Interim summary report EURL-*Salmonella*

Combined interlaboratory comparison study for Food and Primary Production stage (2017)

Detection of *Salmonella* in contaminated hygiene swabs

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Introduction

In October 2017, the combined interlaboratory comparison study for Food and Primary Production stage on the detection of *Salmonella* in hygiene swabs was organised by the EURL-*Salmonella*. In total 56 NRLs participated in this study: 33 NRLs for *Salmonella* in Food matrices and 23 NRLs for *Salmonella* in Primary Production matrices (PPS).
The participants originated from 28 EU-Member States (MS), 4 NRLs from third countries within Europe (EU candidate 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
The samples in this combined interlaboratory study consisted of hygiene swabs contaminated with background flora and different concentrations of *Salmonella*.
The hygiene swabs were moistened with 10 ml of peptone saline solution (PS) and left to saturate at room temperature. Next, the hygiene swabs were contaminated with a high concentration of background flora by adding 1 ml of a cell suspension containing an even mixture of *E.coli* and *Citrobacter freundii* (approx. 10^6 cfu/ml) followed by contamination with three different levels (blank, low and high level) of *Salmonella* Typhimurium (STM). The artificially contaminated samples were stored at 5 °C until the day of transport. On Monday 2 October, the artificially contaminated hygiene swabs 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 9 October.

Table 1 shows the number of background flora by counts of *Enterobacteriaceae* in hygiene swab samples determined by the EURL-*Salmonella* on 9 October 2017.

Table 1 Number of Enterobacteriaceae *in the high, low and blank* Salmonella *hygiene swab samples*

Date of testing	Blank samples cfu	Low level STM samples cfu	High level STM sample cfu
28 Sept 2017	7.7×10^5	1.4×10^6	7.3×10^7
9 October 2017, after storage at 5 °C	7.1×10^6	1.4×10^4	4.7×10^6

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

Table 2 Salmonella *Typhimurium concentration in the inoculum and in the inoculated hygiene swab samples.*

Date of testing	Low level STM (cfu)	High level STM (cfu)
28 Sept 2017 (Inoculum level diluted culture)	5	107
9 Oct 2017 MPN contaminated swabs (95 % confidence limit)	7 (2.3-22)	92 (28-300)

Each NRL analysed in total 20 samples: 18 hygiene swab samples artificially contaminated with background flora and three different levels of *Salmonella* Typhimurium (6 blank samples, 6 low contaminated samples and 6 high contaminated samples). In addition, 2 control samples had to be analysed: 1 procedure control consisting of moistened hygiene swabs contaminated with background flora only, and 1 positive control to which the participants had to add their own positive control strain.

The contaminated hygiene swab samples were individually packed and labelled. The decoding of these samples can be found in the tables with the individual NRL results. The parcels were sent to the participating laboratories on Monday 2 October 2017. In total, 44 parcels were sent.

Two laboratories received the parcel within that same day. Thirty parcels were delivered after 1 day, 9 parcels after two days and 1 parcel arrived after 3 days. 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. Temperature of the parcels during transport was predominantly below 4 °C. Storage temperature of the sample at the laboratories varied between 0 and 9 °C. Start date of the analysis of all laboratories was 9 October 2017, one laboratory started on 8 October 2017.

Results

The prescribed method was preferably 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.). Most laboratories (43) used this method. Six laboratories (all NRLs PPS) used Annex D of ISO 6579:2007 and 7 laboratories (all NRLs Food) used ISO 6579: 2002.

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 hygiene swabs had to be analysed. Almost all laboratories scored both control samples correct.

Procedure control Blank (Hygiene swab + background fora)
All laboratories correctly analysed the procedure control sample negative for *Salmonella*.

Positive control with Salmonella
All but one laboratory (lab code 28) 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* (36 laboratories). Others used a lenticule disc (10), a cultiloop (4), 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 (20), *Salmonella* Typhimurium (13), *Salmonella* Nottingham (8) and others (15).

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 almost 100%.

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

Control samples		Total labs n = 56	NRL-Food n = 33	NRL-PPS n = 23
Procedure control n = 1	No. of sample No. of negative samples **Specificity in %**	56 56 **100%**	33 33 **100%**	23 23 **100%**
Positive control (Own *Salmonella*) n = 1	No. of samples No. of positive samples **Sensitivity in %**	56 55 **98%**	33 32 **97%**	23 23 **100%**
All control samples n = 2	No. of samples No. of correct samples **Accuracy in %**	112 111 **99%**	66 65 **98%**	46 46 **100%**

Contaminated hygiene swab samples

Blank samples
All but one laboratory correctly analysed the blank hygiene swab samples negative for *Salmonella*. Laboratory 24 scored 2 of the 6 blank samples positive for *Salmonella*.

