Interim summary report EURL-*Salmonella*

Proficiency Test (PT) Primary Production 2019

Detection of *Salmonella* in chicken faeces

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Introduction

In October 2019, the *EURL-Salmonella* Proficiency Test for detection of *Salmonella* in samples from the Primary Production Stage (PPS) was organised for the NRLs-*Salmonella.* In total 35 NRLs for *Salmonella* participated in this study: 29 participants originated from 28 EU-Member States (MS), 5 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 25 kg of *Salmonella* free chicken faeces was obtained from a *Salmonella* free chicken farm (Specific Pathogen Free (SPF) farm) of the Animal Health Service (GD) in Deventer, the Netherlands.
The chicken faeces arrived at the EURL-*Salmonella* laboratory on Tuesday 26 August 2019 and was tested negative for *Salmonella*.
The PT samples were prepared by weighing 25 gram of chicken faeces into coded sample bags and placed at -20 °C to avoid growth of yeast and fungi and inactivate small flies. The chicken faeces samples were defrosted in the week of 16 September 2019. Each sample of chicken faeces was artificially contaminated with a low or a high level of *Salmonella* Typhimurium (STm) or not contaminated at all (negative samples). The chicken faeces samples were stored at 5 °C until the day of transport. On Monday 23 September 2019, all samples 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 30 September 2019.

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 27 August and 30 September 2019.

Table 1. Number of aerobic bacteria and Enterobacteriaceae *per gram of chicken faeces*

Date of testing	Aerobic bacteria cfu/g	*Enterobacteriaceae cfu/g*
27 Aug 2019	5,3 x 10^8	4,3 x 10^6
30 Sept 2019 * after storage at -20 °C and 5 °C	7,4 x 10^8	1,1 x 10^6

*After storage for two weeks at -20 °C and one week at 5°C

Table 2 shows the level of the diluted culture of *Salmonella* Typhimurium used as inoculum to contaminate the chicken faeces samples. The contamination level of the low contaminated samples was below our critical limit (results 18 September 2019) and the concentration was increased with a second inoculum (results 19 September 2019). Additionally, the number of *Salmonella* in the artificially contaminated chicken faeces samples was determined using a five-tube Most Probable Number (MPN) test in the week of the Proficiency Test.

Table 2. Salmonella *Typhimurium concentration in the inoculums and in the inoculated chicken faeces samples.*

Date of testing	Low level STm (cfu)	High level STm (cfu)
18 Sept 2019 (first inoculum level diluted culture)	3	30,5
19 Sept 2019 (second inoculum level diluted culture)	16	
30 Sept 2019 MPN contaminated chicken faeces (95 % confidence limit)	13 (4,5-37,5)	35 (11-110)

Each NRL-*Salmonella* analysed in total 16 samples:

- 4 negative chicken faeces samples (no *Salmonella* added);
- 6 low contaminated chicken faeces samples;
- 4 high contaminated chicken faeces samples;
- 1 procedure control (BPW only);
- 1 positive control (each participants used its own positive control strain).

All samples were individually packed and labelled and sent to the participating laboratories on Monday 23 September 2019. The decoding of the samples can be found in the tables with the individual NRL results.
One laboratory received the parcel within the same day of dispatch. Twenty-six parcels were delivered after one day, six parcels after two days, one parcel arrived after three days, and one parcel after seven days of dispatch due to delay at the boarder. 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. The temperature of the parcels during transport was predominantly between -4 °C and 4 °C. The storage temperature of the sample at the laboratories varied between 0 °C and 10 °C. The start date of the analysis for all laboratories was 30

September 2019. Laboratory 28 received their parcel late and started with the analyses on 1 October 2019. The temperature of the parcel during transport stayed below 5 °C up to 25 September, but raised rapidly to 10 °C on 27 September and 16 °C on 29 September. The parcel arrived at the laboratory on 30 September and was put in a refridgerator. The analyses was started on the 1 October. There were two other laboratories which started the analysis already on the day of arrival (24 September 2019) because of national holidays in the starting week.

Results

The prescribed method was 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.). The majority (33 laboratories) used this method. One laboratory used the former version of EN ISO 6579-1: Annex D of ISO 6579:2007. One laboratory used another method.

Control samples

The laboratories were asked to use their own positive control strain normally used in their routine analysis for the detection of *Salmonella*. Additional to this own control, a blank control BPW sample had to be analysed. All but one laboratory scored both control samples correct. Laboratory 6 scored an unsatisfactory performance for reporting their positive control sample negative for *Salmonella* and their negative BPW sample positive.

