Interim summary report EURL-*Salmonella*

19th Interlaboratory Comparison study Primary production (2016) on detection of *Salmonella* in bootsocks contaminated with chicken faeces

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Introduction

In February 2016, the nineteenth EURL-*Salmonella* interlaboratory comparison study on the detection of *Salmonella* in samples from the primary production stage was organised for the NRLs for *Salmonella.*
In total 36 NRLs participated in this study: 29 NRLs from 28 EU-Member States (MS), 6 NRLs from third countries within Europe (EU candidate MSs or potential EU candidate MSs and members of the European Free Trade Association (EFTA)) and on request of DG-Sante, one NRL from a non-European country.

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 are described here.

Materials & Methods

Samples
Each NRL analysed in total 20 samples: 18 samples of bootsocks with 10 g chicken faeces artificially contaminated with three different levels (blank, low and high level) of *Salmonella* Typhimurium (STM) and 2 control samples.
A batch of approximately 10 kg *Salmonella*-free chicken faeces was obtained from a *Salmonella* free chicken farm (SPF-farm) of the Animal Health Service (GD) in Deventer, the Netherlands.

The chicken faeces arrived at EURL-*Salmonella* laboratory on Monday 1 February 2016 and five samples of each 25 gram were tested negative for *Salmonella*. Ten grams of chicken faeces was added to each pair of bootsocks, and then artificially contaminated with three different levels (blank, low and high level) of *Salmonella* Typhimurium (STM). The samples were stored at 5 °C until the day of mailing. On Monday 15 February, the artificially contaminated bootsock samples were packed and send to the NRLs. Upon arrival at the NRLs, samples had to be stored at 5 °C until the start of the analysis. Table 1 shows the number of background flora by counts of aerobic bacteria and *Enterobacteriaceae* in the chicken faeces determined by the EURL-*Salmonella* on 2 and 9 February 2016.

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

Date of testing	Aerobic bacteria cfu/g	*Enterobacteriaceae* cfu/g
2 Feb 2016	7.1×10^7	3.5×10^6
9 Feb 2016, after storage at 5 °C	1.5×10^7	2.8×10^5

Table 2 shows the contamination level of the diluted culture of *Salmonella* Typhimurium used as inoculum to contaminate the chicken faeces samples. Additional, a five tube Most Probable Number (MPN) test was performed on the bootsock samples contaminated with low and high level STM in the week of the interlaboratory comparison study.

Table 2 Salmonella *Typhimurium (STM) concentration in the inoculum and in the test samples of bootsocks with inoculated chicken faeces.*

Date of testing	Low level STM cfu/25g chicken faeces	High level STM cfu/25g chicken faeces
9 February 2016 (Inoculum level diluted culture)	11	95
22 Feb 2016 MPN of bootsocks with artificially contaminated chicken faeces (95 % confidence limit)	5 (1.5 -16.3)	>> (65 - >>)

The NRLs had to analyse the following samples:

6x (25g bootsocks + chicken faeces + low level STM)
6x (25g bootsocks + chicken faeces + high level STM)
6x (25g bootsocks + chicken faeces)

In addition some control samples had to be analysed, being:

1x only BPW (procedure control blank)
1x own control sample with *Salmonella* (own positive control)

The bootsocks with artificially contaminated chicken faeces samples were individually packed and labelled. The decoding of these samples can be found in the tables of the individual NRL results. The parcels were sent to the participating laboratories on Monday 15 February. Two laboratories received the parcels within that same day. Eight and seventeen parcels were delivered after 1 day and 2 days respectively. Seven laboratories received the parcels after three days, one laboratory after four days and one laboratory after 6 days. Parcels were stored at 5 °C upon arrival at the laboratory. Start date of the analysis were 18, 19, 21, 22 and 23 February 2016.

Results

For evaluation of the results a positive result found for selective enrichment medium MSRV in combination with all other isolation media used by the laboratory were taken into account. All laboratories used the prescribed method (Annex D of ISO 6579) with selective enrichment on MSRV.

