Interim summary report EURL-*Salmonella*

Interlaboratory Comparison study FOOD VIII (2016)

Detection of *Salmonella* in minced chicken meat

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Introduction

In September 2016, the eighth EURL-*Salmonella* interlaboratory comparison study on the detection of *Salmonella* in a food matrix (Food VIII) was organised for the European NRLs for *Salmonella.* In total 34 NRLs participated in this study: 30 NRLs from 28 EU-Member States (MS) and four NRLs from third countries (EU candidate MS or potential EU candidate MS, members of the European Free Trade Association (EFTA) or non-European countries).
This interim summary report consists of two parts. One part contains the individual NRL results; the other part contains the overall results of all NRLs which is described here.

Materials & Methods

Samples

Each NRL analysed in total 20 samples: 18 samples of each 25 g minced chicken meat artificially contaminated with three different levels (Blank, low and high level) of *Salmonella* Stanley (SSt) and 2 control samples.
It was initially announced that minced turkey meat would be used as matrix for this study, but as it turned out that this batch was naturally contaminated with *Salmonella* the choice of the matrix was changed (at the very last minute) to minced chicken meat.
A batch of 18 kg minced chicken meat was obtained from, Plukon, Ommel in the Netherlands. The meat arrived at EURL-*Salmonella* on 19 September 2016 and was tested negative for *Salmonella*. The minced chicken meat was packed in portions of 25 gram, after which the test portions were artificially contaminated with three different levels (Blank, low and high level) of *Salmonella* Stanley (SSt) and stored at -20 °C. On Monday 26 September, the meat samples were mailed to the NRLs where they were placed at -20 °C. Table 1 shows the number of aerobic bacteria and *Enterobacteriaceae* in the meat determined by the EURL-*Salmonella* at the date of the interlaboratory comparison study (3 October 2016).

Table 1 Number of aerobic bacteria and Enterobacteriaceae *per gram of minced chicken meat (negative for* Salmonella*)*

Date	*Enterobacteriaceae* CFU/g	Aerobic bacteria CFU/g
3 October 2016 After storage for 3 days at +5 °C followed by 10 days at -20 °C	$4*10^4$	$2*10^6$

Table 2 shows the level of the diluted culture with *Salmonella* Stanley used to contaminate the meat samples. Of the artificially contaminated minced chicken meat samples with low and high level SSt, also a five tube Most Probable Number (MPN) test was performed. These results are also summarised in Table 2.

Table 2 Number of Salmonella *Stanley (SSt) in the inoculum for artificial contamination of the meat and in the meat samples after storage at -20 °C.*

Date of testing	Low level SSt CFU/per sample	High level SSt CFU/per sample
21 September 2016 (Inoculum of meat)	16	73
3 October 2016 MPN of meat, inoculated with SSt (95 % confidence limit) after storage for 10 days at -20 °C	35 (11-110)	55 (16-188)

The NRLs had to analyse the following samples:

6x (25g meat + low level of SSt)
6x (25g meat + high level of SSt)
6x (25g meat)

Furthermore some control samples had to be analysed, being:

1x only BPW (Procedure control Blank)
1x own control sample with *Salmonella* (Own positive control)

The meat samples were individually packed and labelled. The decoding of these samples can be found in the tables of the individual NRL results.

Calculation of specificity, sensitivity and accuracy rates:

$$\text{Specificity rate: } \frac{\text{number of negative results}}{\text{Total number of (negative) samples}} \times 100\%$$

$$\text{Sensitivity rate: } \frac{\text{number of positive results}}{\text{Total number of (expected positive) samples}} \times 100\%$$

$$\text{Accuracy rate: } \frac{\text{number of correct results (positive and negative)}}{\text{Total number of samples}} \times 100\%$$

Analysis of samples according to ISO/FDIS 6579-1

The NRLs had to follow EN ISO 6579 (and the underlying EN ISO documents, e.g. the EN ISO 6887 series for preparation of test samples) according to their normal routine procedure for detection (and confirmation) of *Salmonella* in 'official' samples. The Final Draft International Standard (FDIS) version of ISO 6579-1 is available. This document describes the (final) updated technical steps for the detection of *Salmonella* in food, animal feed and samples from the primary production stage. An important change in this document compared to the current version of ISO 6579, is the possibility to choose between RVS and MSRV for the selective enrichment of *Salmonella* from food and animal feed samples. For that reason, this choice was already introduced in the current study, meaning that additional to MKTTn,

either RVS or MSRV could be used for selective enrichment. It was also allowed to use all three selective enrichment media.

