Interim summary report

EURL-*Salmonella* combined Proficiency Test Food-Feed 2025

Detection of *Salmonella* in flaxseed

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Introduction

In February/March 2025, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised a combined Proficiency Test (PT) for the detection of *Salmonella* in food and feed for the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*). The matrix under analysis is flaxseed, which is used as a food product as well as an ingredient of animal feed. NRLs-*Salmonella* which analyse food samples and NRLs-*Salmonella* which analyse animal feed products were invited to participate in this PT.

There were in total 49 participants in this PT: NRLs-*Salmonella* from 27 EU Member States and 9 NRLs-*Salmonella* from third countries (EU candidate Member State, members of the European Free Trade Association (EFTA), and United Kingdom).

Samples

The samples of this PT consisted of flaxseed with different concentrations of *Salmonella* Typhimurium.
Each NRL-*Salmonella* had to analyse in total 14 samples:

- 4 negative samples of each 25 g flaxseed (no *Salmonella* added);
- 6 samples of each 25 g flaxseed with a low level of *Salmonella* Typhimurium (STm);
- 4 samples of each 25 g flaxseed with a high level of *Salmonella* Typhimurium (STm).

On Monday 24 February 2025, 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'). The NRLs-*Salmonella* could start the analyses of the samples immediately after receipt and at latest by Monday 3 March 2025.

The level of natural background flora in the flaxseed was tested at the EURL-*Salmonella* on 8 November 2024 (shortly after receipt of the flaxseed) and on 3 March 2025, which was the latest date to start the PT. The total number of aerobic bacteria and the number of *Enterobacteriaceae* in the flaxseed was examined according to EN ISO 4833-1:2013 and EN ISO 21528-2:2017, respectively. Table 1 shows the number of aerobic bacteria and *Enterobacteriaceae* in the flaxseed.

Table 1 Number of aerobic bacteria and Enterobacteriaceae *per 25 g flaxseed sample*

Date	Aerobic bacteria (cfu/sample)	*Enterobacteriaceae* (cfu/sample)
8 November 2024	$2{,}4 \times 10^7$	$3{,}9 \times 10^5$
3 March 2025[a]	$7{,}7 \times 10^6$	$1{,}7 \times 10^6$

[a] After storage at room temperature for 15 weeks and at 5 °C for 13 days

Table 2 shows the (low and high) inoculation levels of the diluted culture of *Salmonella* Typhimurium used to artificially contaminate the flaxseed samples. Also a five tube Most Probable Number (MPN) test was performed on the

artificially contaminated PT samples with a low and high level STm. The MPN test was performed at the latest date to start the PT (3 March 2025), at the EURL-*Salmonella*.

Table 2 Number of Salmonella *Typhimurium in the inoculum for artificial contamination of the 25 g flaxseed samples and in the PT samples after storage at 5 °C for 13 days*

Date	Low level STm (cfu/sample)	High level STm (cfu/sample)
18 February 2025 Inoculation of flaxseed	11	72
3 March 2025[a] MPN of flaxseed samples, inoculated with STm (95% confidence limit)	5 (1,5 – 16)	24 (7,8 – 75)

[a] After storage at 5 °C for 13 days

Results

The results per laboratory, when following EN ISO 6579-1:2017(/A1:2020), can be found in Table 3. Nineteen laboratories also reported results of a second detection method. These results can be found in Table 4.

Performance analysis

The performance of the NRLs-*Salmonella* were judged on the results found with EN ISO 6579-1:2017(/A1:2020) only. The criteria for good performance used in the current EURL-*Salmonella* PT for the detection of *Salmonella* in flaxseed are presented in Table 5.

Table 5 Criteria for good performance

Artificially contaminated samples	Percentage positive	# pos samples / total # samples
Negative samples	0%	0 / 4
Low level of *S.* Typhimurium	≥ 50%	≥ 3 / 6
High level of *S.* Typhimurium	≥ 75%	≥ 3 / 4

All laboratories fulfilled the criteria for good performance.

Six laboratories (laboratory codes 13, 32, 34, 40, 43 and 44) reported five of the six low level samples positive for *Salmonella*. One laboratory (laboratory code 7) reported four of the six low level samples as positive. The level of good performance for the low level samples for this PT was set at the detection of *Salmonella* in at least three of the six low level samples. It is expected that not all low level flaxseed samples will test positive for *Salmonella*, because the concentration of the inoculation is low (near the detection limit of the method). In addition, laboratory 32 detected *Salmonella* in only three out of four high level samples, but this is within the criteria for good performance.

*Table 3 Results of the samples of the EURL-*Salmonella *combined PT Food-Feed 2025 per laboratory, following EN ISO 6579-1:2017(/A1:2020)*

Sample number	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**	**A11**	**A12**	**A13**	**A14**
Level of contamination	N	L	N	L	H	N	L	H	L	L	H	N	H	L
Laboratory code														
1	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
2	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
3	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
4	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
5	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
6	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
7	ND	ND[a]	ND	D	D	ND	D	D	D	D	D	ND	D	ND[a]
8	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
9	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
10	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
11	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
12	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
13	ND	ND[a]	ND	D	D	ND	D	D	D	D	D	ND	D	D
14	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
15	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
16	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
17	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
18	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
19	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
20	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
21	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
22	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
23	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
24	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
25	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
26	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
27	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D

Sample number	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14
Level of contamination	N	L	N	L	H	N	L	H	L	L	H	N	H	L
Laboratory code														
28	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
29	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
30	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
31	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
32	ND	D	ND	D	ND[a]	ND	D	D	ND[a]	D	D	ND	D	D
33	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
34	ND	D	ND	D	D	ND	ND[a]	D	D	D	D	ND	D	D
35	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
36	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
37	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
38	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
39	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
40	ND	D	ND	D	D	ND	ND[a]	D	D	D	D	ND	D	D
41	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
42	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
43	ND	D	ND	D	D	ND	D	D	ND[a]	D	D	ND	D	D
44	ND	D	ND	D	D	ND	D	D	ND[a]	D	D	ND	D	D
45	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
46	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
47	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
48	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
49	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D

N = Negative
L = Low level of *S*. Typhimurium (inoculation level 11 cfu/sample)
H = High Level of *S*. Typhimurium (inoculation level 72 cfu/sample)
D = *Salmonella* detected
ND = *Salmonella* not detected
[a] = Deviating from expected result, but within the criteria of good performance

*Table 4 Results of the samples of the EURL-*Salmonella *combined PT Food-Feed 2025 per laboratory, analysed with a second detection method. Only laboratories, which reported results of a second detection method, are presented in this table*

Sample number	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**	**A11**	**A12**	**A13**	**A14**
Level of contamination	N	L	N	L	H	N	L	H	L	L	H	N	H	L
Laboratory code														
1	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
3	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
8	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
15	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
19	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
20	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
21	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
25	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
26	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
27	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
29	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
33	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
39	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
41	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
43	ND	D	ND	D	D	ND	D	D	ND[a]	D	D	ND	D	ND[a]
45	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
46	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
47	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D
49	ND	D	ND	D	D	ND	D	D	D	D	D	ND	D	D

N = Negative
L = Low level of *S*. Typhimurium (inoculation level 11 cfu/sample)
H = High Level of *S*. Typhimurium (inoculation level 72 cfu/sample)
D = *Salmonella* detected
ND = *Salmonella* not detected
[a] = Deviating from expected result, but within the criteria of good performance

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