



National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

EURL-Salmonella

Proficiency Test Primary Production Stage 2025

Detection of *Salmonella* in chicken faeces samples

**EURL-*Salmonella* Proficiency Test Primary
Production Stage 2025**

Detection of *Salmonella* in chicken faeces samples

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Colophon

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Synopsis

EURL-*Salmonella* Proficiency Test Primary Production Stage 2025

Detection of *Salmonella* in chicken faeces samples

Each European Union Member State has a specialised laboratory that examines whether the pathogenic *Salmonella* bacterium is present in food or animals. These are the European National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*). These laboratories are obliged to participate in various annual quality monitoring exercises, known as proficiency tests. One of these proficiency tests has the aim of checking whether NRLs are able to detect the *Salmonella* bacterium in samples from the living environment of animals. This year, it was decided to conduct this test in samples of chicken faeces.

In 2025, 34 out of the 35 NRLs-*Salmonella* obtained a good score in this proficiency test. One NRL-*Salmonella* scored a moderate performance, due to an administrative error. The 35 participants in this proficiency test were from 27 European Union Member States, 7 other European countries and 1 non-European country.

The laboratories used an obligatory, internationally accepted method to detect the presence of *Salmonella* in chicken faeces 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, primary production stage, chicken faeces

Publiekssamenvatting

EURL-*Salmonella* ringonderzoek productiedieren 2025

Detectie van *Salmonella* in kippenmestmonsters

Elke lidstaat van de Europese Unie heeft een speciaal laboratorium dat onderzoekt of de ziekmakende *Salmonella*-bacterie in voedsel of dieren zit. Dit zijn de Europese Nationale Referentie Laboratoria voor *Salmonella* (NRL's-*Salmonella*). Deze laboratoria 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 kunnen aantonen in monsters uit de leefomgeving van dieren. Dit jaar is ervoor gekozen om dat in monsters van kippenmest te doen.

In 2025 hebben 34 van de 35 NRL's-*Salmonella* een goede score gehaald in dit ringonderzoek. Eén NRL-*Salmonella* had een matige score door een administratieve fout. De 35 deelnemers aan dit ringonderzoek kwamen uit 27 lidstaten van de Europese Unie, 7 andere Europese landen en 1 niet-Europees land.

De laboratoria gebruikten een verplichte, internationaal erkende analysemethode om *Salmonella* in kippenmestmonsters 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 Europese Unie Referentie Laboratorium voor *Salmonella* (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, productiedieren, kippenmest

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Summary

In June/July 2025, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised a proficiency test (PT) for the detection of *Salmonella* in the primary production stage for the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*). The matrix under analysis was chicken faeces.

NRLs-*Salmonella* that analyse *Salmonella* in samples from the primary production stage were invited to participate in this PT. A total of 35 NRLs-*Salmonella* participated in this EURL-*Salmonella* PT. The participants included NRLs-*Salmonella* located in 27 EU Member States and 8 third countries (EU candidate countries), members of the European Free Trade Association (EFTA), the United Kingdom, and one non-European country).

The most important objective of the PT was to test the participating laboratories' performance in detecting *Salmonella* in the artificially contaminated chicken faeces 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. To this end, chicken faeces samples were artificially contaminated with different (low-level) concentrations of *Salmonella* Typhimurium (STm) and their stability was tested at 2 storage temperatures (5 °C and 10 °C) for a total of 2 weeks' storage. Additionally, the concentration of the natural background flora (aerobic count and number of *Enterobacteriaceae*) in the chicken faeces was measured.

The number of aerobic bacteria and *Enterobacteriaceae* in the chicken faeces samples remained stable when stored at 5 °C. The number of aerobic bacteria in the samples during storage was approximately 10^{10} cfu/25 g chicken faeces. The number of *Enterobacteriaceae* was approximately 10^8 cfu/25 g chicken faeces for 12 days.

On the basis of the results of the pre-tests and the results from previous EURL-*Salmonella* PTs using chicken faeces as a matrix, the aim was to inoculate the low-level chicken faeces samples for the PT with approximately 20 cfu STm/25 g and the high-level chicken faeces samples with approximately 100 cfu STm/25 g.