Control samples

The laboratories were asked to use their own positive control normally used in their routine analysis for the detection of *Salmonella*. Additional to this own control, a blank control of the BPW had to be analysed. Thirty-four laboratories scored all two control samples correct.

Procedure control Blank (only BPW)
All but two laboratories (3 and 32) correctly analysed the procedure control sample (BPW, no matrix) correctly negative for *Salmonella*.

Positive control with Salmonella
All but two laboratories (3 and 32) scored good results with their own *Salmonella* positive control sample and detected *Salmonella* with all used media.

For the positive control, the majority of the participants used a diluted culture of *Salmonella* (23 laboratories). Others used a lenticule disc (7), a freeze dried ampoule (2) or a cultiloop (1) with *Salmonella* or other. The *Salmonella* serovar used for the positive control sample were *Salmonella* Enteritidis (16), *Salmonella* Typhimurium (7), *Salmonella* Nottingham (3) and *Salmonella* Poona (1) and others (9).

Table 3 shows the specificity, sensitivity and accuracy rates for the control samples with selective enrichment on MSRV in combination with the isolation medium that gave the highest number of positive samples for *Salmonella* (MSRV/x). The laboratories scored good results for the control samples with an accuracy rate of 94 % for MSRV/x.

Table 3 Specificity, sensitivity and accuracy rates of the control samples

Control samples	Laboratories n = 36	**MSRV/X**
Procedure control Blank (BPW) n = 1	No. of samples	36
	No. of negative samples	34
	Specificity in %	**94**
Positive control (Own *Salmonella*) n = 1	No. of samples	36
	No. of positive samples	34
	Sensitivity in %	**94**
All control samples n = 1	No. of samples	72
	No. of correct samples	68
	Accuracy in %	**94**

MSRV/X = any combination of MSRV with an isolation medium giving the highest number of positive results.

Artificially contaminated chicken faeces on bootsocks samples

Blank samples (bootsocks with non- contaminated chicken faeces)
All but one laboratory (28) correctly analysed the blank bootsock samples with chicken faeces negative for *Salmonella*. Laboratory 28 scored 3 of the 6 blank samples positive for *Salmonella*.

Low level contaminated Salmonella Typhimurium *samples*
Almost all laboratories were able to detect *Salmonella* in all 6 low level bootsock samples. Two laboratories (6 and 20) scored 1 of the 6 low level contaminated samples negative for *Salmonella*. See Figure 1.

High level contaminated Salmonella Typhimurium *samples*
All laboratories detected *Salmonella* in all 6 high level bootsock samples with the prescribed method (MSRV).

