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EURL-*Salmonella* combined Proficiency Test food-feed 2025

Detection of *Salmonella* in flaxseed

RIVM report 2025-0038
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Colophon

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Synopsis

EURL-*Salmonella* combined Proficiency Test food-feed 2025

Detection of *Salmonella* in flaxseed

In 2025, 49 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) obtained a good score in this Proficiency Test for the *Salmonella* bacteria.

All European Union (EU) Member States have an NRL-*Salmonella*. These NRLs are obliged to participate in various annual quality monitoring exercises, known as Proficiency Tests. One of these Proficiency Tests checks whether NRLs are able to detect *Salmonella* bacteria in food and feed. This year flaxseed was chosen. Each EU Member State has to appoint an NRL that is responsible for performing these tests in its laboratory. The 49 participants in this Proficiency Test were NRLs-*Salmonella* from 27 EU Member States and 6 other European countries.

The laboratories used an obligatory, internationally accepted method to detect the presence of *Salmonella* in flaxseed samples. Each laboratory received a package containing samples that either had been artificially contaminated with two different concentrations of *Salmonella* Typhimurium or did not contain this bacterium.

This proficiency test was organised by the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*). EURL-*Salmonella* monitors the performance of the NRLs-*Salmonella* in the European Union and is part of the National Institute for Public Health and the Environment (RIVM).

Keywords: *Salmonella*, EURL, NRL, Proficiency Test, *Salmonella* detection method, food, feed, flaxseed

Publiekssamenvatting

EURL-*Salmonella* gecombineerd ringonderzoek voedsel-diervoerder 2025

Detectie van *Salmonella* in lijnzaad

In 2025 hebben 49 Nationale Referentie Laboratoria voor *Salmonella* (NRL's-*Salmonella)* een goede score gehaald in dit ringonderzoek voor de *Salmonella*-bacterie.

Alle lidstaten van de Europese Unie hebben een NRL-*Salmonella*. Deze NRL's zijn verplicht om elk jaar hun kwaliteit te laten toetsen met behulp van verschillende zogeheten ringonderzoeken. Een van de ringonderzoeken controleert of de NRL's de *Salmonella*-bacterie in voedsel en diervoeder kunnen aantonen. Dit jaar is gekozen voor lijnzaad. Elk lidstaat van de EU wijst hiervoor het NRL aan dat verantwoordelijk is voor deze testen in hun laboratorium. De 49 deelnemers aan dit ringonderzoek kwamen uit 27 lidstaten van de Europese Unie en uit 6 andere Europese landen.

De laboratoria gebruikten een verplichte, internationaal erkende analysemethode om *Salmonella* in monsters lijnzaad aan te tonen. Elk laboratorium kreeg een pakket toegestuurd met monsters die kunstmatig besmet waren met twee verschillende concentraties *Salmonella* Typhimurium of zonder deze bacterie.

Het EURL-*Salmonella* organiseerde het ringonderzoek. Het EURL-*Salmonella* ziet toe op de kwaliteit van de NRL's-*Salmonella* in de Europese Unie en is gevestigd bij het RIVM.

Kernwoorden: *Salmonella*, EURL, NRL, ringonderzoek, *Salmonella*-detectiemethode, voedsel, diervoeder, lijnzaad

Contents

Summary

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 was flaxseed.
NRLs-*Salmonella* that analyse *Salmonella* in food samples and NRLs-*Salmonella* that analyse animal feed products were invited to participate in this PT. NRLs-*Salmonella* that perform the analysis of food and feed samples in one and the same laboratory could request two separate laboratory codes with two (similar) sets of samples. In this manner, the laboratory could perform the analysis separately as NRL-*Salmonella* food and as NRL-*Salmonella* feed. However, the latter NRLs-*Salmonella* could also choose to analyse only one set of samples under one laboratory code or under two separate codes.

A total of 49 laboratory codes were generated for this EURL-*Salmonella* Proficiency Test (PT). The participants included NRLs-*Salmonella* located in 27 EU Member States and 9 NRLs-*Salmonella* from third countries (EU candidate Member States, members of the European Free Trade Association (EFTA), and the United Kingdom).

The most important objective of the PT was to test the performance of the participating laboratories' detection of *Salmonella* in the artificially contaminated flaxseed samples. The prescribed method for detecting *Salmonella* species (spp.) was EN ISO 6579-1:2017(/A1:2020). The participants were asked to report *Salmonella* 'detected' or 'not detected' for each sample (following confirmation).

Prior to the start of the PT, pre-tests were conducted to ensure that the samples were fit for use. Flaxseed samples, artificially contaminated with two concentrations of *Salmonella* Typhimurium (STm), were tested for their stability at 2 storage temperatures (5 °C and 10 °C). Additionally, the concentration of the natural background flora (aerobic count and number of *Enterobacteriaceae*) in the flaxseed was measured.

For the pre-tests, flaxseed samples were artificially contaminated with 5 cfu STm/25 g or with 11 cfu STm/25 g. The flaxseed samples that had been artificially contaminated with 11 cfu STm/25 g were stable at 5 °C and at 10 °C during the storage period of 13 days. The samples that had been artificially contaminated with 5 cfu STm/25 g showed only four out of six samples to be positive for *Salmonella* after 13 days and 20 days of storage at 5 °C. All samples stored at 10 °C were positive after 13 days of storage. On the basis of these results and the results from previous PTs with flaxseed as a matrix, the aim was to inoculate the low-level flaxseed samples with approximately 8 cfu STm/25 g.

The number of aerobic bacteria and *Enterobacteriaceae* in the flaxseed samples remained relatively stable when stored at 5 °C and at 10 °C. The number of aerobic bacteria in the flaxseed samples during storage

was approximately 10^7 cfu/25 g flaxseed. The number of *Enterobacteriaceae* was approximately 10^6 cfu/25 g flaxseed.

On Monday 24 February 2025, the EURL-*Salmonella* sent the PT samples to all participants. Each laboratory received 14 samples, each containing 25 g flaxseed. These samples consisted of four negative samples (no *Salmonella* added), six samples with a low level of STm (inoculum 11 cfu/25 g) and four samples with a high level of STm (inoculum 72 cfu/25 g). The PT samples had been artificially contaminated with a diluted culture of *Salmonella* Typhimurium at the EURL-*Salmonella* laboratory. NRLs-*Salmonella* could start the analysis of the samples immediately upon arrival of the parcel, or in the following days. The latest date to start the analysis was Monday 3 March 2025.

All 49 participants fulfilled the criteria for good performance for the EURL-*Salmonella* Proficiency Test for the detection of *Salmonella* in flaxseed samples.

The specificity rate of the negative flaxseed samples was 100%. The sensitivity rates of the flaxseed samples artificially contaminated with *Salmonella* Typhimurium was 98%. The accuracy rate of all flaxseed samples for all participating laboratories was 99%.

The NRLs-*Salmonella* were given the opportunity to analyse the samples using a second detection method as well, if this method was (routinely) used in their laboratories. The results obtained with the second method were not used to assess the performance of the NRLs-*Salmonella*. Nineteen participants also used a second detection method (real-time PCR, VIDAS and PCR) to analyse the samples. Bar one, the results of the second detection methods were all comparable to the reported results obtained with EN ISO 6579-1:2017(/A1:2020). One laboratory tested four out of six low-level contaminated flaxseed samples positive for *Salmonella* using the second detection method, while they tested five out of the six low-level contaminated flaxseed samples positive for *Salmonella* using EN ISO 6579-1:2017(/A1:2020).

1 Introduction

One important task of the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*), as described in Commission Regulation EC No 2017/625 (EC, 2017), is the organisation of Proficiency Tests (PTs) to evaluate the performance of the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*). The history of the PTs for the detection of *Salmonella*, as organised by EURL-*Salmonella* since 1995, is summarised on the EURL-*Salmonella* website (EURL-*Salmonella*, 2025a).

The objective of the current study was to test whether the participating laboratories could detect *Salmonella* species (spp.) in flaxseed. This is important in order to verify that the examination of samples is carried out uniformly in all European Union (EU) Member States (MS), and that all NRLs-*Salmonella* obtain comparable results.

The PT was carried out in February/March 2025. For this combined PT, the matrix to be analysed was flaxseed, which is used as a food product as well as (an ingredient of) animal feed. Hence, both NRLs-*Salmonella* that analyse food samples and NRLs-*Salmonella* that analyse animal feed products were invited to participate in this PT.

