NordVal International has studied the enclosures to the application and evaluated the results obtained in the validations conducted by the expert laboratories l’Institut Pasteur de Lille and ADRIA Développement, France, respectively. The validations have been carried out according to ISO 16140:2003 with additional studies for the inclusivity/exclusivity tests. The calculations of the sensitivity acceptance criteria are carried out according to ISO 16140-2:2016. NordVal International concludes that it has been satisfactorily demonstrated that results document no difference in the performances between the iQ-Check® Salmonella II kit and the reference method.

The production of the iQ-Check® Salmonella II kit is fulfilling the requirements given in ISO 9001.

Date: 20 December 2019

Yours sincerely,

Hilde Skår Norli
Chair of NordVal International

Nina Skall Nielsen
NMKL Secretary General
**PRINCIPLE OF THE METHOD**

The iQ-Check *Salmonella* II is a qualitative method allowing the detection of *Salmonella* spp specific DNA sequences after enrichment by culture in buffered peptone water. It is based upon polymerase chain reaction and real time detection using fluorescent probes.

iQ-Check *Salmonella* II describes the following four procedures, differing from each other in preliminary enrichment and lysis steps:

- Standard Protocol I: 18h ± 2h at 37°C ± 1°C enrichment in buffered peptone water followed by the standard lysis protocol.
- Easy Protocol I in micro plates: 21 h ± 1h at 37°C ± 1°C enrichment in buffered peptone water, followed by a simplified extraction protocol, no longer requiring the first centrifugation step.
- Standard Protocol II: specific for raw meat: 10h ± 2h at 37°C ± 1°C enrichment in buffered peptone water followed by the standard lysis protocol.
- Easy Protocol II in microplates, specific for raw beef: 21 h ± 1h at 37°C ± 1°C enrichment in buffered peptone water, followed by a simplified extraction protocol, no longer requiring the first centrifugation step.
- Easy Protocol II, specific for meat products, 18h ± 2h at 37°C ± 1°C enrichment in buffered peptone water followed by the standard lysis protocol.

**FIELD OF APPLICATION**

The method is applicable for the detection of *Salmonella* spp in a broad range of food, animal feed and primary production samples.

**HISTORY**

In 2004, a study was conducted on 362 samples on different matrices showed satisfactory results for iQ-Check Salmonella.

The expert laboratories l’Institut Pasteur de Lille and ADRIA Développement, France carried out extensive studies in 2007 and 2008.

For the 2011 renewal: In addition to the four previously validated NordVal protocols for DNA extraction, the following changes were approved:

- a modification of the extraction of DNA from meat products, using a new “Deepwell plate” was introduced. The expert laboratory ADRIA Développement, France had provided comparative data for the use of the new extraction protocol. The data showed that the extraction modification had no impact on the analytical result
- a new protocol of extraction for meat products, Easy Protocol II, 18h ± 2h was validated by ADRIA.

For the 2017 renewal, the obtained results were evaluated according to the acceptance criteria of ISO 16140-2:2016.

In 2019, the results have been recalculated according to ISO 16140-2 and the NordVal International protocol 1.
COMPARISON STUDY

COMPLIANCE BETWEEN iQ-CHECK SALMONELLA II AND THE REFERENCE METHOD:
Studies in 2007 and 2008 were conducted on 582 product samples, hereof 64 naturally contaminated, 219 artificially contaminated and 299 non-contaminated. Samples from the following main food categories were tested:
Meat products, dairy products, fish-based and vegetable products, egg products, animal feed, primary production samples.
All samples were analysed in single by both the alternative and the reference method.

The comparison studies show that there are no significant differences between the results obtained by using one of the four protocols of the alternative method and the reference method. The following results were obtained:

