Interim summary report

EURL-*Salmonella* Proficiency Test food 2021

Detection of *Salmonella* in liquid whole egg

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Introduction

In March 2021, an EURL-*Salmonella* Proficiency Test for detection of *Salmonella* in food was organised for the National Reference Laboratories-*Salmonella* (NRLs-*Salmonella*). The matrix under analysis was liquid whole egg. NRLs-*Salmonella* which analyse food, were invited to participate in this Proficiency Test (PT).
In total 33 NRLs-*Salmonella* participated in this PT: 28 NRLs from 27 EU Member States (MS) and 5 NRLs from third countries (EU candidate MS, members of the European Free Trade Association (EFTA), and United Kingdom). 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.

Materials & Methods

Samples

The samples for this PT consisted of liquid whole egg with different concentrations of *Salmonella* Enteritidis.

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

- 4 samples of each 25 g liquid whole egg with a high level of *Salmonella* Enteritidis (SE)
- 6 samples of each 25 g liquid whole egg with a low level of *Salmonella* Enteritidis (SE)
- 4 negative samples of 25 g liquid whole egg (no *Salmonella* added)
- 2 control samples (procedure control and own positive control)

In total, 16 packages of 1 litre pasteurised liquid whole egg of the brand Eggstra were obtained on 12-02-2021. All packages had an identical expiration date: 02-05-2021. All packages were stored at 5 °C until sample preparation.
Samples from five different packages were tested for the absence of *Salmonella*. *Salmonella* was not detected in any of the tested samples.

By the end of February 2021, the samples for the PT were prepared. For this, approximately 500 subsamples of each 25 g liquid whole egg were weighed into (plastic) sample bags. Each subsample was individually, artificially contaminated with a low or a high level of SE or no *Salmonella* at all (negative samples). The decoding of these samples can be found in the tables of the individual NRL results. Next, the samples were stored at 5 °C until shipment.

On Monday 1 March 2021, the PT samples were shipped to the NRLs-*Salmonella*. During transport the samples were kept cool by using frozen cooling elements and the temperature during transport was registered by an electronic temperature device ('temperature probe'). Upon arrival, the NRLs were requested to store the samples, together with the temperature probe, at 5 °C until the start of the analysis on Monday 8 March 2021.

The level of natural background flora in the liquid whole egg was tested on 16 February 2021 (shortly after receipt of the liquid whole egg) and on 9 March 2021 (during the PT). Table 1 shows the number of aerobic bacteria and *Enterobacteriaceae* in the liquid whole egg.

Table 1 Number of aerobic bacteria and Enterobacteriaceae *per gram liquid whole egg*

Date	Aerobic bacteria (cfu/g)	*Enterobacteriaceae* (cfu/g)
16 February 2021	$9{,}6 \times 10^2$	<1
9 March 2021[a]	$3{,}2 \times 10^3$	<1

[a] After storage at 5 °C for 3 weeks

Table 2 shows the inoculation levels of the diluted culture of *Salmonella* Enteritidis used to artificially contaminate the liquid whole egg samples. Also a five tube Most Probable Number (MPN) test was performed on the artificially contaminated PT samples with low and high level SE. The MPN test was performed at the start of the PT.

Table 2 Number of Salmonella *Enteritidis in the inoculum for artificial contamination of the liquid whole egg samples and after storage at 5 °C for 1,5 week*

Date	Low level SE in cfu per sample	High level SE in cfu per sample
25 February 2021 Inoculation of liquid whole egg	10	69
8 March 2021[a] MPN of liquid whole egg samples, inoculated with SE (95% confidence limit)	3,3 (1,1-10,3)	160 (52,5-500)

[a] After storage at 5 °C for 1,5 week

Analysis of samples following EN ISO 6579-1

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.) and the underlying EN ISO documents, e.g. the EN ISO 6887 series for preparation of test samples. In 2020, Amendment 1 of EN ISO 6579-1:2017 was published (EN ISO 6579-1:2017/A1:2020), allowing incubation of selective media at a broader temperature range (34 °C to 38 °C instead of 37 °C ± 1 °C). The participants were free to choose for this broader temperature range or to retain to 37 °C ± 1 °C.
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. EN ISO 6579-1(/A1:2020) prescribes the use of two selective enrichment media. In addition to Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn) either Rappaport-Vassiliadis with Soya (RVS) broth or Modified semi-solid Rappaport-Vassiliadis agar (MSRV) agar shall be used. For the PT it was also allowed to use all three selective enrichment media.

