Interim summary report EURL-*Salmonella*

Interlaboratory Comparison study FEED III (2014)

Detection of *Salmonella* in chicken feed

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Introduction

In September 2014, the third EURL-*Salmonella* interlaboratory comparison study on the detection of *Salmonella* in an animal feed matrix (Feed III) 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-Europe 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 21 samples: 18 samples of each 25 g chicken feed artificially contaminated with three different levels (Blank, low and high level) of *Salmonella* Senftenberg (SSE) and 3 control samples.
A batch of 25 kg mixed flour for laying hens (free-range) was obtained from the retail sector and was produced by De Heus Voeders, Ede in the Netherlands. The chicken feed arrived at EURL-*Salmonella* on 25 August 2014 and five samples of each 25 gram were tested negative for *Salmonella.* The chicken feed was repacked in portions of 25 gram, after which they were artificially contaminated with three different levels (Blank, low and high level) of *Salmonella* Senftenberg (SSE) and stored at 5 °C. On Monday 29 September the chicken feed samples, each packed in separate numbered plastic bags, were mailed to the NRLs where it was placed at 5 °C. Table 1 shows the number of aerobic bacteria and *Enterobacteriaceae* in the chicken feed determined by the EURL-*Salmonella* on 25 August and on 6 October 2014 (Table 1).

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

Date	*Enterobacteriaceae* CFU/g	Aerobic bacteria CFU/g
25 August 2014	$9*10^2$	$5*10^4$
6 October 2014 After 5 weeks at roomtemperature and 1 week at 5 °C	$2*10^2$	$5*10^4$

Table 2 shows the level of the diluted culture with *Salmonella* Senftenberg used to contaminate the chicken feed samples. Of the artificially contaminated chicken feed with low and high level SSE, also a five tube Most Probable Number (MPN) test was performed.

Table 2 Number of Salmonella *Senftenberg (SSE).*

Date of testing	Low level SSE CFU/per sample	High level SSE CFU/per sample
26 September 2014 (Inoculum of chicken feed)	20	61
20 October 2014 MPN of chicken feed, inoculated with SSE (95 % confidence limit) after storage for 3 weeks at 5 °C	2 (0.8-7)	11 (3.75-30)

The NRLs had to analyse the following samples:

6x (25g chicken feed + low level of SSE)
6x (25g chicken feed + high level of SSE)
6x (25g chicken feed)

Furthermore some control samples had to be analysed, being:

1x only BPW	(Procedure control Blank)
1x 25g chicken feed	(Matrix control Blank)
1x own control sample with *Salmonella*	(Own positive control)

The chicken feed 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:

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

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

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

Criteria for good performance

For determining good performance per laboratory, all combinations of the prescribed and requested selective enrichment media and isolation media used by the laboratory were taken into account. For example if a laboratory found for the SSE low level with matrix 5/6 positive with RVS/XLD, but no positives with any other selective enrichment medium or isolation medium this was still considered as a good result. The opposite was used for the blank samples. Here also all combinations of media used per laboratory were taken into account. If for example a laboratory found 2/6 blank samples positive with MKTTn/BGA but no positives with the other media, this was still considered a ‘no-good’ result.

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

Table 3 Used criteria for good performance in the Feed III study (2014)

Control samples	Minimum result	
	Percentage positive	No. of positive samples/ total No. of samples
Own control with *Salmonella*	100 %	1 /1
Chicken feed	0 %	0 /1
BPW	0 %	0 /1

Samples : chicken feed artificially contaminated	Minimum result	
	Percentage positive	No. of positive samples / total No. of samples
Blank[1]	20 % at max[1]	1/6 at max[1]
SSE high	80 %	5/6
SSE 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 29 September 2014 (week 40) the samples were sent to 34 laboratories. Most samples were delivered at the NRLs within 2-3 days.
All laboratories performed the study as requested in week 41, starting on 5 or 6 October. All laboratories used the prescribed method (ISO 6579) and all, except laboratory 18, used the requested method (Annex D of ISO 6579).

Controls

The laboratories were asked to use an own positive control which they normally use when analysing routine samples for the detection of *Salmonella*. Additional to this own control a blank control of the BPW and of the matrix had to be analysed.

Thirty-two laboratories scored all three control samples correctly.

Procedure control Blank (only BPW)
Thirty-three laboratories correctly analysed the one procedure control sample (no matrix, only BPW) correctly negative for *Salmonella*. Laboratory 17 reported this sample positive for *Salmonella* with all selective enrichment media.