On Monday 2 June 2025, the EURL-*Salmonella* sent the PT samples to all participants. Each laboratory received 14 samples, each containing 25 g chicken faeces. These samples consisted of four negative samples (no *Salmonella* added), six samples with a low level of STm (inoculum 21 cfu/25 g) and four samples with a high level of STm (inoculum 79 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 their analysis of the samples immediately upon arrival of the parcel, or in the following days. The latest possible date to start the analysis was Tuesday 10 June 2025.

Thirty-four NRLs-*Salmonella* scored a good performance in this PT. One NRL-*Salmonella* (laboratory 33) scored a moderate performance, because of an administrative error.

The specificity rate of the negative chicken faeces samples was 100%. Based on the results of all laboratories, the sensitivity rate of the chicken faeces samples artificially contaminated with *Salmonella* Typhimurium was 95%.

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

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 chicken faeces. 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 June/July 2025. For this PT, the matrix to be analysed was chicken faeces. NRLs-*Salmonella* that are responsible for the detection of *Salmonella* in samples from the primary production stage (PPS) were invited to participate.

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 chicken faeces samples were artificially contaminated with a diluted culture of *Salmonella* Typhimurium (STm) at the EURL-*Salmonella* laboratory.

Fourteen chicken faeces samples were tested by each NRL-*Salmonella*: four negative samples (chicken faeces 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* PT PPS 2025.

Table 2.1 List of participants in the EURL-Salmonella proficiency test primary production stage 2025

Country	City	Institute / NRL-<i>Salmonella</i>
Austria	Graz	Institute for Medical Microbiology and Hygiene Graz, Public Health
Belgium	Brussels	Sciensano, Food Pathogens
Bosnia and Herzegovina	Ilidza	Veterinary Institute - Veterinary Faculty, Laboratory for bacteriology and mycology
Bulgaria	Sofia	National Diagnostic Research Veterinary Institute (NDRVI), NRL- <i>Salmonella</i>
Croatia	Zagreb	Croatian Veterinary Institute, Poultry Centre, Laboratory for Bacteriology
Cyprus	Nicosia	Veterinary Services of Cyprus, EPABP - Pathology, Bacteriology, Parasitology Laboratory
Czech Republic	Prague	Státní veterinární ústav Praha, Bacteriology
Denmark	Ringsted	Danish Veterinary and Food Administration, Microbiology
Estonia	Tartu	The National Centre for Laboratory Research and Risk Assessment (LABRIS), Bacteriology-pathology department
Finland	Kuopio	Finnish Food Authority, Laboratory and Research Division
France	Ploufragan	Anses, Ploufragan – Unité HQPAP (NRL- <i>Salmonella</i>)
Germany	Berlin	German Federal Institute for Risk Assessment (BfR), Biological Safety
Greece	Chalkida	Veterinary Laboratory of Chalkida, NRL- <i>Salmonella</i>
Hungary	Budapest	National Food Chain Safety Office, Food Chain Safety Laboratory Directorate, National Food Microbiological Reference Laboratory
Iceland	Reykjavik	Matis ohf
Ireland	Celbridge, Co Kildare	Department of Agriculture Food and the Marine Laboratories (DAFM), NRL- <i>Salmonella</i>
Israel	Masmiya	Laboratory of the Israel Poultry and Egg Board, <i>Salmonella</i> Surveillance Department

Country	City	Institute / NRL-<i>Salmonella</i>
Italy	Legnaro, Padova	Istituto Zooprofilattico Sperimentale delle Venezie, Centro di Referenza Nazionale per le Salmonellosi
Latvia	Riga	Institute of Food Safety, Animal Health and Environment BIOR, Laboratory of Microbiology and Pathology
Lithuania	Vilnius	National food and veterinary risk assessment institute, Bacteriology section
Luxembourg	Dudelange	Laboratoire vétérinaire et alimentaire, LVA, Microbiologie alimentaire, vétérinaire et OGM
Malta	Valletta	Public Health Laboratory, Health Regulation, Env. Health Directorate
Moldova	Chisinau	National Centre of Animal Health, Plant and Food Safety (NRL) Head of the Animal Health Laboratory, Department of Bacteriology and Parasitology
Netherlands, the	Bilthoven	National Institute for Public Health and the Environment (RIVM), Centre for Zoonoses and Environmental Microbiology (Z&O)
Northern Ireland	NRL tasks carried out by the NRL- <i>Salmonella</i> in Ireland	
Norway	Ås	Norwegian Veterinary Institute, Section of bacteriology
Poland	Puławy	National Veterinary Research Institute (NVRI), Department of Bacteriology and Bacterial Animal Diseases
Portugal	Vairão	Instituto Nacional de Investigação Agrária e Veterinária, I. P., Food Microbiology Laboratory
Romania	Bucharest	Institute for Diagnosis and Animal Health, Bacteriology
Serbia	Novi Sad	Scientific Veterinary Institute "Novi Sad", Department for Clinical Bacteriology, Mycology and parasitology
Slovak Republic	Dolný Kubín	State Veterinary and Food Institute Dolný Kubín, Bacteriology department
Slovenia	Ljubljana	UL, VF, National Veterinary Institute, NRL- <i>Salmonella</i> , Institute of Microbiology and Parasitology
Spain	Algete, Madrid	Laboratorio Central de Veterinaria, Bacteriología 1
Sweden	Uppsala	National Veterinary Agency, Department of Microbiology

