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EURL-*Salmonella* Proficiency Test Food-Feed 2023

Detection of *Salmonella* in flaxseed

RIVM report 2023-0340
R. Diddens | K. Mooijman

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Synopsis

EURL-*Salmonella* Proficiency Test Food-Feed 2023

Detection of *Salmonella* in flaxseed

In 2023, 50 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) obtained a good score in a Proficiency Test for *Salmonella* bacteria. One laboratory initially scored a moderate performance due to an administrative error. Eventually, this laboratory also obtained a good score by providing the correct result with raw data.

The NRLs-*Salmonella* of the European Union (EU) Member States are obliged to participate in annual quality monitoring exercises, known as Proficiency Tests. One of these Proficiency Tests checks whether NRLs can detect *Salmonella* bacteria in food and feed. The matrix chosen for this year's Proficiency Test was flaxseed. Each EU Member State has to appoint a NRL that is responsible for performing these analysis in its laboratory. The 51 participants in this Proficiency Test came from 27 EU Member States and from seven other European countries.

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

This Proficiency Test is organised by the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*). This is part of the Dutch National Institute for Public Health and the Environment (RIVM). An important task of the EURL-*Salmonella* is to monitor the performance of the NRLs for *Salmonella* in Europe.

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

Publiekssamenvatting

EURL-*Salmonella* ringonderzoek Voedsel-Diervoeder 2023

Detectie van *Salmonella* in lijnzaad

Vijftig Nationale Referentie Laboratoria voor *Salmonella* (NRL's-*Salmonella)* hebben in 2023 een goede score gehaald in het ringonderzoek voor de *Salmonella*-bacterie. Eén laboratorium haalde eerst een matige score door een administratieve fout. Dit laboratorium haalde uiteindelijk ook een goede score door met ruwe data het juiste resultaat te geven.

De NRL's-*Salmonella* uit de lidstaten van de Europese Unie zijn verplicht om elk jaar hun kwaliteit te laten toetsen met zogeheten ringonderzoeken. Een van de ringonderzoeken controleert of de NRL's de *Salmonella*-bacterie in voedsel en diervoeder kunnen aantonen; dit keer in lijnzaad. Elk lidstaat van de EU wijst hiervoor het NRL aan dat verantwoordelijk is voor deze testen in hun laboratorium. De 51 deelnemers in dit ringonderzoek kwamen uit 27 lidstaten van de Europese Unie en uit zeven 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 ringonderzoek is georganiseerd door het Europese Unie Referentie Laboratorium voor *Salmonella* (EURL-*Salmonella*). Dit is gevestigd bij het RIVM. Een belangrijke taak van het EURL-*Salmonella* is toezien op de kwaliteit van de NRL's-*Salmonella* in Europa.

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

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Summary

In March 2023, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised a 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* which analyse *Salmonella* in food samples and NRLs-*Salmonella* which analyse animal feed products were invited to participate in this PT. NRLs-*Salmonella* which perform the analysis of food and feed samples in one and the same laboratory could request two different 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, these latter NRLs-*Salmonella* could choose to analyse only one set of samples under one laboratory code or two different codes. Requesting two different codes gives the laboratory the opportunity to receive two separate performance reports.

In total, 51 laboratory codes were generated for this EURL-*Salmonella* Proficiency Test (PT). The participants in this PT were NRLs from 27 EU Member States and nine NRLs-*Salmonella* from seven different third countries (EU candidate Member States, members of the European Free Trade Association (EFTA), and United Kingdom).

The most important objective 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 (after 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 different concentrations of *Salmonella* Typhimurium (STm), were tested for their stability at different 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 7 cfu STm/25 g or with 11 cfu STm/25 g. The flaxseed samples 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 artificially contaminated with 7 cfu STm/25 g were less stable. After 13 days only three out of six samples were still positive for *Salmonella* when stored at 5 °C or 10 °C. Based on these results, the aim was to inoculate the low level flaxseed samples for the PT with approximately 9 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 concentration of both flora remained approx. 10^7 cfu/g when stored at both temperatures for up to three weeks.

Each laboratory received 14 samples of each 25 g flaxseed. These samples consisted of four negative samples (no *Salmonella* added), six samples with a low level of STm (inoculum 9 cfu/sample) and four samples with a high level of STm (inoculum 52 cfu/sample). The PT samples were artificially contaminated with a diluted culture of *Salmonella* Typhimurium at the EURL-*Salmonella* laboratory. In addition, each participating laboratory had to test two control samples: a procedure control (Buffered Peptone Water only) and a positive control with *Salmonella*.

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

One laboratory scored a 'Good performance*', with additional explanation. The laboratory made an administrative error when reporting the result of a negative sample. The laboratory communicated this error with the EURL-*Salmonella* after the reporting deadline of the PT, but before the intended results were shared with the laboratories. An explanation was given how this error came to light and raw data confirmed the correct result.

The accuracy rate of all control samples was 100%. The sensitivity rates of the flaxseed samples artificially contaminated with *Salmonella* Typhimurium was 96%. The accuracy rate of all flaxseed samples for all participating laboratories was 97%. The specificity rate of the negative flaxseed samples was 100%.

The NRLs-*Salmonella* were given the opportunity to analyse the samples with 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 for assessing the performance of the NRL-*Salmonella*. Nineteen participants also used a second detection method (real-time PCR, VIDAS and PCR) for analysing the samples. The results of the second detection methods were all similar to the reported results obtained with EN ISO 6579-1:2017(/A1:2020) by the laboratories.

1 Introduction

An 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 on the detection of *Salmonella*, as organised by EURL-*Salmonella* since 1995, is summarised on the EURL-*Salmonella* website (EURL-*Salmonella*, 2023).

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 comparable results are obtained by all NRLs-*Salmonella*.

The PT was organised in March 2023. For this PT, flaxseed was chosen as matrix to be analysed, which is used as a food product as well as (an ingredient of) animal feed. Hence, NRLs-*Salmonella,* which analyse food samples, as well as NRLs-*Salmonella,* which 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(/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 samples artificially contaminated with a high level of STm, six samples artificially contaminated with a low level of STm, and four negative samples (flaxseed samples without *Salmonella*). Additionally, two control samples (procedure control and own positive control with *Salmonella*) had to be tested by the NRLs-*Salmonella*.

2 Participants

Table 2.1 displays a list of participants in the EURL-*Salmonella* PT Food-Feed 2023.