Matrix control Blank (chicken feed)
All laboratories correctly analysed the one chicken feed control sample (25 g of matrix) negative for *Salmonella*.

Positive control with Salmonella
Thirty-three laboratories scored good results with their own *Salmonella* positive control sample and detected *Salmonella* with all used media. Laboratory 3 could not detect *Salmonella* in their positive control with all used selective enrichment media.

For the positive control, the majority of the participants used a diluted culture of *Salmonella* (20 laboratories). Others used a lenticule disc (8), a freeze dried ampoule (2) or a culti loop (2) with *Salmonella.* The *Salmonella* serovars used for the positive control sample were *Salmonella* Enteritidis (15) and *Salmonella* Typhimurium (9).

In Table 4 the correct scores are given for the control samples for the different selective enrichment media RVS, MKTTn and MSRV, in combination with the isolation medium that gives the highest number of positives. All selective enrichment media gave the same results with accuracy rates of 98%.

Artificially contaminated chicken feed

Blank samples
Thirty-two laboratories correctly scored all 6 blank chicken feed samples negative for *Salmonella* with all used media. Two laboratories (lab codes 2 and 30) found one blank sample out of six positive for *Salmonella* with the selective enrichment medium RVS while they found the same sample correctly negative with the other selective enrichment media (MKTTn and MSRV) inoculated from the same pre-enriched sample in BPW.
All blanks should be tested negative. However, as no 100 % guaranty about the *Salmonella* negativity of chicken feed 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 have been caused by cross-contamination or by misinterpretation of the results.

High level contaminated Salmonella *Senftenberg samples*
All laboratories detected *Salmonella* in all six high level contaminated chicken feed samples with at least one of the used media. One laboratory (lab code 24) could not detect *Salmonella* in one out of six high level contaminated samples with the selective enrichment medium MKTTn while they found the same sample positive with the other selective enrichment media (RVS and MSRV) inoculated from the same pre-enriched sample in BPW.

Low level contaminated Salmonella *Senftenberg samples*
Thirty laboratories detected *Salmonella* in all six low level contaminated chicken feed samples with all selective enrichment media in combination with at least one of the used isolation media. Three laboratories (lab codes 7, 14 and 33) could not detect *Salmonella* in one out of six low level contaminated samples. Laboratory 4 could not detect *Salmonella* in two out of six low level contaminated samples with any of the used media (RVS, MKTTn and MSRV).

Figure 1 and 2 give the number of positive results per laboratory for the low and high level (SSE) contaminated chicken feed samples after pre-enrichment in BPW, selective enrichment in respectively RVS, MKTTn and on MSRV in combination with the isolation medium giving the highest number of positive results and all possible combinations of media giving the highest number of positive results (x).

In Table 5 the specificity, sensitivity and accuracy rates are given for the artificially contaminated chicken feed samples. The rates were comparable for the different selective enrichment media: specificity rates 99-100%, sensitivity rates 97-100% and accuracy rates of 99%.

Good performance

Thirty-two laboratories fulfilled the criteria of good performance.

Two laboratories scored below the level of good performance for one of the control samples:
-Laboratory 3 could not detect *Salmonella* in their own positive control sample.
-Laboratory 17 found false positive blank results with the procedure control sample (only BPW).

Both laboratories showed correct results for the samples with animal feed contaminated with *Salmonella* as well for the blank animal feed samples. However, those results may not be reliable because of deviations in the positive or negative control samples.

The EURL-*Salmonella* will contact these laboratories to discuss their results.

Table 4 Correct scores of the control samples

Control samples	n=34	**RVS/X**	**MKTTn/X**	**MSRV/X***
Procedure control	No. of samples	34	34	33
Blank (BPW)	No. of negative samples	33	33	32
n=1	**Correct score in %**	**97**	**97**	**97**
Matrix control Blank	No. of samples	34	34	33
Blank chicken feed	No. of negative samples	34	34	33
n=1	**Correct score in %**	**100**	**100**	**100**
Positive control	No. of samples	34	34	33
(Own *Salmonella*)	No. of positive samples	33	33	32
n=1	**Correct score in %**	**97**	**97**	**97**
All	No. of samples	102	102	99
Control samples	No. of correct samples	100	100	97
	Accuracy in %	**98**	**98**	**98**

X = isolation medium with the highest number of positives of all used isolation media.
*Results without Laboratory 18: they did not use MSRV