The method prescribed for the detection of *Salmonella* spp. is set out in EN ISO 6579-1:2017 and EN ISO 6579-1:2017/A1:2020.

For the current PT, the flaxseed samples were artificially contaminated with a diluted culture of *Salmonella* Typhimurium (STm) at the EURL-*Salmonella* laboratory.

Fourteen flaxseed samples were tested by each NRL-*Salmonella*: four negative samples (flaxseed samples without *Salmonella*), six samples artificially contaminated with a low level of STm, and four samples artificially contaminated with a high level of STm.

2 Participants

Table 2.1 displays a list of participants in the EURL-*Salmonella* combined PT food-feed 2025.

*Table 2.1 List of participants in the EURL-*Salmonella *combined Proficiency Test food-feed 2025*

Country	**City**	**Product(s) under analysis at the NRL-*Salmonella***	**Institute / NRL-*Salmonella***
Albania	Tirana	Animal feed and food	Food Safety and Veterinary Institute (FSVI), Department of Food Microbiology
Austria	Graz	Food	AGES – Food Microbiology
Austria	Linz	Animal feed	AGES GmbH, Animal nutrition and feed, MOKA
Belgium	Brussels	Animal feed and food	Sciensano, Food Pathogens
Bulgaria	Sofia	Animal feed and food	National Diagnostic Research Veterinary Institute (NDRVI), NRL-*Salmonella*
Croatia	Zagreb	Animal feed	Croatian Veterinary Institute Zagreb, Veterinary Public Health
Croatia	Zagreb	Food	Croatian Veterinary Institute, Veterinary Public Health
Cyprus	Nicosia	Animal feed and food	Veterinary Services of Cyprus, Laboratory for the Control of Food of Animal Origin (LCFAO)
Czech Republic	Prague	Animal feed and food	Státní veterinární ústav Praha, Bacteriology
Denmark	Ringsted	Animal feed and food	Danish Veterinary and Food Administration, Section of Microbiology
Estonia	Tartu	Animal feed and food	The National Centre for Laboratory Research and Risk Assessment (LABRIS), Bacteriology-pathology department
Finland	Helsinki	Animal feed and food	Finnish Food Authority, Microbiology Unit
France	Ploufragan	Animal feed and food	Anses, Ploufragan – Unité HQPAP (NRL-*Salmonella*)

Country	City	Product(s) under analysis at the NRL-*Salmonella*	Institute / NRL-*Salmonella*
Germany	Berlin	Animal feed and food	German Federal Institute for Risk Assessment (BfR), Biological Safety
Greece	Chalkida	Animal feed and food	Veterinary Laboratory of Chalkida, NRL-*Salmonella*
Hungary	Budapest	Animal feed and food	National Food Chain Safety Office, Food Chain Safety Laboratory Directorate, Microbiological NRL
Iceland	Reykjavik	Animal feed and food	Matís ohf, Microbiology
Ireland	Celbridge, Co Kildare	Animal feed and food	Department of Agriculture Food and the Marine Laboratories (DAFM), NRL-*Salmonella*
Italy	Legnaro, Padova	Animal feed and food	Istituto Zooprofilattico Sperimentale delle Venezie, Centro Nazionale per le Salmonellosi
Latvia	Riga	Animal feed and food	Institute of Food Safety, Animal Health and Environment BIOR, Laboratory of Microbiology and Pathology
Lithuania	Vilnius	Animal feed and food	National food and veterinary risk assessment institute, Bacteriology section
Luxembourg	Dudelange	Animal feed and food	Laboratoire National de Santé, Health Protection – Food Monitoring
Malta	Valletta	Animal feed and food	Public Health Laboratory, Health Regulation, Env. Health Directorate
Netherlands, the	Bilthoven	Animal feed and food	National Institute for Public Health and the Environment (RIVM), Centre for Zoonoses and Environmental Microbiology (Z&O)
Netherlands, the	Wageningen	Food	Wageningen Food Safety Research (WFSR), Microbiology
Northern Ireland	NRL tasks carried out by the NRL-*Salmonella* in Belgium		

Country	City	Product(s) under analysis at the NRL-*Salmonella*	Institute / NRL-*Salmonella*
Norway	Ås	Animal feed and food	Norwegian Veterinary Institute, Section of bacteriology
Poland	Puławy	Animal feed	National Veterinary Research Institute (NVRI), Department of Hygiene of Animal Feeding stuffs
Poland	Puławy	Food	National Veterinary Research Institute (NVRI), Department of Food Safety
Portugal	Vairão	Animal feed and food	Instituto Nacional de Investigação Agrária e Veterinária, I. P., Food Microbiology Laboratory
Romania	Bucharest	Animal feed and food	Institute for hygiene and veterinary public health, Microbiology
Serbia	Novi Sad	Animal feed	Scientific Veterinary Institute "Novi Sad", Department of Microbiological examination of feed from animals
Serbia	Novi Sad	Food	Scientific Veterinary Institute "Novi Sad", Department of food safety
Slovak Republic	Dolný Kubín	Animal feed and food	State Veterinary and Food Institute Dolný Kubín, Bacteriology department
Slovenia	Ljubljana	Animal feed and food	UL, Veterinary faculty, National Veterinary Institute (NVI), NRL-*Salmonella,* Institute of Microbiology and Parasitology and Institute of Food Safety, Feed and Environment
Spain	Algete, Madrid	Animal feed	Laboratorio Central de Veterinaria, Bacteriología 1
Spain	Lugo, Galicia	Vegetal matrices, soil and water	National Plant Health Laboratory and Hygiene Laboratory, Hygiene
Spain	Majadahonda, Madrid	Food	Centro Nacional de Alimentación (CNA), Microbiología Alimentaria I
Sweden	Uppsala	Animal feed and food	National Veterinary Agency, Department of Microbiology

Country	**City**	**Product(s) under analysis at the NRL-*Salmonella***	**Institute / NRL-*Salmonella***
Switzerland	Zürich	Food	Institute for Food Safety and Hygiene, National Centre for Enteropathogenic Bacteria and Listeria (NENT)
United Kingdom	Addlestone	Animal feed	Animal and Plant Health Agency Weybridge, Bacteriology
United Kingdom	Wiltshire	Food	UK Health Security Agency, Food, Water & Environmental Laboratory

3 Materials and methods

3.1 Preparation of artificially contaminated flaxseed samples

3.1.1 General

The matrix used for this PT was (ground) flaxseed. A batch of 25 kg flaxseed was obtained on 23-10-2024 from a mill in the Netherlands. The batch was used for performing pre-tests and preparing the PT samples. The batch of flaxseed was stored at room temperature until sample preparation.

Following receipt of the batch of flaxseed at the EURL-*Salmonella*, the absence of *Salmonella* was checked by analysing a total of ten randomly taken samples of 25 g, following EN ISO 6579-1:2017(/A1:2020). In brief: to each test portion of 25 g, 225 ml of Buffered Peptone Water (BPW) was added and left to stand for 20 to 30 minutes at laboratory ambient temperature (18 °C to 27 °C) in order to assist resuscitation of any damaged organisms. The samples were then mixed by hand for 30 seconds. Following pre-enrichment in BPW, selective enrichment was carried out in Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn) and on Modified semi-solid Rappaport-Vassiliadis (MSRV) agar. Subsequently, the MKTTn tubes and any suspect growth on the MSRV plates were plated out on Xylose Lysine Deoxycholate (XLD) agar and on Brilliance *Salmonella* Agar (BSA). Suspect colonies were confirmed biochemically and serologically.

3.1.2 Pre-tests for the preparation of flaxseed samples

Experiments were performed to test the stability of the flaxseed samples artificially contaminated with *Salmonella* during storage and transport. Samples were contaminated with two levels of *Salmonella* and stored at 5 °C and 10 °C. After 0, 6, and 13 days, 6 artificially contaminated samples were tested for the presence of *Salmonella* following EN ISO 6579-1:2017(/A1:2020) (see section 3.3). This was carried out for each inoculation level and storage temperature. The samples stored at 5 °C were tested after 20 days of storage as well.

Salmonella Typhimurium (STm) from the American Type Culture Collection (ATCC 14028, Manassas, USA) was chosen to artificially contaminate the 25 g flaxseed samples for the pre-test. The *Salmonella* strain was inoculated in Brain Heart Infusion broth (BHI) and incubated at 34-38 °C for 18 h ± 2 h. Next, tenfold dilutions of the (overnight) culture were prepared in peptone saline solution in order to inoculate the flaxseed samples with approximately 5 cfu STm/25 g and 10 cfu STm/25 g. The inoculum concentration was determined by streaking the inoculum onto XLD agar and incubating the plates at 34-38 °C for 24 h ± 3 h.

