

Interim summary report

EURL-*Salmonella* Proficiency Test Primary Production Stage 2023

Detection of *Salmonella* in chicken faeces

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1. Introduction

In October 2023, the EURL-*Salmonella* Proficiency Test (PT) for detection of *Salmonella* in samples from the Primary Production Stage (PPS) was organised for the National Reference Laboratories (NRLs) for *Salmonella*. The matrix for this PT was chicken faeces. In total, 37 NRLs-*Salmonella* participated in this study originating from 27 EU-Member States (MS), 9 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, which was sent separately to the NRLs-*Salmonella*. The other part contains the overall results of all NRLs-*Salmonella*, which is described here.

2. Materials & Methods

2.1 Samples

The samples in this PT consisted of chicken faeces samples contaminated with *Salmonella* Typhimurium.

Each NRL-*Salmonella* had to analyse in total 16 blindly coded samples:

- 4 negative samples of each 25 g chicken faeces (no *Salmonella* added);
- 6 samples of each 25 g chicken faeces with a low level of *Salmonella* Typhimurium (STm);
- 4 samples of each 25 g chicken faeces with a high level of *Salmonella* Typhimurium (STm);
- 1 procedure control (BPW only)
- 1 positive control (laboratories' own *Salmonella* control strain)

The chicken faeces originated from a specific pathogen free farm (SPF farm) and absence of *Salmonella* was tested in ten different samples prior to the PT. The chicken faeces was stored at 5 °C until further use. Shortly before the PT, the samples were prepared by weighing 25 g of chicken faeces into coded sample bags and each sample was artificially contaminated with a low or a high level of STm, or not contaminated at all (negative samples). The artificially contaminated samples and the negative samples were stored at 5 °C ± 3 °C until the day of transport, approximately 5-6 days after inoculation. On Monday 25 September 2023, the artificially contaminated chicken faeces samples were packed with frozen cooling elements and sent to the NRLs-*Salmonella*. Upon arrival, the NRLs were requested to store the samples at 5 °C ± 3 °C until the start of the analysis on Monday 2 October 2023. The decoding of the samples can be found in the tables with the individual NRL results.

The level of natural background flora in the chicken faeces was determined one month before the start of the PT and on the day of the performance of the PT (2 October 2023). Table 2.1 shows the number of aerobic bacteria and *Enterobacteriaceae* in the chicken faeces.

Table 2.1 Number of aerobic bacteria and Enterobacteriaceae per gram chicken faeces

Date	Aerobic bacteria (cfu/g)	Enterobacteriaceae (cfu/g)
5 September 2023	1,3 x 10 ⁸	2,5 x 10 ⁵
2 October 2023^a	5,4 x 10 ⁸	1,5 x 10 ⁶

^a After storage at 5 °C for 4 weeks

Table 2.2 shows the level of the diluted culture of *S. Typhimurium* used to contaminate the chicken faeces samples. Also, the number of *Salmonella* in the chicken faeces samples was determined using a five-tube Most Probable Number (MPN) test at the start of the PT (2 October 2023) and one week later (9 October 2023).

Table 2.2 Number of *Salmonella Typhimurium* in the inoculum for artificial contamination of the chicken faeces samples and in the samples after storage at 5 °C for 13 and 20 days

Date of testing	Low level STm (cfu/sample)	High level STm (cfu/sample)
19 Sept 2023 Inoculum chicken faeces samples	17	50
2 Oct 2023^a MPN of artificially contaminated chicken faeces samples (95 % confidence limit)	8 (2,475 – 25)	8 (2,475 – 25)
9 Oct 2023^b MPN of artificially contaminated chicken faeces samples (95 % confidence limit)	0 (0 – 0,675)	1,7 (0,725 – 4)

^a After storage of the inoculated samples at 5 °C for 13 days

^b After storage of the inoculated samples at 5 °C for 20 days

2.2 Analysis of samples following EN ISO 6579-1/A1:2020

The prescribed method was EN ISO 6579-1:2017, including Amendment 1 (EN ISO 6579-1:2017/A1:2020), and the underlying EN ISO documents, e.g. the EN ISO 6887 series for preparation of test samples. EN ISO 6579-1:2017(/A1:2020) describes the technical steps for the detection of *Salmonella* in food, animal feed samples, environmental samples from the food production area, and samples from the primary production stage.

In summary, for samples from the primary production stage samples:

- pre-enrichment in:
buffered peptone water (BPW);
- selective enrichment on:
modified semi-solid Rappaport-Vassiliadis (MSRV) agar
- plating-out on two isolation media:
first isolation medium: xylose lysine deoxycholate agar (XLD);
second isolation medium (obligatory): medium of choice;
- confirmation by means of:

appropriate biochemical and serological tests (EN ISO 6579-1:2017(/A1:2020)) or reliable, commercially available identification kits.