Country	City	Institute / NRL-<i>Salmonella</i>
Switzerland	Zürich	Institute for Food Safety and Hygiene, University of Zurich, National Reference Centre for Poultry and Rabbit Diseases (NRGK)
United Kingdom	Addlestone	Animal and Plant Health Agency Weybridge, Bacteriology

3 Materials and methods

3.1 Preparation of artificially contaminated chicken faeces samples

3.1.1 *General*

The matrix used for this PT was chicken faeces. A 25 kg batch of chicken faeces was collected from a *Salmonella*-free broiler breeder flock, arriving at the EURL-*Salmonella* on 7 April 2025. The batch was used to perform the pre-tests as well as to prepare the PT samples. The batch chicken faeces was stored at -20 °C until sample preparation.

Following receipt of the batch of chicken faeces at the EURL-*Salmonella*, the absence of *Salmonella* was checked by analysing a total of ten randomly taken samples of 25 g (defrosted) chicken faeces each, following EN ISO 6579-1:2017(/A1:2020). In brief, to each 25 g test portion, 225 ml of buffered peptone water (BPW) was added. The samples were then mixed by hand for 30 seconds. Following pre-enrichment in BPW, selective enrichment was carried out on modified semi-solid Rappaport-Vassiliadis (MSRV) agar. Subsequently, 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 chicken faeces samples*

Experiments were performed to test the stability of the chicken faeces samples artificially contaminated with a low level of *Salmonella* during storage and transport. The aim was to contaminate the samples with two levels of *Salmonella* and test the stability after 0, 6, 13, and 20 days' storage at 5 °C and 10 °C. For each inoculation level, storage temperature, and timepoint, six artificially contaminated samples were tested for the presence of *Salmonella* following EN ISO 6579-1:2017(/A1:2020) (see section 3.3).

Salmonella Typhimurium (STm) from the American Type Culture Collection (ATCC 14028, Manassas, USA) was chosen to artificially contaminate the 25 g chicken faeces 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 chicken faeces samples with approximately 10 cfu STm/25 g and 15 cfu STm/25 g. The inoculum concentration was determined by streaking the inoculum onto BSA and incubating the plates at 34-38 °C for 24 h ± 3 h.

In addition, negative chicken faeces samples (no *Salmonella* added) were stored at 5 °C and 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 aim was to analyse the samples after 0, 6, 13, and 20 days' storage.

3.1.3 *Preparation of chicken faeces samples for the proficiency test*

In May 2025, the EURL-*Salmonella* weighed approximately 560 subsamples of 25 g chicken faeces each into (plastic) sample bags. Next, 240 and 160 subsamples were individually and artificially contaminated with a low and with a high level of the diluted overnight culture of *S. Typhimurium*, respectively. One hundred sixty 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 chicken faeces (no *Salmonella* added);
- 6 samples, each containing 25 g of chicken faeces with a low level of *Salmonella* Typhimurium, aimed at 20 cfu/25 g;
- 4 samples, each containing 25 g of chicken faeces with a high level of *Salmonella* Typhimurium, aimed at 100 cfu/25 g;

Following artificial contamination, the samples were mixed by hand and stored at 5 °C until transport to the NRLs-*Salmonella* on Monday 2 June 2025.

The inoculum concentration used to artificially contaminate the 25 g chicken faeces samples was determined by streaking the inoculum onto BSA and incubating the plates at 34-38 °C for 24 h ± 3 h.