In addition, negative flaxseed samples (no *Salmonella* added) were stored at 5 °C and at 10 °C. The concentration of the natural background flora was determined in these samples by analysing the number of aerobic bacteria and *Enterobacteriaceae* (see section 3.1.4).

The samples were analysed after 0, 6, and 13 days of storage, while the samples stored at 5 °C were tested after 20 days of storage as well.

3.1.3 Preparation of flaxseed samples for the Proficiency Test

In February 2025, the EURL-*Salmonella* weighed approximately 740 subsamples of 25 g flaxseed each into (plastic) sample bags. Next, 320 subsamples were individually and artificially contaminated with a low level, and 210 subsamples were contaminated with a high level of the diluted overnight culture of *S*. Typhimurium (ATCC 14028, Manassas, USA); 210 subsamples were not contaminated with *Salmonella* (negative samples).

The following set of samples was prepared for each participant:

- 4 negative samples, each containing 25 g of flaxseed (no *Salmonella* added);
- 6 samples, each containing 25 g of flaxseed with a low level of *Salmonella* Typhimurium, aimed at 10 cfu/25 g;
- 4 samples, each containing 25 g of flaxseed with a high level of *Salmonella* Typhimurium, aimed at 50 cfu/25 g;

Following artificial contamination, the samples were mixed by hand and stored at 5 °C until transport to the NRLs-*Salmonella* on 24 February 2025.
The inoculum concentration used to artificially contaminate the 25 g flaxseed samples was determined by streaking the inoculum onto XLD agar and incubating the plates at 34-38 °C for 24 h ± 3 h.

3.1.4 Determination of level of background flora in flaxseed

The total number of aerobic bacteria and the number of *Enterobacteriaceae* in the flaxseed were examined by following EN ISO 4833-1:2013 and EN ISO 21528-2:2017, respectively. For this purpose, an initial suspension was prepared by adding 225 ml of peptone saline solution to 25 g of flaxseed. The tenfold dilutions of the initial suspension were analysed on Plate Count Agar (PCA) and on Violet Red Bile Glucose (VRBG) Agar.

3.1.5 Determination of the number of Salmonella *in flaxseed samples by MPN*

The number of *Salmonella* in the flaxseed samples was determined at the start of the PT. This was done by using a five-tube, most probable number (MPN) technique. The MPN technique entails a tenfold dilution of five artificially contaminated flaxseed samples of each contamination level, representing 25 g, 2,5 g, and 0,25 g of the original sample. The presence of *Salmonella* was determined in each dilution by following EN ISO 6579-1:2017(/A1:2020). From the number of confirmed positive dilutions, the MPN of *Salmonella* in the original sample was calculated using freely available Excel-based MPN software (Jarvis et al., 2010).

3.2 Design of the Proficiency Test

3.2.1 Number and type of samples

Each participant received 14 (artificially contaminated) flaxseed samples, numbered A1 to A14.

Table 3.1 gives an overview of the number and types of samples tested by the participants.

Table 3.1 Overview of the number and types of samples tested per laboratory in the combined Proficiency Test food-feed 2025

Contamination level of the flaxseed samples	Number of samples (n=14)
Negative sample (no *Salmonella* added)	4
S. Typhimurium low level	6
S. Typhimurium high level	4

3.2.2 Shipment of samples and temperature recording during shipment

Each set of 14 PT samples per participant was packed in a large safety bag. Each safety bag was then placed in a parcel with four frozen cooling elements for shipment.
To monitor exposure to excessive temperatures during shipment and storage, a temperature button was included in each safety bag that records the temperature. These buttons are tiny units sealed in a stainless-steel case, 16 mm in diameter and 6 mm deep. The loggers were programmed by the EURL-*Salmonella* to measure the temperature every hour. Each NRL-*Salmonella* had to return the temperature recorder to the EURL-*Salmonella* on the day the laboratory started the PT. At the EURL-*Salmonella*, the loggers were read using a computer programme, and all recorded temperatures during transport and storage were inputted to an Excel sheet.

The parcel was sent to the participants on 24 February 2025 as 'biological substances category B (UN3373)' (IATA, 2025) using a door-to-door courier service.

Further details about the shipping and handling of the samples and the reporting of the test results can be found in the protocol (EURL-*Salmonella*, 2025b) and in (a printout of) the result form (EURL-*Salmonella,* 2025c).

3.3 Methods

NRLs-*Salmonella* could start the analysis of the samples immediately upon arrival of the parcel, or on one of the following days. The latest date to start the analysis was Monday 3 March 2025.

The prescribed method was EN ISO 6579-1:2017(/A1:2020) and the underlying EN ISO documents, for example, 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 samples, animal feed samples, environmental samples from the food production area, and samples from the primary production stage.
For this Proficiency Test, the laboratories could follow their own procedure for the preparation of the test samples for this type of matrix, or follow the procedure described below. This procedure was slightly

adjusted from EN ISO 6887-4:2017, to avoid punctures in the plastic sample bags:

- add 225 ml Buffered Peptone Water (BPW) to the 25 g test sample (instead of accurately weighing the sample into a pre-dispense volume of BPW);
- resuscitate the sample for 20 to 30 minutes at 18 °C to 27 °C (room temperature);
- mix for 30 seconds (± 5 seconds) by hand;
- continue with the non-selective pre-enrichment procedure as described in EN ISO 6579-1:2017(/A1:2020).

The procedure described in EN ISO 6579-1:2017(/A1:2020) in summary:

- pre-enrichment in:
  Buffered Peptone Water;
- selective enrichment in/on:
  Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn);
  Modified semi-solid Rappaport-Vassiliadis agar (MSRV) 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 flaxseed.

Additionally, the NRLs-*Salmonella* were given the opportunity to analyse the samples using a second detection method if this method was (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-*Salmonella*.

3.4 Statistical analysis of the data

From the results of all laboratories, the specificity, sensitivity and accuracy rates were calculated using the following formulae:

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:
Number of correct results (positive and negative)
———————————————— x 100%
Total number of samples

3.5 Criteria for good performance

The performance of the NRLs-*Salmonella* were judged on the basis of the results found using 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 3.2.

Table 3.2 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

4 Results and discussion

4.1 Preparation of artificially contaminated flaxseed samples

4.1.1 Pre-tests for the preparation of flaxseed samples

Before performing the pre-test, the absence of *Salmonella* in the batch of flaxseed was checked by analysing a total of ten randomly taken samples of 25 g. *Salmonella* was not detected in any of the tested samples.

For the pre-tests, subsamples of each 25 g flaxseed were artificially contaminated with two different concentrations of *Salmonella* Typhimurium. The actual inoculation levels were 5 cfu/25 g of flaxseed and 11 cfu/25 g of flaxseed. Figures 4.1 and 4.2 show the results of the stability tests.

Figure 4.1 Stability test of flaxseed samples (n=6) artificially contaminated with S. *Typhimurium at (initial) levels of 5 cfu/25 g and 11 cfu/25 g, stored at 5 °C*

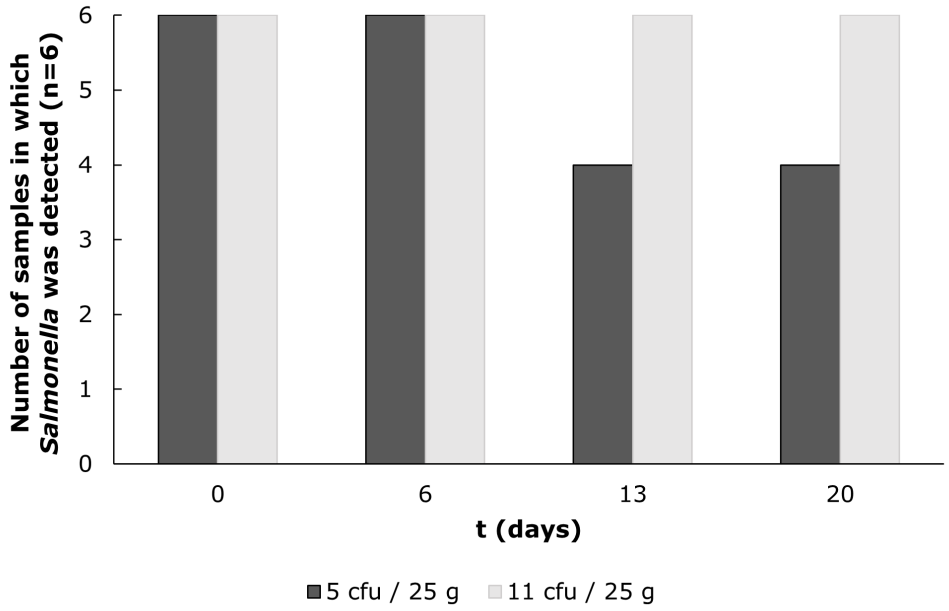


Figure 4.2 Stability test of flaxseed samples (n=6) artificially contaminated with S. Typhimurium at (initial) levels of 5 cfu/25 g and 11 cfu/25 g, stored at 10 °C

