td>
<td>0.5</td>
<td>4.0</td>
<td>Day 1</td>
</tr>
</tbody>
</table>

All the labs received their package at Day 1 and no problem was encountered during the shipping.

3.3.4 Calculation and summary of data

The raw data are given in Appendix 9.

3.3.4.1 Results obtained by the expert Lab.

The results obtained by the expert Lab. are the following (See table 18).

Table 18 – 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>8/8</td>
</tr>
<tr>
<td>L2</td>
<td>8/8</td>
<td>8/8</td>
</tr>
</tbody>
</table>
3.3.4.2 Results obtained by the collaborators

Mesophilic aerobic flora

The enumeration of the mesophilic aerobic flora varies from $< 10$ to $> 3.0 \times 10^7$ CFU/g.

Reference method

The positive results by the reference method are provided in Table 19.

Table 19 - Positive results by the reference method
(ALL the collaborators)

<table>
<thead>
<tr>
<th>Laboratory</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>2</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>0</td>
</tr>
<tr>
<td>H</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>
<tr>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>$P_0 = 2$</td>
</tr>
</tbody>
</table>

Alternative method

The positive results of the alternative method are given in table 20.
Table 20 - Positive results (before and after confirmation) by the alternative method (ALL the collaborators)

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Contamination level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L0</td>
</tr>
<tr>
<td></td>
<td>Before confirmation</td>
</tr>
<tr>
<td>A</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
</tr>
<tr>
<td>E</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>0</td>
</tr>
<tr>
<td>H</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>
<tr>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>P₀ = 5</td>
</tr>
</tbody>
</table>

According to the AFNOR technical rules, it is possible to include the results from a collaborator with a maximum of one cross contamination at Level 0. For this study, this rule was applied and results from 2 labs were not kept: C and D.

The results from 10 Labs were kept: A, B, E, G, H, J, K, L, M and N. The results obtained by the 10 individual collaborators in the inter-laboratory study are summarised in Tables 21 and 22.

Table 21 – Positive results by the reference method (with 10 Labs)

<table>
<thead>
<tr>
<th>Laboratory</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>E</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>0</td>
</tr>
<tr>
<td>H</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>
<tr>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>P₀ = 0</td>
</tr>
</tbody>
</table>
Table 22 – Positive results (before and after confirmation) by the alternative method (with 10 Labs)

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Contamination level</th>
<th>Before confirmation</th>
<th>After confirmation</th>
<th>Before confirmation</th>
<th>After confirmation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L0</td>
<td></td>
<td></td>
<td>L1</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>E</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>G</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>H</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>J</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>K</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>L</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>M</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>N</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>P₀ = 0</td>
<td>CP₀ = 0</td>
<td>P₁ = 80</td>
<td>CP₁ = 80</td>
</tr>
</tbody>
</table>

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:

- Specificity for the reference method
  \[ SP_{ref} = \left(1 - \frac{P₀}{N₀}\right) \times 100\% = 100\% \]

- Specificity for the alternative method
  \[ SP_{alt} = \left(1 - \frac{CP₀}{N₀}\right) \times 100\% = 100\% \]

With the 10 Collaborators retained for interpretation, the following results were obtained for Level 1 and level 2 (See table 23).

Table 23 – Summary of the obtained results with the reference method and the alternative method for Level 1 and level 2

<table>
<thead>
<tr>
<th>Response</th>
<th>Reference method positive (R+)</th>
<th>Reference method negative (R-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alternative method positive (A+)</td>
<td>Positive agreement (A+/R+)</td>
<td>Positive deviation (R-/A+)</td>
</tr>
<tr>
<td>PA = 80</td>
<td></td>
<td>PD = 0</td>
</tr>
<tr>
<td>Alternative method negative (A-)</td>
<td>Negative deviation (A-/R-)</td>
<td>Negative agreement (A-/R-)</td>
</tr>
<tr>
<td>ND = 0</td>
<td></td>
<td>NA = 0</td>
</tr>
<tr>
<td>Level 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alternative method positive (A+)</td>
<td>Positive agreement (A+/R+)</td>
<td>Positive deviation (R-/A+)</td>
</tr>
<tr>
<td>PA = 80</td>
<td></td>
<td>PD = 0</td>
</tr>
<tr>
<td>Alternative method negative (A-)</td>
<td>Negative deviation (A-/R-)</td>
<td>Negative agreement (A-/R-)</td>
</tr>
<tr>
<td>ND = 0</td>
<td></td>
<td>NA = 0</td>
</tr>
</tbody>
</table>
Based on the data summarised in table 23, the sensitivity values of the alternative and reference methods, as well as the relative trueness and false positive ratio for the alternative method taking account of the confirmations, are the following (See table 24):

### Table 24

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity for the alternative method: ( SE_{alt} = \frac{(PA+PD)}{(PA+PD+ND)} \times 100% = )</td>
<td>100.0 %</td>
<td>100.0 %</td>
</tr>
<tr>
<td>Sensitivity for the reference method: ( SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)} \times 100% = )</td>
<td>100.0 %</td>
<td>100.0 %</td>
</tr>
<tr>
<td>Relative trueness: ( RT = \frac{(PA+NA)}{N} \times 100% = )</td>
<td>100.0 %</td>
<td>100.0 %</td>
</tr>
<tr>
<td>False positive ratio for the alternative method ( FPR = \frac{FP}{NA} \times 100% = )</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>