For the reporting of the results, the participants were asked to report what would have been reported in case these samples would have been routine samples, meaning that the indication 'positive' (1) or 'negative' (0) per sample (after confirmation) was sufficient (independent of the combination of selective enrichment medium and isolation medium).

Criteria for good performance

For determining good performance per laboratory, all combinations of selective enrichment media (MKTTn and/or RVS and/or MSRV) and isolation media used by the laboratory were taken into account.

For the determination of good performance, the criteria as indicated in Table 3 were used.

Table 3 Used criteria for good performance in the Food VIII study (2016)

	Minimum result	
	Percentage positive	No. of positive samples/ total No. of samples
Control samples		
Own control with *Salmonella*	100 %	1 /1
BPW	0 %	0 /1
Samples : minced chicken meat artificially contaminated		
Blank[1]	20 % at max[1]	1/6 at max[1]
SSt high	80 %	5/6
SSt low	50 %	3/6

1: All should be negative. However, as no 100 % guarantees about the *Salmonella* negativity of the matrix can be given, 1 positive out of 6 blank samples (20 % pos.) will still be considered as acceptable.

Results

General

On Monday 26 September 2016 (week 39) the samples were sent to 34 laboratories. The majority of the parcels were delivered at the NRLs within 1- 2 days.
All laboratories performed the study as requested in week 40. Most of them started on 3 October 2016, three participants performed the study 1 or 2 days later. All laboratories used as requested MKTTn as selective enrichment medium. Nineteen participants used additional both selective enrichment media RVS and MSRV. Six laboratories used only RVS in addition to MKTTn and nine laboratories used only MSRV in combination with MKTTn.

Controls

Procedure control Blank (only BPW)
All laboratories analysed the one procedure control sample (no matrix, only BPW) correctly negative for *Salmonella*.

Positive control with Salmonella
All laboratories scored good results with their own *Salmonella* positive control sample.

For the positive control, the majority of the participants used a diluted culture of *Salmonella* (20 laboratories). Other participants used a lenticule disc (7) or a freeze dried ampoule (4) with *Salmonella*. The *Salmonella* serovars used for the positive control sample were *Salmonella* Enteritidis (14), *Salmonella* Typhimurium (7) and *Salmonella* Nottingham (5).

Table 4 gives the correct scores for the control samples with accuracy rates of 100%.

Artificially contaminated meat samples

Blank samples
Thirty-one laboratories correctly scored all 6 blank meat samples negative for *Salmonella*. Three laboratories (lab code 10, 19 and 23) found one blank sample out of six positive for *Salmonella.* All blanks should be tested negative. However, as no 100 % guaranty about the *Salmonella* negativity of meat can be given, one positive out of six blank samples (80 % neg.) will still be considered as acceptable. A false positive result for a blank sample may also been caused by cross-contamination, exchange of samples or by misinterpretation of the results.

High level contaminated Salmonella *Stanley samples*
Thirty-three laboratories detected *Salmonella* in all six high level contaminated meat samples. One laboratory (lab code 10) could not detect *Salmonella* in one out of six high level contaminated samples.

Low level contaminated Salmonella *Stanley samples*
All laboratories detected *Salmonella* in all six low level contaminated meat samples.

Figure 1 and 2 give all possible combinations of media (MKTTn and RVS or/and MSRV) giving the highest number of positive results per laboratory for respectively the low and high level (SSt) contaminated meat samples.

In Table 5 the specificity, sensitivity and accuracy rates are given for the artificially contaminated meat samples. The specificity and accuracy rates were 99 % and sensitivity rates for low contaminated meat samples was 100%.