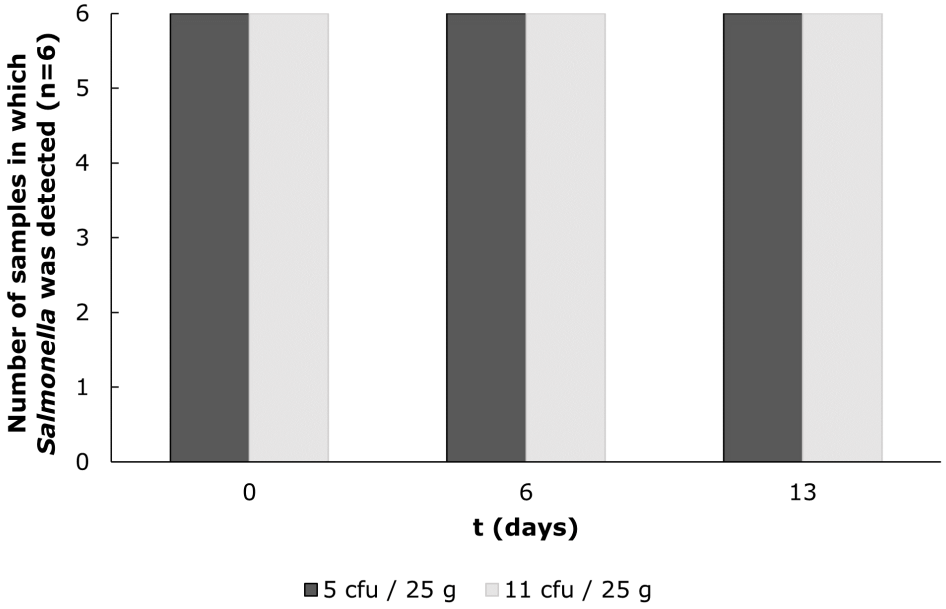


Figure 4.1 shows that the flaxseed samples artificially contaminated with 11 cfu of *Salmonella* Typhimurium per 25 g flaxseed were stable during 20 days of storage at 5 °C. Of the samples artificially contaminated with 5 cfu of *Salmonella* Typhimurium, only four out of six samples tested positive for *Salmonella* after 13 days and 20 days of storage at 5 °C.

Figure 4.2 shows that all 6 flaxseed samples artificially contaminated with 5 cfu/25 g and 11 cfu/25 g still tested positive for *Salmonella* after 13 days of storage at 10 °C.

On the basis of these results and the results from previous PTs using flaxseed as a matrix (Diddens and Mooijman, 2019; Diddens and Mooijman, 2023), the aim was to inoculate the low-level flaxseed samples of the PT with approximately 8 cfu STm/25 g.

Figure 4.3 shows the number of aerobic bacteria in the flaxseed samples during storage at 5 °C and at 10 °C. The number of aerobic bacteria remained stable at approximately 10^7 cfu/25 g flaxseed when stored at either temperature.

Figure 4.4 shows the number of *Enterobacteriaceae*, which remained stable at approximately 10^6 cfu/25 g flaxseed when stored at either temperature.

Figure 4.3 Number of aerobic bacteria per 25 gram flaxseed (negative for Salmonella*) after storage at 5 °C and 10 °C for 0, 6, 13, and 20 days*

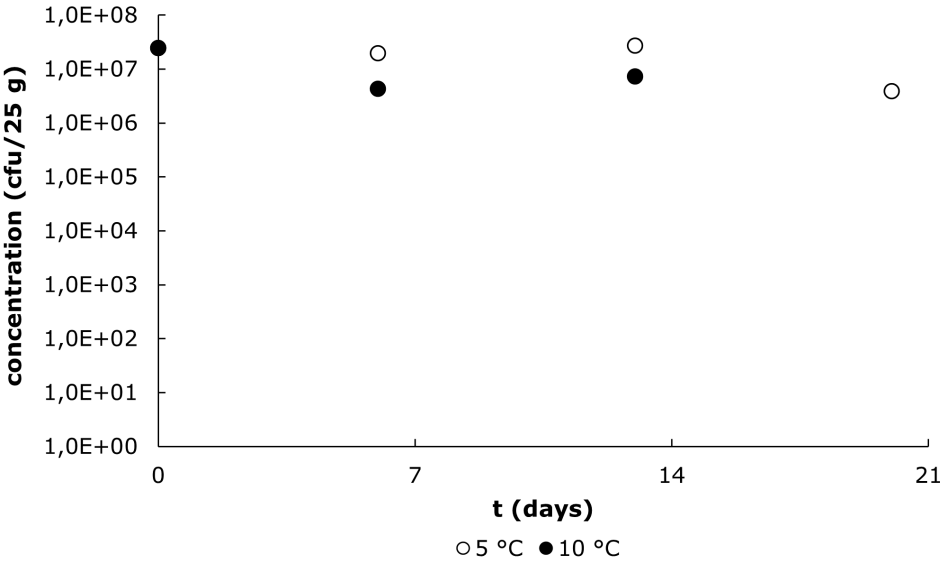
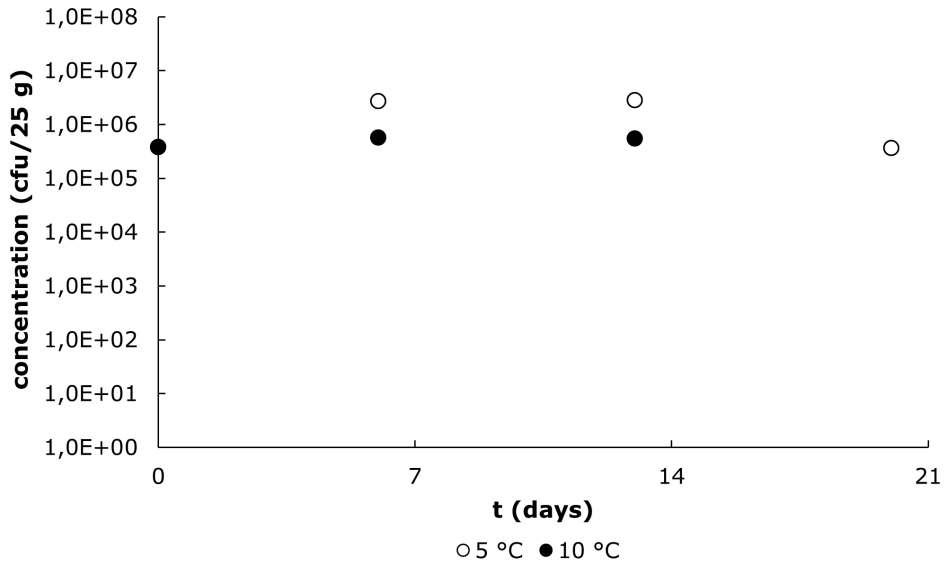


Figure 4.4 Number of Enterobacteriaceae *per 25 gram flaxseed (negative for* Salmonella*) after storage at 5 °C and 10 °C for 0, 6, 13, and 20 days*



4.1.2 *Preparation of flaxseed samples for the Proficiency Test*

The same batch of flaxseed used for the pre-tests was also used for the preparation of the PT samples. This batch was tested negative for *Salmonella*.

The samples were prepared as described in section 3.1.3. The PT samples were artificially contaminated on 18 February 2025 and all samples were stored at 5 °C until shipment on 24 February 2025.

4.1.3 Background flora in flaxseed

The level of natural background flora in the flaxseed was tested at the EURL-*Salmonella* on 8 November 2024 (shortly following receipt of the flaxseed) and on 3 March 2025, which was the latest date to start the PT. Table 4.1 shows the number of aerobic bacteria and *Enterobacteriaceae* in the flaxseed samples.

