Interim summary report EURL-*Salmonella*

20th Interlaboratory Comparison study Primary production (2017) on detection of *Salmonella* in contaminated chicken faeces

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Introduction

In March 2017, the twentieth 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-Santé, 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
A batch of approximately 25 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 6 March 2017 and ten samples of each 25 gram were tested negative for *Salmonella.* Twenty-five grams of chicken faeces was artificially contaminated with three different levels (blank, low and high level) of *Salmonella* Infantis (SI). The samples were stored at 5 °C until the day of transport. On Monday 13 March, the artificially contaminated chicken faeces samples were packed and send to the NRLs. Upon arrival, the NRLs were asked to store the samples 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 7 and 20 March 2017.

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
7 March 2017	4.2×10^8	8.7×10^4
20 March 2017, after storage at 5 °C	1.0×10^8	5.5×10^4

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

Table 2 Salmonella *Infantis (SI) concentration in the inoculum and in the inoculated chicken faeces samples.*

Date of testing	Low level SI cfu/25g chicken faeces	High level SI cfu/25g chicken faeces
7 March 2017 (Inoculum level diluted culture)	17	55
20 March 2017 MPN of artificially contaminated chicken faeces (95 % confidence limit)	22 (8.5 -56)	35 (11 - 110)

Each NRL analysed in total 20 samples: 18 samples of 25 g chicken faeces artificially contaminated with three different levels (6 blank samples, 6 low contaminated samples and 6 high contaminated. In addition, 2 control samples had to be analysed: 1 procedure control consisting of BPW only, and 1 positive control to which the participants had to add their own positive control strain.

The 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 13 March. One laboratory received the parcel within that same day. Twenty nine parcels were delivered after 1 day and 3 parcels took 2 days to arrive. Three laboratories received the parcels after three days, one laboratory after four days and one laboratory after 6 days. Parcels had to be stored at 5 °C upon arrival at the laboratory. The temperature during storage was registered using a temperature probe. Temperature of the parcels was predominantly below 6 °C, with two parcels that experienced temperatures up to 12 and 17 °C (labcodes 13 and 36 respectively). Storage temperature of the sample, upon arrival at the laboratories varied between 0 and 9 °C. Two parcels were stored at 17 and 23 °C (labcode 13 and 18 respectively). Start date of the analysis of all laboratories was 20 March 2017, one laboratory started on 17 march 2017.

Results

All laboratories used the prescribed method (Annex D of ISO 6579:2007 or ISO 6579-1: 2017) with selective enrichment on MSRV and isolation on XLD and a second medium free of choice.

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-five laboratories scored all two control samples correct.

Procedure control Blank (only BPW)
All but one laboratory (labcode 16) correctly analysed the procedure control sample (BPW, no matrix) negative for *Salmonella*.

Positive control with Salmonella
All but one laboratory (labcode 16) 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* (23 laboratories). Others used a lenticule disc (6), a freeze dried ampoule (2) or a capsule (1) with *Salmonella* or other (3). The *Salmonella* serovars used for the positive control sample were *Salmonella* Enteritidis (12), *Salmonella* Typhimurium (9), *Salmonella* Nottingham (5) and *Salmonella* Tranaroa (1) and others (9).

Table 3 shows the specificity, sensitivity and accuracy rates of the control samples after selective enrichment on MSRV and isolation on XLD and a second medium of choice (MSRV/x). The laboratories scored good results for the control samples with an accuracy rate of 100 % 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	35
	Specificity in %	**97**
Positive control (Own *Salmonella*) n = 1	No. of samples	36
	No. of positive samples	35
	Sensitivity in %	**97**
All control samples n = 1	No. of samples	72
	No. of correct samples	70
	Accuracy in %	**97**

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

Artificially contaminated chicken faeces samples

Blank samples (non- contaminated chicken faeces)
All but one laboratory (labcode 18) correctly analysed the blank chicken faeces samples negative for *Salmonella*. Laboratory 18 scored 3 of the 6 blank samples positive for *Salmonella*.

Low level contaminated Salmonella Infantis *samples*
Almost all laboratories were able to detect *Salmonella* in all 6 low level samples. Three laboratories (labcodes 9, 34 and 36) each scored 1 of the 6 low level contaminated samples negative for *Salmonella*. See Figure 1.

High level contaminated Salmonella Infantis *samples*
Almost all laboratories detected *Salmonella* in all 6 high level samples. Two laboratories (labcodes 3 and 21) scored 1 of the 6 low level contaminated samples negative for *Salmonella*. See Figure 2.