*Table 2.1 List of participants in the EURL-*Salmonella *Proficiency Test Food-Feed 2023*

Country	City	Product(s) under analysis at the NRL-*Salmonella*	Institute / NRL-*Salmonella*
Austria	Graz	Food	AGES - Institute for Medical Microbiology and Hygiene, NRC *Salmonella*
Austria	Linz	Animal feed	AGES GmbH, Animal nutrition and feed
Belgium	Brussels	Animal feed and food	Sciensano, Food Pathogens
Bulgaria	Sofia	Animal feed and food	National Diagnostic and Research Veterinary Institute (NDRVMI), NRL-*Salmonella*
Croatia	Zagreb	Animal feed	Croatian Veterinary Institute Zagreb, Veterinary Public Health
Croatia	Zagreb	Food	Croatian Veterinary Institute, Laboratory for Food Microbiology
Cyprus	Nicosia	Animal feed and food	Cyprus Veterinary Services, Laboratory for the Control of Food of Animal Origin
Czech Republic	Prague	Animal feed and food	Státní veterinární ústav Praha, dep. of Bacteriology
Denmark	Ringsted	Animal feed and food	Danish Veterinary and Food Administration, Sektion of Microbiology
Estonia	Tartu	Animal feed and food	Estonian Veterinary and Food Laboratory, Department of food microbiology
Finland	Helsinki	Animal feed and food	Finnish Food Authority, Microbiology Unit
France	Ploufragan	Animal feed and food	Anses, Ploufragan - Unité HQPAP
Germany	Berlin	Animal feed and food	German Federal Institute for Risk Assessment (BfR), Biological Safety

Country	City	Product(s) under analysis at the NRL-*Salmonella*	Institute / NRL-*Salmonella*
Greece	Chalkida	Animal feed and food	Veterinary Laboratory of Chalkis, NRL-*Salmonella* Greece
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, NRL-*Salmonella*
Italy	Legnaro, Padova	Animal feed and food	Istituto Zooprofilattico Sperimentale delle Venezie, National Reference Centre/OIE Reference Laboratory for Salmonellosis
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 Unit
Luxembourg	Dudelange	Animal feed and food	Laboratoire national de santé, Laboratoires de Protection de la Santé
Malta	Valletta	Animal feed and food	Malta Public Health Laboratory, Health Regulation
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	Animal feed	Wageningen Food Safety Research (WFSR), Microbiology
Northern Ireland	NRL tasks carried out by NRL-*Salmonella* in Belgium		
Norway	Ås	Animal feed and food	Norwegian Veterinary Institute, Microbiology

Country	City	Product(s) under analysis at the NRL-*Salmonella*	Institute / NRL-*Salmonella*
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 Hygiene of Food of Animal Origin
Portugal	Vairão	Animal feed and food	Instituto Nacional de Investigação Agrária e Veterinária, I. P., Food Microbiology Laboratory
Republic of Moldova	Chisinau	Animal feed	Republican Centre for Veterinary Diagnostic, Bacteriology
Republic of North Macedonia	Skopje	Food	Faculty of veterinary medicine, Food institute
Republic of Serbia	Novi Sad	Animal feed	Scientific Veterinary Institute "Novi Sad", Department of Microbiological Examination of Feed
Republic of Serbia	Novi Sad	Food	Scientific Veterinary Institute "Novi Sad", Department of microbiology and sensory analysis of food
Romania	Bucharest	Animal feed and food	Hygiene and Veterinary Public Health Institute, Microbiology
Slovak Republic	Dolný Kubín	Animal feed and food	State Veterinary and Food Institute Dolný Kubín, Section of Bacteriology
Slovenia	Ljubljana	Animal feed and food	UL, Veterinary faculty, National Veterinary Institute, Institute of Microbiology and Parasitology
Spain	Algete, Madrid	Animal feed	Laboratorio Central de Veterinaria, Bacteriología 1
Spain	Lugo, Galicia	Vegetal matrices, soil and water	National Plant Health Laboratory, Plant hygiene
Spain	Majadahonda, Madrid	Food	Centro Nacional de Alimentación, Food Microbiology laboratory
Sweden	Uppsala	Animal feed and food	National Veterinary Institute, Department of Microbiology

Country	City	Product(s) under analysis at the NRL-*Salmonella*	Institute / NRL-*Salmonella*
Switzerland	Zürich	Food	Institut für Lebensmittelsicherheit und -hygiene der Universität Zürich, NENT / Diagnostiklabor
United Kingdom	Addlestone	Animal feed	Animal and Plant Health Agency, Bacteriology
United Kingdom	York	Food	UK Health Security Agency, Food, Water & Environmental Laboratory - York 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 09-01-2023 from a mill in the Netherlands. The batch was used for performing pre-tests and for preparation of the PT samples. The batch of flaxseed was stored at room temperature until sample preparation.

After 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. To each test portion, 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 damaged organisms. The sample was then mixed by hand for 30 seconds. After 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. The MKTTn tubes and the suspect growth on the MSRV plates were then 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. The inoculated samples were stored at 5 °C and at 10 °C and after 0, 6 and 13 days, six artificially contaminated samples were tested for the presence of *Salmonella* following EN ISO 6579-1:2017(/A1:2020) (see 3.3). This was done for each inoculation level and storage temperature. The samples stored at 5 °C were also tested after 20 days of storage.

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 level of the inoculum was confirmed 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 also stored at 5 °C and at 10 °C. The level of the natural background flora was determined in these samples by analysing the number of aerobic bacteria and *Enterobacteriaceae* (see 3.1.4). The samples were analysed after 0, 6 and 13 days of storage and the samples stored at 5 °C were also tested after 20 days of storage.

3.1.3 *Preparation of flaxseed samples for the Proficiency Test*

In February and March 2023, the EURL-*Salmonella* weighed 784 subsamples of each 25 g flaxseed into (plastic) sample bags. Next, 224 subsamples were individually and artificially contaminated with a high level, and 336 subsamples with a low level of the diluted overnight culture of *S*. Typhimurium (ATCC 14028, Manassas, USA); 224 subsamples were not contaminated with *Salmonella* (negative samples).

The following set of samples were prepared for each participant:
- four negative samples, each containing 25 g of flaxseed (no *Salmonella* added);
- six samples, each containing 25 g of flaxseed with a low level of *Salmonella* Typhimurium, aimed at 10 cfu/25 g;
- four samples, each containing 25 g of flaxseed with a high level of *Salmonella* Typhimurium, aimed at 50 cfu/25 g;
- two control samples consisting of empty filter sample bags for the procedure control (BPW only) and an own positive control.

After artificial contamination, the samples were mixed by hand and stored at 5 °C until transport to the NRLs-*Salmonella* on 20 March 2023.

The level of the inoculum used to artificially contaminate the 25 g flaxseed samples was confirmed 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 investigated by following EN ISO 4833-1:2013 and EN ISO 21528-2:2017 respectively. This procedure entails that an initial suspension was prepared by adding 225 ml of peptone saline solution to 25 g of flaxseed. Next, 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 were tested, 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. Additionally, two control samples (CTRL1 and CTRL2) had to be tested by the laboratories: a procedure control (CTRL1) consisting of only BPW and a positive control sample (CTRL2).

The laboratories had to use their own positive control that they normally use when analysing routine samples for the detection of *Salmonella*. The participants could analyse the control samples either way, but reporting of the results should be as described: 'CTRL1' being the procedure control (BPW only) and 'CTRL2' being the positive control with own *Salmonella*.