#### 3.3.5 Interpretation of data

For an **unpaired** data study, the difference between (ND – PD) is calculated for the level(s) where fractional recovery is obtained (so \( L_1 \) and possibly \( L_2 \)). The observed value found for (ND – PD) shall not be higher than the AL. The AL is defined as \([ (ND – PD)_{max} ]\) and calculated per level where fractional recovery is obtained as described below using the following three parameters:

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

where

- \( P_x = \) number of samples with a positive result obtained with the reference method at level \( x \) (\( L_1 \) or \( L_2 \)) for all laboratories
- \( N_x = \) number of samples tested at level \( x \) (\( L_1 \) or \( L_2 \)) with the reference method by all laboratories.

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

where

- \( CP_x = \) number of samples with a confirmed positive result obtained with the alternative method at level \( x \) (\( L_1 \) or \( L_2 \)) for all laboratories;
- \( N_x = \) number of samples tested at level \( x \) (\( L_1 \) or \( L_2 \)) with the alternative method by all laboratories.

\[
(ND-PD)_{max} = \sqrt{3N_x \times (p^+)_{ref} \times (p^+)_{alt} - 2((p^+)_{ref} \times (p^+)_{alt})}
\]

where

- \( N_x = \) the total number of samples tested for level \( x \) (\( L_1 \) or \( L_2 \)) by all laboratories.
The AL is not met when the observed value is higher than the AL. When the AL is not met, investigations should be made (e.g. root cause analysis) in order to provide an explanation of the observed results. Based on the AL and the additional information, it is decided whether the alternative method is regarded as not fit for purpose. The reasons for acceptance of the alternative method when the AL is not met shall be stated in the study report.

In this study, fractional recovery was not observed. The calculations were done for the two inoculated levels according to the EN ISO 16140-2:2016; they are the following:

<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N_x$</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>$(p^+)_\text{ref}$</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>$(p^+)_\text{alt}$</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>$\text{AL} = (\text{ND} - \text{PD})_{\text{max}}$</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>$\text{ND - PD}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Conclusion</strong></td>
<td>ND - PD ≤ AL</td>
<td>ND - PD ≤ AL</td>
</tr>
</tbody>
</table>

The EN ISO 16140-2:2016 requirements are fulfilled as (ND - PD) is equal to the AL.

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

The relative levels of detection (RLOD) is calculated according to Annexe F of the EN ISO 16140-2:2016 using the excel spreadsheet available at [http://standards.iso.org/iso/16410](http://standards.iso.org/iso/16410). The results are used only for information (see table 25)

**Table 25**

<table>
<thead>
<tr>
<th>RLOD</th>
<th>RLODL</th>
<th>RLODV</th>
<th>b=ln(RLOD)</th>
<th>sd(b)</th>
<th>2 Test statistic</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>0.453</td>
<td>2.208</td>
<td>0.000</td>
<td>0.396</td>
<td>0.000</td>
<td>1.000</td>
</tr>
</tbody>
</table>
### 3.4 Conclusion

The *methods comparative study conclusions* are:

In the sensitivity study, seven categories were tested:

- Raw beef meats (25 g sample size)
- Meat Products
- Dairy products
- Egg products
- Seafood and Vegetables
- Feed stuffs
- Raw beef meats (375 g sample size) (10 h and 20 h incubation times)

The alternative method shows respectively 12 positive deviations (PD) and 13 negative deviations (ND) for the seven categories, taking into account all the confirmatory tests applied. The (ND - PD) and (ND + PD) values are below or equal to the acceptability limits (AL) whatever the categories and for all the tested categories.

The RLOD are below the AL fixed at 2.5 for unpaired studies and 1.5 for paired studies for the nine tested matrix/strain pairs.

For the inclusivity study, two enrichment protocols were tested: BPW for 8 h at 41.5°C, and BPW + acriflavine for 16 h at 37°C. All the strains gave a positive PCR result with the two enrichment protocols tested.

For the exclusivity study, no cross reaction was observed on the 30 tested strains.

It is possible to store the primary enrichment broth for 24 h at 2-8°C.

The negative results are obtained the day of analysis for raw beef meats, and within one day for all the products, while three days are required with the reference method.

The positive results are obtained in one or three days with the alternative method, and in four to six days with the reference method.

