Interim summary report EURL-*Salmonella*

Combined Proficiency Test Primary Production Stage and Food 2020

Detection of *Salmonella* in Hygiene swab samples

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Introduction

In October 2020, the combined *EURL-Salmonella* Proficiency Test (PT) for detection of *Salmonella* in samples from the Primary Production Stage (PPS) and Food was organised for the NRLs-*Salmonella.* The matrix for this PT was Hygiene swabs. This matrix is also suitable to mimic a sample from the food production environment.. Since EURL-*Salmonella* did not organise a PT for detection of *Salmonella* in food in 2020 (only a PT for detection of *Salmonella* in mussels was organised), the NRLs-*Salmonella* analysing food samples were also invited to participate in this Proficiency Test. In total 65 NRLs for *Salmonella* participated in this study: 38 NRLs PPS and 27 NRLs Food originating from 34 countries. 27 participants originated from 27 EU-Member States (MS), 7 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

The samples in this combined Proficiency Test consisted of Hygiene swabs contaminated with background flora and different concentrations of *Salmonella* Typhimurium (STm).
The hygiene swabs were moistened with 10 ml of peptone saline solution (PS) and left to saturate at room temperature for at least 30 minutes. Next, all hygiene swabs were contaminated with a high concentration of background flora by adding 1 ml of a cell suspension containing an even mixture of *Escherichia coli* and *Citrobacter freundii* (approx. 10^8 cfu/ml). Additionally, one third of the total number of hygiene swabs with background flora was contaminated with a low level of STm, on third with a high level of STm and one third was not inoculated with *Salmonella* (negative samples). The artificially contaminated samples were stored at 5 °C until the day of transport, approximately 4 days after inoculation. On Monday 28 September 2020, the artificially contaminated hygiene swab 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 5 October 2020.

The total amount of background flora in the hygiene samples was $1{,}2 \times 10^8$ cfu/swab. Table 2 shows the level of the diluted culture of *Salmonella* Typhimurium used to inoculate the samples. Additionally, the number of *Salmonella*

in the artificially contaminated hygiene swab 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 hygiene swab samples.*

Date of testing	Low level STm (cfu)	High level STm (cfu)
23 Sept 2020 (inoculum level diluted culture)	7	47
5 Oct 2020 MPN contaminated hygiene swab (95 % confidence limit)	3,3 (1,1-10,35)	35 (11-110)

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

- 4 negative hygiene swab samples containing background flora only (no *Salmonella* added;
- 6 hygiene swab samples containing a low level of STm;
- 4 hygiene swab samples containing a high level of STm;
- 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 28 September 2020. The decoding of the samples can be found in the tables with the individual NRL results.
Two laboratories received the parcel within the same day of dispatch. Forty-two parcels were delivered after one day, ten parcels after two days, five parcels after three days and three parcels arrived after four days of dispatch. Three parcels arrived very late due to delay at the borders. One parcel (lab code 66) arrived after eight days and one parcel (lab code 30) after nine days of dispatch. The third parcel (lab code 54) arrived only after 17 days of dispatch due to serious delays at the border. 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 7 °C. The storage temperature of the sample at the laboratories varied between 0 °C and 10 °C. The temperature of the parcels arriving late was checked in more detail. The parcel of laboratory 66 arrived at 5 October 2020 with a temperature still below 5 °C. The parcel of laboratory 30 was exposed to very high temperatures during transport for quite some time. The temperature of the samples stayed around 1 °C for three days. From 30 September the temperature raised quickly to 10 °C on 1 October and to 18 °C on 2 October 2020. After 4 October the temperature raised to 24 °C and stayed this high until arrival at the laboratory on 6 October 2020. Also the parcel of laboratory 54 was exposed to elevated temperatures. The samples stayed cool at 1 °C until 30 September 2020. The temperature increased to 10 °C on 1 October and to 18 °C on 2 October. The temperature remained at 18 °C for three more days until 5 October 2020 when temperature dropped to 10 °C for the remaining days until the parcel arrived at the laboratory on 15 October 2020.
Most laboratories started the analyses on 5 October 2020. However five laboratories started the analysis one day later because of national Holidays on 5 October. Laboratory 54 started the analyses the day after arrival of the parcel on 15 October. One laboratory (lab code 37) started already on the day of arrival of the parcel (29 September 2020).

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 (45 laboratories) used this method. There were 12 laboratories that indicated that they already followed the recently published amendment of EN ISO 6579-1 (EN-ISO 6579-1:2017/A1:2020). One laboratory reported to have used only a PCR method but did report results for two methods including the selective enrichment media MKTTn and RVS.