In summary:
- pre-enrichment in:
  Buffered Peptone Water (BPW);
- selective enrichment in/on:
  Muller-Kauffmann tetrathionate-novobiocin (MKTTn) broth;
  Modified semi-solid Rappaport-Vassiliadis (MSRV) agar and/or;
  Rappaport-Vassiliadis with Soya (RVS);
- 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.

NRLs-*Salmonella* had to report the final confirmed results of the samples by indicating if *Salmonella* was 'detected' or 'not detected' per 25 g liquid whole egg.

Additionally, the NRLs-*Salmonella* were allowed to analyse the 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

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

Sensitivity rate

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

Accuracy rate

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

Performance analysis

Criteria for good performance used in the current EURL-*Salmonella* PT for detection of *Salmonella* in liquid whole egg are shown in Table 3.

Table 3 Criteria for good performance

Contaminated samples	**Percentage positive**	**# pos samples/ total # samples**
Negative samples	0%	0 / 4
Low level of *S*. Enteritidis	≥ 50%	≥ 3 / 6
High level of *S*. Enteritidis	≥ 75%	≥ 3 / 4
Control samples	**Percentage positive**	**# pos samples/ total # samples**
Procedure control	0%	0 / 1
Positive control with *Salmonella*	100%	1 / 1

Results

General

On Monday 1 March 2021 the liquid whole egg samples were sent to 33 laboratories. Thirty-two of the parcels were delivered at the NRLs within one or two days. The parcel of laboratory 23 was held at customs and arrived after nine days of transport. This laboratory started with the PT on 11 March 2021.

The temperature during transport and storage was registered using a temperature probe. The temperature of all parcels during transport was below 5 °C, except for the parcel of laboratory 23. The temperature of this parcel reached a maximum of 11,5 °C, when held at customs.
The measured storage temperature of the samples at the laboratories varied between 0 and 7 °C.

All laboratories used the prescribed method EN ISO 6579-1:2017. Two of the thirty-three laboratories indicated that they followed EN ISO 6579-1, including the amendment (EN ISO 6579-1:2017/A1:2020).
Thirty-two laboratories used MKTTn and RVS and/or MSRV as selective enrichment media. One laboratory used RVS and MSRV as selective media (laboratory 30). This laboratory did not used MKTTn as selective enrichment medium, which is prescribed in addition to MSRV and/or RVS in EN ISO 6579-1:2017(/A1:2020) for analysis of food and feed samples.

Twelve laboratories also used a second detection method for analysing the samples. These additional methods concerned PCR, BAX system (standard PCR assay), qPCR and mini VIDAS. One laboratory incubated MKTTn in parallel at 41,5 °C and reported the results as second detection method. The results of the second detection methods were all similar to the reported results obtained with EN ISO 6579-1:2017.

Artificially contaminated liquid whole egg samples

Samples with a high level of *Salmonella* Enteritidis
All laboratories detected *Salmonella* in all four high contaminated liquid whole egg samples. See Figure 1.

Samples with a low level of *Salmonella* Enteritidis
Thirty-two laboratories detected *Salmonella* in all six low contaminated liquid whole egg samples. One laboratory (laboratory 24) detected *Salmonella* in five out of six low level contaminated samples, which still fulfils the criteria of good performance. See Figure 2.

Negative samples
All thirty-three laboratories scored all four negative samples correctly: *Salmonella* was not detected.