The alternative method fulfils all the EN ISO 16140-2:2016 and AFNOR technical rules requirements.
The inter-laboratory study conclusions are:

The data and interpretations comply with the EN ISO 16140-2:2016 requirements. The GeneDisc method is considered equivalent to the ISO standard.
Appendix 1 – EN ISO 6579: 2002: Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

25 g or 375 g of sample + 225 ml BPW

↓

Incubation

18 h ± 2 h at 37°C ± 1°C

0.1 ml BPW

1 ml BPW

10 ml RVS

10 ml MKTTn

Incubation 24 h ± 3 h at 41.5°C ± 1°C

Incubation 24 h ± 3 h at 37°C ± 1°C

Streak on XLD and a second medium

Incubation 24 h ± 3 h at 37°C ± 1°C

Streak 1 characteristic colony onto Nutrient agar

*(Take 4 other colonies if the first one is negative)*

Incubation 24 h ± 3 h at 37°C ± 1°C

Biochemical and serological confirmation
Appendix 2 – Flow diagrams of the alternative method

Raw beef meat

**Enrichment step**
- 25 g + 225 ml pre-warmed BPW at 41.5°C
  - 10 h ± 2 h at 41.5°C ± 1°C
- 375 g + 1.5 l pre-warmed BPW at 41.5°C
  - 10 h - 20 h at 41.5°C ± 1°C

**Extraction step**
- 1 ml Pack Food 1
- 50 µl Pack Food 1 or 2
  - PCR
- 50 µl Pack Food 1 or 2
  - PCR

**Confirmation step**
- Direct streaking onto Brilliance Salmonella Agar, Latex test after a purification step
- Subculture in RVS prior streaking onto Brilliance Salmonella Agar, Latex test after a purification step
**Dairy products**

**Enrichment step**
25 g + 225 ml BPW + Acriflavine (10 mg/l)
18 h ± 2 h at 37°C ± 1°C

**Extraction step**
- 1 ml Pack Food 1
- 50 µl Pack Food 1 or 2

**Confirmation step**
- Direct streaking onto Brilliance Salmonella Agar, Latex test after a purification step
- Subculture in RVS prior streaking onto Brilliance Salmonella Agar, Latex test after a purification step

**All food / Feed samples including raw beef meat samples**

**Enrichment step**
25 g + 225 ml BPW
18 h ± 2 h at 37°C ± 1°C

**Extraction step**
50 µl Pack Food 1 or 2

**Confirmation step**
- Direct streaking onto Brilliance Salmonella Agar, Latex test after a purification step
- Subculture in RVS prior streaking onto Brilliance Salmonella Agar, Latex test after a purification step
Extraction protocols

Extraction Pack Food 1 - Protocol for 1 ml of enriched sample

Extraction Pack Food 1 - Protocol for 50 µl of enriched sample
Extraction Pack Food 2 - Protocol for 50 µl of enriched sample
### Appendix 3 – Artificial contamination of the samples (Initial validation and extension studies, 2008, 2010, 2014 and 2016) in French