3.1.4 *Determination of the level of background flora in chicken faeces*

The total number of aerobic bacteria and the number of *Enterobacteriaceae* in the chicken faeces 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 chicken faeces. 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 chicken faeces samples by MPN*

The number of *Salmonella* in the chicken faeces samples was determined at the latest possible date to start the performance of the PT (10 June 2025). This was done by using a five-tube, most probable number (MPN) technique. The MPN technique entails a tenfold dilution of five artificially contaminated chicken faeces 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 version 6 of the freely available Excel-based MPN software (Jarvis et al., 2010).

3.2 **Design of the proficiency test**

3.2.1 *Number and types of samples*

Each participant received 14 (artificially contaminated) chicken faeces 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 proficiency test primary production stage 2025

Contamination level of the chicken faeces samples	Number of samples (n=14)
Negative sample (no <i>Salmonella</i> added)	4
Low level of <i>S. Typhimurium</i>	6
High level of <i>S. Typhimurium</i>	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 into 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 to record 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 into an Excel sheet.

The parcel was sent to the participants on 2 June 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

The NRLs-*Salmonella* could start the analysis of the samples immediately upon arrival of the parcel, or on one of the following days. The latest possible date to start the analysis was Tuesday 10 June 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, it was advised to carry out the following procedure for preparation of the samples (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);

- mix for 30 seconds by hand;
- continue with the non-selective pre-enrichment procedure as described in EN ISO 6579-1:2017(/A1:2020).

The procedure for detection of *Salmonella* in samples from the primary production stage is described in EN ISO 6579-1:2017(/A1:2020); in summary:

- pre-enrichment in:
buffered peptone water (BPW);
- selective enrichment on:
Modified semi-solid Rappaport-Vassiliadis agar (MSRV);
- 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 for each 25 g chicken faeces sample whether *Salmonella* was 'detected' or 'not detected'.

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:

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

3.5 Criteria for good performance

The performance of the NRLs-*Salmonella* was assessed 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 chicken faeces samples 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 <i>S. Typhimurium</i>	≥ 50%	≥ 3 / 6
High level of <i>S. Typhimurium</i>	≥ 75%	≥ 3 / 4

4 Results and discussion

4.1 Preparation of artificially contaminated chicken faeces samples

4.1.1 Pre-tests for the preparation of chicken faeces samples

Prior to performing the pre-tests, the absence of *Salmonella* in the batch of chicken faeces was examined by analysing a total of ten randomly taken 25 g samples. *Salmonella* was not detected in any of these tested samples.

For the pre-tests, subsamples of 25 g chicken faeces each were artificially contaminated with two different concentrations of *Salmonella* Typhimurium. The actual inoculation levels were 11 cfu/25 g of chicken faeces and 14 cfu/25 g of chicken faeces. Figures 4.1 and 4.2 show the results of the stability tests.

Figure 4.1 Stability tests of chicken faeces samples (n=6) artificially contaminated with *S. Typhimurium* at (initial) levels of 11 cfu/25 g and 14 cfu/25 g, stored at 5 °C