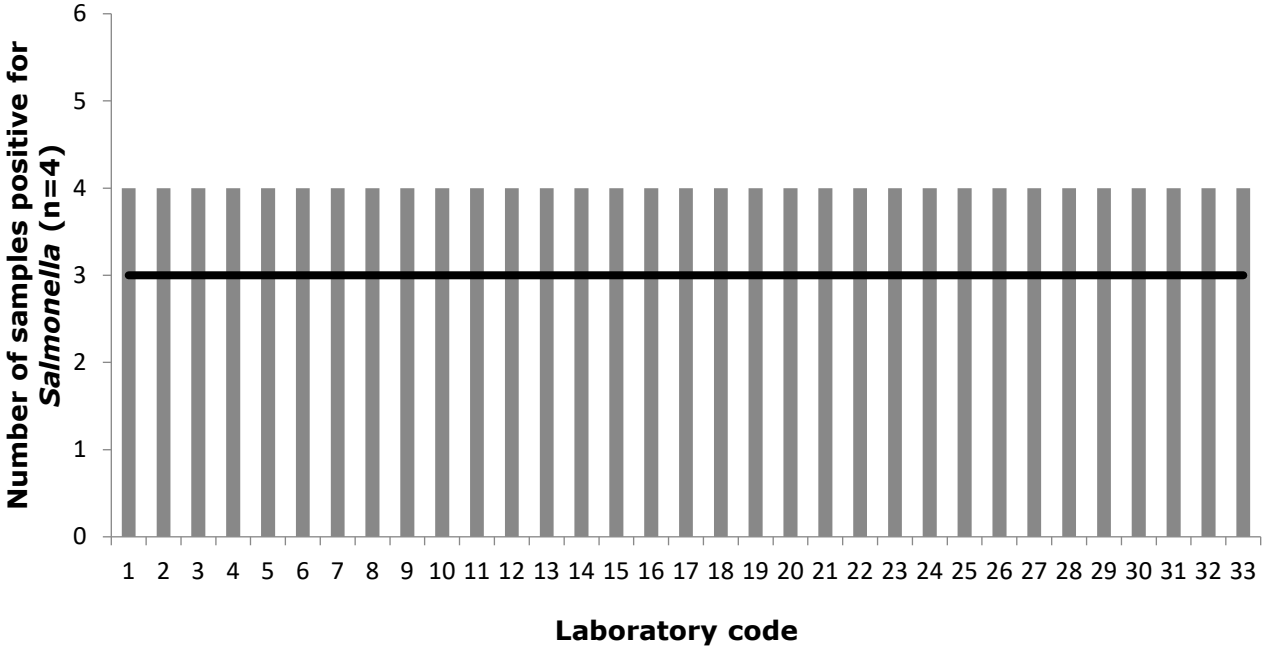
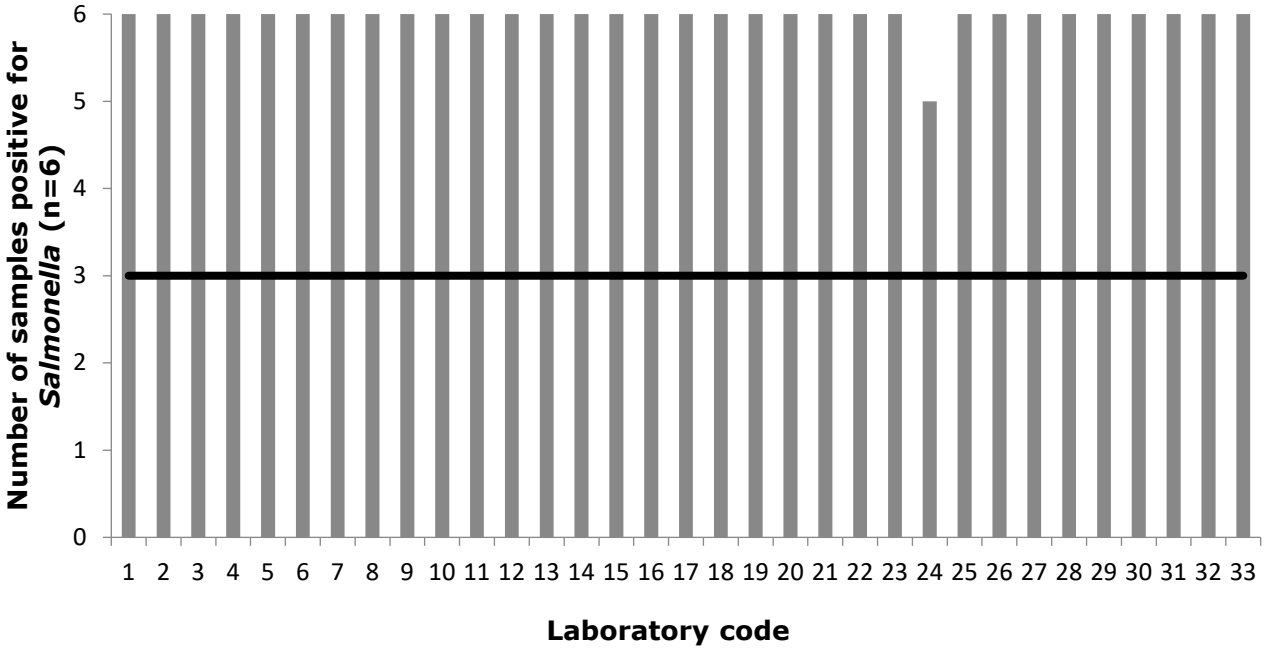