<table>
<thead>
<tr>
<th>Analysis date</th>
<th>Sample No</th>
<th>Product (French name)</th>
<th>Artificial contaminations</th>
<th>Global result</th>
<th>Category</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>513</td>
<td>Entrecôte à griller</td>
<td>Contamination croisée avec viande</td>
<td>+</td>
<td>1</td>
<td>a</td>
</tr>
<tr>
<td>2008</td>
<td>531</td>
<td>Viande bovine à bourguignon</td>
<td>Contamination croisée avec viande</td>
<td>-</td>
<td>1</td>
<td>a</td>
</tr>
<tr>
<td>2008</td>
<td>516</td>
<td>Boulettes de bœuf surgelées</td>
<td>Contamination croisée avec viande</td>
<td>+</td>
<td>1</td>
<td>b</td>
</tr>
<tr>
<td>2008</td>
<td>542</td>
<td>Steak haché pur bœuf surgelé</td>
<td>Contamination croisée avec viande</td>
<td>+</td>
<td>1</td>
<td>b</td>
</tr>
<tr>
<td>2008</td>
<td>508</td>
<td>Steak haché</td>
<td><em>Salmonella Typhimurium A00C060</em> Bœuf</td>
<td>+</td>
<td>1</td>
<td>a</td>
</tr>
<tr>
<td>2008</td>
<td>509</td>
<td>Steak haché</td>
<td><em>Salmonella Bredeney 396</em> Bœuf</td>
<td>+</td>
<td>1</td>
<td>a</td>
</tr>
<tr>
<td>2008</td>
<td>510</td>
<td>Steak haché</td>
<td><em>Salmonella Typhimurium A00C060</em> Bœuf</td>
<td>+</td>
<td>1</td>
<td>a</td>
</tr>
<tr>
<td>2008</td>
<td>511</td>
<td>Entrecôte à griller</td>
<td><em>Salmonella Typhimurium A00C060</em> Bœuf</td>
<td>+</td>
<td>1</td>
<td>a</td>
</tr>
<tr>
<td>2008</td>
<td>512</td>
<td>Entrecôte à griller</td>
<td><em>Salmonella Bredeney 396</em> Bœuf</td>
<td>+</td>
<td>1</td>
<td>a</td>
</tr>
<tr>
<td>2008</td>
<td>529</td>
<td>Viande bovine à bourguignon</td>
<td><em>Salmonella Newbrunswick 436</em> Bœuf</td>
<td>+</td>
<td>1</td>
<td>a</td>
</tr>
<tr>
<td>2008</td>
<td>530</td>
<td>Viande bovine à bourguignon</td>
<td><em>Salmonella Bredeney 396</em> Bœuf</td>
<td>+</td>
<td>1</td>
<td>a</td>
</tr>
<tr>
<td>2008</td>
<td>575</td>
<td>Steak haché</td>
<td><em>Salmonella Newbrunswick 586</em> Bœuf</td>
<td>+</td>
<td>1</td>
<td>a</td>
</tr>
<tr>
<td>2008</td>
<td>576</td>
<td>Viande haché fraîche pur bœuf</td>
<td><em>Salmonella Newbrunswick 436</em> Bœuf</td>
<td>+</td>
<td>1</td>
<td>a</td>
</tr>
<tr>
<td>2008</td>
<td>577</td>
<td>Tartare de bœuf</td>
<td><em>Salmonella Newbrunswick 436</em> Bœuf</td>
<td>+</td>
<td>1</td>
<td>a</td>
</tr>
<tr>
<td>2008</td>
<td>578</td>
<td>Viande bovine faux filet à griller</td>
<td><em>Salmonella Panamá 195</em> Bœuf</td>
<td>+</td>
<td>1</td>
<td>a</td>
</tr>
<tr>
<td>2008</td>
<td>515</td>
<td>Boulettes de bœuf surgelées</td>
<td><em>Salmonella Bredeney 396</em> Bœuf</td>
<td>+</td>
<td>1</td>
<td>b</td>
</tr>
<tr>
<td>2008</td>
<td>517</td>
<td>Steak haché surgelé</td>
<td><em>Salmonella Typhimurium A00C060</em> Bœuf</td>
<td>+</td>
<td>1</td>
<td>b</td>
</tr>
<tr>
<td>2008</td>
<td>518</td>
<td>Steak haché surgelé</td>
<td><em>Salmonella Newbrunswick 586</em> Bœuf</td>
<td>+</td>
<td>1</td>
<td>b</td>
</tr>
<tr>
<td>2008</td>
<td>519</td>
<td>Steak haché surgelé</td>
<td><em>Salmonella Dublin 529</em> Bœuf</td>
<td>-</td>
<td>1</td>
<td>b</td>
</tr>
<tr>
<td>2008</td>
<td>520</td>
<td>Steak haché surgelé</td>
<td><em>Salmonella Newbrunswick 436</em> Bœuf</td>
<td>+</td>
<td>1</td>
<td>b</td>
</tr>
<tr>
<td>2008</td>
<td>521</td>
<td>Steak haché surgelé</td>
<td><em>Salmonella Newbrunswick 586</em> Bœuf</td>
<td>+</td>
<td>1</td>
<td>b</td>
</tr>
<tr>
<td>2008</td>
<td>522</td>
<td>Steak haché surgelé</td>
<td><em>Salmonella Dublin 529</em> Bœuf</td>
<td>+</td>
<td>1</td>
<td>b</td>
</tr>
<tr>
<td>2008</td>
<td>523</td>
<td>Steak haché pur bœuf surgelé</td>
<td><em>Salmonella Dublin 529</em> Bœuf</td>
<td>+</td>
<td>1</td>
<td>b</td>
</tr>
<tr>
<td>Analysis date</td>
<td>Sample No</td>
<td>Product (French name)</td>
<td>Artificial contaminations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>-----------</td>
<td>-----------------------</td>
<td>---------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>524</td>
<td>Steak haché pur boeuf surgelé</td>
<td>Salmonella Newbrunswick 436 &lt;br&gt; Bœuf &lt;br&gt; -20°C puis 2 jours à 4°C &lt;br&gt; 0,6 &lt;br&gt; 4-3-2-5-2(3,2) &lt;br&gt; + &lt;br&gt; 1 &lt;br&gt; b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>525</td>
<td>Steak haché pur boeuf surgelé</td>
<td>Salmonella Newport 586 &lt;br&gt; Bœuf &lt;br&gt; -20°C &lt;br&gt; 1,12 &lt;br&gt; 3-1-0-7-1(2,4) &lt;br&gt; + &lt;br&gt; 1 &lt;br&gt; b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>526</td>
<td>Steak haché pur boeuf surgelé</td>
<td>Salmonella Typhimurium A00C060 &lt;br&gt; Bœuf &lt;br&gt; 4°C 2 jours puis -20°C 2 jours &lt;br&gt; 0,73 &lt;br&gt; 15-15-15-13-8(13,2) &lt;br&gt; + &lt;br&gt; 1 &lt;br&gt; b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>527</td>
<td>Steak haché pur boeuf surgelé</td>
<td>Salmonella Bredeney 396 &lt;br&gt; Bœuf &lt;br&gt; 4°C 2 jours puis -20°C 2 jours &lt;br&gt; 0,97 &lt;br&gt; 1-4-1-5-7(3,6) &lt;br&gt; + &lt;br&gt; 1 &lt;br&gt; b</td>
<td></td>
<td></td>
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<td><em>Salmonella Heidelberg</em></td>
<td>Poussières de laiterie</td>
<td>pH3 / 4°C / 25 jours</td>
<td>0,75</td>
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<td>2008</td>
<td>1279</td>
<td>Gros lait</td>
<td><em>Salmonella Montevideo Ad912</em></td>
<td>Lait cru</td>
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<td>2008</td>
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<td><em>Salmonella Anatum Ad298</em></td>
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<td>1522</td>
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<td><em>Salmonella arizonae Ad453</em></td>
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<td>Mayonnaise</td>
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<td>Environnement</td>
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<td>56°C 15 min</td>
<td>1,83e</td>
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<td>Salmonella Derby F81</td>
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<td>Pommes frites Salmonella Havana Ad390</td>
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<td>1679</td>
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<td>Salmonella Typhimurium Adria 305</td>
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<td>Environnement  10% NaCl 30 jours</td>
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<td>Environnement  4°C 33 jours</td>
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<td>Salmonella Kedougou</td>
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## Artificial contaminations

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#### Table: Raw BEEF MEATS (25 g sample size)

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*Analyses performed according to the COFRAC accreditation.