Table 4.1 Number of aerobic bacteria and Enterobacteriaceae *per 25 gram flaxseed*

Date	**Aerobic bacteria (cfu/25 g)**	***Enterobacteriaceae* (cfu/25 g)**
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

4.1.4 Number of Salmonella *in the artificially contaminated flaxseed samples*

Table 4.2 shows the inoculation levels of *Salmonella* Typhimurium that has been used to artificially contaminate the flaxseed samples. Additionally, this table shows the results of the five-tube MPN test performed on the artificially contaminated PT samples with low- and high-level STm at the latest date to start the PT. The results show that the low and high inoculation contamination levels of *Salmonella* Typhimurium in the flaxseed samples were as intended.

Table 4.2 Number of Salmonella *Typhimurium in the inoculum for artificial contamination of the flaxseed samples and in the samples*

Date	**Low-level STm (cfu/25 g)**	**High-level STm (cfu/25 g)**
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] Following storage at 5 °C for 13 days

4.2 Technical data on the Proficiency Test

4.2.1 General

NRLs-*Salmonella* that perform the analysis of food and feed samples in one and the same laboratory could request two separate laboratory codes with two (similar) sets of samples. Thus, these laboratories could perform the analysis separately as NRL-*Salmonella* food and as NRL-*Salmonella* feed. However, these NRLs-*Salmonella* could also choose to

analyse only one set of samples under one laboratory code or two different codes.
In total, 49 laboratory codes were generated for this EURL-*Salmonella* PT. The NRLs-*Salmonella* originated from 27 EU Member States and 9 third countries (EU candidate Member State, members of the European Free Trade Association (EFTA), and the United Kingdom).

4.2.2 Accreditation and methods used

Forty-two participants indicated to be accredited for EN ISO 6579-1:2017, including amendment 1 from 2020. Five participants did not mention the amendment of ISO 6579-1:2017 (Amd.1:2020) for their accreditation, but indicated they were accredited for EN ISO 6579-1:2017 only. Two participants (originating from the same EU MS) were no longer accredited, but these laboratories indicated a planning for their re-accreditation.
Nine laboratories specified they also had other *Salmonella* methods under accreditation, such as real-time PCR and Vidas.

All laboratories used the prescribed method EN ISO 6579-1:2017(/A1:2020) to analyse the PT samples.

4.2.3 Shipment of samples and start of the Proficiency Test

On Monday 24 February 2025, the PT samples were shipped to all participants. NRLs-*Salmonella* that requested two sets of samples, received one parcel with both sets of samples.
Forty-six parcels were delivered at the NRLs-*Salmonella* within one or two days. One parcel was delivered after three days (laboratory code 13). The parcels sent to the NRLs-*Salmonella* with laboratory codes 40 and 44 were held at customs and arrived at the laboratories after fifteen days of transport.
The temperature during transport and storage was registered using a temperature probe. The temperature of all parcels during transport was below 4 °C, except for the parcels of the NRLs-*Salmonella* with laboratory codes 40 and 44. The temperature of these parcels reached a maximum of 11 °C while they were held at customs.

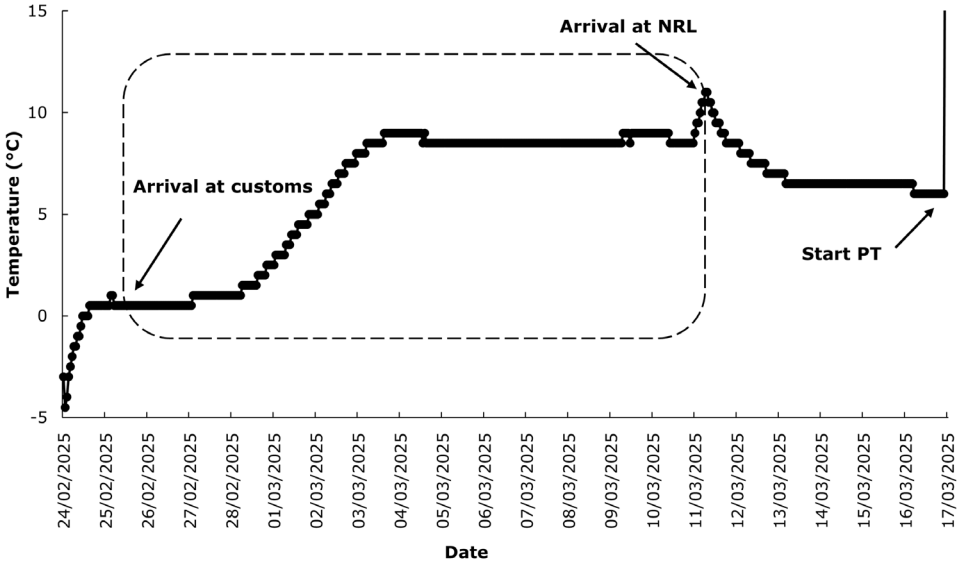
Sixteen NRLs-*Salmonella* started the analysis of the samples immediately upon arrival of the samples on 25, 26 or 27 February 2025 (laboratory codes: 1, 4, 5, 9, 11, 12, 13, 15, 20, 21, 22, 25, 28, 29, 33, and 38).
Fifteen laboratories started the PT after one, two, or three day(s) following the arrival of the parcel, on 26, 27, or 28 February 2025 (laboratory codes: 3, 14, 26, 27, 31, 34, 35, 36, 37, 39, 43, 45, 46, 48, and 49).
The prescribed latest date to start the PT was on 3 March 2025. Fifteen laboratories started on this date (laboratory codes: 2, 6, 7, 8, 10, 16, 17, 19, 23, 24, 30, 32, 41, 42, and 47).
Laboratory 18 started the analysis of the samples on 4 March 2025, following consultation with the EURL-*Salmonella*.
Laboratories 40 and 44, which received their samples after fifteen days of transport, started on 17 March 2025.

The measured storage temperature at the laboratories that did not start the PT immediately upon the arrival of the samples, ranged between

0 °C and 8 °C. Except for laboratories 23 and 24, where the storage temperature was 15,5 °C for 2,5 days prior to starting the analysis.

Figure 4.5 shows the temperature record of the parcel for laboratory 40, until the receipt and the start of the PT on 17 March 2025. During the period at customs, the parcel eventually reached a temperature of 11 °C, before it was delivered to the laboratory. At the NRL-*Salmonella,* the samples were stored at 6 °C, until the start of the analysis. The temperature record for laboratory 44, which had a separate temperature button, showed a comparable temperature curve over time.

Figure 4.5 Temperature record of the parcel with PT samples for laboratory 40



4.2.4 Media and confirmation methods

For this PT, the prescribed method for the detection of *Salmonella* in flaxseed was EN ISO 6579-1:2017(/A1:2020), which prescribes the use of MKTTn and RVS and/or MSRV as selective enrichment media.

Seventeen laboratories used MKTTn and RVS as selective enrichment media (laboratory codes: 1, 2, 3, 6, 8, 10, 15, 19, 21, 22, 23, 25, 27, 31, 40, 41, and 44). Sixteen laboratories used MKTTn and MSRV as selective enrichment media (laboratory codes: 4, 5, 7, 12, 14, 16, 17, 18, 20, 24, 28, 29, 32, 39, 43, and 49). Fifteen laboratories used three selective enrichment media: MKTTn, MSRV and RVS (laboratory codes: 9, 11, 13, 26, 30, 33, 34, 35, 36, 37, 38, 45, 46, 47, and 48). Laboratory 42 only used MSRV (and not also the prescribed MKTTn) as a selective enrichment medium.

Table 4.3 presents the reported values of the incubation times, the concentrations of novobiocin, pH, and the incubation temperatures of the various media. Only the laboratories that reported deviating values from EN ISO 6579-1:2017(/A1:2020) are shown.

One laboratory (laboratory code: 15) used a longer incubation time than prescribed for the pre-enrichment in BPW. Five laboratories (laboratory codes: 3, 19, 31, 32, and 48) reported a concentration of novobiocin in

MKTTn that deviated from what was prescribed. Two laboratories (laboratory codes: 33 and 44) did not report all their details concerning the media used.

The selective enriched culture had to be plated-out on two selective solid isolation media: XLD and an obligatory second selective isolation medium. The choice of the second isolation medium for the various laboratories can be found in Table 4.4. Laboratory 27 did not report the use of XLD. Most laboratories used Brilliant Green Agar (BGA), Rambach or Rapid *Salmonella* agar as a second isolation medium. Six laboratories used XLD and two additional selective isolation media.