Figure 1 Number of liquid whole egg samples artificially contaminated with a high level of Salmonella *Enteritidis (n=4) in which* Salmonella *was detected per laboratory*



: level of good performance

Figure 2 Number of liquid whole egg samples artificially contaminated with a low level of Salmonella *Enteritidis (n=6) in which* Salmonella *was detected per laboratory*

In Table 4 the specificity, sensitivity and accuracy rates are given for the liquid whole egg samples.

Table 4 Specificity, sensitivity and accuracy rates of the liquid whole egg samples

Samples		**All participants n = 33**
High level of *Salmonella* Enteritidis n = 4	No. of samples	132
	No. of positive samples	132
	Sensitivity in %	100%
Low level of *Salmonella* Enteritidis n = 6	No. of samples	198
	No. of positive samples	197
	Sensitivity in %	99,5%
Negative samples n=4	No. of samples	132
	No. of negative samples	132
	Specificity in %	100%
All liquid whole egg samples artificially contaminated with *Salmonella*	No. of samples	330
	No. of positive samples	329
	Sensitivity in %	99,7%
All liquid whole egg samples	No. of samples	462
	No. of correct samples	461
	Accuracy in %	99,8%

Control samples

Procedure control (BPW only)
All laboratories analysed the procedure control sample (BPW only) correctly: *Salmonella* was not detected.

Own positive control with *Salmonella*
The laboratories were asked to use their own positive control normally used in their routine analysis for the detection of *Salmonella*.
All laboratories detected *Salmonella* in their own *Salmonella* positive control sample.

The *Salmonella* serovars used by the majority of the participants for the positive control sample were: *S.* Enteritidis (9), *S.* Typhimurium (8), *S.* Nottingham (5), *S*. Abaetetuba (3) and eight participants used other *Salmonella* serovars.

Table 5 gives the correct scores for the control samples with an accuracy rate of 100%.

Table 5 Correct scores of the control samples

Control samples		**All participants n = 33**
Procedure control (BPW only) n=1	No. of samples	33
	No. of negative samples	33
	Correct score in %	100%
Positive control with *Salmonella* n=1	No. of samples	33
	No. of positive samples	33
	Correct score in %	100%
All control samples n=2	No. of samples	66
	No. of correct samples	66
	Accuracy in %	100%

Performance of the participants

All thirty-three laboratories fulfilled the criteria of good performance.

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
MKTTn	Muller-Kauffmann tetrathionate-novobiocin broth
MPN	Most Probable Number
MS	Member State
MSRV	Modified semi-solid Rappaport-Vassiliadis
NRL	National Reference Laboratory
PCR	Polymerase Chain Reaction
PT	Proficiency Test
qPCR	quantitative Polymerase Chain Reaction
RVS	Rappaport-Vassiliadis medium with Soya
SE	*Salmonella* Enteritidis
XLD	Xylose Lysine Deoxycholate agar

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 & -4: 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 4: Specific rules for the preparation of miscellaneous products.

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