ADRIA Développement
Summary Report (Version 0)
GeneDisc Salmonella

Pall GeneDisc Technologies

52/110
January 16, 2017
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**Notes:**
- **XL:** XLD; **MKT:** Hektoen; **RVS:** Compass; **MKT:** Brilliance.
- **PPNA:** Purpurina.
- **PP:** Pall GeneDisc Technologies.
- **ND:** Not determined.
- **PA:** Present.
- **NA:** Not applicable.
- **+:** Present.
- **-:** Absent.
- **0:** Not detected.
- **b:** Positive control.
- **c:** Negative control.
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### MEAT PRODUCTS

**Reference method : ISO 6579**

**Alternative method: GeneDisc Salmonella**

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**GeneDisc STEC2**

**Compass Salmonelle**

**Brilliance Salmonelle**

**Alternative method: GeneDisc Salmonella**

**DS/Compass Salmonella**

**Brilliance Salmonelle**

**Agreement**

**Final result**

**All confirmatory tests**

**Case**

**Type**

* Analyses performed according to the COFRAC accreditation

ADRA Développement

Summary Report (Version 0)

GeneDisc Salmonella

January 16, 2017
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ADRIA Développement
Summary Report (Version 0)
GeneDisc Salmonella

Pall GeneDisc Technologies

57/110
January 16, 2017
### DAIRY PRODUCTS

**Alternative method: GeneDisc Salmonella**

**BPW + Aciflavin for 16 h at 37°C**

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* Analyses performed according to the COFRAC accreditation

ADRIA Développement

Summary Report (Version 0)

GeneDisc Salmonella

ADRIA Développement

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January 16, 2017

Pall GeneDisc Technologies
| Year of the study | Sample N° | Product (French name) | GeneDisc\(\text{\textregistered}\) Salmonella RVS | MKT\(\text{\textregistered}\) Salmonella | Compass Salmonella Typical colonies Latex | Final result | Agreement\(\text{\textsuperscript{1}}\) to\(\text{\textsuperscript{2}}\) Compass Salmonella Typical colonies Latex | Final result | Agreement\(\text{\textsuperscript{1}}\) to\(\text{\textsuperscript{2}}\) Compass Salmonella Typical colonies Latex | Final result | Agreement\(\text{\textsuperscript{1}}\) to\(\text{\textsuperscript{2}}\) Compass Salmonella Typical colonies Latex | Final result | Agreement\(\text{\textsuperscript{1}}\) to\(\text{\textsuperscript{2}}\) Compass Salmonella Typical colonies Latex | Final result | Agreement\(\text{\textsuperscript{1}}\) to\(\text{\textsuperscript{2}}\) Compass Salmonella Typical colonies Latex | Final result | Agreement\(\text{\textsuperscript{1}}\) to\(\text{\textsuperscript{2}}\) Compass Salmonella Typical colonies Latex |
|------------------|------------|------------------|---------------------------------|----------------|-----------------|----------------|---------------------------------|----------------|---------------------------------|----------------|---------------------------------|----------------|---------------------------------|----------------|---------------------------------|----------------|---------------------------------|----------------|---------------------------------|----------------|---------------------------------|----------------|---------------------------------|----------------|
| 2008             | 1049       | Crème \(\text{\textsuperscript{1}}\) \(\text{\textsuperscript{2}}\) |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1071       | Poudre \(\text{\textsuperscript{1}}\) \(\text{\textsuperscript{2}}\) |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1273       | Lait ribot       |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1274       | Lait ribot       |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1295       | Lait ribot       |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1276       | Lait ribot       |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1277       | Lait ribot       |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1278       | Lait ribot       |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1279       | Lait ribot       |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1510       | Lait ribot       |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1517       | Lait ribot       |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1518       | Lait ribot       |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1520       | Créme \(\text{\textsuperscript{1}}\) \(\text{\textsuperscript{2}}\) épaisse |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1521       | Créme \(\text{\textsuperscript{1}}\) \(\text{\textsuperscript{2}}\) épaisse liquide |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1522       | Créme \(\text{\textsuperscript{1}}\) \(\text{\textsuperscript{2}}\) épaisse bio |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1523       | Créme \(\text{\textsuperscript{1}}\) \(\text{\textsuperscript{2}}\) épaisse 15% MS |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1524       | Gros \(\text{\textsuperscript{1}}\) \(\text{\textsuperscript{2}}\) lait fermier |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1525       | Créme \(\text{\textsuperscript{1}}\) \(\text{\textsuperscript{2}}\) épaisse \(\text{\textsuperscript{3}}\) |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1530       | Gros \(\text{\textsuperscript{1}}\) \(\text{\textsuperscript{2}}\) lait fermier |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1550       | Lait ribot       |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1560       | Gros \(\text{\textsuperscript{1}}\) \(\text{\textsuperscript{2}}\) lait fermier |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
| 2008             | 1567       | Créme \(\text{\textsuperscript{1}}\) \(\text{\textsuperscript{2}}\) épaisse \(\text{\textsuperscript{3}}\) |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |                |