Low level contaminated Salmonella Typhimurium *samples*
Almost all laboratories were able to detect *Salmonella* in all 6 low level samples. One laboratory (lab code 11) scored 1 of the 6 low level contaminated samples negative for *Salmonella*. See Figure 1 for results NRL Food and Figure 2 for results NRL PPS.

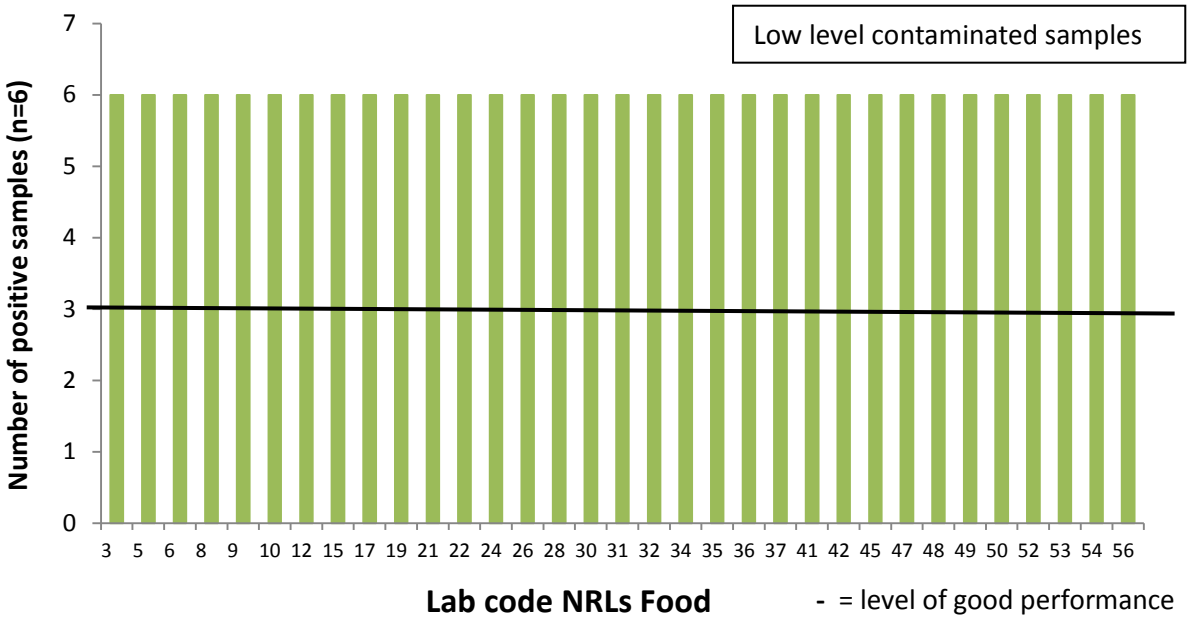

Figure 1 *Number of positive* Salmonella *isolations per laboratory found by NRLs Food in hygiene swab samples contaminated with low level* Salmonella *Typhimurium (n=6).*