Good performance

All laboratories fulfilled the criteria of good performance.

Table 4 Correct scores of the control samples

Control samples	n=34	**MKTTn and RVS or/and MSRV/ XLD or 2nd plate**
Procedure control Blank (BPW) n=1	No. of samples	34
	No. of negative samples	34
	Correct score in %	**100**
Positive control (Own *Salmonella*) n=1	No. of samples	34
	No. of positive samples	34
	Correct score in %	**100**
All Control samples	No. of samples	68
	No. of correct samples	68
	Accuracy in %	**100**

Table 5 Specificity, sensitivity and accuracy rates of the artificially contaminated minced chicken meat samples

Meat	n=34	**MKTTn and RVS or/and MSRV/ XLD or 2nd plate**
Blank n=6	No. of samples	204
	No. of negative samples	201
	Specificity in %	**99**
Low level n=6	No. of samples	204
	No. of positive samples	204
	Sensitivity in %	**100**
High level n=6	No. of samples	204
	No. of positive samples	203
	Sensitivity in %	**99**
All meat samples with *Salmonella*	No. of samples	408
	No. of positive samples	407
	Sensitivity in %	**99**
All meat samples	No. of samples	612
	No. of correct samples	608
	Accuracy in %	**99**

List of abbreviations

BL	Blank-No colony forming units
BPW	Buffered Peptone Water
CFU	colony forming units
EFTA	European Free Trade Associations
EURL	European Union Reference Laboratory
FDIS	Final Draft International Standard
ISO	International Standardisation Organisation
MKTTn	Mueller Kauffmann Tetrathionate novobiocin broth
MPN	Most Probable Number
MS	Member State
MSRV	Modified Semi-solid Rappaport Vassiliadis
NRL	National Reference Laboratory
RVS	Rappaport Vassiliadis Soya broth
SSt	*Salmonella* Stanley
XLD	Xylose Lysine Deoxycholate agar

References

International Standard – ISO 6579: 2002(E)
Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

Amendment ISO 6579:2002/Amd 1 2007
Amendment 1 Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage.

International Standard – ISO 6887-4: 2003
Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 4: Specific rules for the preparation of products other than milk and milk products, meat and meat products and fish and fishery products.

International Standard – ISO/FDIS 6579-1: November 2015
Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Horizontal method for the detection of *Salmonella* spp.

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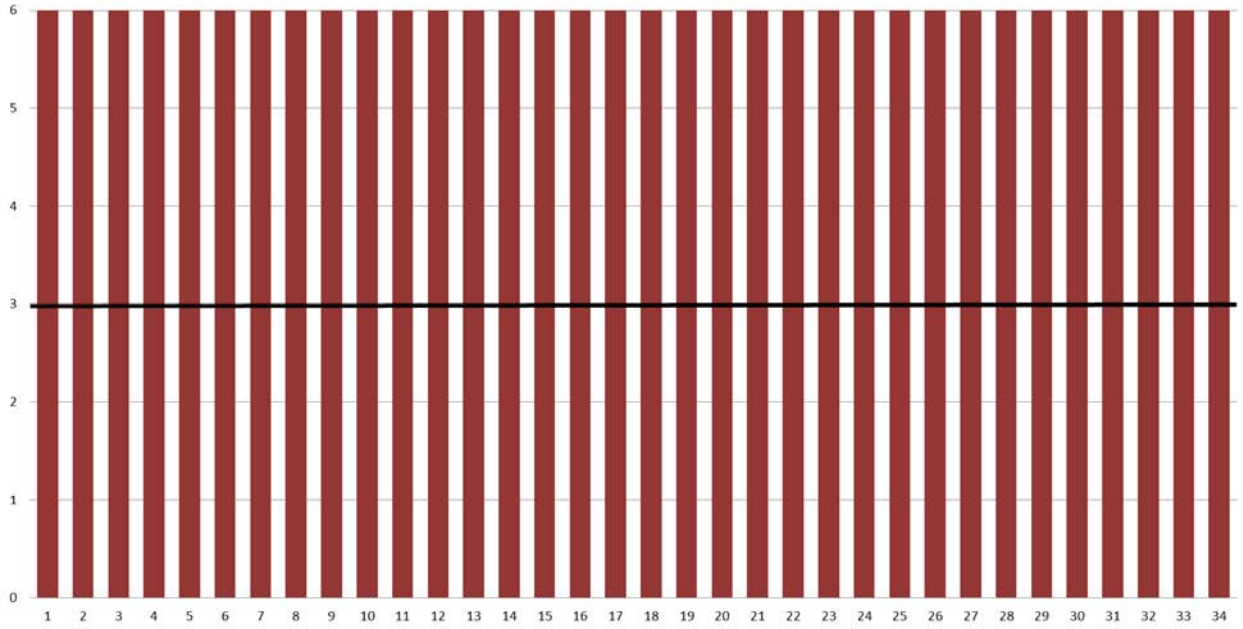