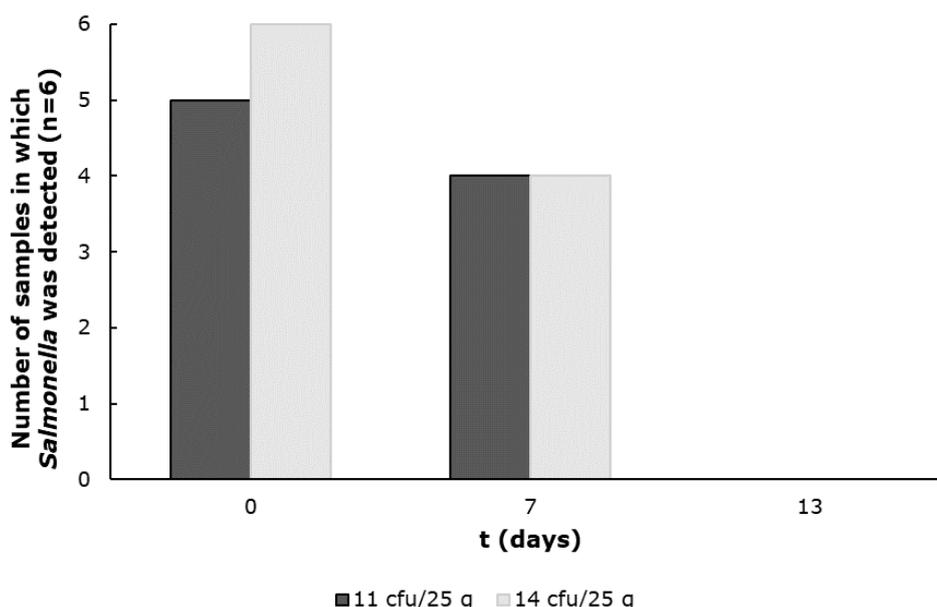
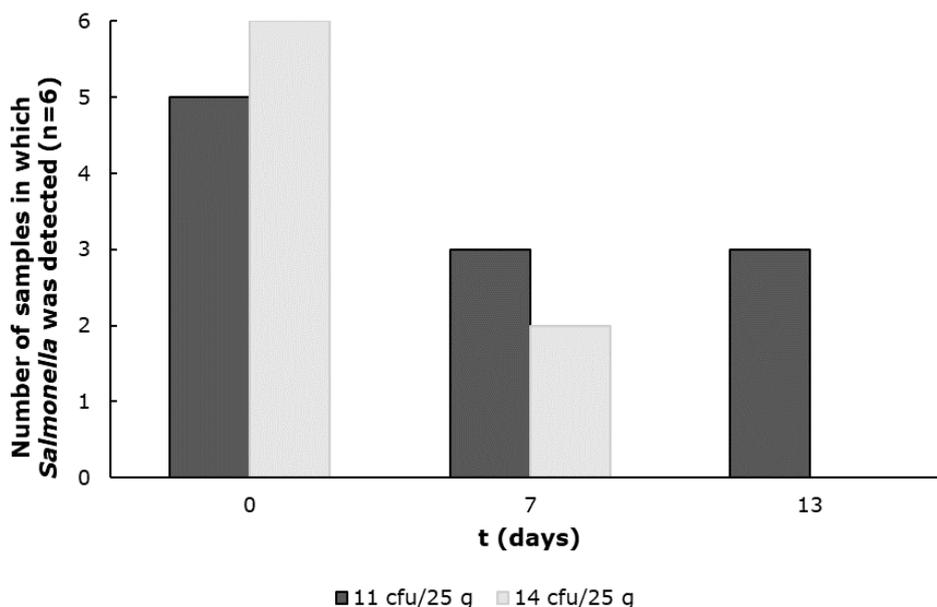


Figure 4.1 shows that none of the chicken faeces samples, for either concentration (11 cfu STm/25 g and 14 cfu STm/25 g), tested positive for *Salmonella* after 13 days' storage at 5 °C.

Similar results are seen for the samples stored at 10 °C. Figure 4.2 shows that after 13 days' storage, only 3 samples at a level of 11 cfu STm/25 g were still found positive for *Salmonella*. However, no *Salmonella* was detected in any of the 6 samples artificially contaminated with 14 cfu STm/25 g.

On the basis of these results, it was decided not to analyse the samples at t = 20 days, and to perform a second pre-test.

Figure 4.2 Stability test of chicken faeces samples (n=6) artificially contaminated with *S. Typhimurium* at (initial) levels of 11 cfu/25 g and 14 cfu/25 g, stored at 10 °C



During the first pre-test, the total number of aerobic bacteria and the number of *Enterobacteriaceae* in the chicken faeces remained stable at approximately 10^{11} cfu/25 g and 10^9 cfu/25 g respectively.

For the second pre-test, the samples were artificially contaminated with a higher concentration of *Salmonella* Typhimurium. The chicken faeces samples were artificially contaminated with 16 cfu/25 g and 27 cfu/25 g. Due to a narrow time frame, the samples were stored at 5 °C only and analysed for the presence of *Salmonella* on t = 0, 5, and 12 days. At every timepoint, the background flora was also tested. The results can be found in Figures 4.3 and 4.4.

In this second pre-test, 3-6 samples still tested positive for *Salmonella* after 12 days' storage at 5 °C (Figure 4.3), whereas in the first pre-test, none of the samples was found positive after 13 days' storage.

Figure 4.4 shows the number of aerobic bacteria and *Enterobacteriaceae* in the chicken faeces samples during storage at 5 °C. The number of aerobic bacteria remained stable at approximately 10^{10} cfu/25 g chicken faeces and the *Enterobacteriaceae* concentration had remained stable at approximately 10^8 cfu/25 g chicken faeces for 12 days.