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

Table 3.1 Overview of the number and type of samples tested per laboratory in the Proficiency Test Food-Feed 2023

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

Control samples	**Number of samples (n=2)**
Procedure control (BPW only)	1
Positive control with *Salmonella*	1

3.2.2 *Shipment of parcels and temperature recording during shipment*

A set of 14 PT samples and two empty safety bags, which could be used for the control samples, were packed in a large safety bag. The set of samples was placed in a parcel with four frozen cooling elements for shipment.

To monitor exposure to excessive temperatures during shipment and storage, temperature buttons were included in the large safety bag to record the temperature. These buttons are tiny units sealed in a stainless-steel case, 16 mm in diameter and 6 mm deep. One button was packed together with the PT samples in the large safety bag. 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 program, and all recorded temperatures from transport and storage were transferred to an Excel sheet.

The parcel was sent to the participants on 20 March 2023 as 'biological substances category B (UN3373)' (IATA, 2023) 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*, 2023a) and in (a printout of) the result form (EURL-*Salmonella,* 2023b).

3.3 Methods

The prescribed method was EN ISO 6579-1:2017/A1:2020 and the underlying EN ISO documents, e.g. 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, 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 preparation of the test samples for this type of matrix, or follow the procedure described below. This procedure is slightly adjusted from EN ISO 6887-4:2017 to avoid punctures in the plastic sample bags:

- add the Buffered Peptone Water (BPW) to the 25 g test sample (instead of weighing accurately 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 with 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

The specificity, sensitivity, and accuracy rates were calculated for the flaxseed samples artificially contaminated with STm. For the control

samples, only the accuracy rates were calculated. The 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

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

3.5 Criteria for good performance

Criteria for good performance used in the current EURL-*Salmonella* PT for detection of *Salmonella* in flaxseed are shown in Table 3.2.

In the past, the tools for subtyping isolates from 'false-positive' samples were limited, but nowadays it is well possible to precisely distinguish the inoculum strain from other *Salmonella* strains by using Whole Genome Sequencing (WGS). For that reason, the new criterion for negative samples will become: 'No *Salmonella* detected in any of the negative samples. However, as 100% *Salmonella*-free matrix cannot be guaranteed, an incidental positive result with a *Salmonella* strain different from the inoculum strain may still be acceptable.'

Therefore, the EURL-*Salmonella* asks the NRLs-*Salmonella* to conserve (at least) one *Salmonella* confirmed colony from each positive sample, so that in case of deviating results, additional tests can be performed.

*Table 3.2 Criteria for good performance for the EURL-*Salmonella *PT Food-Feed 2023*

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

* 100% *Salmonella*-free matrix cannot be guaranteed, so that an incidental positive result with a *Salmonella* strain different from the inoculation strain is still considered as acceptable.

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 flaxseed was checked by analysing in total 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 7 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.

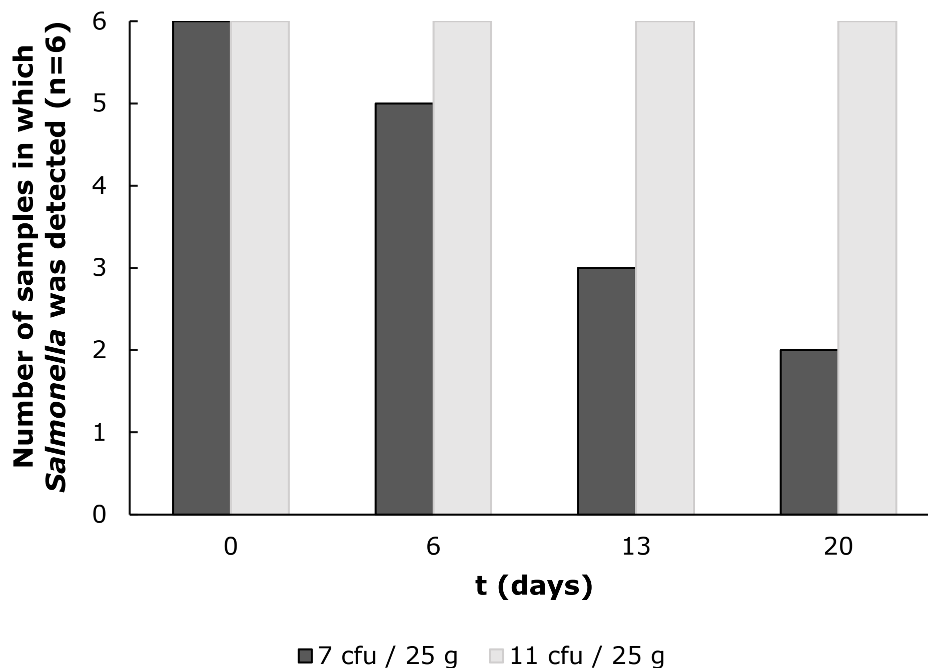

Figure 4.1 Stability test of flaxseed samples (n=6) artificially contaminated with S. *Typhimurium at (initial) levels of 7 cfu/25 g and 11 cfu/25 g, stored at 5 °C*

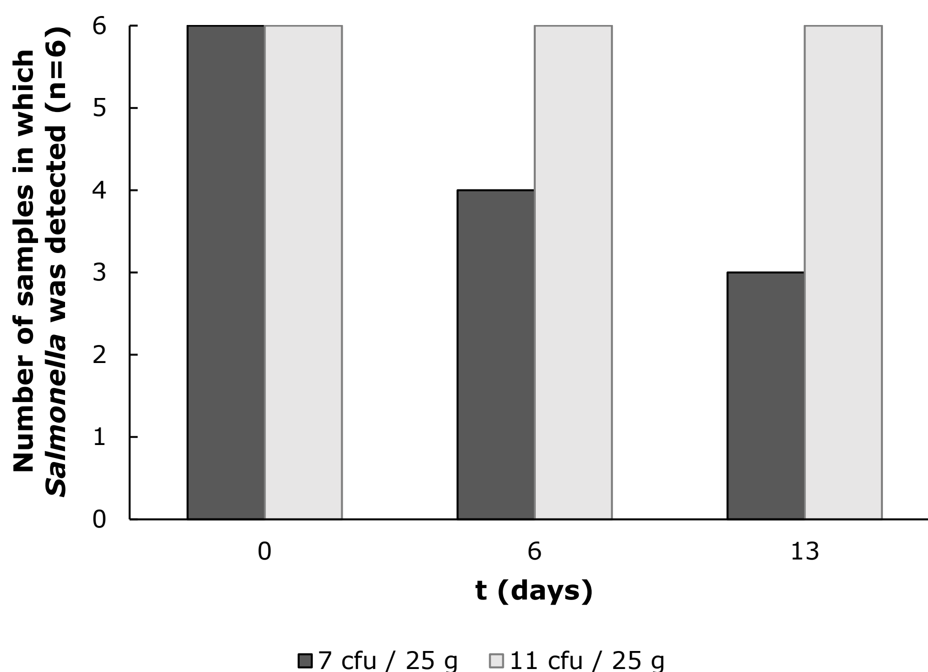

Figure 4.2 Stability test of flaxseed samples (n=6) artificially contaminated with S. *Typhimurium at (initial) levels of 7 cfu/25 g and 11 cfu/25 g, stored at 10 °C*

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. The samples artificially contaminated with 7 cfu of *Salmonella* Typhimurium were less stable: after 20 days only two out of six samples were still positive for *Salmonella*.