For the positive control, the majority of the participants used *Salmonella* Enteritidis as their positive control (12), followed by *Salmonella* Typhimurium (9), and *Salmonella* Nottingham (6). Eight participants used other strains.

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 97%.

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

Control samples		Total labs n = 35
Procedure control n = 1	No. of samples No. of negative samples **Specificity in %**	35 34 **97,1%**
Positive control (Own *Salmonella*) n = 1	No. of samples No. of positive samples **Sensitivity in %**	35 34 **97,1%**
All control samples n = 2	No. of samples No. of correct samples **Accuracy in %**	70 68 **97,1%**

Artificially contaminated chicken faeces samples

Negative samples
All laboratories correctly analysed the negative chicken faeces samples negative for *Salmonella*.

Low-level chicken faeces samples with Salmonella *Typhimurium*
Almost all laboratories were able to detect *Salmonella* in all six low level samples. Two laboratories (lab codes 21 and 22) scored one respectively two of the six low level contaminated 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).

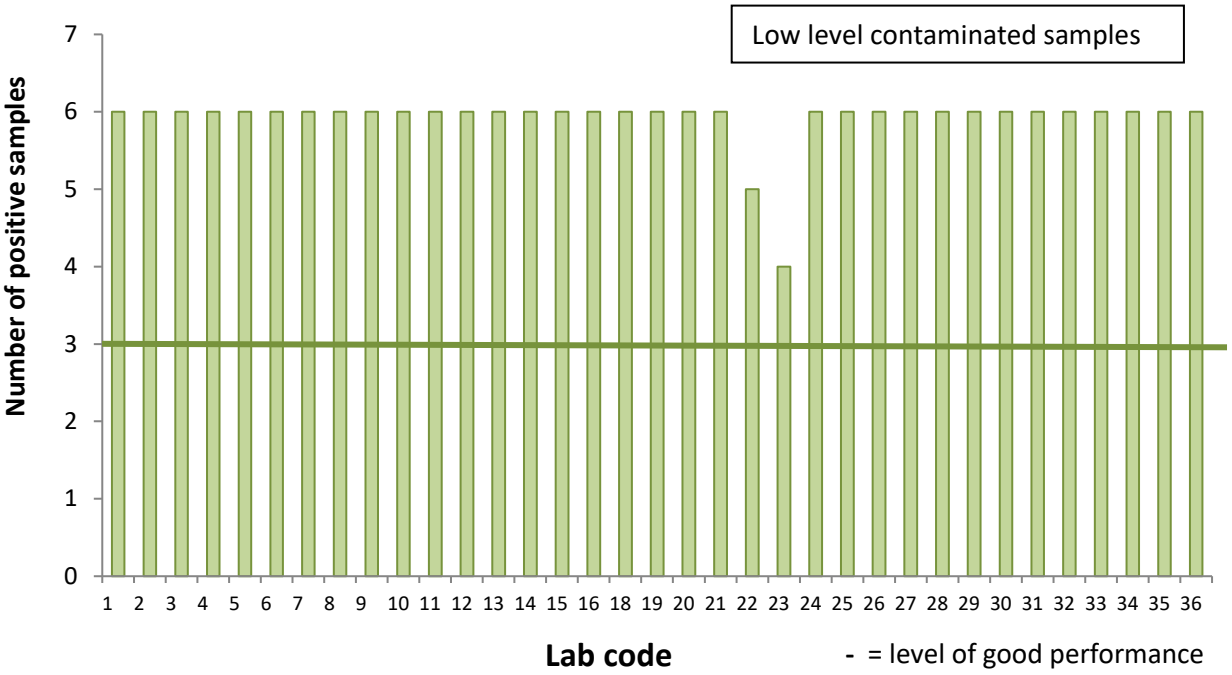

Figure 1. Number of positive Salmonella *isolations per laboratory found in the chicken faeces samples contaminated with low level of* Salmonella *Typhimurium (n=6).*

High-level chicken faeces samples with Salmonella *Typhimurium*
Almost all laboratories detected *Salmonella* in all four high level samples. One laboratory (lab code 22) scored one sample out of the four high level contaminated samples negative. See Figure 2 for results. The level of good performance allows for one sample out of the four high level contaminated samples to be scored negative.