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

Chicken feed	n=34	**RVS/X**	**MKTTn/X**	**MSRV/X***
Blank	No. of samples	204	204	198
n=6	No. of negative samples	202	204	198
	Specificity in %	**99**	**100**	**100**
Low level	No. of samples	204	204	198
n=6	No. of positive samples	199	199	193
	Sensitivity in %	**98**	**98**	**97**
High level	No. of samples	204	204	198
n=6	No. of positive samples	204	203	198
	Sensitivity in %	**100**	**99**	**100**
All feed samples with	No. of samples	408	408	396
Salmonella	No. of positive samples	403	402	391
	Sensitivity in %	**99**	**99**	**99**
All feed samples	No. of samples	612	612	594
	No. of correct samples	605	606	589
	Accuracy in %	**99**	**99**	**99**

X = isolation medium with the highest number of positives of all used isolation media.
*Results without Laboratory 18: they did not use MSRV

List of abbreviations

BL	Blank-No colony forming units
BPW	Buffered Peptone Water
BGA	Brilliant Green Agar
CFU	colony forming units
EFTA	European Free Trade Associations
EURL	European Union Reference Laboratory
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
SSE	*Salmonella* Senftenberg
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.

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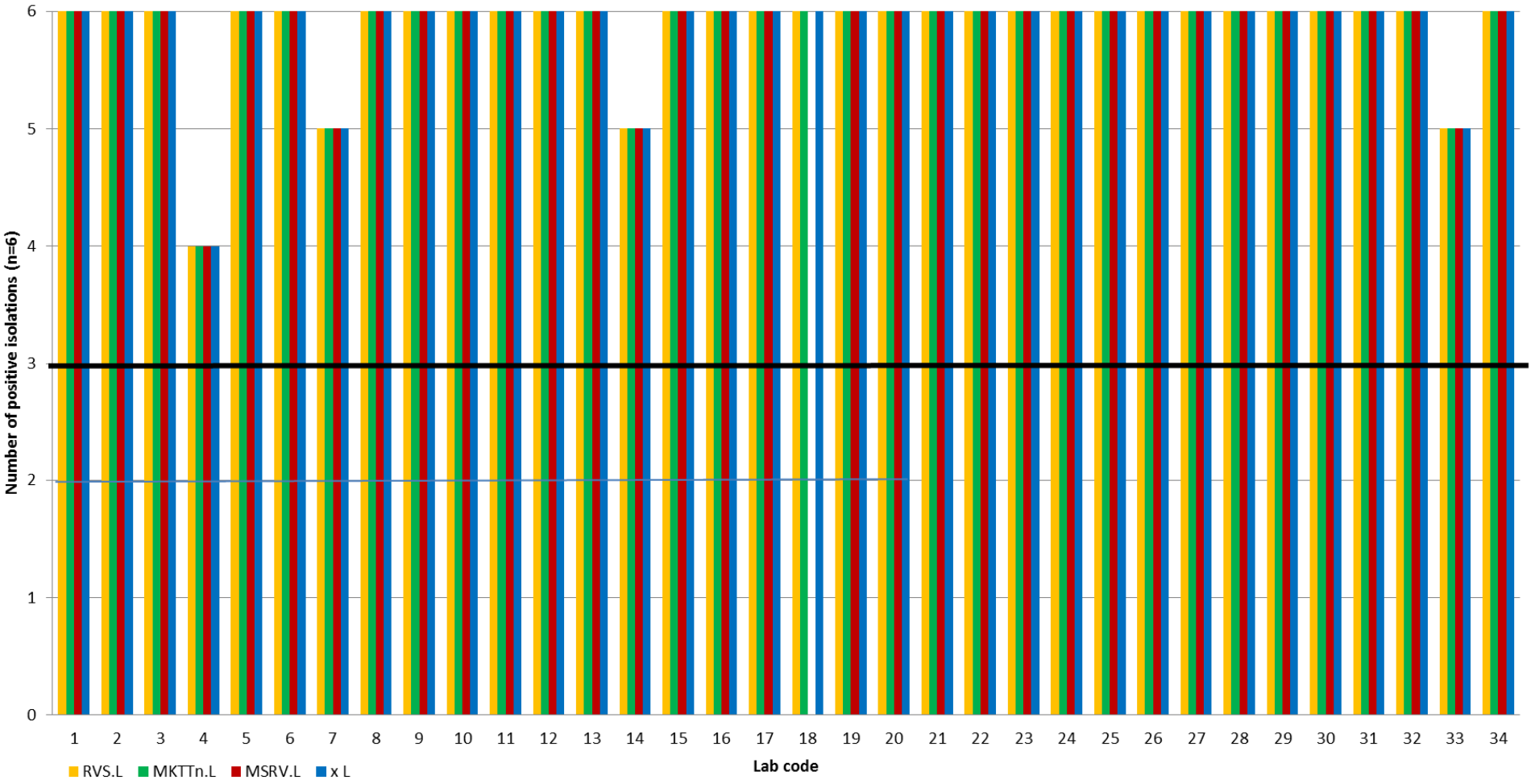