Figure 4.2 shows that the same flaxseed samples stored at 10 °C have similar results compared to the samples stored at 5 °C. After 13 days, only three out of six samples inoculated with 7 cfu STm/25 g were still positive for *Salmonella*. All samples with an inoculation level of 11 cfu STm/25 g were still positive for *Salmonella* after 13 days of storage at 10 °C.

Based on these results, the aim was to inoculate the low level flaxseed samples of the PT with approximately 9 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 approx. 10^7 cfu/g flaxseed when stored at both temperatures.

Figure 4.4 shows the number of *Enterobacteriaceae*, which also remained at approx. 10^7 cfu/g flaxseed when stored at both temperatures.

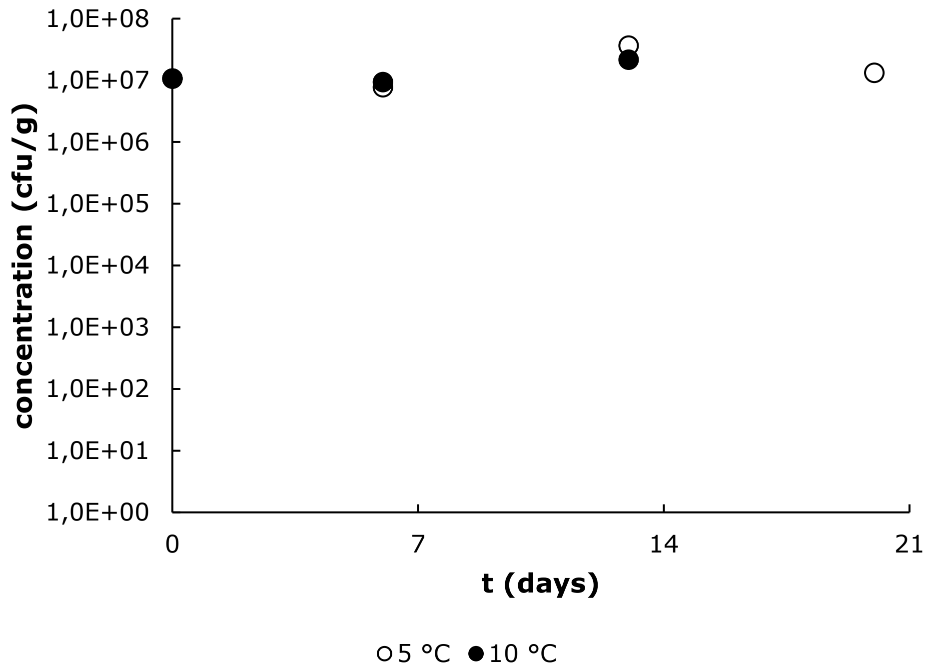

Figure 4.3 Number of aerobic bacteria per gram flaxseed (negative for Salmonella*) after storage at 5 °C and at 10 °C for 0, 6, 13 and 20 days*

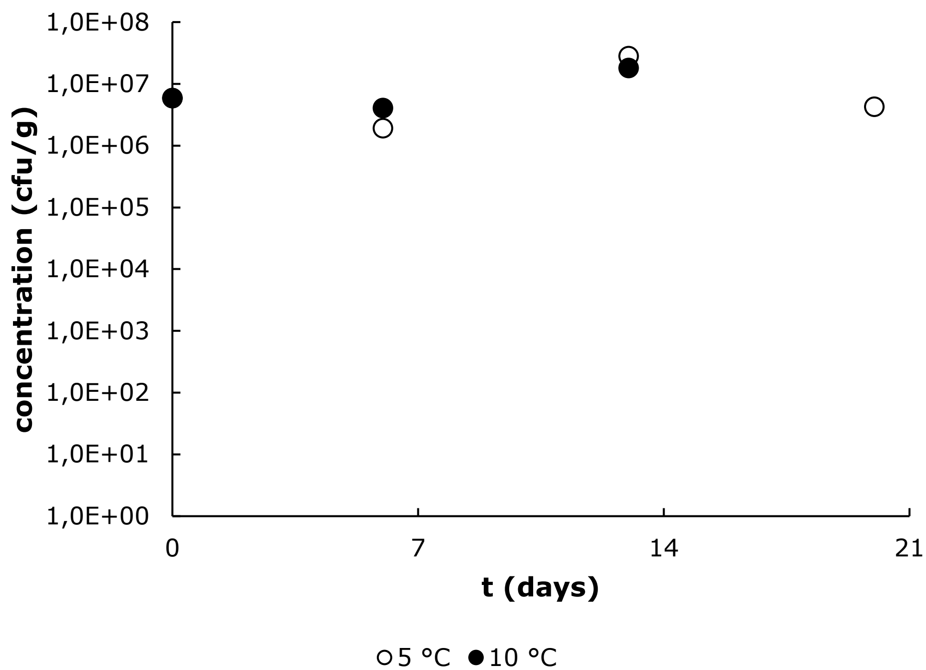

Figure 4.4 Number of Enterobacteriaceae *per gram flaxseed (negative for* Salmonella*) after storage at 5 °C and at 10 °C for 0, 6, 13 and 20 days*

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 preparation of the samples of the PT. This batch was tested negative for *Salmonella*.

The samples were prepared as described in 3.1.3. The PT samples were artificially contaminated on 13 March 2023 and all samples were stored at 5 °C until shipment on 20 March 2023.

4.1.3 *Background flora in flaxseed*

The level of natural background flora in the flaxseed was tested on 10 January 2023 (shortly after receipt of the flaxseed) and on 27 March 2023 (during the PT). Table 4.1 shows the number of aerobic bacteria and *Enterobacteriaceae* in the flaxseed.

Table 4.1 Number of aerobic bacteria and Enterobacteriaceae *in the flaxseed*

Date	Aerobic bacteria (cfu/g)	*Enterobacteriaceae* (cfu/g)
10 January 2023	1,1 x 10^7	5,9 x 10^6
27 March 2023[a]	4,0 x 10^6	2,1 x 10^6

a After storage at room temperature for 9 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 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 start of 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 after storage at 5 °C for 13 days*

Date	Low level STm in cfu per sample	High level STm in cfu per sample
14 March 2023 Inoculation of flaxseed	9	52
27 March 2023[a] MPN of flaxseed samples, inoculated with STm (95% confidence limit)	3,25 (1,1-10,3)	7 (2,3-21,5)

a After storage at 5 °C for 13 days

4.2 Technical data of the Proficiency Test

4.2.1 *General*

NRLs-*Salmonella* which perform the analysis of food and feed samples in one and the same laboratory could request two different 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, these latter NRLs-*Salmonella* could also choose to analyse only one set of samples under one laboratory code or two different codes. Requesting two different codes gives the laboratory the opportunity to receive two separate performance reports. In total, 51 laboratory codes were generated for this EURL-*Salmonella* PT.