Accuracy, sensitivity, specificity

Standard Protocol I

<table>
<thead>
<tr>
<th>Matrix</th>
<th>PA</th>
<th>NA</th>
<th>PD</th>
<th>ND</th>
<th>FP</th>
<th>Sum</th>
<th>Relative Trueness RT</th>
<th>Sensitivity alternative method SE_{alt} (%)</th>
<th>Sensitivity reference method SE_{ref} (%)</th>
<th>FPR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat products</td>
<td>29</td>
<td>30</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>60</td>
<td>98.3</td>
<td>96.7</td>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>Dairy products</td>
<td>43</td>
<td>41</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>88</td>
<td>95.5</td>
<td>95.7</td>
<td>95.7</td>
<td>4.9</td>
</tr>
<tr>
<td>Fish based and vegetable</td>
<td>32</td>
<td>39</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>71</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>products</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg products</td>
<td>29</td>
<td>31</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>61</td>
<td>98.4</td>
<td>100</td>
<td>96.7</td>
<td>3.2</td>
</tr>
<tr>
<td>Animal feed</td>
<td>30</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>63</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>Primary prod. samples</td>
<td>28</td>
<td>54</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>84</td>
<td>97.6</td>
<td>93.3</td>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>191</td>
<td>228</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>427</td>
<td>98.1</td>
<td>97.5</td>
<td>98.5</td>
<td>1.3</td>
</tr>
</tbody>
</table>

* see definitions of the abbreviations below.

The Acceptability Limits (AL) for the sensitivity for six categories are ND-PD ≤ 6 and ND+PD ≤ 16, respectively.

For Standard Protocol I, confirmation: ND-PD = 5-3 = 2 and ND+PD = 5+3 = 8

NordVal International requires that there should be a very good agreement between the methods, i.e. kappa > 0.80. Kappa ranged between 0.91 and 1.0.

The acceptance criteria are fulfilled for Standard Protocol I.
### Easy Protocol I

<table>
<thead>
<tr>
<th>Matrix</th>
<th>PA</th>
<th>NA</th>
<th>PD</th>
<th>ND</th>
<th>FP</th>
<th>Sum</th>
<th>Relative Trueness RT</th>
<th>Sensitivity alternative method SE_{alt} (%)</th>
<th>Sensitivity reference method SE_{ref} (%)</th>
<th>FPR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat products</td>
<td>29</td>
<td>30</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>60</td>
<td>98.3</td>
<td>96.7</td>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>Dairy products</td>
<td>42</td>
<td>42</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>88</td>
<td>95.5</td>
<td>93.5</td>
<td>97.8</td>
<td>2.4</td>
</tr>
<tr>
<td>Fish based and vegetable products</td>
<td>30</td>
<td>39</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>71</td>
<td>97.2</td>
<td>93.8</td>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>Egg products</td>
<td>29</td>
<td>31</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>61</td>
<td>98.4</td>
<td>100</td>
<td>96.7</td>
<td>3.2</td>
</tr>
<tr>
<td>Animal feed</td>
<td>29</td>
<td>32</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>63</td>
<td>96.8</td>
<td>96.8</td>
<td>96.8</td>
<td>3.1</td>
</tr>
<tr>
<td>Primary prod samples</td>
<td>29</td>
<td>54</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>84</td>
<td>98.8</td>
<td>96.7</td>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>188</td>
<td>228</td>
<td>3</td>
<td>8</td>
<td>3</td>
<td>427</td>
<td>97.4</td>
<td>96.0</td>
<td>98.5</td>
<td>1.3</td>
</tr>
</tbody>
</table>

* see definitions of the abbreviations below.

The Acceptability Limits (AL) for the sensitivity for six categories are ND-PD ≤ 6 and ND+PD ≤ 16, respectively.

For Easy Protocol I, confirmation: ND-PD = 8-3 = 5 and ND+PD = 8+3 =11

NordVal International requires that there should be a very good agreement between the methods, i.e. kappa > 0.80. Kappa ranged between 0.91 and 1.0.

The acceptance criteria are fulfilled for Easy Protocol I.

### Standard Protocol II (raw meat)

<table>
<thead>
<tr>
<th>Matrix</th>
<th>PA</th>
<th>NA</th>
<th>PD</th>
<th>ND</th>
<th>FP</th>
<th>Sum</th>
<th>Relative Trueness RT</th>
<th>Sensitivity alternative method SE_{alt} (%)</th>
<th>Sensitivity reference method SE_{ref} (%)</th>
<th>FPR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw meat</td>
<td>51</td>
<td>36</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>89</td>
<td>97.8</td>
<td>98.1</td>
<td>98.1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* see definitions of the abbreviations below.