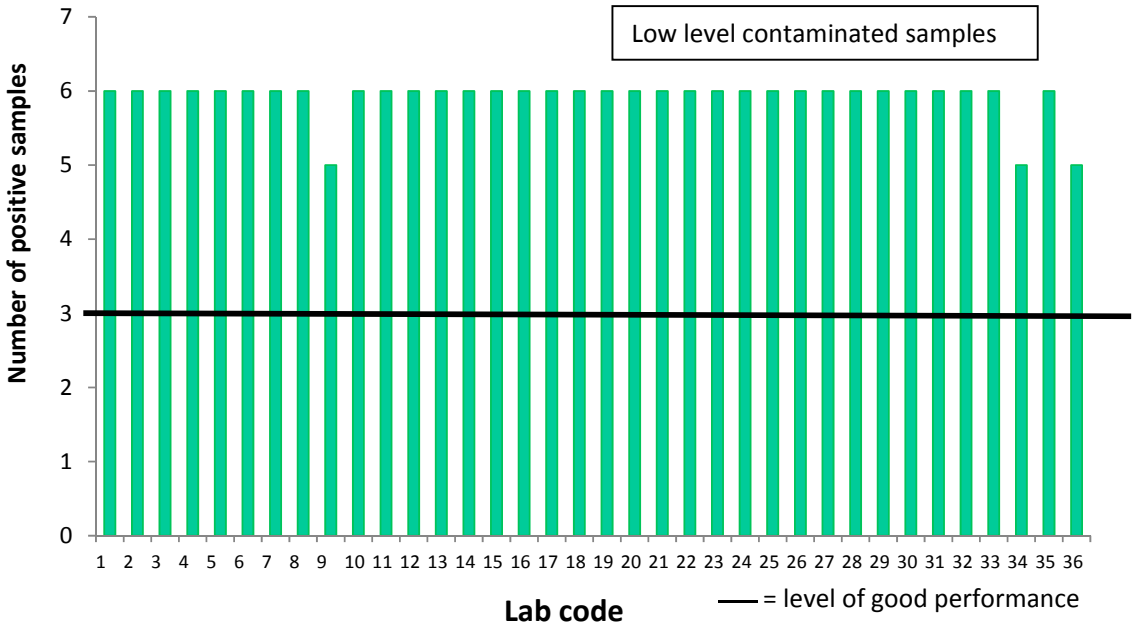

Figure 1 *Number of positive* Salmonella *isolations per laboratory found in chicken faeces samples artificially contaminated with low levels of* Salmonella *Infantis (n=6).*

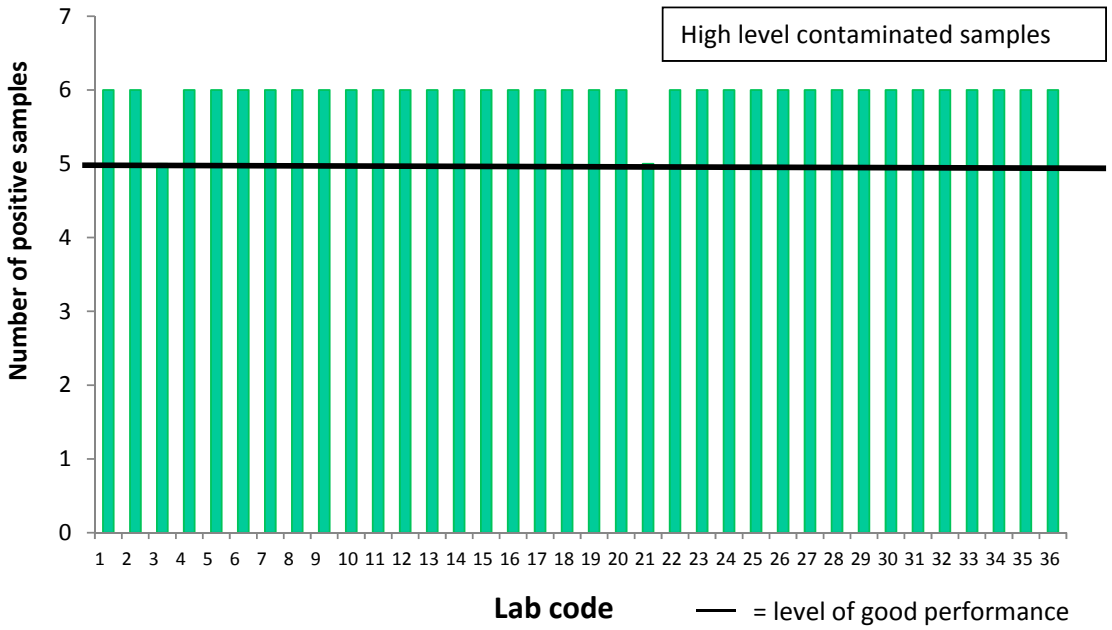

Figure 2 *Number of positive* Salmonella *isolations per laboratory found in chicken faeces samples artificially contaminated with high levels of* Salmonella *Infantis (n=6).*

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

Chicken faeces samples	**Laboratories n = 36**	**MSRV/X**
Blank n=6	No. of samples No. of negative samples **Specificity in %**	216 213 **99**
Low level (SI) n=6	No. of samples No. of positive samples **Sensitivity in %**	216 213 **99**
High level (SI) n=6	No. of samples No. of positive samples **Sensitivity in %**	216 214 **99**
All faeces samples with SI	No. of samples No. of positive samples **Sensitivity in %**	432 427 **99**
All faeces samples (positive and negative)	No. of samples No. of correct samples **Accuracy in %**	648 640 **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 artificially contaminated chicken faeces samples. 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-four laboratories fulfilled the criteria of good performance for the prescribed method (MSRV). Laboratories 16 scored a poor performance because they used C1 as their own positive control sample and C2 as a process control instead of vice versa. In addition, laboratory 18 detected *Salmonella* in 3 of the 6 blank chicken faeces samples and also received a poor performance. The EURL-*Salmonella* will contact this 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
SI	*Salmonella* Infantis
SPF	Specified pathogen free

References

International Standard – 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.

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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