Interim summary report EURL-*Salmonella*

Interlaboratory comparison study for samples from the Primary Production stage (2018)

Detection of *Salmonella* in contaminated bootsocks samples with chicken faeces

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Introduction

In October 2018, the interlaboratory comparison study for samples from the Primary Production Stage (PPS) on the detection of *Salmonella* in bootsocks samples with chicken faeces was organised by the EURL-*Salmonella*. In total 36 NRLs participated in this study: 29 participants originated from 28 EU-Member States (MS), six NRLs from third countries within Europe (EU candidate or potential EU candidate MSs and members of the European Free Trade Association (EFTA)) and 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 participants.

Materials & Methods

Samples
A batch of 10 kg of *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 the EURL-*Salmonella* laboratory on Tuesday, 11th of September 2018 and were tested negative for *Salmonella*. The chicken faeces was added to the bootsocks (10 g per pair of bootsocks), which were pre-moisturised with 15 ml peptone saline solution and artificially contaminated with three different concentrations of *Salmonella* (blank, low and high level). The artificially contaminated samples were stored at 5 °C until the day of transport. On Monday, 24th of September 2018, the artificially contaminated bootsocks samples with chicken faeces were packed and sent to the NRLs. Upon arrival, the NRLs were requested to store the samples at 5 °C until the start of the analysis on Monday 1st of October.

Table 1 shows the number of background flora by counts of aerobic bacteria and *Enterobacteriaceae* in the chicken faeces samples determined by the EURL-*Salmonella* on the 17th of September and first of October 2018.

Table 1 Number of aerobic bacteria and Enterobacteriaceae *in chicken faeces adhering to bootsocks*

Date of testing	Aerobic bacteria cfu/g	*Enterobacteriaceae cfu/g*
17 Sept 2018	4.9×10^8	2.8×10^6
1 October 2018, after storage at 5 °C	1.2×10^8	1.9×10^5

Table 2 shows the contamination level of the diluted culture of *Salmonella* Infantis used as inoculum to contaminate the bootsocks samples with chicken faeces. Additionally, the number of *Salmonella* in the artificially contaminated bootsocks samples was determined using a five-tube Most Probable Number (MPN) test in the week of the interlaboratory comparison study.

Table 2 Salmonella Infantis concentration in the inoculum and in the inoculated bootsocks samples with chicken faeces.

Date of testing	Low level SI (cfu)	High level SI (cfu)
18 Sept 2018 (Inoculum level diluted culture)	10	53
1 Oct 2018 MPN contaminated chicken faeces (95 % confidence limit)	3,25 (1,08-10,25)	17,25 (6,5-45)

Each NRL analysed in total 20 samples: 18 bootsocks samples with chicken faeces, artificially contaminated with three different levels of *Salmonella* Infantis (six blank samples, six low contaminated samples and six high-contaminated samples). In addition, two control samples had to be analysed: one procedure control consisting of moistened bootsocks (without chicken faeces), and one positive control (consisting of dry bootsocks sample) to which the participants had to add their own positive control strain.

The artificially contaminated bootsocks samples with chicken faeces were individually packed and labelled. The decoding of these samples can be found in the tables with the individual NRL results. The parcels were sent to the participating laboratories on Monday 24th of September 2018.

One laboratory received the parcel within the same day of dispatch. Twenty-five parcels were delivered after one day, six parcels after two days, two parcels arrived after three days, and two parcels after more than seven days of dispatch. Parcels had to be stored at 5 °C upon arrival at the laboratory. The temperature during transport and storage was registered using a temperature probe. Temperature of the parcels during transport was predominantly below 5 °C. Storage temperature of the sample at the laboratories varied between 0 and 7 °C. The start date of the analysis of all laboratories was 1st of October 2018, the two laboratories receiving their parcels late, started one or two days later. Laboratory 22 received the parcel on the 4th of October and started the analysis immediately. The temperature of the parcels was below 5 °C until the 1st of October. After that date, the temperature increased to 8 °C on the 2nd of October, to 8,5 °C on the 3rd of October and to 16,5 °C after arrival at the laboratory. Lab 35 received the parcel on the 2nd of October. However, the temperature of the parcel was between 26 and 28 °C already for several days. For that reason, the quality of the samples could not be guaranteed. Results of

laboratory 35 are shown in Figure 1 and Figure 2 but will not be included in the performance evaluation in Table 3 and Table 4.

Results

The prescribed method was EN ISO 6579 and preferably the latest version EN ISO 6579-1:2017 (Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp.). Most laboratories (31) used this method. Two laboratories used Annex D of ISO 6579:2007 and three laboratories used another method.

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 boot sock samples had to be analysed. All laboratories scored both control samples correct.

