EURL Salmonella
Interlaboratory comparison study
FOOD VIII 2016

Detection of Salmonella in minced chicken meat

Angelina Kuijpers
### Time Table

<table>
<thead>
<tr>
<th>Week (2016)</th>
<th>Dates</th>
<th>Subject</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>19 – 23 September</td>
<td>Mailing of the protocol and instructions for the web based test report and link to the NRLs by email.</td>
</tr>
<tr>
<td>39</td>
<td>26 September</td>
<td>Mailing of parcels to the NRLs</td>
</tr>
<tr>
<td>40</td>
<td>3 October</td>
<td>Performance of the study</td>
</tr>
<tr>
<td>43</td>
<td>Before 25 October</td>
<td>Deadline for completing the electronic submission of results: <strong>25 October 2016</strong> (23:59h CET)</td>
</tr>
</tbody>
</table>
EURL *Salmonella* detection study

Comparable to earlier studies

The number of samples to be tested and the type of samples are comparable to the studies organised since 2013:

- Artificial contamination of samples at laboratory EURL-*Salmonella* before sending to NRLs
- Use of webbased testreport
- Less control samples: one blank control & one own positive control
- No Standard Operating Procedure (SOP), Follow EN ISO 6579 (and the underlying EN ISO documents) according to normal routine procedure for detection of *Salmonella* in ‘official’ samples.
- Reporting results positive or negative per sample
Pre-test: stability of samples (I) minced meat with *Salmonella*

PRE-TEST

- Artificially contamination of minced turkey meat with a diluted *Salmonella* culture

- *Salmonella* Stanley (SSt) and S. Typhimurium (STM) tested: isolated from human & chicken

- Level of contamination/25 g meat: 10-15 CFU and 100 CFU

- Stability of samples during:
  - storage (-20 °C & 5 °C)
  - transport (10 °C)
  - *Salmonella* detection and influence of backgroundflora

- BRO food 2013 storage at NRL 5 °C high amount of backgroundflora
  - if storage at -20 °C is possible
Pre-test: stability of samples (II)
minced turkey meat

**4 samples with SS\textit{t}6 and STM\textit{1}2 tested**

3 weeks at -20 °C, 5 °C & 10 °C
Test 4 samples after
1, 2 and 3 weeks of storage
All samples positive : stable

**Backgroundflora:**
**Enterobacteriacea** $10^2$ CFU/g
**Aerobic** $10^7$ CFU/g
Increase at 5 °C & 10 °C
Entero$>\log4$ - $5$
Aerobic $>\log2$ - $3$

Stable for 3 weeks at -20 °C
Pre-test: stability of samples (III)
minced turkey meat with SSr

6 samples with SSr12 and SSr42 tested

3 weeks -20 °C
2 weeks at -20 °C, 1 week 5 °C
1 week -20 °C, 1w 5 °C, 1w -20 °C
All samples positive : stable

Backgroundflora:
Enterobacteriacea $10^3$ CFU/g
Aerobic $10^6$ CFU/g
Stable at -20 °C
Increase at 5 °C
Entero > log 3
Aerobic > log 2
Pre-test: stability of samples (IV) minced meat with *Salmonella* Stanley

CONCLUSION

- **Matrix:** minced turkey meat

- **Artificial contamination of meat with a diluted culture:**
  - SSSt low level (15-20 CFU/25g meat)
  - SSSt high level (50-100 CFU/25g meat)
  - Blank

- **After contamination of the meat with SSSt**
  Storage at the EURL and the NRLs at -20 °C
  But at the very last minute..
  change of matrix because of naturally contamination of minced turkey meat
  New Matrix: minced chicken meat
## Study design

<table>
<thead>
<tr>
<th>Artificially contaminated</th>
<th>Samples (n=18): 25 g minced meat</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Stanley low</td>
<td>6</td>
</tr>
<tr>
<td>S. Stanley high</td>
<td>6</td>
</tr>
<tr>
<td>Blank</td>
<td>6</td>
</tr>
</tbody>
</table>