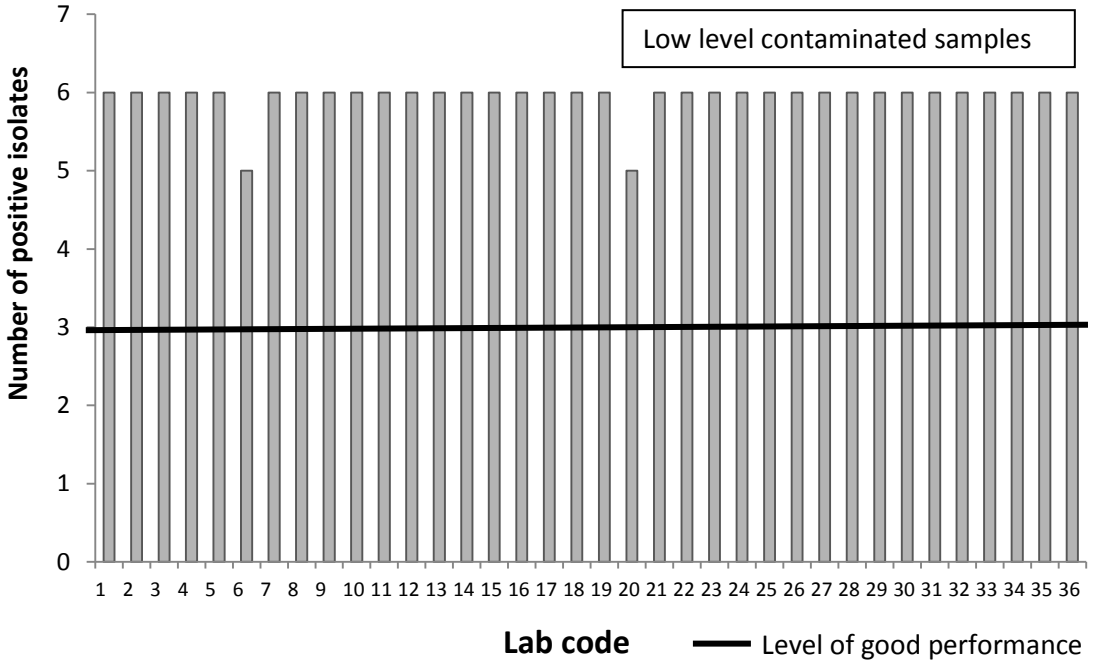

Figure 1 *Number of positive* Salmonella *isolations per laboratory found in bootsock samples with chicken faeces artificially contaminated with low levels of* Salmonella *Typhimurium (n=6). The best results (highest number of positive samples) of all used isolation media after selective enrichment MSRV were taken into account (MSRV/x).*

Table 4 *Specificity, sensitivity and accuracy rates of the bootsock samples with chicken faeces artificially contaminated with* Salmonella *Typhimurium*

Bootsocks with chicken faeces	Laboratories n = 36	**MSRV/X**
Blank	No. of samples	216
n=6	No. of negative samples	213
	Specificity in %	**99**
Low level (STM)	No. of samples	216
n=6	No. of positive samples	214
	Sensitivity in %	**99**
High level (STM)	No. of samples	216
n=6	No. of positive samples	216
	Sensitivity in %	**100**
All faeces samples	No. of samples	432
with STM	No. of positive samples	430
	Sensitivity in %	**99,5**
All faeces samples	No. of samples	648
(positive and negative)	No. of correct samples	643
	Accuracy in %	**99**

MSRV/X = any combination of MSRV with an isolation medium giving the highest number of positive results.

Table 4 shows the specificity, sensitivity and accuracy rates for the bootsock samples with artificially contaminated chicken faeces. Laboratories have scored good results in both the high and low contaminated chicken faeces samples as shown by the high sensitivity and accuracy rates of 99%.

Good performance

Criteria for good performance used in EURL studies for detection of *Salmonella* are shown in Table 5.

Table 5 Criteria for good performance

Control samples	**Percentage positive**	**# pos samples/ total # samples**
BPW	0%	0/1
Own positive control	100%	1/1

Contaminated samples	**Percentage positive**	**# pos samples/ total # samples**
Blank *	20% max	1/6 max
Low level contamination	50%	3/6
High level contamination	80%	5/6

*100% *Salmonella* free matrix cannot be guaranteed, 1 positive out of 6 blank samples is still considered as acceptable (20%).

In this study, thirty-three laboratories fulfilled the criteria of good performance for the prescribed method (MSRV). Laboratories 3 and 32 scored below the criteria of good performance because they could not detect *Salmonella* in their own positive control sample and additional they tested blank BPW positive for *Salmonella*. Furthermore, laboratory 28 detected *Salmonella* in 3 of the 6 blank chicken faeces samples. The EURL-*Salmonella* will contact these laboratories to discuss their results.

List of abbreviations

Blank	No colony forming units per sample
BPW	Buffered Peptone Water
cfu	colony forming units
EFTA	European Free Trade Associations
EURL	European Union Reference Laboratory
ISO	International Standardisation Organisation
MPN	Most Probable Number
MS	Member State
MSRV	Modified Semi-solid Rappaport Vassiliadis
NRL	National Reference Laboratory
STM	Salmonella Typhimurium
SPF	Specified pathogen free

References

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

ISO 6579:2002/Amd 1 2007: Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.
Amendment 1 Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage.

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