### Easy Protocol II (raw beef)

<table>
<thead>
<tr>
<th>Matrix</th>
<th>PA</th>
<th>NA</th>
<th>PD</th>
<th>ND</th>
<th>FP</th>
<th>Sum</th>
<th>Relative Trueness RT</th>
<th>Sensitivity alternative method SE_{alt} (%)</th>
<th>Sensitivity reference method SE_{ref} (%)</th>
<th>FPR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw beef</td>
<td>31</td>
<td>34</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>66</td>
<td>98.5</td>
<td>96.9</td>
<td>100</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* see definitions of the abbreviations below.

The overall sensitivity and the agreement between the methods are satisfactory.
Easy Protocol II (meat products)

In 2011, a total of 67 meat samples were analysed: 17 naturally contaminated, 13 artificial contaminated and 37 non-contaminated samples. The following results were obtained:

<table>
<thead>
<tr>
<th>Matrix</th>
<th>PA</th>
<th>NA</th>
<th>PD</th>
<th>ND</th>
<th>FP</th>
<th>Sum</th>
<th>Relative Trueness RT</th>
<th>Sensitivity alternative method SE alt (%)</th>
<th>Sensitivity reference method SE ref (%)</th>
<th>FPR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat products</td>
<td>30</td>
<td>37</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>67</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* see definitions of the abbreviations below.

The overall sensitivity and the agreement between the methods are satisfactory.

PA = number of obtained results that are positive with both the alternative and the reference method
NA = number of obtained results that are negative with both the alternative and the reference method
PD = number of obtained results that are positive with the alternative method and negative with the reference method (possible false positive)
ND = number of obtained results that are negative with the alternative method and positive with the reference method (possible false negative)
FP = Positive result by the tested method that is actually confirmed as a negative result
RT = Closeness of agreement between the obtained average of several numbers of replicate measured by the alternative method and the reference method
FRP = Number of false positive test results related to the total number of samples tested.

The Acceptability Limits (AL) for the sensitivity for one category are ND-PD ≤ 3 and ND+PD ≤ 6, respectively.

NordVal International requires that there should be a very good agreement between the methods, i.e. kappa > 0.80. Kappa ranged between 0.97 and 1.0.

The acceptance criteria are fulfilled for all protocols.

The study conducted in 2004 showed satisfactory results for iQ-Check Salmonella. The following was obtained:

- Relative accuracy: 98.9%
- Relative specificity: 100.0%
- Relative sensitivity: 97.5%
- Agreement between the methods, kappa: 0.98

Detection Level

The different matrices have been analysed 6 times at 4 different contamination levels by both methods. The limit of detection was found to be 1-10 cfu in a sample of 25g or 25 ml for all matrices and method protocols.
Inclusivity /exclusivity

The studies carried out in 2007 and 2008, respectively, for the alternative method showed that:
  • 156 strains of *Salmonella* were detected out of the 156 tested, regardless of the lysis protocol used.
  • The study of 30 non-*Salmonella* strains resulted in no cross-reactions regardless of the lysis protocol used.

In addition, the study carried out in 2004 for the alternative method showed that:
  • 51 strains of *Salmonella* were detected out of the 51 tested, regardless of the protocol used.
  • The study of 31 non-*Salmonella* strains resulted in no cross reactions, regardless of the protocol used.

COLLABORATIVE STUDY:
The collaborative study was conducted in 2008 using Easy Protocol I.

Number of participating laboratories was 19. Results from 8 laboratories were excluded due to intralaboratory contamination of the samples, and one laboratory received the samples too late and hence was unable to perform the tests.

The analyses were performed on samples of pasteurized milk, artificially contaminated with a strain of *Salmonella typhimurium* at the following three contamination levels:
  • 0 cfu/25 ml
  • 1-10 cfu/25 ml
  • 5-50 cfu/25 ml

The laboratories analysed 8 replicates for each level using both the alternative and the reference method. The following results were obtained:
  • Sensitivity: 100%
  • Specificity: 95%
  • Relative accuracy: 100%
  • Kappa: 1.00

Thus, there is no statistical difference between the results obtained by the two methods.

CONCLUSION:
According to the comparison and the collaborative study no statistical differences were found between the iQ-check *Salmonella* II test and the reference methods, ISO 6579:2002, for the detection of *Salmonella* in foods, animal feeds and primary production samples.