Additionally, the NRLs-*Salmonella* were allowed to analyse the chicken faeces samples with a second detection method, if this is (routinely) used in their laboratories. These results could also be reported, but only the results obtained with EN ISO 6579-1:2017(/A1:2020) were used to assess the performance of each NRL.

From the results of all laboratories the specificity, sensitivity and accuracy rates were calculated, as follows:

Specificity rate

$$\frac{\text{Number of negative results}}{\text{Total number of (expected) negative samples}} \times 100\%$$

Sensitivity rate

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

Accuracy rate

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

2.3 Performance analysis

Criteria for good performance used in the current EURL-*Salmonella* PT for detection of *Salmonella* in chicken faeces are shown in Table 2.3.

Table 2.3 Criteria for good performance

Chicken faeces samples	Percentage positive	# pos. samples / total # samples
Negative samples	0 *	0 / 4
Low level of STm	≥ 50%	≥ 3 / 6
High level of STm	≥ 75%	≥ 3 / 4
Control samples	Percentage positive	# pos. samples / total # samples
Procedure control	0%	0 / 1
Positive control with <i>Salmonella</i>	100%	1 / 1

* 100% *Salmonella*-free chicken faeces cannot be guaranteed, so that an incidental positive result with a *Salmonella* strain different from the inoculation strain is still considered as acceptable

3. Results

3.1 General

On Monday 25 September 2023, the chicken faeces samples were sent to the participating laboratories. Two parcels arrived within the same day of dispatch. Twenty-two parcels were delivered after one day, ten after two days, one after four days and one after seven days of dispatch. Laboratory 17 received the samples on 10 October 2023 and started the analyses the next day.

The temperature during transport and storage was registered using a temperature probe. The temperature of the parcels during transport was predominantly between -3,5 °C and 4 °C. The storage temperature of the samples at the laboratories varied between -1,5 °C and 8,5 °C.

The temperature of the parcels arriving with (substantial) delay were checked in more detail. The parcel for laboratory 9 experienced an elevated storage temperature with a day and night rhythm of 6,5 °C and 13 °C until 30 September. The temperature remained stable at 11-13 °C until the start of the analyses at 2 October 2023.

The parcel for laboratory 17 was held at the customs for almost 2 weeks. The temperature of the samples stayed at -1 °C until 29 September followed by a slow increase to 8,5 °C at 3 October and a fast increase to 15,5 °C on 9 October 2023, when the parcel arrived at its destination.

The temperature profile of the parcel of laboratory 20 showed a strange pattern of a sharp, high peak in storage temperature of 25 °C on 27 September, followed by a stable temperature profile of 5 °C. From 28 September onwards, there was a second sharp increase in temperature to, again, 24 °C, followed by a slow decrease to 20,5 °C until the start of the analyses on 2 October 2023.

Also the parcel of laboratory 37 arrived late (on 2 October 2023). Still, the samples stayed cool during transport (-2,5 °C – 0,5 °C).

All laboratories but 1 (lab code 7) were accredited for the prescribed method EN ISO 6579-1:2017. The majority of the laboratories also indicated that they followed Amendment 1 of EN ISO 6579-1 (EN ISO 6579-1:2017/A1:2020). Seven laboratories also used a second detection method for analysing the samples. All those laboratories found identical results using the alternative method compared to the results found with EN ISO 6579-1:2017(/A1:2020).

3.2 Chicken faeces samples

Negative samples

The negative samples consisted of chicken faeces only. All thirty-seven laboratories tested all four chicken faeces samples correctly negative for *Salmonella* (see figure 3.1).