Number and level of samples according to ISO/TS 22117 (2010)

<table>
<thead>
<tr>
<th>Control samples</th>
<th>n=2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procedure control blank: BPW</td>
<td>1</td>
</tr>
<tr>
<td>Positive control: Own control with <em>Salmonella</em></td>
<td>1</td>
</tr>
</tbody>
</table>
Analytical methods in study

● Follow FDIS ISO 6579-1 (choose between RVS and MSRV)
  - Selective enrichment in: MKTTn and RVS or MKTTn and MSRV or MKTTn, RVS and MSRV

● Isolation medium
  - XLD and 2nd agar for choice

● Confirmation
Participants

34 Laboratories

- 30 NRLs from 28 EU-Member States (MS)
- 4 NRLs from third countries
  - EU candidate MSs or potential EU candidate MSs
  - members of EFTA countries
  - non-European countries
Transport of samples

Cooling devices & Temperature logger
Packages all transported as Biological Substances Cat. B UN3373 by DHL door-to-door

- **Transport time:**
  - 27 parcels: 1 day
  - 6 parcels: 2 days
  - 1 parcel (non-EU MS): 3 days delay at the customs
Transport of samples

Cooling devices & Temperature logger
Packages all transported as Biological Substances Cat. B UN3373 by DHL door-to-door

**Temperature** during
- **transport:** all parcels /NRLs at least **-1 °C**
- **storage at the NRL**
  31 NRLs between **-15 °C** and **-28 °C**
  1 NRL **-7 °C**
  1 NRL **1 °C**
  1 NRL **room Temp** after arrival ?

![Graph showing temperature changes](image-url)
Material & Methods

Follow as much as possible your routine procedure

● Addition of 225 ml BPW to plastic bags (samples)  
  (BPW at least at room temperature)

● Homogenisation  
  (according ISO 6887-2)

● Incubation BPW sample at 37 °C for 18 h

● Selective enrichment media MKTTn and RVS and/or MSRV

● Isolation on XLD and 2nd plate & Confirmation
## Level of contamination S. Stanley

<table>
<thead>
<tr>
<th>Date of testing</th>
<th>Low level SST CFU/sample 25 g</th>
<th>High level SST CFU/sample 25 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 September 2016</td>
<td>16</td>
<td>73</td>
</tr>
<tr>
<td>Inoculum of meat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 October 2016</td>
<td>35 MPN (11-110)</td>
<td>55 MPN (16-188)</td>
</tr>
<tr>
<td>Meat inoculated with SST and stored at -20 °C for 10 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Performance of the study 3 October 2016
MPN: Most Probable Number (5 tube), (95 % confidence interval)
**Backgroundflora minced chicken meat**

<table>
<thead>
<tr>
<th>Date of testing</th>
<th>Enterbacteriaceae CFU/g</th>
<th>Aerobic bacteria CFU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 October 2016 After storage for 3 days at +5 °C followed by 10 days at -20 °C</td>
<td>$4 \times 10^4$</td>
<td>$2 \times 10^6$</td>
</tr>
</tbody>
</table>

Performance of the study 3 October 2016
Accreditation according to ISO/IEC 17025

All 34 participants are accredited

Accredited method:
32 NRLs ISO 6579: 28 NRLs also Annex D of ISO 6579
2 NRLs (non-EU-MS) only Annex D of ISO 6579
Media/ Methods used

● Media for selective enrichment:
  – 19 NRLs MKTTn, RVS & MSRV (2015:27)
  – 6 NRLs MKTTn & RVS
  – 9 NRLs MKTTn & MSRV (2015:3)

● Media for 2nd isolation medium:
  - BGA mod 8
  - Rambach 6
  - BPLS & RS 5
  - BGA 3
  - SM(ID)2& BSA 2
  - ASAP, Compas S, Chrom S 1