NRLs-*Salmonella* from 27 EU MS and nine NRLs-*Salmonella* from seven different third countries (EU candidate Member States, members of the European Free Trade Association (EFTA), and United Kingdom) participated.

The number of samples (approximately) analysed in 2022 with the prescribed method, EN ISO 6579-1:2017(/A1:2020), per participating laboratory is summarised in Table 4.3.

Table 4.3 Number of samples (approximately) analysed in 2022 with EN ISO 6579-1:2017(/A1:2020) per participating laboratory

Laboratory code	Samples (approx.) analysed
01	100
02	6 000
03	100
04	100
05	20 000
06	20
07	20
08	700
09	5 500
10	10
11	10
12	5 128
13	850
14	2 500
15	190
16	1 300
17	2 500
18	3 000
19	6 000
20	485
21	485
22	19 527
23	4 300
24	1 289
25	-
26	0*
27	30
28	2 362
29	2 362
30	100
31	500
32	500
33	10 000
34	1 500
35	5 100
36	500
37	3 200
38	3 200

Laboratory code	Samples (approx.) analysed
39	0*
40	6 000
41	1 386
42	500
43	1 460
44	1 500
45	3 500
46	-
47	3 962
48	2 300
49	600
50	34
51	300

- : no information reported

*: No routine samples, only Proficiency Test samples

Forty-seven participants performed the Proficiency Test on 27 March 2023 as prescribed. Two participants started the PT on 22 March 2023 and two participants started on 28 March 2023 due to a delay in parcel transportation.

4.2.2 *Accreditation*

Forty-two participants are accredited for EN ISO 6579-1:2017/A1:2020. Seven participants indicated to only be accredited for EN ISO 6579-1:2017. Two participants (originating from the same EU MS) were not accredited, but the laboratories indicated to plan the re-accreditation. Two laboratories also have other *Salmonella* methods under accreditation: NF U 47-100; NF U 47-101; NF U 47-102; Vidas and SureTect Real-time PCR.

4.2.3 *Transport of samples*

On Monday 20 March 2023, the PT samples were sent to all participants. Forty parcels were delivered at the NRLs-*Salmonella* within one or two days. NRLs-*Salmonella* which requested two sets of samples received one parcel with both sets of samples. The parcel sent to the NRL-*Salmonella* with laboratory codes 47 and 48 was held at customs and arrived after eight days of transport at the laboratory. This laboratory started the performance of the PT on 28 March 2023.

The temperature during transport and storage was registered using a temperature probe. The temperature of all parcels during transport was below 2,5 °C, except for the parcel of the NRL-*Salmonella* with laboratory codes 47 and 48. The temperature of this parcel reached a maximum of 11 °C when held at customs.

The measured storage temperature of the samples at the laboratories varied between 0 °C and 9 °C.

Figure 4.4 shows the temperature record of the parcel for laboratory 47, until receipt and start of the PT on 28 March 2023. The temperature record for laboratory 48, which had a separate temperature button, showed a comparable curve.

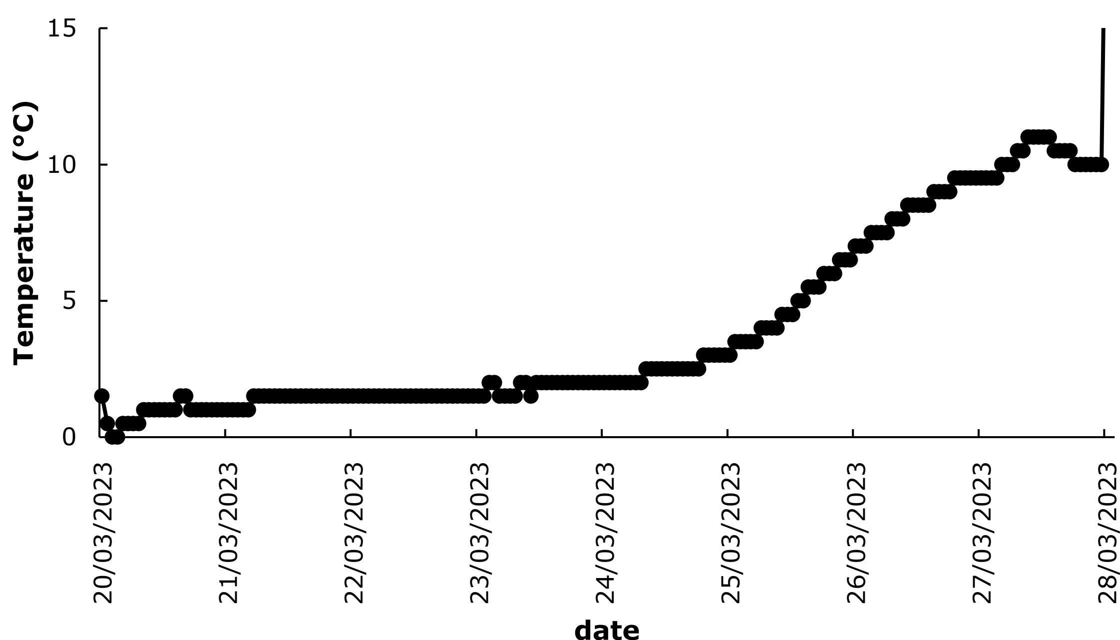

Figure 4.4 Temperature record of the parcel with PT samples for laboratory 47

4.2.4 *Media*

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. Fifty laboratories used MKTTn and RVS and/or MSRV as selective enrichment media.

Eighteen laboratories used MKTTn and RVS as selective enrichment media (laboratories 01, 06, 07, 08, 12, 13, 14, 22, 23, 24, 33, 35, 39, 40, 41, 47, 48 and 50). Sixteen laboratories used MKTTn and MSRV as selective enrichment media (laboratories 05, 10, 11, 15, 17, 18, 19, 26, 31, 32, 34, 37, 38, 43, 45 and 49). Sixteen laboratories used all three prescribed selective enrichment media: MKTTn, MSRV and RVS (laboratories 02, 03, 04, 09, 16, 20, 21, 27, 28, 29, 30, 36, 42, 44, 46 and 51). Laboratory 25 used only MSRV (and not the prescribed MKTTn) as selective enrichment medium.

Table 4.4 shows the reported values of the incubation times, the concentrations of novobiocin, pH, and the incubation temperatures of the different media. Only the laboratories which reported deviating values from EN ISO 6579-1:2017(/A1:2020) are shown. Laboratory 24 initially reported the incubation temperature for MKTTn and RVS wrongly, but indicated that the incubation temperatures of these two media were reported inversed.