Table 4.4 Second selective solid isolation media used by the participating laboratories

Media	**No. of users**
ASAP	1
BGA	10
BGA(mod)	7
BPLS	5
BSA	2
Chromogenic *Salmonella* Agar	3
Hectoen Enteric Agar	1
Iris agar	1
Rambach	10
Rapid *Salmonella* Agar	8
Salmonella Differential Agar	2
SM(ID)2	2

Explanations of the abbreviations used are provided in the 'List of abbreviations'.

Table 4.3 Reported technical deviations from the prescribed method EN ISO 6579-1:2017(/A1:2020)

	BPW	MKTTn			RVS		MSRV		
Laboratory code	**incubation (h)**	**concentration novobiocin (mg /L)**	**pH**	**Temperature (°C)**	**pH**	**Temperature (°C)**	**concentration novobiocin (mg/L)**	**pH**	**Temperature (°C)**
EN ISO 6579-1:2017 (/A1:2020)	18 ± 2 h	40 mg /L	7 – 8,2	34 °C - 38 °C	5,2 ± 0,2	41,5 °C ± 1 °C	10 mg / L	5,1 – 5,4	41,5 °C ± 1 °C
3	18	20[a]	8,0	37	5,2	41,5			
8	18 h 15 min	40	6,6[a]	36	5,2	41,5			
15	21[a]	40	7,86	37	5,22	41,5			
19	18	20[a]	8,13	37	5,23	41,5			
21	18	40	6,8[a]	37	5,3	41,5			
31	20	20[a]	8,0	37	5,15	41,5			
32	18 h 30 min	10 mg/ml[a]	8,2	37			10	5,2	41,5
33	19	40	-	37	5,3	41,5	10	5,4	41,5
42	18	[a]	[a]	[a]			10	5,2	41,5
44	18	-	-	37	-	41,5			
48	20	0,04[a]	8,00	37	5,20	41,5	10	5,2	41,5

[a] : deviations from EN ISO 6579-1:2017(/A1:2020)
- : no information reported

The last step in the procedure for *Salmonella* detection is the confirmation step. All participating laboratories performed one or several confirmation tests for *Salmonella*. An overview can be found in Table 4.5.

Thirty-two laboratories performed a biochemical test and performed one or more additional confirmation test(s). In addition to serological confirmation tests, serotyping and PCR, nineteen laboratories (also) used MALDI-TOF.

Table 4.5 Number of participants using different (combinations of) confirmation tests

Biochemical	Serological	Serotyping	PCR	Other	Number of participants
x					2
x	x				12
x	x	x			5
x	x		x		2
x	x			x	3
x		x			4
x		x		x	2
x			x	x	1
x				x	3
	x				1
	x	x			2
	x	x		x	1
		x		x	4
			x		2
				x	5

4.3 Control samples

4.3.1 General

Each NRL-*Salmonella* was expected to include (process) control samples according to its own Standard Operating Procedure and quality system. The *Salmonella* serovars mainly used by the participants for the positive control sample were: *S*. Typhimurium (thirteen participants), *S*. Enteritidis (eleven participants) and *S*. Nottingham (seven participants). Eighteen participants used another *Salmonella* serovar. More details are presented in Table 4.6.

Table 4.6 Salmonella *serovars used by participants for the positive control samples*

***Salmonella* serovar**	**Number of participants**
S. Abaetetuba	3
S. Adabraka	1
S. Agbeni	2
S. Agona	1
S. Alachua	1
S. Blegdam	2
S. Enteritidis	11
S. Harleystreet	1
S. Infantis	3
S. Nottingham	7
S. Poona	1
S. Tranoroa	1
S. Typhimurium	13
S. bongori serovar 66:z41:-	2

The concentration of *Salmonella* in the positive control samples used by the various participants varied widely. For example, fourteen participants used a concentration between 1 – 10 cfu/sample and six participants used a concentration of 1 000 cfu/sample or higher (see Table 4.7). Five laboratories did not determine the concentration of *Salmonella* used for their positive control sample.

A positive control sample for a detection method should demonstrate that media are capable of supporting the growth of the target organisms in low numbers. To obtain information on the sensitivity of a method, the concentration of a positive control sample should preferably be just above the detection limit of the method. For a positive control, it may be advisable to use a *Salmonella* serovar rarely isolated from the routine samples analysed in the laboratory. Thus, possible cross-contamination can be detected more easily.

Table 4.7 Concentration of Salmonella *in the positive control samples*

Concentration *Salmonella* (cfu/sample)	**Number of participants**
1 - 10	14
11 - 20	3
21 - 120	19
500	2
≥ 1 000	6
Not Determined	5

Additionally, a more realistic control of the procedure is obtained when the positive control is added to a *Salmonella*-free matrix similar to the tested samples. Eight laboratories (laboratories 7, 13, 25, 28, 30, 32, 33, and 43) reported the use of a matrix with their positive control. These laboratories used the following matrices with their positive control: chia seeds, feed, milk, minced meat, a mixture of 'meat, milk and egg', pig faeces, rapeseed meal, and seed.

4.4 Artificially contaminated flaxseed samples

4.4.1 General

Table 4.8 shows the results of the tested flaxseed samples. It shows that the temperature abuse of the samples for laboratories 23, 24, 40, and 44, or the technical deviations (see section 4.2.4), did not heavily influence the final results.

Table 4.8 Number of (artificially contaminated) flaxseed samples tested positive for Salmonella *at each laboratory*

	Number of samples in which *Salmonella* is detected		
Laboratory code	**Negative n = 4**	**Low-level STm n = 6**	**High-level STm n = 4**
7	0	4	4
32	0	5	3
13, 34, 40, 43, 44	0	5	4
All other NRLs-*Salmonella* (n = 42)	0	6	4
Criteria for good performance	**0**	**≥3**	**≥3**

4.4.2 Negative samples

All 49 laboratories scored all four negative samples correctly: *Salmonella* was not detected.

4.4.3 Low-level contaminated Salmonella *flaxseed samples*

Forty-two laboratories detected *Salmonella* in all six low-level contaminated flaxseed samples. Six laboratories detected *Salmonella* in five out of six low-level contaminated samples, and one laboratory detected *Salmonella* in four out of six low-level contaminated samples. Both cases still fulfil the criteria for good performance. The level for good performance for the low-level samples for this PT was set at the detection of *Salmonella* in at least three of the six samples. See Figure 4.6.

4.4.4 High-level contaminated Salmonella *flaxseed samples*

All laboratories, except one laboratory, detected *Salmonella* in all four flaxseed samples artificially contaminated with a high level of *Salmonella* Typhimurium. Laboratory 32 detected *Salmonella* in three out of four high-level contaminated samples, which is still within the criteria for good performance. The results by all laboratories are shown in Figure 4.7.

Figure 4.6 Number of flaxseed samples artificially contaminated with a low level of Salmonella *Typhimurium (n=6) that tested positive for* Salmonella, *per laboratory*