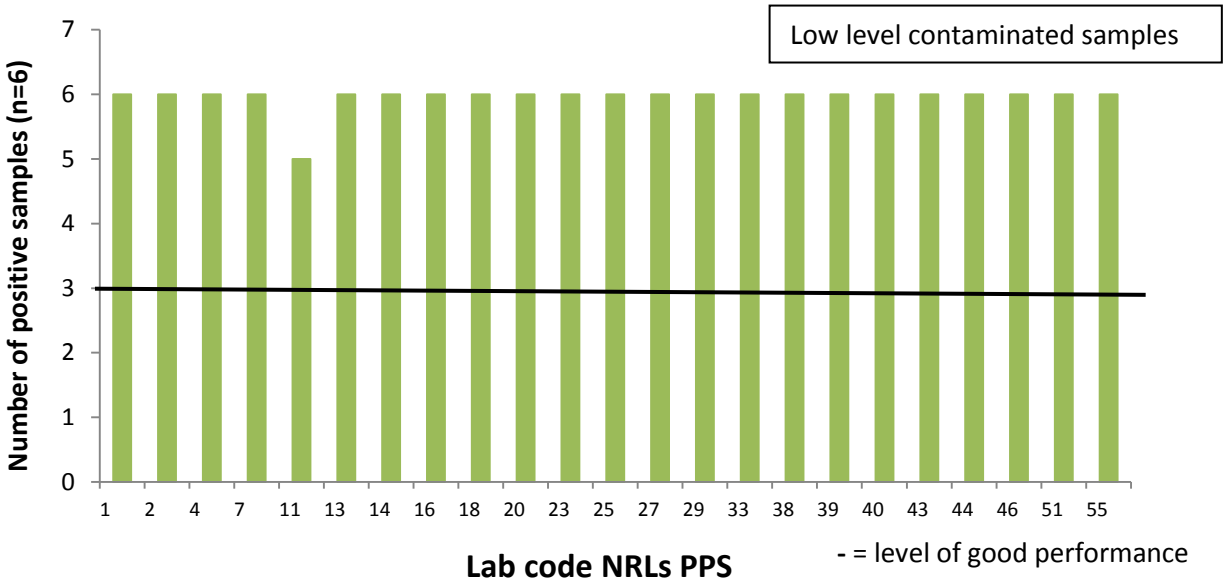

Figure 2 *Number of positive* Salmonella *isolations per laboratory found by NRLs PPS in hygiene swab samples contaminated with low level* Salmonella *Typhimurium (n=6)*

High level contaminated Salmonella Typhimurium *samples*
All laboratories detected *Salmonella* in all 6 high level samples. See Figure 4 for results NRL Food and Figure 5 for results NRL PPS.

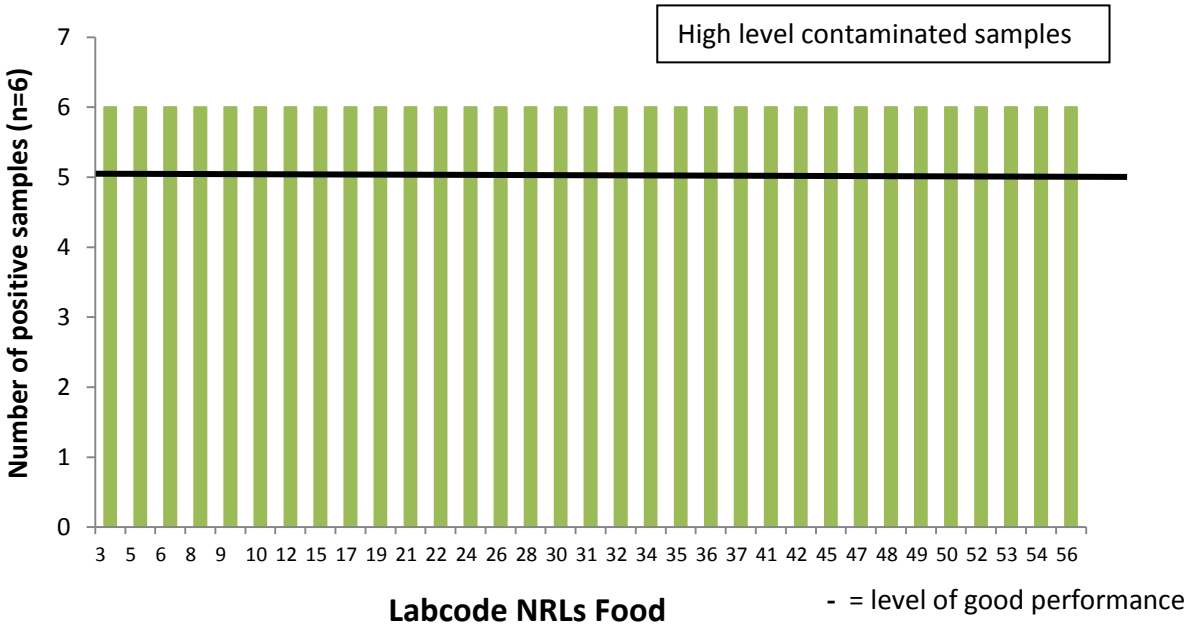

Figure 3 *Number of positive* Salmonella *isolations per laboratory found by NRLs Food in hygiene swabs samples contaminated with high level* Salmonella *Typhimurium (n=6).*