On the basis of all the results from both pre-tests and of the results from previous PTs using chicken faeces as a matrix (Pol-Hofstad and Mooijman, 2020; Pol-Hofstad and Mooijman, 2024), the aim was to inoculate the low-level chicken faeces samples for the PT with approximately 20 cfu STm/25 g. This relatively high level was also chosen because the concentration of background flora in this batch of chicken faeces was higher than in previous PTs.

For the high-level chicken faeces samples, the aim was 5 times the low-level concentration, i.e. approximately 100 cfu STm/25 g.

Figure 4.3 Stability test of chicken faeces samples (n=6) artificially contaminated with *S. Typhimurium* at (initial) levels of 16 cfu/25 g and 27 cfu/25 g, stored at 5 °C

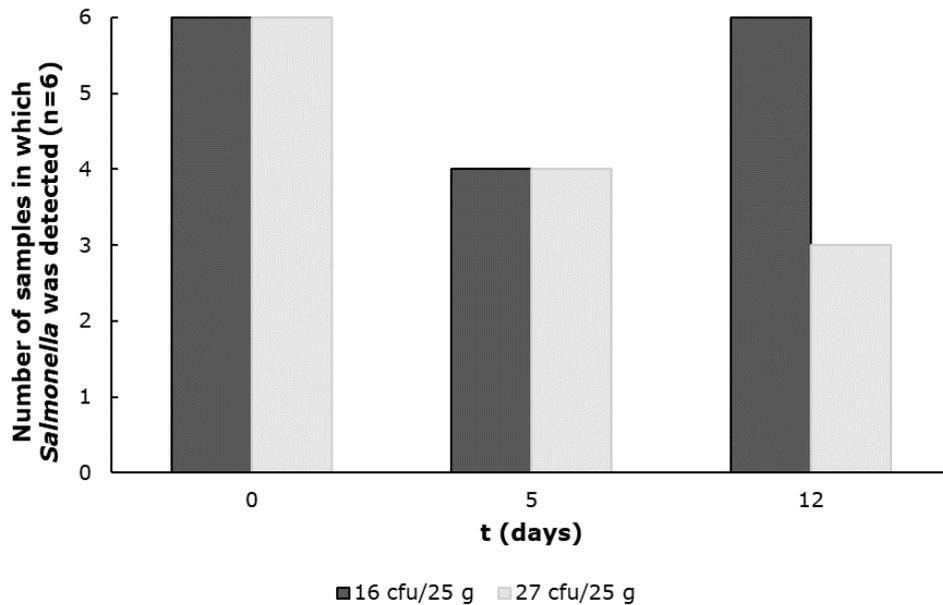
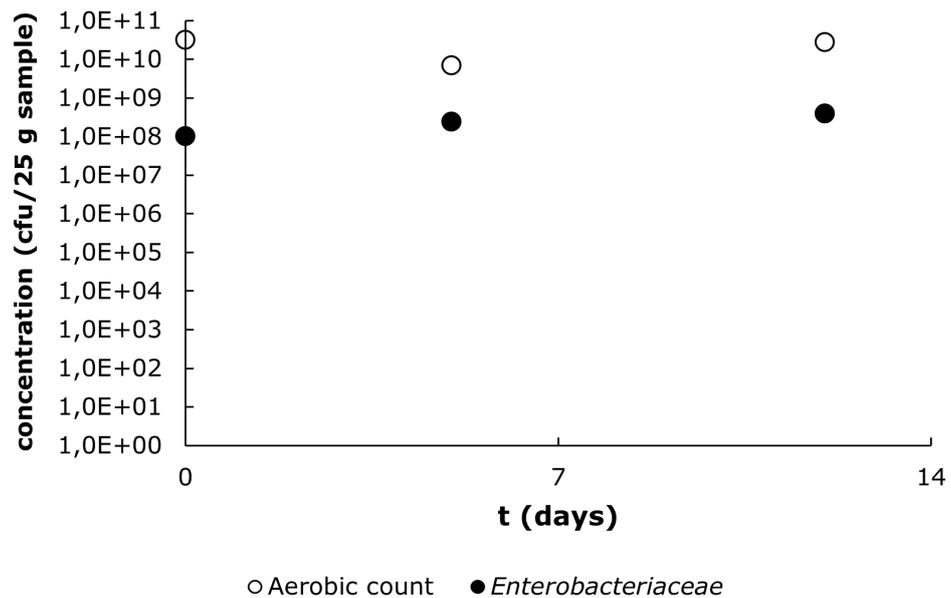


Figure 4.4 The concentration of aerobic bacteria, and Enterobacteriaceae, per 25 g of chicken faeces (negative for Salmonella) after storage at 5 °C for 0, 5, and 12 days



4.1.2 Preparation of chicken faeces samples for the proficiency test

The batch of chicken faeces used for the pre-tests was also used for the preparation of the PT samples. This batch tested negative for *Salmonella* (see section 4.1.1).