● Additional own method: PCR 9 participants (8 real time PCR)
## Criteria for Good performance

### Samples (n=18)

<table>
<thead>
<tr>
<th>Minced meat</th>
<th>Percentage positive</th>
<th>No of positive samples/Total No. Of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Stanley low</td>
<td>50 %</td>
<td>3/6</td>
</tr>
<tr>
<td>S. Stanley high</td>
<td>80 %</td>
<td>5/6</td>
</tr>
<tr>
<td>Blank</td>
<td>20 % at max</td>
<td>1/6 at max</td>
</tr>
</tbody>
</table>

### Control samples (n=2)

<table>
<thead>
<tr>
<th>Procedure control blank: BPW</th>
<th>0 %</th>
<th>0/1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control: Own control with <em>Salmonella</em></td>
<td>100%</td>
<td>1/1</td>
</tr>
</tbody>
</table>
Control samples
The laboratories used their own routine positive control.

Positive controls:  
diluted culture 20
lenticule disc 7
freeze dried ampoule 4
culti-loop, kwik-stik, Vitroid 1

Salmonella serovar.
S. Enteritidis 14
S. Typhimurium 7
S. Nottingham 5
S. Blegdam, S. Abaetetuba, S. Senftenberg, S. Dublin
S. Bongori, S. Tennessee, S. Harleystreet, S. Alachua
Results control samples

Procedure control Blank (BPW)
- All NRLs analysed this sample correctly negative

Positive control (own) with *Salmonella*
- All NRLs analysed this sample correctly positive

Correct scores (%)

<table>
<thead>
<tr>
<th>Control samples</th>
<th>MKTTn and RVS or/and MSRV XLD or 2nd plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procedure control (BPW)</td>
<td>100</td>
</tr>
<tr>
<td>Positive control (own)</td>
<td>100</td>
</tr>
<tr>
<td>All control samples</td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
Results meat samples (I)

Blank
- 31 NRLs scored all 6 blank meat samples correctly negative.
- 3 laboratories found 1 sample positive for *Salmonella*.

Low SSt
- All NRLs detected *Salmonella* in all 6 samples.

High SSt
- 33 NRLs detected *Salmonella* in all 6 samples.
- 1 laboratory found 1 sample negative for *Salmonella*.

*All participants scored a good performance !!!!
Results meat samples (II)

Blank
● 3 laboratories found 1 sample positive for *Salmonella*.

High SST
● 1 laboratory found 1 sample negative for *Salmonella*.
(and 1 blank sample positiv for *Salmonella*)

- exchange, *cross-contamination, misinterpretation of the results* ??

No other *S*. *Infantis* at the laboratories (positive control, samples...)
Possible clarification:
Naturally contamination of meat with *S*. *Infantis* at a very low level because all other tested Blanks by NRLs and EURL (>200) were tested negative.
Results minced chicken meat samples (III)
Low level SSt
Results minced chicken meat samples (IV)

High level SSt
### Specificity, sensitivity & accuracy (%)

Minced chicken meat samples

<table>
<thead>
<tr>
<th></th>
<th>MKTTn and RVS or/and MSRV XLD or 2nd plate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specificity</strong></td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>99</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td></td>
</tr>
<tr>
<td>Low SSSt</td>
<td>100</td>
</tr>
<tr>
<td>High SSSt</td>
<td>99</td>
</tr>
<tr>
<td>All SSSt samples</td>
<td>99</td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td></td>
</tr>
<tr>
<td>All samples</td>
<td><strong>99</strong></td>
</tr>
</tbody>
</table>
Performance of the NRLs

- All 34 NRLs achieved the level of good performance

Suggestion from participant

- Stomacher bags with filter to contain the samples.
Conclusions

- All participants achieved the level of **good performance**

- The **accuracy** rates for the artificially contaminated minced chicken meat samples were high **99%**.
THANKS for your participation & cooperation in this study

Angelina Kuijpers