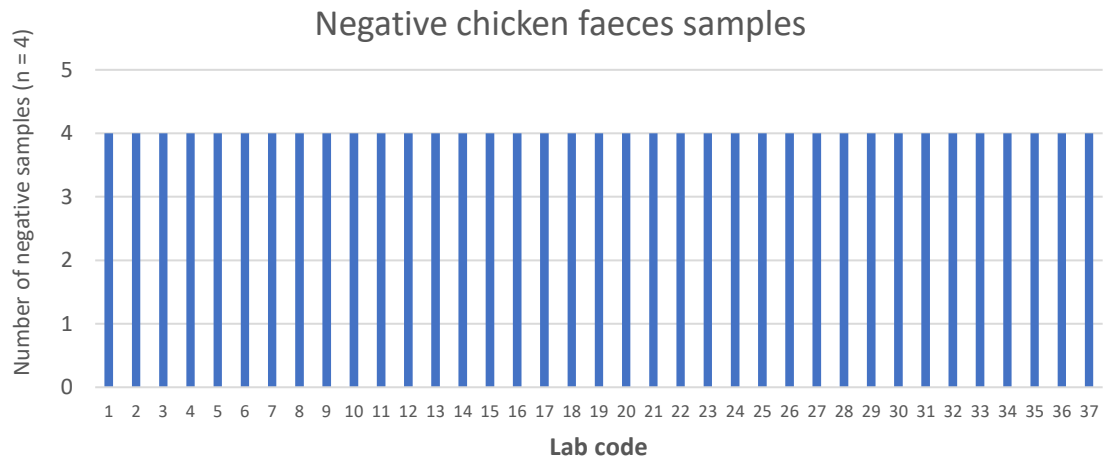


Figure 3.1. Number of negative chicken faeces samples (n=4) tested negative for Salmonella, per participant.

Samples with a low level of Salmonella Typhimurium

Almost all laboratories were able to detect *Salmonella* Typhimurium in all six low level chicken faeces samples. See Figure 3.2 for results. Four laboratories scored 1 of the 6 low level samples negative for *Salmonella*. One laboratory (lab code 1) could not detect *Salmonella* Typhimurium in four of the six low level samples. This result is not within the limits of good performance, which allows for three out of the six low contaminated samples to be scored negative (see Table 2.3).

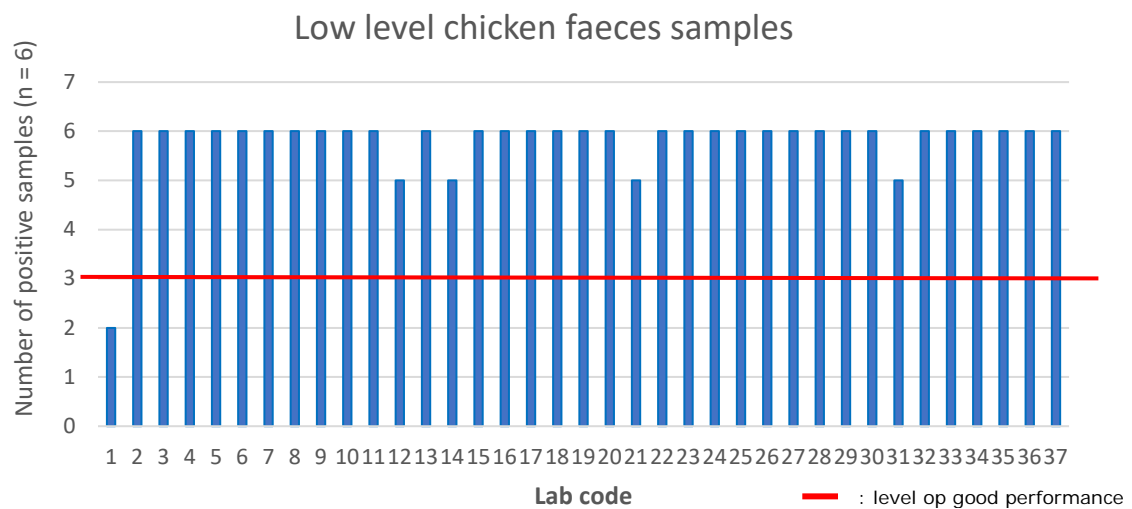


Figure 3.2. Number of positive Salmonella isolations per laboratory found in the chicken faeces samples artificially contaminated with a low level of *Salmonella* Typhimurium (n=6).

Samples with a high level of Salmonella Typhimurium

All laboratories but 1 (lab code 1) were able to detect *Salmonella* in all four high level chicken faeces samples. See Figure 3.3 for results. Laboratory 1 tested three out of four high level chicken faeces positive, which is still within the limits of good performance (see Table 2.3).