\(\text{\textsuperscript{1}}\) GeneDisc Technologies

**Reference method:** ISO 6579*

**Alternative method:** GeneDisc Salmonella

**Confirmation by direct streaking**

**BPW + Aciflavin for 16 h at 37°C**

**Subculture in RVS prior streaking**

**Confirmation by reference tests**

**All confirmatory tests**

**Final result**

**Agreement All confirmatory tests**

**GeneDisc Salmonella**

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**ADIRA Développement**

Summary Report (Version 0)

January 16, 2017

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* Analyses performed according to the COFRAC accreditation
ADRU A Développement
Summary Report (Version 0)
GeneDisc Salmonella

January 16, 2017
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* Analyses performed according to the COFRAC accreditation

ADIRA Développement
Summary Report (Version 0)
GeneDisc Salmonella

January 16, 2017
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* Analyses performed according to the COFRAC accreditation

ADRIA Développement
Summary Report (Version 0)
GeneDisc Salmonella

January 16, 2017
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ADRA Développement Summary Report (Version 0)
GeneDisc Salmonella

January 16, 2017

65/110
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* Analyses performed according to the COFRAC accreditation
ADRIA Développement
Summary Report (Version 0)
GeneDisc Salmonella

Pall GeneDisc Technologies

January 16, 2017

68/110
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**RAW BEEF (25 g sample size)**

**Protocol: pre-warmed BPW 8h at 41.5°C**

**Confirmation tests**

**All confirmatory tests**

**Agreement**

**GeneDisc Salmonella**

**Type**

**Category**

**ADRIA Développement**

Summary Report (Version 0)

GeneDisc Salmonella

January 16, 2017
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### Alternative method: GeneDisc Salmonella<br>
**Protocol:** pre-warmed BPW 8h at 41.5°C<br>
**BPW storage for 24 h at 2-8°C**

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### Confirmatory tests<br>
- Typical colonies
- Reference method tests
- Final result

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* Analyses performed according to the COFRAC accreditation
ADRA Développement
Summary Report (Version 0)
GeneDisc Salmonella
### RAW BEEF MEATS (375 g sample size)