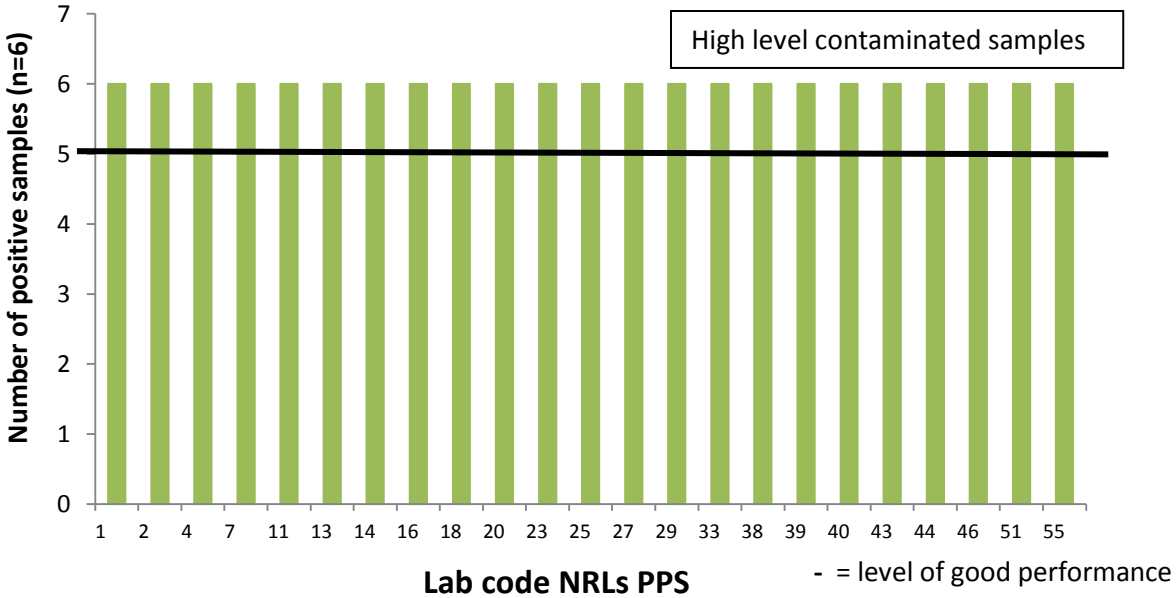

Figure 4 *Number of positive* Salmonella *isolations per laboratory found by NRLs PPS in hygiene swabs samples contaminated with high level* Salmonella *Typhimurium (n=6).*

Table 4 shows the specificity, sensitivity and accuracy rates for the contaminated hygiene swab samples. Laboratories have scored good results in both the high and low contaminated hygiene swab samples as shown by the high sensitivity and accuracy rates of 99-100%.

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

Hygiene swab samples		Total labs n = 56	NRL-Food n = 33	NRL-PPS n = 23
Blank n=6	No. of samples	336	198	138
	No. of negative samples	334	196	138
	Specificity in %	**99%**	**98,9%**	**100%**
Low level (STM) n=6	No. of samples	336	198	138
	No. of positive samples	335	198	137
	Sensitivity in %	**99,7%**	**100%**	**99,3%**
High level (STM) n=6	No. of samples	336	198	138
	No. of positive samples	336	198	138
	Sensitivity in %	**100%**	**100%**	**100%**
All swab samples with STM	No. of samples	672	396	276
	No. of positive samples	671	396	275
	Sensitivity in %	**99,9%**	**100%**	**99,6%**
All swab samples (positive and negative)	No. of samples	1008	594	414
	No. of correct samples	1005	592	413
	Accuracy in %	**99,7%**	**99,7%**	**99,8%**

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 however, sterile hygiene swab samples were used in which the absence of *Salmonella* can be guaranteed. Therefore an exception is made for this study and all blanc samples need to be scored as negative. In total, 55 laboratories fulfilled the criteria of good performance for the prescribed method. Laboratory 24 scored a poor performance for detecting *Salmonella* in two blanc hygiene swab samples. In addition, laboratory 28 could not detect *Salmonella* in the control sample to which they had added their own positive control and received a poor performance. The EURL-*Salmonella* will contact these two 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
EU	European Union
EURL	European Union Reference Laboratory
ISO	International Standardisation Organisation
MPN	Most Probable Number
MS	Member State
NRL	National Reference Laboratory
PS	Peptone saline Solution
PPS	Primary Production Stage
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.

EN ISO 6579: 2002. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

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