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 procedure control sample (BPW only) had to be analysed. All but two laboratory scored both control samples correct. Laboratory 63 and 66 both scored an unsatisfactory performance by reporting their positive control sample negative for *Salmonella*.

For the positive control, the majority of the participants used as their positive control *Salmonella* Typhimurium (19), followed by *Salmonella* Enteritidis (17), and *Salmonella* Nottingham (8). Twenty-one participants used other *Salmonella* serovars.

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 98,5%.

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

Control samples		Total no of labs n = 65
Procedure control (BPW only) n = 1	No. of samples No. of negative samples **Specificity in %**	65 65 **100%**
Positive control (Own *Salmonella* control) n = 1	No. of samples No. of positive samples **Sensitivity in %**	65 63 **96,9%**
All control samples n = 2	No. of samples No. of correct samples **Accuracy in %**	130 128 **98,5%**

Artificially contaminated hygiene swab samples

Negative samples
All laboratories correctly analysed the negative hygiene swab samples negative for *Salmonella*.

Low-level hygiene swab samples with Salmonella *Typhimurium*
Almost all laboratories were able to detect *Salmonella* in all six low level samples. See Figure 1 for results. Three laboratories (lab codes 1, 3 and 39) scored one of the six low level contaminated samples negative for *Salmonella*. One laboratory (lab code 67) scored three of the six low level samples negative. This is just within the limits of good performance, which allows for three out of the six low level contaminated samples to be scored negative (see Table 5).

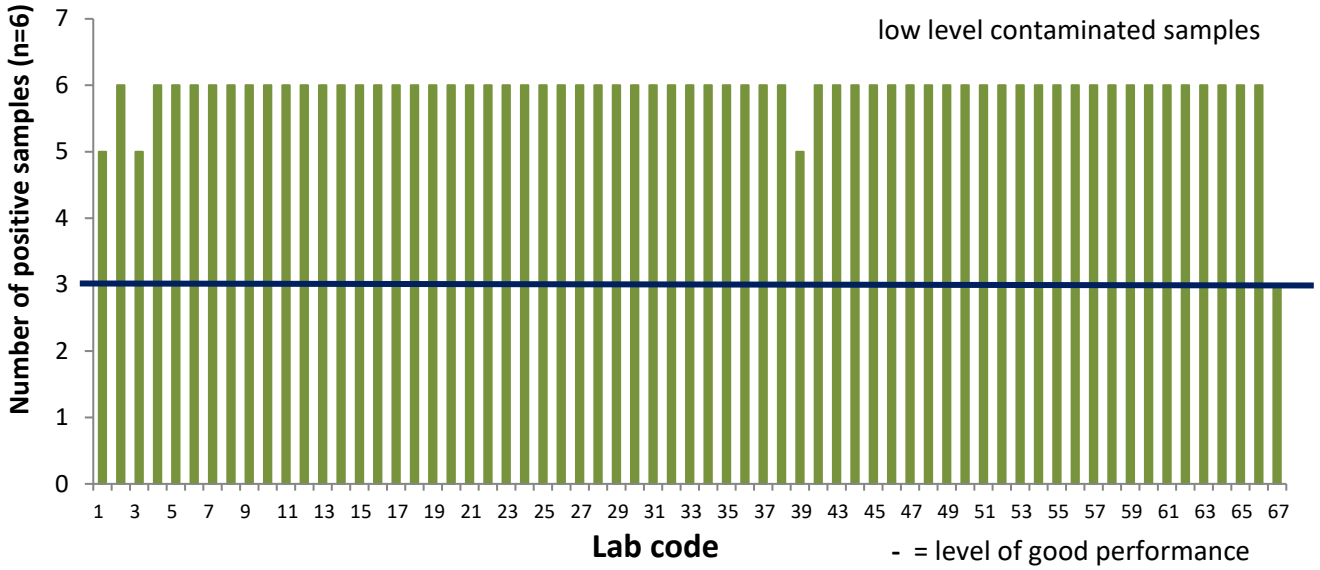

Figure 1. Number of positive Salmonella *isolations per laboratory found in the hygiene swab samples contaminated with a low level of* Salmonella *Typhimurium (n=6).*