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<th>Year</th>
<th>Study</th>
<th>N° Sample</th>
<th>French Name</th>
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<th>Conformity tests</th>
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<th>Result (Ct)</th>
<th>Reference method tests</th>
<th>Conformity tests</th>
<th>Agreement</th>
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</table>
| 2014 | 5846  | Hache de boeuf à l'huile surgelé | Frozen seasoned ground beef | Frozen seasoned ground beef | +31 | +M | + | +(34,1) | +1/2 | + | + | + | + | PA | PA | PA | +(30,7) | +M | + | + | M+ | + | + | PA | PA | PA | T | b
| 2014 | 407   | Steak haché congelé | Frozen ground beef | Frozen ground beef | - | - | - | - | - | - | - | - | - | NA | NA | NA | - | - | - | - | - | - | NA | NA | NA | T | b
| 2014 | 408   | Steak haché congelé | Frozen ground beef | Frozen ground beef | - | - | - | - | - | - | - | - | - | NA | NA | NA | - | - | - | - | - | - | NA | NA | NA | T | b
| 2014 | 409   | Steak haché congelé | Frozen ground beef | Frozen ground beef | - | - | - | - | - | - | - | - | - | NA | NA | NA | - | - | - | - | - | - | NA | NA | NA | T | b
| 2014 | 410   | Steak haché et panmesse surgelés | Frozen seasoned ground beef | Frozen seasoned ground beef | - | - | - | - | - | - | - | - | - | NA | NA | NA | - | - | - | - | - | - | NA | NA | NA | T | b
| 2014 | 411   | Steak haché et boulettes surgelées | Frozen beef balls | Frozen beef balls | - | +1 (NC) | - | - | - | - | - | - | - | NA | NA | NA | - | - | - | - | - | - | NA | NA | NA | T | b
| 2014 | 412   | Steak haché et boulettes | Frozen beef balls | Frozen beef balls | - | - | - | - | - | - | - | - | - | NA | NA | NA | - | - | - | - | - | - | NA | NA | NA | T | b
| 2014 | 508   | Hache de boeuf surgelé | Frozen ground beef | Frozen ground beef | - | - | - | - | - | - | - | - | - | NA | NA | NA | - | - | - | - | - | - | NA | NA | NA | T | b
| 2014 | 510   | Steak haché et HAM surgelé | Frozen ground beef | Frozen ground beef | - | - | - | - | - | - | - | - | - | NA | NA | NA | - | - | - | - | - | - | NA | NA | NA | T | b
| 2014 | 511   | Steak haché et saucisson de porc | Frozen ground beef | Frozen ground beef | - | - | - | - | - | - | - | - | - | NA | NA | NA | - | - | - | - | - | - | NA | NA | NA | T | b
| 2014 | 512   | Steak haché et saucisson de porc surgelé | Frozen ground beef | Frozen ground beef | - | - | - | - | - | - | - | - | - | NA | NA | NA | - | - | - | - | - | - | NA | NA | NA | T | b
| 2014 | 5715  | Carapaccio au porc mariné | Carapaccio | Seasoned beef trim | +12 | +M | + | +(36,9) | +(37,4) | + | +(30,0) | +(XLD) | - | - | + | NA | + | ND | ND | PA | +(32,9) | +(XLD) | + | + | +(XLD) | + | + | PA | PA | PA | T | b
| 2014 | 5716  | Carapaccio intégral | Seasoned ground beef | Seasoned ground beef | +3 | +M | + | +(25,0) | +(XLD) | - | - | + | + | +(36,0) | +(XLD) | + | + | + | PPND | PA | PA | +(35,2) | +MSRV(XLD) | + | + | + | PPND | PA | PA | T | b
| 2014 | 5834  | Surimi à la provençale | Seasoned beef trim | Seasoned beef trim | +(12) | +M | + | +(36,0) | +(XLD) | - | - | + | + | + | + | + | PA | PA | PA | +(31,0) | +M | + | + | + | + | PA | PA | PA | T | b
| 2014 | 5835  | Surimi intégral | Seasoned ground beef | Seasoned ground beef | +3 | +M | + | +(36,0) | +(XLD) | - | - | + | + | + | + | + | PA | PA | PA | +(30,0) | +M | + | + | + | + | PA | PA | PA | T | b
| 2014 | 5837  | Carapaccio au saumon | Carapaccio | Seasoned ground beef | +(12) | +M | + | +(36,0) | +(XLD) | - | - | + | + | + | + | + | PA | PA | PA | +(31,7) | +(XLD) | - | - | + | + | + | PPND | PA | PA | T | b
| 2014 | 5838  | Carapaccio sardines | Carapaccio | Seasoned ground beef | +(12) | +M | + | +(36,0) | +(XLD) | - | - | + | + | + | + | + | PA | PA | PA | +(30,0) | +M | + | + | + | + | PA | PA | PA | T | b
| 2014 | 5839  | Carapaccio aux crevettes | Carapaccio | Seasoned ground beef | +(5) | +M | + | +(31,0) | +(XLD) | - | - | + | + | + | + | + | PA | PA | PA | +(24,0) | +M | + | + | + | + | PA | PA | PA | T | b
| 2014 | 389   | Carapaccio au thon | Carapaccio | Seasoned ground beef | +(5) | +M | + | +(31,0) | +(XLD) | - | - | + | + | + | + | + | PA | PA | PA | +(24,0) | +M | + | + | + | + | PA | PA | PA | T | b
| 2014 | 399   | Carapaccio au saumon mariné | Carapaccio | Seasoned ground beef | +(5) | +M | + | +(31,0) | +(XLD) | - | - | + | + | + | + | + | PA | PA | PA | +(24,0) | +M | + | + | + | + | PA | PA | PA | T | b
| 2014 | 400   | Surimi à l'italienne | Seasoned beef trim | Seasoned beef trim | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | T | b
| 2014 | 401   | Surimi à l'italienne | Seasoned beef trim | Seasoned beef trim | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | T | b
| 2014 | 402   | Hache intégrale | Seasoned beef trim | Seasoned beef trim | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | T | b
| 2014 | 403   | Hache intégrale | Seasoned beef trim | Seasoned beef trim | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | T | b
| 2014 | 406   | Hache congelé au thon | Seasoned ground beef | Seasoned ground beef | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | T | b
| 2014 | 499   | Hache intégrale | Seasoned ground beef | Seasoned ground beef | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | T | b

**Reference method ISO 6579**

- **RVS broth**
- **MRTH broth**
- **XLD**

**Alternative method: GeneDisc Salmonella**

- **Pre-warmed BPW for 10h at 41°C**
- **Pre-warmed BPW for 20h at 41°C**
- **PPND**
- **MSRV**
- **Brilliance XLD**
- **Brilliance RVS**
- **Brilliance MSRV**
- **Brilliance RVS/MSRV**
- **Brilliance Brilliance**
- **Brilliance PPND**

**Confidentiality**

- **T**
- **b**
- **c**

**Summary Report (Version 0)**

GeneDisc Salmonella

Pall GeneDisc Technologies

January 16, 2017

72/110
## RAW BEEF MEATS (375 g sample size)

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* An2014ayles performed according to the COFRAC accreditation
ADIR Development
Summary Report (Version 0)
GeneDisc Salmonella

Pall GeneDisc Technologies

January 16, 2017

74/110
# DAIRY PRODUCTS

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* Analyses performed according to the COFRAC accreditation

ADIRA Développement
Summary Report (Version 0)
GeneDisc Salmonella
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* Analyses performed according to the COFRAC accreditation

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Summary Report (Version 0)
GeneDisc Salmonella

Pall GeneDisc Technologies
77/110
January 16, 2017
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## Appendix 5 – Relative level of detection study: raw data (initial validation and extension studies)

**Study realized in 2008**

**Raw beef meat**

Salmonella infantis 128

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Summary Report (Version 0)