Two laboratories (laboratories 40 and 50) used a longer incubation time than prescribed for the pre-enrichment in BPW. Five laboratories (laboratories 20, 21, 36, 41 and 50) reported a lower concentration of novobiocin in MKTTn than prescribed. Laboratory 19 also incubated MKTTn at a higher temperature than prescribed. According to EN ISO 6579-1:2017(/A1:2020), the pH of the base medium of MKTTn broth should be 7,8 – 8,2. In addition, it indicates that the complete medium should no longer be used if, after storage, the pH is <7. Two laboratories (laboratories 14 and 24) reported a pH lower than 7. Laboratories 20, 21 and 46 reported for RVS a higher pH than prescribed in EN ISO 6579-1:2017(/A1:2020). Laboratories 43 and 45 reported a higher pH for MSRV than prescribed. One laboratory (laboratory 51) used a incubation temperature of 37 °C for MSRV instead of 41,5 °C ± 1 °C.

The selective enriched culture had to be plated-out on two isolation media: XLD and an obligatory second isolation medium. The choice of the second isolation medium for the different laboratories can be found in Table 4.5. Most laboratories used Rambach or Brilliant Green Agar (BGA) as a second isolation medium. Five laboratories used two selective isolation media in addition to XLD and one laboratory used three selective isolation media in addition to XLD.

Table 4.4 Reported technical deviations from prescribed method EN ISO 6579-1:2017(/A1:2020)

	BPW	MKTTn			RVS		MSRV		
Laboratory code	**incubation (hours)**	**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 hours	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
14	20	40	6,6	36	5,2	41,5			
19	18	40	8,2	41			10	5,3	41
20	20	20	8,02	37	5,48	41,5	10	5,43	41,5
21	20	20	8,02	37	5,48	41,5	10	5,43	41,5
24	18	40	6,8	37	5,2	41,5			
25	18						-	5,3	41,5
36	18	10	7,7	37	5,2	42	10	5,2	42
40	24	40	7,8	37	5,2	41,5			
41	19	20	8,05	37	5,00	41,5			
43	18	40	8,2	37			10	5,6	41,5
46	20	40	8,08	37	5,45	41,5	10	5,6	41,5
50	21 h 40 min.	39,02	7,3	37	5,3	41,5			
51	20	40	8	37	5,2	41,5	10	5,2	37

Grey cells are deviations from EN ISO 6579-1:2017(/A1:2020)
- : no information reported

Table 4.5 Second isolation media used by the laboratories

Media	No. of users
ASAP	1
BGA	11
BGA(mod)	6
BPLS	5
BSA	2
Chromogenic *Salmonella* Agar	4
Compass *Salmonella*	2
Rambach	13
Rapid *Salmonella* Agar	9
Salmonella Differential Agar	2
SM(ID)2	3

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

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 4.6.

Thirty-eight laboratories performed a biochemical test and performed one or more additional confirmation test(s). In addition to serological confirmation tests, serotyping and PCR, fifteen laboratories (also) used MALDI-TOF and one laboratory used WGS as confirmation method.

Table 4.6 Number of participants using different (combinations of) confirmation tests

Biochemical	Serological	Serotyping	PCR	Other	Number of participants
x					1
x	x				12
x	x	x			1
x	x	x		x	1
x	x		x		4
x	x			x	2
x		x			7
x		x	x		2
x		x		x	3
x			x	x	1
x				x	5
	x	x			2
		x			2
		x		x	2
			x		2
				x	4

4.3 Control samples

4.3.1 General

Two empty safety bags were sent to each participating NRL-*Salmonella* to be used for the control samples, being:

- a procedure control consisting only of BPW (CTRL1);
- a positive control with the laboratories' own *Salmonella* control strain (CTRL2).

Procedure control (BPW only)
All laboratories reported the procedure control sample (no matrix, BPW only) correctly negative for *Salmonella*.

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

The *Salmonella* serovars used by the participants for the positive control sample were: *S.* Typhimurium (fifteen participants), *S.* Enteritidis (thirteen participants), *S.* Nottingham (seven participants), *S*. Abaetetuba (three participants), *S.* Infantis (three participants) and ten participants used another *Salmonella* serovar. More details are given in Table 4.7.

Table 4.7 Salmonella *serovars used by participants for the positive control samples*

***Salmonella* serovar**	**Number of participants**
S. Abaetetuba	3
S. Agbeni	2
S. Alachua	1
S. Blegdam	2
S. Enteritidis	13
S. Harleystreet	2
S. Infantis	3
S. Nottingham	7
S. Poona	1
S. Tranoroa (*Salmonella enterica* subsp. *salamae*)	1
S. Typhimurium	15
Salmonella bongori serovar 66 : z41: -	1

The concentration of *Salmonella* in the positive control samples used by the different participants varied between 1 and 10^8 cfu/sample (see Table 4.8). Two laboratories did not determine the concentration of *Salmonella* added to their positive control sample.

Table 4.8 Concentration of Salmonella *in the positive control samples*

Concentration *Salmonella* (cfu/sample)	Number of participants
1 - 10	14
11 - 20	6
21 - 120	18
250 - 500	4
10^3 – 10^8	7
Not Determined	2

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. Additionally, for a positive control, it may be advisable to use a *Salmonella* serovar rarely isolated from the routine samples analysed in the laboratory. In this way, possible cross-contamination can be detected more easily. 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. Five laboratories (laboratories 5, 18, 19, 26 and 45) also used a matrix with their positive control. The matrices used were: fishmeal, milk, minced meat, rapeseed meal and ‘raw meat, milk, eggs’.

4.3.2 *Correct scores of the control samples*

Table 4.9 shows the number of correct scores found with the control samples. The calculations were performed for the results of all participants and for the participants from EU MS only.

Table 4.9 Correct scores found with the control samples by all participants (‘All’) and by the participants from EU Member States only (‘EU MS’)

Control samples		All n = 51	EU MS n = 42
Procedure control (BPW only) n=1	No. of samples	51	42
	No. of negative samples	51	42
	Correct score	100%	100%
Positive control with *Salmonella* n=1	No. of samples	51	42
	No. of positive samples	51	42
	Correct score	100%	100%
All control samples n = 2	No. of samples	102	84
	No. of correct samples	102	84
	Accuracy	100%	100%

4.4 Artificially contaminated flaxseed samples

4.4.1 General

Table 4.10 shows the results of the tested flaxseed PT samples. It shows that the temperature abuse of the parcel for laboratories 47 and 48, as well as the technical deviations (see section 4.2.4), did not influence the final results.

Table 4.10 Number of (artificially contaminated) flaxseed samples tested positive for Salmonella *at each laboratory**

Laboratory code	**Number of samples tested positive for *Salmonella* per laboratory**		
	Negative n = 4	**Low level STm n = 6**	**High level STm n = 4**
Criteria for good performance	**0**	**≥3**	**≥3**
08, 13, 17, 21, 28, 29, 30, 32, 33, 34, 35, 36, 37, 38, 44, 45	0	5	4
43	0	4	4
All other NRLs-*Salmonella* (n = 34)	0	6	4

* Laboratory 14 made an administrative error when reporting a result of a negative sample. This table is based on the correct raw data.

Negative samples

All 51 laboratories scored all four negative samples correctly: *Salmonella* was not detected. Laboratory 14 reported one of the negative samples mistakenly as positive for *Salmonella*. This laboratory made an administrative error and the result had to be 'Not detected'. This NRL-*Salmonella* communicated the error with the EURL-*Salmonella* after the deadline of reporting of the PT, but before the intended results were shared with the laboratories. An explanation was given how this error came to light after the deadline of reporting and raw data confirmed the (negative) result of this sample.