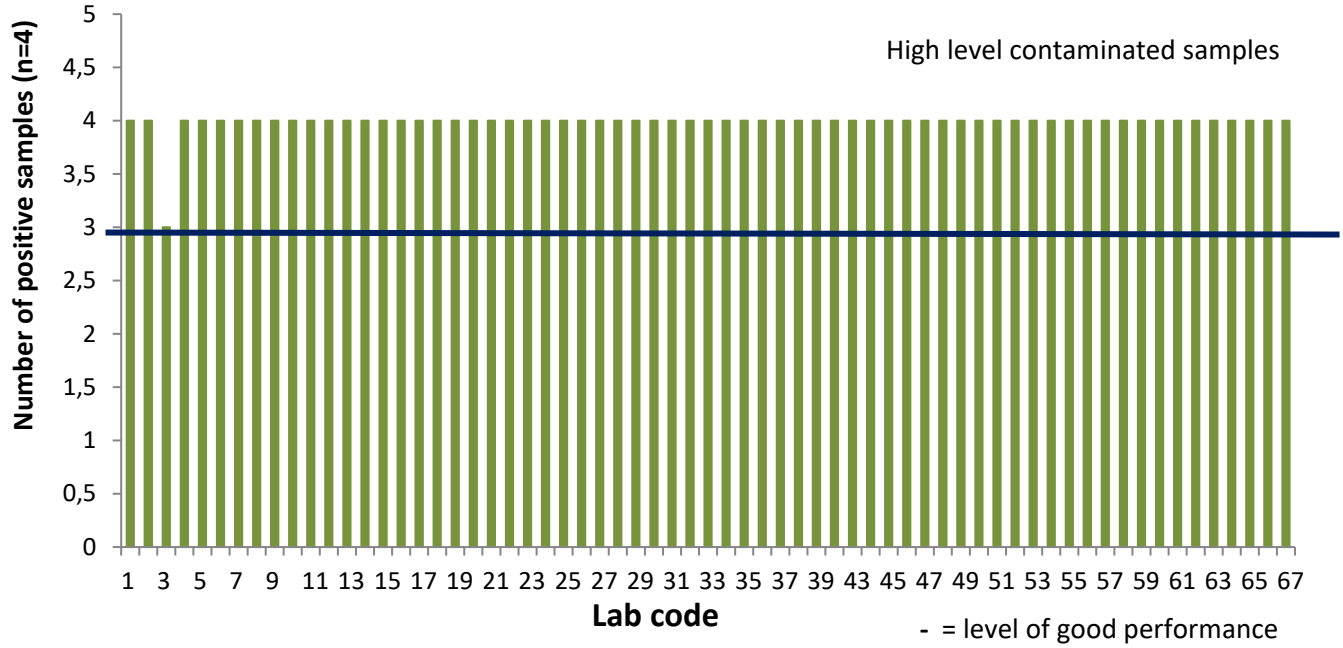

Figure 2. Number of positive Salmonella *isolations per laboratory found in the hygiene swab samples contaminated with a high level of* Salmonella *Typhimurium (n=4).*

High-level hygiene swab samples with Salmonella *Typhimurium*
Almost all laboratories detected *Salmonella* in all four high level samples. See Figure 2 for results. One laboratory (lab code 3) scored one sample out of the four high level contaminated samples negative. This is still within the level of good performance which allows for one sample out of the four high level contaminated samples to be scored negative (see Table 5).

Table 4 shows the specificity, sensitivity and accuracy rates for the hygiene swab samples. The laboratories have scored good results with all hygiene swab samples (negative, low and high contaminated) as shown by the high rates for specificity, sensitivity and accuracy (> 98%). Two laboratories (lab code 30 and 54) received samples which had experienced elevated temperature during a prolonged period of time. This temperature abuse did not affect the quality of the samples, since these laboratories have scored a good performance in this PT.

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

Hygiene swab samples		**Total no of labs n = 65**
Negative n=4	No. of samples No. of negative samples **Specificity in %**	260 260 **100%**
Low level (STm) n=6	No. of samples No. of positive samples **Sensitivity in %**	390 384 **98,5%**
High level (STm) n=4	No. of samples No. of positive samples **Sensitivity in %**	260 259 **99,6%**
All hygiene swab samples with STm	No. of samples No. of positive samples **Sensitivity in %**	650 643 **98,9%**
All hygiene swab samples (positive and negative)	No. of samples No. of correct samples **Accuracy in %**	910 903 **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	0%	0/4
Low level contamination	≥ 50%	≥ 3/6
High level contamination	≥ 80%	≥ 3/4

In total 63 laboratories fulfilled the criteria of good performance for the prescribed method.
Two laboratory scored their own positive control negative resulting in an unsatisfactory performance. These laboratories 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 6579-1/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).

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