GeneDisc Salmonella

January 16, 2017

Pall GeneDisc Technologies
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Pall GeneDisc Technologies
### Raw milk

**Mesophilic aerobic flora: 1,3.10^4/ml**

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A+ : autoagglutinable strains

* Analyses performed according to the COFRAC accreditation
**Study realized in 2010**

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* Analyses performed according to the COFRAC accreditation

ADRIA Développement
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GeneDisc Salmonella
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* Analyses performed according to the COFRAC accreditation
### Study realized in 2014

**Bolognaise ground beef**
**Salmonella Infantis 128**

Mesophilic aerobic flora: $1.6 \times 10^3$ CFU/g

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* Analyses performed according to the COFRAC accreditation

ADRIA Développement
Summary Report (Version 0)
GeneDisc Salmonella

January 16, 2017
# Summary Report (Version 0)

**Ground beef (375 g)**  
**Salmonella Typhimurium AOOC060**  
**Mesophilic aerobic flora:** 3.0.10^3/g  

## GeneDisc Salmonella Protocol

**GeneDisc Salmonella Protocol:**  
- **pre-warmed BPW 41.5°C 10h at 41.5°C**  
- **GeneDisc Salmonella Protocol:**  
  - **pre-warmed BPW 41.5°C 20h at 41.5°C**

### Analyses performed according to the COFRAC accreditation

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* Analyses performed according to the COFRAC accreditation
### Raw milk cheese
**Salmonella Mbandaka Ad 1722**

Mesophilic aerobic flora: 1.2.10⁸ CFU/g

### GeneDisc Salmonella Protocol: BPW with 10mg/L acriflavine 41.5°C 16h at 37°C

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* Analyses performed according to the COFRAC accreditation

Pall GeneDisc Technologies

ADRIA Développement
Summary Report (Version 0)
GeneDisc Salmonella

January 16, 2017

88/110
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### Appendix 6 – Inclusivity and exclusivity: raw data
(Initial validation study - 2008)

#### Raw beef meats protocol (pre-warmed BPW, 8 h at 41.5°C)

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<th>COMPASS Salmonella</th>
<th>Latex</th>
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<td>40 (+34.05/33.99)</td>
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<td>+ microscopic colonies</td>
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<td>18</td>
<td>Chair à merquez</td>
<td>35 (+22.14/22.01)</td>
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<td>Volaille</td>
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## Positive strains

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<td>Typhi</td>
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<td>Lagos</td>
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<td>Newbrunswick</td>
<td>436</td>
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<td>Sternhauze</td>
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<td>Produit alimentaire</td>
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<td>+</td>
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<td>Wayne</td>
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<td>+ micro-colonies</td>
<td>+</td>
<td>+/- weak</td>
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### Raw beef meats protocol (BPW, 24 h at 37°C)

#### Negative strains

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<td>3</td>
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<td>VSM de canard</td>
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<td>4</td>
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<td>Légumes surgelés</td>
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<tr>
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<td>Fromage</td>
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<tr>
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<td>Jambon</td>
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(1) Growth in the broth
### Dairy products protocol (BPW + acriflavine for 16 h at 37°C)

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<th>Reference</th>
<th>Origin (in French)</th>
<th>Inoculation level cfu/225ml</th>
<th>PCR</th>
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### Dairy products protocol (BPW + acriflavine for 16 h at 37°C)

<table>
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<tr>
<th>N°</th>
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<td>Cerro</td>
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<td>Fromage</td>
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<td>Ad 600</td>
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<td>Salmonella</td>
<td>Give</td>
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<td>Blockley</td>
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<td>Poule</td>
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<td>Paella</td>
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### Appendix 7 – Inclusivity study: raw data (renewal study - 2012)

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<th>Strain</th>
<th>Reference</th>
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<th>Inoculation level (cfu/225ml)</th>
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<th>Dairy products protocol (BPW + acriflavin 16 h at 37°C)</th>
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## Appendix 8 – Inclusivity: raw data (Extension study - 2016)

### Strains previously required by AFNOR

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## Appendix 9 - Results obtained by the expert laboratory and the collaborative laboratories (Initial validation study, 2008) in French

Laboratoire: ADRIA

Flore mésophile: 2,3.10^7/g

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* Essai effectué sous le couvert de l’accréditation

ADRIA Développement 98/110 January 16, 2017
Summary Report (Version 0)
GeneDisc Salmonella
### Summary Report

**Laboratoire:** A  
**Flore mésophile:** 2,3.10^7/g  

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2 Isolement direct sur Brilliance *Salmonella*
3 Repiquage en RVS puis isolement sur Brilliance *Salmonella*
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Flore mésophile: >3.0.10^7/g

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4 Isolement direct sur Brilliance Salmonella

5 Repiquage en RVS puis isolement sur Brilliance Salmonella
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Flore mésophile: <10/g

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**Laboratoire: H**

Flore mésophile: 3,1.10°/g

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Laboratoire: J
Flore mésophile: 5,5.10²/g

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Flore mésophile: 3,1.10^6/g
**Laboratoire: L**

**Flore mésophile: 6,2.10⁶/g**

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**GeneDisc Salmonella**

**Laboratoire: M**

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* Analyses performed according to the COFRAC accreditation