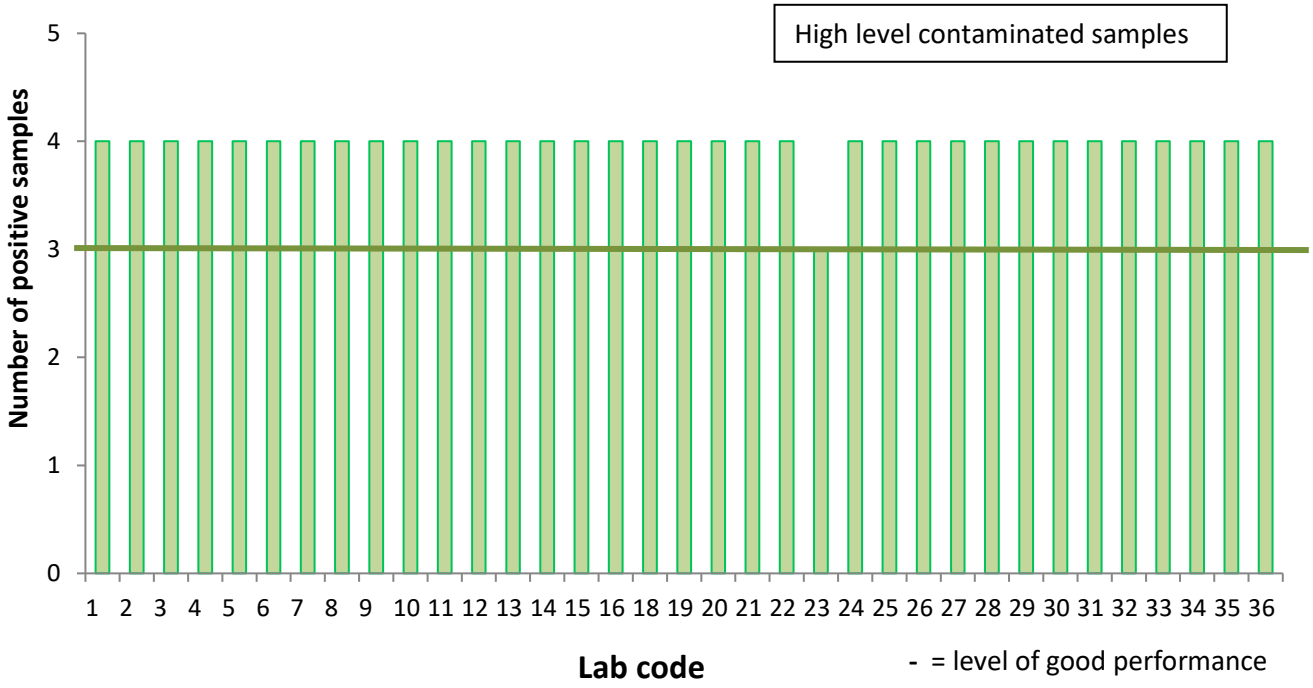

Figure 2. Number of positive Salmonella *isolations per laboratory found in the chicken faeces samples contaminated with high level of* Salmonella *Typhimurium (n=4).*

Table 4 shows the specificity, sensitivity and accuracy rates for the chicken faeces samples. The laboratories have scored good results with all chicken faeces samples (negative, low and high contaminated) as shown by the high rates for specificity, sensitivity and accuracy (> 98%).

Table 4. Specificity, sensitivity and accuracy rates of the chicken faeces samples artificially contaminated with Salmonella *Typhimurium (STm)*

Chicken faeces samples		**Total labs n = 35**
Negative n=4	No. of samples No. of negative samples **Specificity in %**	140 140 **100%**
Low level (STm) n=6	No. of samples No. of positive samples **Sensitivity in %**	210 207 **98,6%**
High level (STm) n=4	No. of samples No. of positive samples **Sensitivity in %**	140 139 **99,3%**
All chicken faeces samples with STm	No. of samples No. of positive samples **Sensitivity in %**	350 346 **98,9%**
All chicken faeces samples (positive and negative)	No. of samples No. of correct samples **Accuracy in %**	490 486 **99,2%**

Good performance

Criteria for good performance used in this EURL-*Salmonella* PT 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**
Negative*	20% max	→ 1/4 max
Low level contamination	≥ 50%	→ ≥ 3/6
High level contamination	≥ 80%	→ ≥ 3/4

*100% *Salmonella* free matrix cannot be guaranteed, 1 positive out of 4 negative samples is still considered as acceptable (20% max).

In total 34 laboratories fulfilled the criteria of good performance for the prescribed method.
One laboratory scored their own positive control negative and their negative control positive for *Salmonella,* resulting in an unsatisfactory performance. This laboratory will be contacted for additional explanation of this result.

List of abbreviations

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
PT	Proficiency Test
STm	*Salmonella* Typhimurium

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.

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