Procedure control Blank (moisturised bootsocks)
All laboratories correctly analysed the procedure control sample negative for *Salmonella*.

Positive control with Salmonella
All laboratories correctly scored their own *Salmonella* positive control sample as positive.

For the positive control, the majority of the participants used a diluted culture of *Salmonella* (24 laboratories). Others used a lenticule disc (8) or a freeze-dried ampoule (2) with *Salmonella* or other (2). The *Salmonella* serovars used for the positive control sample were *Salmonella* Enteritidis (15), *Salmonella* Typhimurium (7), *Salmonella* Nottingham (6) and others (8).

Table 3 shows the specificity, sensitivity and accuracy rates of the control samples. The laboratories scored good results for the control samples with an accuracy rate of 100%.

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

Control samples		Total labs n = 35
Procedure control n = 1	No. of sample No. of negative samples **Specificity in %**	35 35 **100%**
Positive control (Own *Salmonella*) n = 1	No. of samples No. of positive samples **Sensitivity in %**	35 35 **100%**
All control samples n = 2	No. of samples No. of correct samples **Accuracy in %**	70 70 **100%**

Artificially contaminated bootsocks samples with chicken faeces

Blank samples
All laboratories correctly analysed the blank bootsocks samples negative for *Salmonella*.

Low-level bootsocks samples with Salmonella Infantis
Almost all laboratories were able to detect *Salmonella* in all six low level samples. Two laboratories (lab codes 1 and 3) scored one of the six low level contaminated samples negative for *Salmonella*. Laboratory 35 scored five of the six low-level samples negative for *Salmonella*. See Figure 1 for results. The level of good performance allows for three out of the six low level contaminated samples to be scored negative (see Table 5). The performance of laboratory 35 will not be scored due to transport temperature abuse.

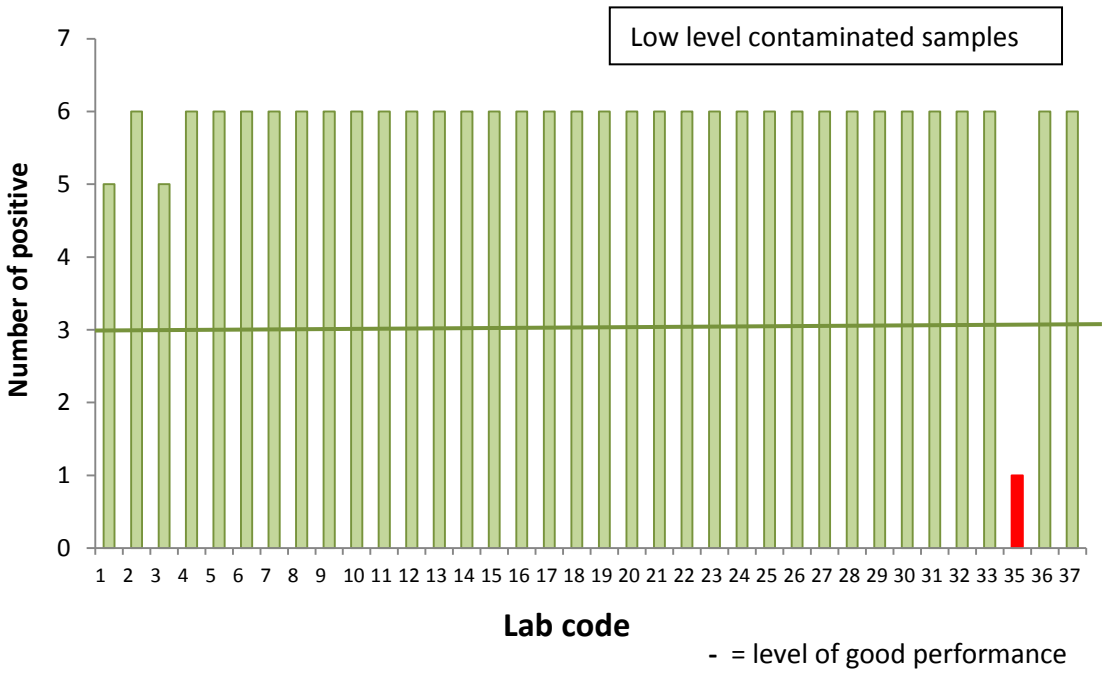

Figure 1 *Number of positive* Salmonella *isolations per laboratory found in the bootsocks samples contaminated with low level* Salmonella *Infantis (n=6).*

High-level bootsocks samples with Salmonella Infantis
Almost all laboratories detected *Salmonella* in all six high level samples. Two laboratories (lab codes 26 and 35) scored one sample out of the six high level contaminated samples negative. See Figure 2 for results. The level of good performance allows for one sample out of the six high level contaminated samples to be scored negative.