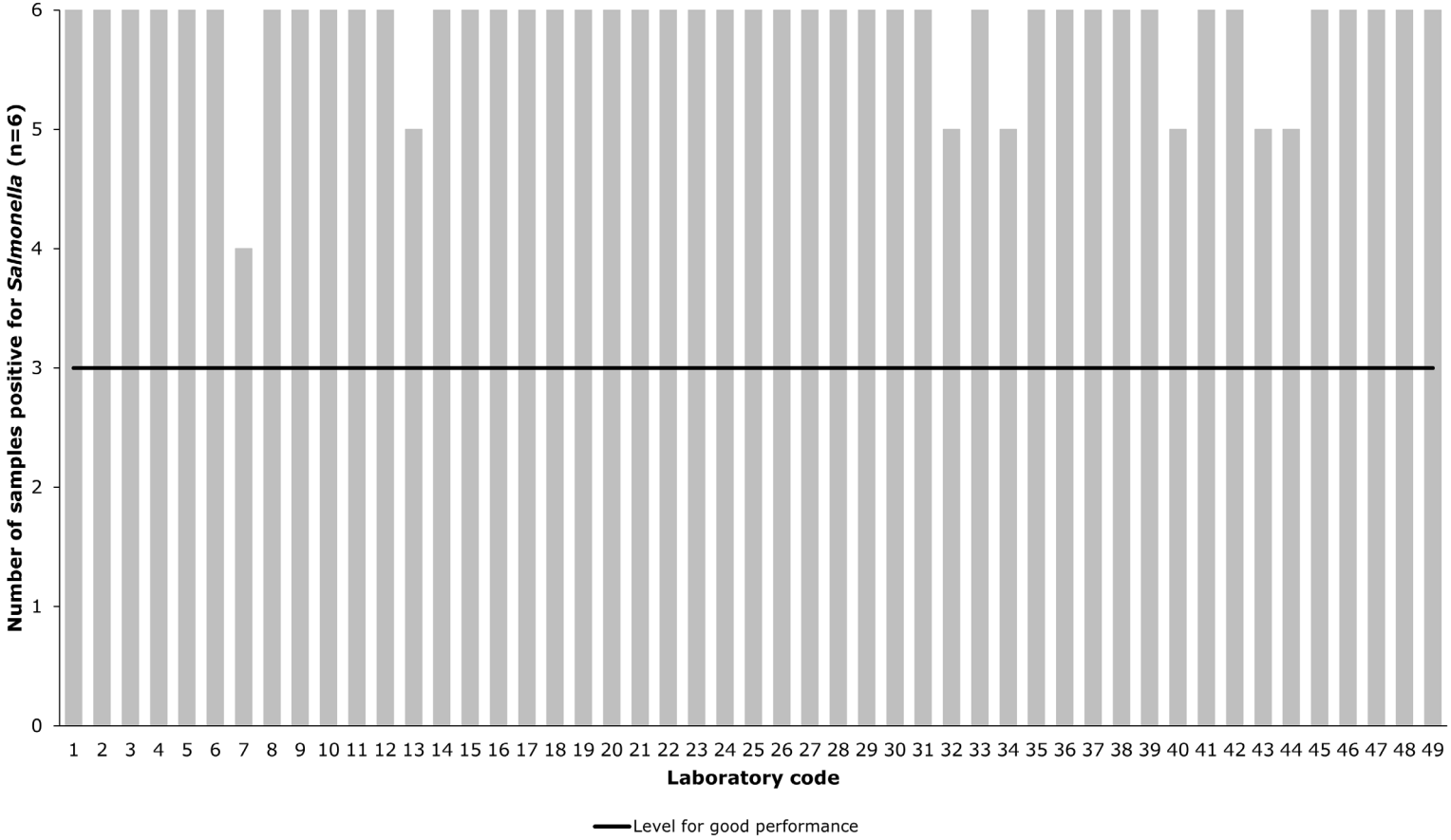
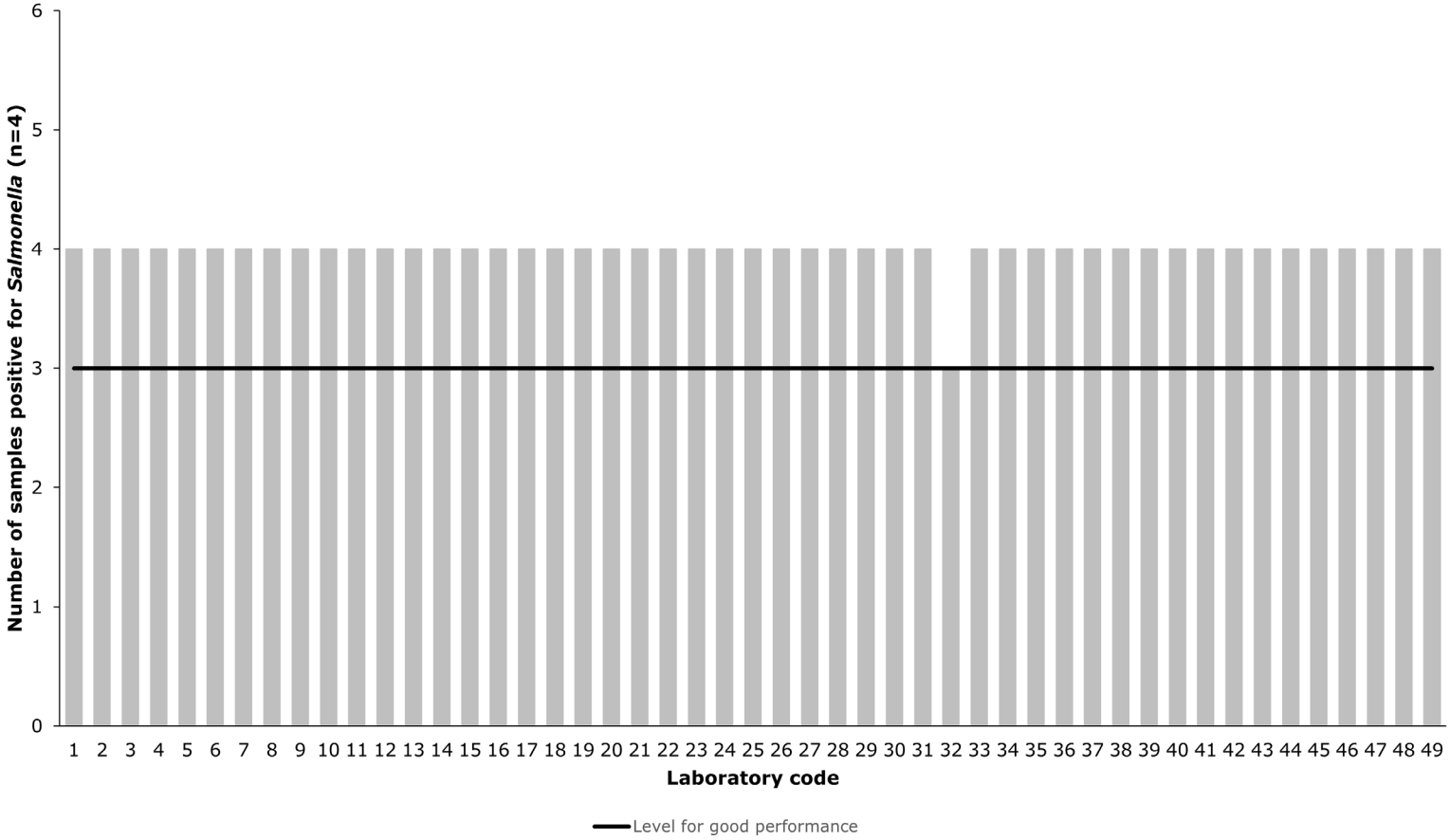


Figure 4.7 Number of flaxseed samples artificially contaminated with a high level of Salmonella *Typhimurium (n=4) that tested positive for* Salmonella, *per laboratory*



4.4.5 *Specificity, sensitivity and accuracy rates of the (artificially contaminated) flaxseed samples*

Table 4.9 shows the specificity, sensitivity, and accuracy rates of the flaxseed samples tested in this PT. The calculations were performed on the results of all participating laboratories and on those of the participants from EU Member States only.

*Table 4.9 Specificity, sensitivity, and accuracy rates calculated from the results found by all participants ('All') and by the NRLs-*Salmonella *from EU Member States ('EU MS') only, with the artificially contaminated flaxseed samples*

Flaxseed samples		All n = 49	EU MS n = 40
Negative (n = 4)	No. of samples	196	160
	No. of negative samples	196	160
	Specificity	**100%**	**100%**
Low-level STm (n = 6)	No. of samples	294	240
	No. of positive samples	286	234
	Sensitivity	**97%**	**98%**
High-level STm (n = 4)	No. of samples	196	160
	No. of positive samples	195	159
	Sensitivity	**99%**	**99%**
All flaxseed samples artificially contaminated with *Salmonella*	No. of samples	490	400
	No. of positive samples	481	393
	Sensitivity	**98%**	**98%**
All flaxseed samples	No. of samples	686	560
	No. of correct samples	677	553
	Accuracy	**99%**	**99%**

4.5 Second detection method

Nineteen participants also used a second detection method for analysing the samples, but the results of this second method were not used to assess the performance of these laboratories. An overview of the methods used per laboratory is given in Table 4.10.
Thirteen laboratories used a real-time PCR as an additional method, four laboratories used VIDAS, and two laboratories used a PCR. Bar one, the results of the second detection methods were all similar to the reported results obtained with EN ISO 6579-1:2017(/A1:2020). Laboratory 43 tested four out of six low-level contaminated flaxseed samples positive for *Salmonella* with the second detection method, while they tested five out of the six low-level contaminated flaxseed samples positive with EN ISO 6579-1:2017(/A1:2020).