Low-level contaminated Salmonella *flaxseed samples*

Thirty-four laboratories detected *Salmonella* in all six low contaminated flaxseed samples. Sixteen 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 of good performance. 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 samples. See Figure 4.5.

High-level contaminated Salmonella *flaxseed samples*

All laboratories detected *Salmonella* in all four flaxseed samples artificially contaminated with a high level of *Salmonella* Typhimurium. The results are shown in Figure 4.6

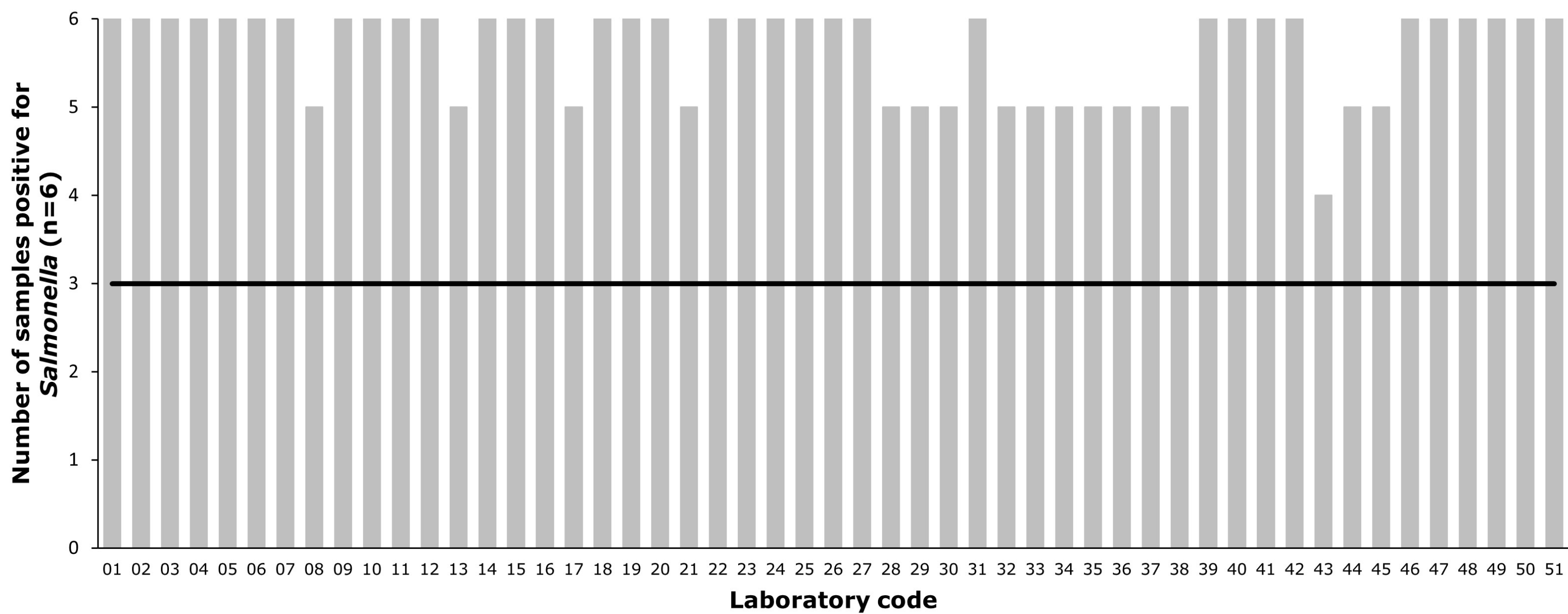

Figure 4.5 Number of flaxseed samples artificially contaminated with a low level of Salmonella *Typhimurium (n=6) that tested positive per laboratory*

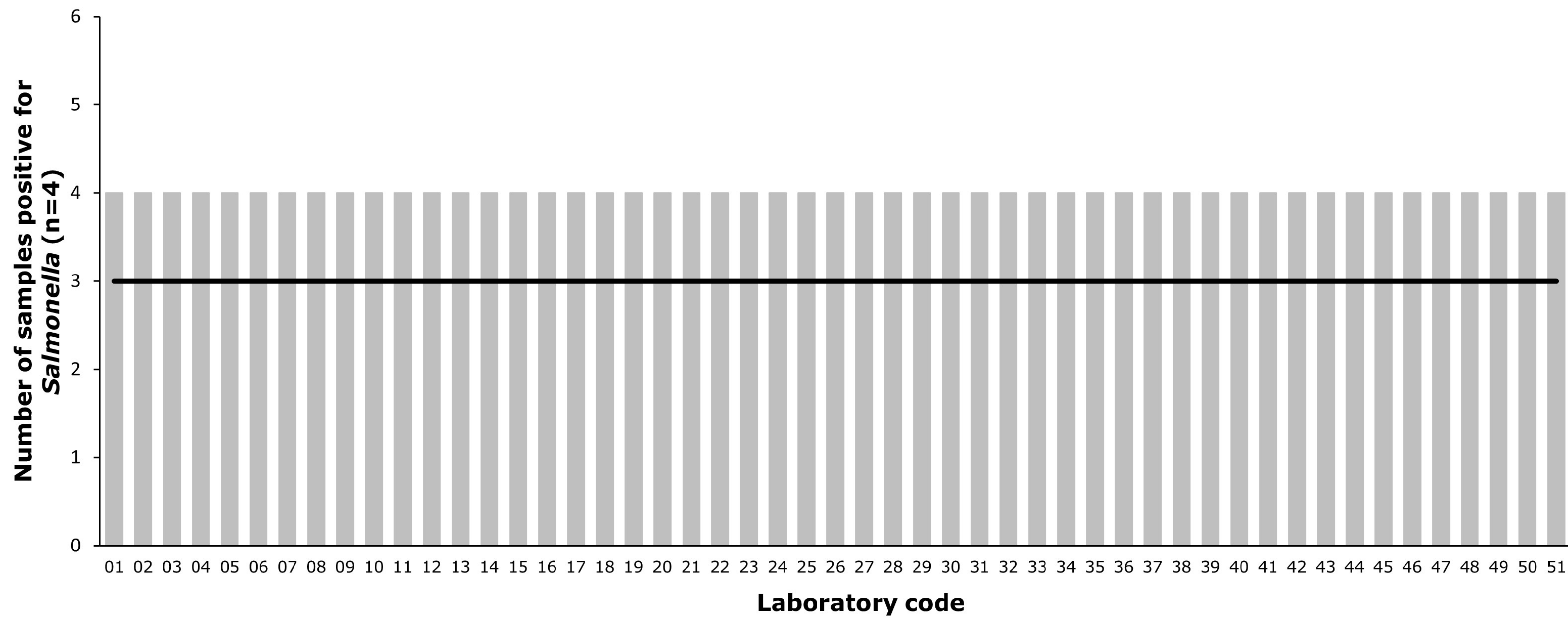

Figure 4.6 Number of flaxseed samples artificially contaminated with a high level of Salmonella *Typhimurium (n=4) that tested positive per laboratory*