Table 4 shows the specificity, sensitivity and accuracy rates for the bootsocks samples.
Laboratories have scored good results in both the high and low contaminated bootsocks samples as shown by the high sensitivity and accuracy rates of more than 99%.

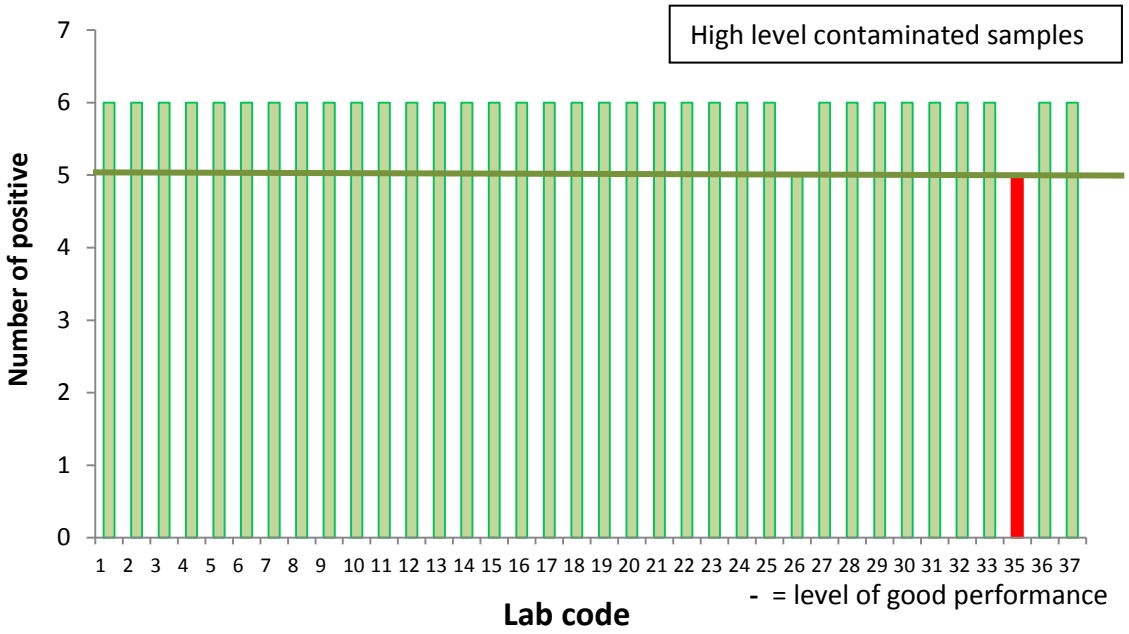

Figure 2 Number of positive Salmonella *isolations per laboratory found in the bootsocks samples contaminated with high level* Salmonella *Infantis (n=6).*

Table 4 *Specificity, sensitivity and accuracy rates of the bootsocks samples artificially contaminated with* Salmonella *Infantis*

Bootsocks samples with chicken faeces		**Total labs n = 35**
Blank n=6	No. of samples No. of negative samples **Specificity in %**	210 210 **100%**
Low level (SI) n=6	No. of samples No. of positive samples **Sensitivity in %**	210 208 **99%**
High level (SI) n=6	No. of samples No. of positive samples **Sensitivity in %**	210 209 **99,5%**
All bootsocks samples with SI	No. of samples No. of positive samples **Sensitivity in %**	420 417 **99,3%**
All bootsocks samples (positive and negative)	No. of samples No. of correct samples **Accuracy in %**	630 627 **99,5%**

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 total, 35 laboratories fulfilled the criteria of good performance for the prescribed method.

One laboratory was facing some problems with the contaminated bootsocks samples, scoring five of the six low-level samples negative for *Salmonella* and one of the six high-level samples negative. Most likely, this was caused by the high temperature this parcel experienced during transport, which negatively affected the concentration of *Salmonella* in the bootsocks samples with chicken faeces. Due to the poor temperature conditions in the parcel during the seven days of transport, the quality of the samples could not be guaranteed and results of this laboratory cannot be evaluated.

List of abbreviations

Blank	No colony forming units per sample
BPW	Buffered Peptone Water
cfu	colony forming units
EFTA	European Free Trade Associations
EU	European Union
EURL	European Union Reference Laboratory
ISO	International Standardisation Organisation
MPN	Most Probable Number
MS	Member State
NRL	National Reference Laboratory
PPS	Primary Production Stage
SI	*Salmonella* Infantis

References

EN ISO 6579-1: 2017. Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* spp. Part 1: Detection of *Salmonella* spp.

EN 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.

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

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