Figure 1 Number of positive isolations per laboratory after analysing 6 samples of each 25 g minced chicken meat artificially contaminated with low level Salmonella *Stanley. Results concern all possible combinations of media (MKTTn and RVS or/and MSRV) giving the highest number of positive samples.*

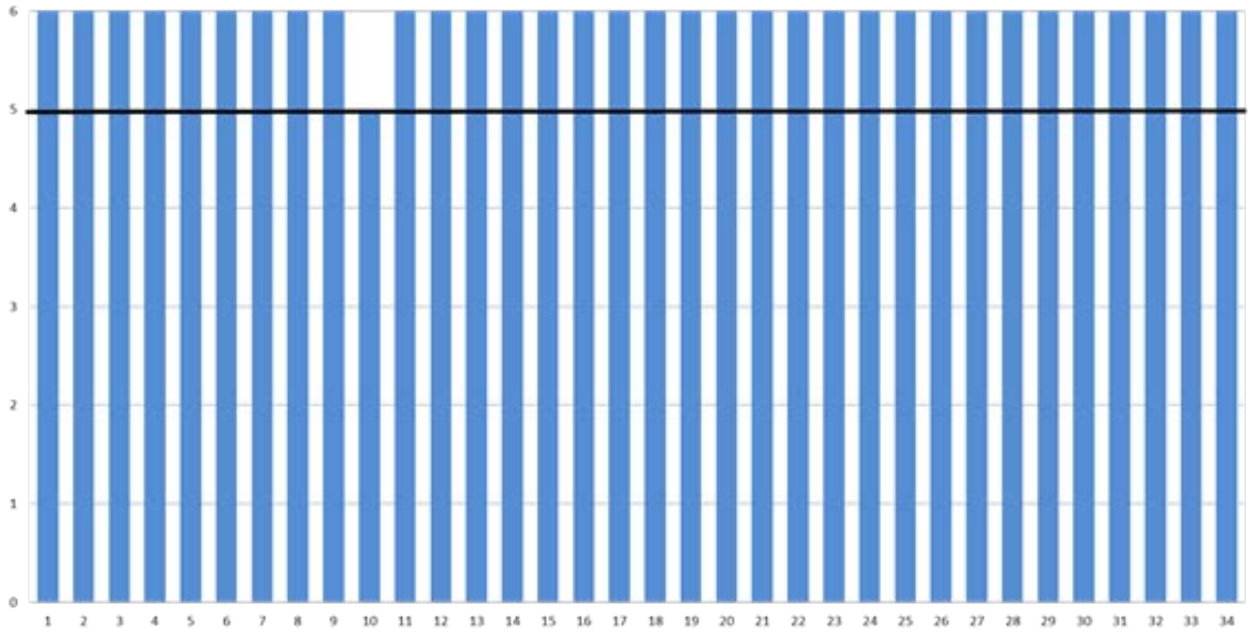

Figure 2 Number of positive isolations per laboratory after analysing 6 samples of each 25 g minced chicken meat artificially contaminated with high level Salmonella *Stanley. Results concern all possible combinations of media (MKTTn and RVS or/and MSRV) giving the highest number of positive samples.*