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

Table 4.11 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 4.11 Specificity, sensitivity, and accuracy rates calculated from the results found by all participants ('All') and by the participants from EU Member States ('EU MS') only, with the artificially contaminated flaxseed samples**

Flaxseed samples		**All n = 51**	**EU MS n = 42**
Negative (n = 4)	No. of samples	204	168
	No. of negative samples	204	168
	Specificity	**100%**	**100%**
Low level STm (n = 6)	No. of samples	306	252
	No. of positive samples	288	239
	Sensitivity	**94%**	**95%**
High level STm (n = 4)	No. of samples	204	168
	No. of positive samples	204	168
	Sensitivity	**100%**	**100%**
All flaxseed samples artificially contaminated with *Salmonella*	No. of samples	510	420
	No. of positive samples	492	407
	Sensitivity	**96%**	**97%**
All flaxseed samples	No. of samples	714	588
	No. of correct samples	696	575
	Accuracy	**97%**	**98%**

* Laboratory 14 made an administrative error when reporting a result of a negative sample. The specificity and accuracy in this table were calculated using the correct raw data.

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 4.12. Fifteen laboratories used a real-time PCR as an additional method, three laboratories used VIDAS, and one laboratory a PCR. The results of the second detection methods were all similar to the reported results obtained with EN ISO 6579-1:2017(/A1:2020) by the laboratories.

Table 4.12 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
01	SureTect Real-time PCR	Yes	Thermo Fischer Scientific	AOAC 051303, AFNOR UNI 03/07-11/13	2000
02	VIDAS SLM TEST	Yes	AFNOR	BIO 12/10-09/02	N/A
05	qPCR	Yes	-	ABI 29/02-09/10	302
06	VIDAS Rapid *Salmonella*	Yes	National Accreditation Board	AFNOR Bio-12/10-09/02	1900
07	PCR BAX System	Yes	AFNOR	QUA 18/03-11/02 (expiry date: 28-11-2026)	N/A
12	Real-time PCR	Yes	In-house validation, primers according to Josefsen et al. 2007	-	221
13	ELISA - VIDAS UP *Salmonella* (SPT)	Yes	ADRIA Développement - AFNOR certification	Bio 12/32- 10/11	N/A
14	Real-time PCR	Yes	AFNOR	BRD 07/06-07/04	2000
17	qPCR	No	N/A	N/A	N/A
24	qPCR	Yes	AFNOR	BRD 07/06-07/04	N/A
25	qPCR	Yes	AFNOR	BRD 07/06-07/04	N/A
31	AFNOR BRD 07/06 - 07/04	Yes	AFNOR	Certificate No.: BRD 07/06 -07/04	11000
32	AFNOR BRD 07/06 - 07/04	Yes	AFNOR	Certificate No.: BRD 07/06 -07/04	11000
35	Thermo Fisher *Salmonella* Rapidfinder Spp, ST SE	Yes	AFNOR	UNI 03/12-1/18	N/A

Laboratory code	Second detection method	Validated	Validated by	Reference	Number of tests/year, when routinely used
36	Real-time PCR	Yes	§64 of the National Food and Feed Code	Malorny et al.(2004) AEM 70:7046-7052	264
40	PCR	Yes	AFNOR	TRA 02/12-01/09	250
44	Real-time PCR	Yes	In-house validation	ISO 20837:2006, MKS EN ISO 22119-2012	N/A
45	qPCR	No	N/A	N/A	N/A
50	qPCR	Yes	AFNOR	BRD 07/06 -07/04	45

- : no information reported

N/A: Not Applicable

4.6 Performance of the NRLs

4.6.1 General

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

One laboratory (laboratory 14) initially scored a moderate performance, because the laboratory made an administrative error when reporting the results of a negative sample. One of the (negative) samples was mistakenly reported as positive for *Salmonella*. The error was detected by laboratory 14 and communicated with the EURL-*Salmonella* after the reporting-deadline of the PT, but before the intended results were shared with the laboratories. An explanation was given how this error came to light and raw data confirmed the (negative) result of this sample. It was therefore decided to give laboratory 14 a 'Good performance*', with the above additional explanation.

The participants were informed of their results in an individual laboratory Performance report (Annex I) and an interim summary report containing the results of all participants (Diddens and Mooijman, 2023) within 2 months after performing the PT.

5 Conclusions

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

One laboratory scored a 'Good performance*', with additional explanation. The laboratory made an administrative error when reporting the result of a negative sample. The communication was done after the reporting-deadline of the PT, but before the intended results were shared with the laboratories. An explanation was given how this error came to light and raw data confirmed the result.

The accuracy rate of the control samples was 100%.

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

The sensitivity rates of the flaxseed samples artificially contaminated with *Salmonella* Typhimurium was 96%.

The accuracy rate of all flaxseed samples for all participating laboratories was 97%.

Nineteen participants also used a second detection method (real-time PCR, VIDAS and PCR) for analysing the samples. The results of the second detection methods were all similar to the reported results obtained with EN ISO 6579-1:2017(/A1:2020) by the laboratories.

Acknowledgements

The authors would like to thank the technical assistance by Wendy van Overbeek and Ellen Delfgou-van Asch (RIVM) during the preparation of all sample materials.

List of abbreviations

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
STm	*Salmonella* Typhimurium
VRBG	Violet Red Bile Glucose agar
WGS	Whole Genome Sequencing
XLD	Xylose Lysine Deoxycholate agar

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Annex I Example of an individual laboratory Performance report of the EURL-*Salmonella* PT Food-Feed 2023

Performance

EURL-*Salmonella* PT Food-Feed 2023

Detection of *Salmonella* in flaxseed

	Number of positive samples / Total number of samples per level				
	Flaxseed samples			Control samples	
Labcode	High	Low	Negative	BPW	Pos control
#	4/4	6/6	0/4	0/1	1/1

Evaluation: Good performance

Sample	Level	Your detection results	Method and media
A1	Negative	Not detected	Detection method:
A2	High	Detected	EN ISO 6579-1:2017/A1:2020
A3	Negative	Not detected	
A4	Low	Detected	Selective enrichment:
A5	Low	Detected	MKTTn, MSRV and RVS
A6	High	Detected	
A7	Low	Detected	Selective isolation media:
A8	Negative	Not detected	XLD and Rambach
A9	Low	Detected	
A10	High	Detected	
A11	Negative	Not detected	
A12	High	Detected	
A13	Low	Detected	
A14	Low	Detected	
CTRL1	BPW	Not detected	
CTRL2	Pos control	Detected	

High = High concentration *S*. Typhimurium (Inoculation level: 52 cfu / sample)
Low = Low concentration *S*. Typhimurium (Inoculation level: 9 cfu / sample)
Negative = Negative flaxseed sample (no *Salmonella* added)
BPW = Buffered Peptone Water (procedure control)
Pos control = own positive control with *Salmonella*

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