Figure 1 Number of positive isolations per laboratory after analysing 6 samples of each 25 g chicken feed artificially contaminated with low level S. Senftenberg, for selective enrichment media RVS, MKTTn and MSRV in combination with the isolation medium giving the highest number of positive results and all possible combinations of media giving the highest number of positive results (x).(Laboratory 18 did not use MSRV)

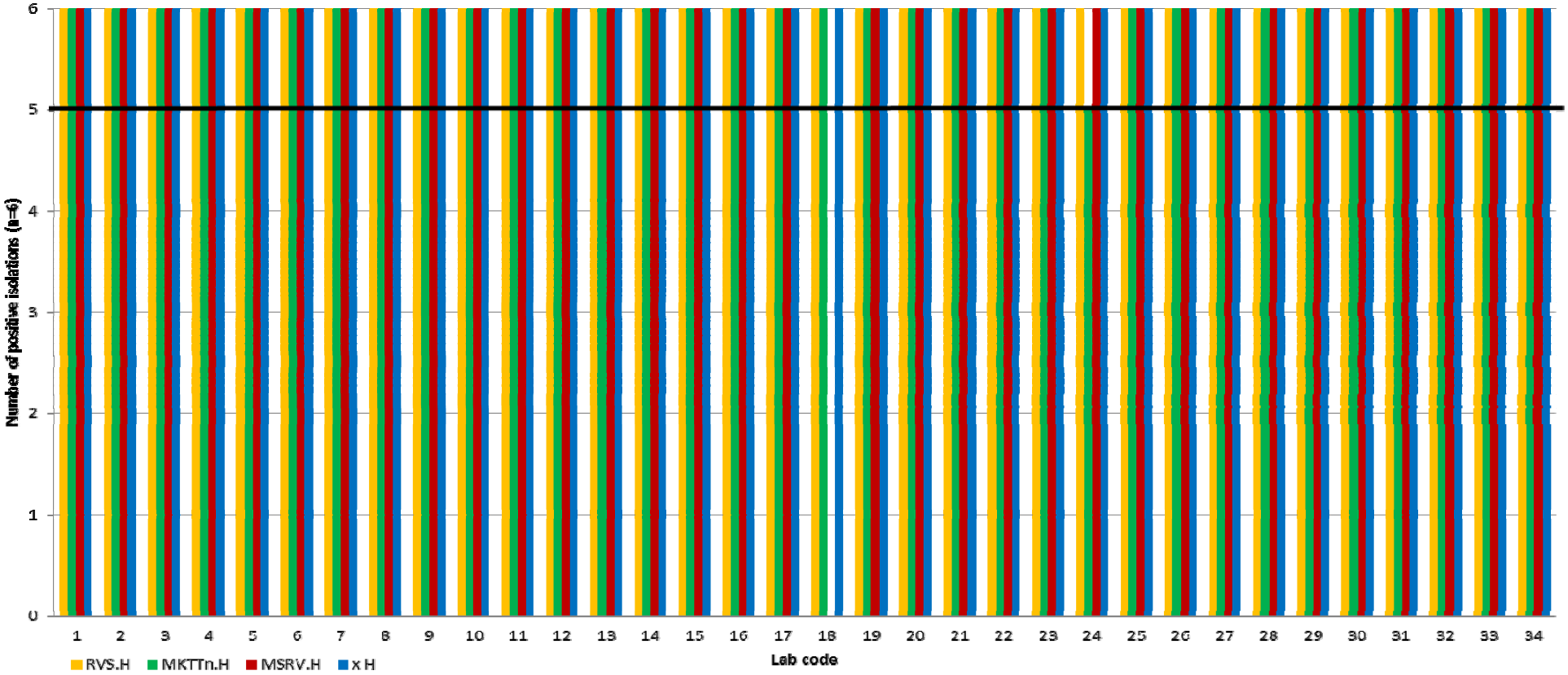

Figure 2 Number of positive isolations per laboratory after analysing 6 samples of each 25 g chicken feed artificially contaminated with high level S. Senftenberg, for selective enrichment media RVS, MKTTn and MSRV in combination with the isolation medium giving the highest number of positive results and all possible combinations of media giving the highest number of positive results (x). (Laboratory 18 did not use MSRV)