High level chicken faeces samples

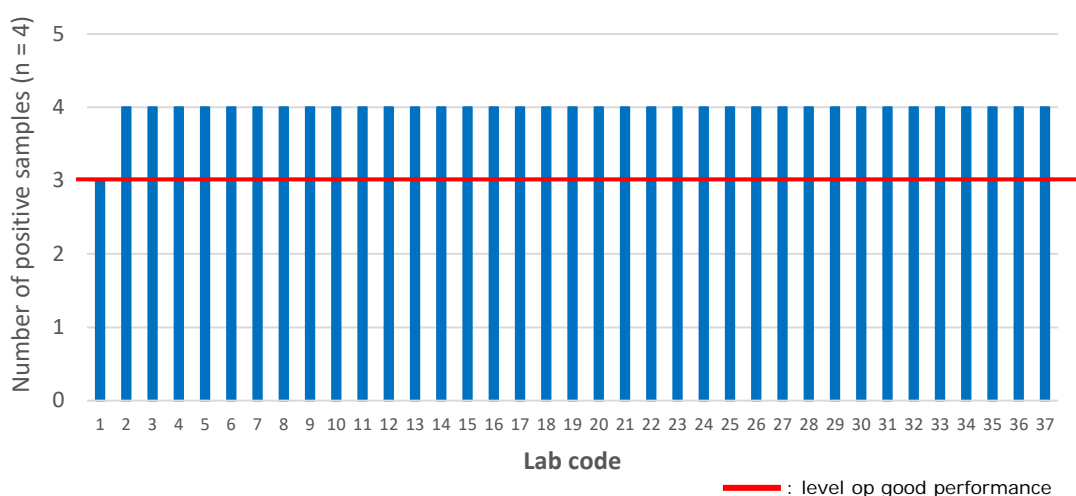


Figure 3.3 Number of positive *Salmonella* isolations per laboratory found in the chicken faeces samples artificially contaminated with a high level of *Salmonella* Typhimurium (n=4)

In table 3.1 the specificity, sensitivity and accuracy rates are given for the chicken faeces samples. The laboratories scored good results with all chicken faeces samples, as shown by the high rates for sensitivity, specificity and accuracy.

Table 3.1 Specificity, sensitivity and accuracy rates of the chicken faeces samples

Chicken faeces samples		Total no of labs n = 37	EU NRLs only n=27
Negative n=4	No. of samples	148	108
	No. of neg. samples	148	108
	Specificity in %	100%	100%
Low level (STm) n=6	No. of samples	222	162
	No. of pos. samples	214	155
	Sensitivity in %	96,4%	95,7%
High level (STm) n=4	No. of samples	148	108
	No. of positive samples	147	107
	Sensitivity in %	99,3%	99,1%
All chicken faeces with <i>Salmonella</i> (STm)	No. of samples	370	270
	No. of positive samples	361	262
	Sensitivity in %	97,6%	97%
All chicken faeces (pos. and neg.)	No. of samples	518	378
	No. of correct samples	509	370
	Accuracy in %	98,3%	97,9%

3.3 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 procedure control sample (BPW only) had to be analysed. Almost all

laboratories scored both control samples correct. One laboratory scored their procedure control positive and their positive control negative. This laboratory (lab code 21) scored unsatisfactory performance because of that.

For the positive control, the majority of the participants used *Salmonella* Enteritidis (10) as their positive control, followed by *Salmonella* Typhimurium (9) and *Salmonella* Nottingham (5). Thirteen participants used other *Salmonella* serovars.

Table 3.2 shows the correct scores for each control sample and the accuracy rate for both control samples. The laboratories scored good results for the control samples with an accuracy rate of 97%.

Table 3.2 Correct scores of the control samples

Control samples		Total no of labs n = 37
Procedure control (BPW only) n = 1	No. of samples	37
	No. of negative samples	36
	Correct score in %	97%
Positive control (Own <i>Salmonella</i> control) n = 1	No. of samples	37
	No. of positive samples	36
	Correct score in %	97%
All control samples n = 2	No. of samples	74
	No. of correct samples	72
	Accuracy in %	97%

3. Performance of the participants

The results of 35 of the 37 participating laboratories fulfilled the criteria of good performance.

One laboratory (lab code 21) scored their own positive control negative and their procedure control positive for *Salmonella*, resulting in an unsatisfactory performance. Laboratory 1 scored 4 of the 6 low level samples negative, resulting in an unsatisfactory performance. Both laboratories will be contacted for additional explanation of these results.

4. 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 Organization for Standardization
MPN	Most Probable Number
MS	Member State
MSRV	Modified semi-solid Rappaport-Vassiliadis
NRL	National Reference Laboratory
PPS	Primary Production Stage
PT	Proficiency Test
STm	<i>Salmonella</i> Typhimurium
XLD	Xylose Lysine Deoxycholate agar

5. 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 6579-1/A1:2020. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp. - Amendment 1 - Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC (ISO 6579-1:2017/Amd 1:2020).

[EN ISO 6887-1 & -6]: 2017. Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - series for preparation of test samples. Part 1: General rules for the preparation of the initial suspension and decimal dilutions. Part 6: Specific rules for the preparation of samples taken at the primary production stage.

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