Table 4.10 Details of the second detection methods used by participants during the Proficiency Test on detection of Salmonella *in flaxseed samples*

Labo-ratory code	Second detection method	Vali-dated	Validated by	Reference	Number of tests/year, when routinely used
1	Automated PCR System	Yes	AFNOR and AOAC	AFNOR: QUA 18/03 -11/02 (Expiry date: 28-11-2026) ; AOAC: Certificate Nº 100201 (Expiration date: December 31, 2025)	N/A
3	qPCR for aceA gene	No	N/A	N/A	N/A
8	Real-time PCR	Yes	AFNOR	BRD 07/06-07/04	3 000
15	Real-time PCR	Yes	AFNOR	TRA 02/12-01/09	300
19	Rapid finder PCR	Yes	National Accreditation Board	FNES153 (M15)	20
20	Real-time PCR	Yes	National Accreditation Board	10135	1 100
21	qPCR	Yes	AFNOR	BRD 07/06-07/04	N/A
25	Real-time PCR	Yes	AFNOR	BRD 07/06 -07/04	109
26	VIDAS SLM TEST; Lot: 1010775240; exp: 2025-05-31	Yes	AFNOR	BIO 12/1-04/94	N/A
27	Real-time PCR	Yes	In-house	MA-VP-15	138
29	Real-time PCR	No	N/A	N/A	N/A
33	Real-time PCR	Yes	AFNOR	Reference: ABI 29/02-09/10	200
39	Real-time PCR	Yes	AFNOR	AFNOR BRD-07-06-07-04	560
41	VIDAS rapid *Salmonella*	Yes	AFNOR	AFNOR BIO-12/10-09/02	2 100

Labo-ratory code	Second detection method	Vali-dated	Validated by	Reference	Number of tests/year, when routinely used
43	Real-time PCR	No	N/A	N/A	N/A
45	VIDAS® Up *Salmonella* (SPT) (ELISA based)	Yes	AFNOR	Certificate No.: BIO 12/32-10/11 Certificate No.: BIO 12/32-10/11 Renewal decision dated: 15-06-2023 Expiry date: 06-10-2027	14 215
46	VIDAS® Up *Salmonella* (SPT) (ELISA based)	Yes	AFNOR	Certificate No.: BIO 12/32-10/11 Certificate No.: BIO 12/32-10/11 Renewal decision dated: 15-06-2023 Expiry date: 06-10-2027	14 215
47	Real-time PCR	Yes	§64 of the National Food and Feed Code	Malorny et al.(2004) AEM 70:7046-7052	180
49	Real-time PCR	Yes	AFNOR	AFNOR BRD-07-06-07-04	560

N/A: Not Applicable

4.6 Performance of the NRLs-*Salmonella*

All 49 laboratories fulfilled the criteria for good performance in the EURL-*Salmonella* Proficiency Test for detection of *Salmonella* in flaxseed samples.

One month after the deadline of the PT, the participants were informed of their results in an interim summary report containing the results of all participants (Diddens and Mooijman, 2025).

5 Conclusion

All 49 NRLs-*Salmonella* fulfilled the criteria for good performance in the EURL-*Salmonella* Proficiency Test for the detection of *Salmonella* in flaxseed samples. The NRLs-*Salmonella* originated from 27 EU Member States and 9 third countries (EU candidate Member States, members of the European Free Trade Association (EFTA), and the United Kingdom).

The specificity rate of the negative flaxseed samples was 100%.

The sensitivity rate of the flaxseed samples artificially contaminated with *Salmonella* Typhimurium was 97%.

The accuracy rate of all flaxseed samples for all participating laboratories was 99%.

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References

Diddens, R.E. and Mooijman, K.A. (2019). Final report – EURL-*Salmonella* Proficiency Test Food-Feed 2019 – Detection of *Salmonella* spp. in flaxseed. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM report 2019-0134. https://www.rivm.nl/bibliotheek/rapporten/2019-0134.pdf (access date April 2025).

Diddens, R.E. and Mooijman, K.A. (2023). EURL-*Salmonella* Proficiency Test Food-Feed 2023 – Detection of *Salmonella* in flaxseed. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM report 2023-0340. https://www.rivm.nl/bibliotheek/rapporten/2023-0340.pdf (access date April 2025).

Diddens, R.E. and Mooijman, K.A. (2025). Interim summary report – EURL-*Salmonella* combined Proficiency Test Food-Feed 2025 – Detection of *Salmonella* spp. in flaxseed. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. Z&O letter report: 2025-0036. https://www.eurlsalmonella.eu/en/documenten/interim-summary-report-eurl-salmonella-combined-pt-food-feed-2025 (access date June 2025).

EC (2017). Regulation (EU) 2017/625 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products. Journal of the European Union L 95 of 7 April. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L:2017:095:TOC (access date April 2025).

EN ISO 4833-1:2013. Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Part 1: Colony count at 30 °C by the pour plate technique. International Organization for Standardization, Geneva, Switzerland.

EN ISO 6579-1:2017. Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Horizontal method for the detection of *Salmonella* spp. International Organization for Standardization, Geneva, Switzerland.

EN ISO 6579-1:2017/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. International Organization for Standardization, Geneva, Switzerland.

EN ISO 6887-1:2017. 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. International Organization for Standardization, Geneva, Switzerland.

EN ISO 6887-4:2017. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – series for preparation of test samples. Part 4: Specific rules for the preparation of miscellaneous products. International Organization for Standardization, Geneva, Switzerland.

EN ISO 21528-2:2017. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Enterobacteriaceae* – Part 2: Colony-count technique. International Organization for Standardization, Geneva, Switzerland.

EURL-*Salmonella* (2025a). History of EURL-*Salmonella* Proficiency Tests on the detection of *Salmonella*. https://www.eurlsalmonella.eu/en/documenten/history-of-eurl-salmonella-pts-on-detectionversion-march-2025 (access date April 2025).

EURL-*Salmonella* (2025b). Protocol – EURL–*Salmonella* combined Proficiency Test Food-Feed 2025 – Detection of *Salmonella* spp. in flaxseed. https://www.eurlsalmonella.eu/en/documenten/protocol-eurl-salmonella-combined-pt-food-feed-2025 (access date April 2025).

EURL-*Salmonella* (2025c). Result form – EURL–*Salmonella* combined Proficiency Test Food-Feed 2025 – Detection of *Salmonella* spp. in flaxseed. https://www.eurlsalmonella.eu/en/documenten/result-form-eurl-salmonella-combined-pt-food-feed-2025 (access date April 2025).

IATA (2025). Dangerous Goods Regulations, 66th edition 2025. https://www.iata.org/en/publications/dgr/ (access date April 2025).

Jarvis B, Wilrich C, Wilrich P-T. (2010). Reconsideration of the derivation of most probable numbers, their standard deviations, confidence bounds and rarity values. Journal of Applied Microbiology. 109:1660–7. Link to MPN calculation programme: https://www.wiwiss.fu-berlin.de/fachbereich/vwl/iso/ehemalige/wilrich/index.html (access date April 2025).

Malorny, B., Paccassoni, E., Fach, P., Bunge, C., Martin, A. and Helmuth, R. (2004). Diagnostic real-time PCR for detection of *Salmonella* in food, Applied and Environmental Microbiology, 70:7046-52.

List of acronyms

AEM	Applied and Environmental Microbiology
AFNOR	Association Française de Normalisation (French Standardization Association)
AOAC	Association of Analytical Communities
ASAP	AES *Salmonella* Agar Plate
ATCC	American Type Culture Collection
BGA	Brilliant Green Agar
BGA(mod)	Brilliant Green Agar (modified)
BHI	Brain Heart Infusion broth
BPLS	Brilliant green Phenol-red Lactose Sucrose
BPW	Buffered Peptone Water
BSA	Brilliance *Salmonella* Agar
CEN	European Committee for Standardization
cfu	colony-forming units
DG-SANTE	Directorate-General for Health and Consumer Protection
EC	European Commission
EFTA	European Free Trade Association
EU	European Union
EURL	European Union Reference Laboratory
ISO	International Organization for Standardization
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization – Time Of Flight
MKTTn	Muller-Kauffmann tetrathionate-novobiocin broth
MPN	most probable number
MS	Member State
MSRV	Modified semi-solid Rappaport-Vassiliadis
NRL	National Reference Laboratory
PCA	Plate Count Agar
PCR	Polymerase Chain Reaction
PT	Proficiency Test
qPCR	quantitative Polymerase Chain Reaction
RIVM	Rijksinstituut voor Volksgezondheid en het Milieu (National Institute for Public Health and the Environment)
RVS	Rappaport-Vassiliadis Soya broth
SM(ID)2	*Salmonella* Detection and Identification-2
Spp.	Species
STm	*Salmonella* Typhimurium
VRBG	Violet Red Bile Glucose agar
XLD	Xylose Lysine Deoxycholate agar

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