The batch of chicken faeces was stored at -20 °C, until one day before the samples were prepared as described in section 3.1.3. The batch of chicken faeces was then stored at 5 °C. The PT samples were artificially contaminated on Tuesday 27 May 2025 and all samples were stored at 5 °C until shipment on Monday 2 June 2025.

4.1.3 Background flora in chicken faeces

The level of natural background flora in the chicken faeces was tested at the EURL-*Salmonella* on 15 April 2025 (one week following receipt of the chicken faeces) and on 10 June 2025, which was the latest possible date to start the PT. Table 4.1 shows the number of aerobic bacteria and *Enterobacteriaceae* in the chicken faeces samples.

Table 4.1 Number of aerobic bacteria and *Enterobacteriaceae* per 25 g of chicken faeces

Date	Aerobic bacteria (cfu/25 g)	<i>Enterobacteriaceae</i> (cfu/25 g)
15 April 2025	$7,7 \times 10^{10}$	$1,3 \times 10^9$
10 June 2025 ^a	$1,0 \times 10^9$	$1,1 \times 10^7$

^a After storage at -20 °C for 5 weeks and at 5 °C for 4 weeks

4.1.4 Number of *Salmonella* in the artificially contaminated chicken faeces samples for the PT

Table 4.2 shows the inoculation levels of the *Salmonella* Typhimurium that has been used to artificially contaminate the chicken faeces samples for the PT. 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 possible date to start the PT. The results show that the concentration of *Salmonella* in the faeces samples, decreased in the course of two weeks' storage at 5 °C. Still, the concentration of *Salmonella* was sufficiently high for use in the PT.

Table 4.2 Number of *Salmonella Typhimurium* in the inoculum for artificial contamination of 25 g chicken faeces samples and in the PT samples after storage at 5 °C for 14 days

Date	Low level STm (cfu/25 g)	High level STm (cfu/25 g)
27 May 2025 Inoculation of chicken faeces samples	21	79
10 June 2025 ^a MPN of chicken faeces samples, inoculated with STm (95% confidence limit)	0,8 (0,2 – 2,5)	8,8 (2,8 – 27,5)

^a Following storage at 5 °C for 2 weeks

4.2 Technical data on the proficiency test

4.2.1 General

In total, 35 NRLs-*Salmonella* participated in this PT, originating from 27 EU Member States and 8 third countries (EU candidate countries, members of the European Free Trade Association (EFTA), the United Kingdom, and one non-European country).

4.2.2 Accreditation and methods used

Thirty-three participants stated they were accredited for EN ISO 6579-1:2017, including amendment 1 published in 2020. One participant stated they were accredited for EN ISO 6579-1:2017 only. One participant (originating from an EU MS) was not accredited, but this laboratory indicated it envisages accreditation in 2026. 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 2 June 2025, the PT samples were shipped to all participants.

Thirty-three parcels were delivered at the NRLs-*Salmonella* within one or two days. One parcel was delivered after seven days on 9 June 2025 (laboratory code 34) and one parcel after eight days on 10 June 2025 (laboratory code 24). Details can be found in Table 4.3.

The temperature during transport and storage was registered using a temperature probe. The temperature of all parcels during transport was below 8,5 °C, except for the parcel for laboratory code 24. The temperature of this parcel was below 8,5 °C, but reached a temperature of 21 °C in the last day of transport to the laboratory. The temperature recorder of laboratory 28 was lost during transport back to the EURL-*Salmonella* and no data could be retrieved.

Table 4.3 Date of arrival of the samples and start of the proficiency test per laboratory

Laboratory code	Date of arrival of the samples	Date of start of PT	Number of days of storage
01	3-6-2025	3-6-2025	-
02	4-6-2025	4-6-2025	-
03	3-6-2025	3-6-2025	-
04	3-6-2025	6-6-2025	3 days
05	4-6-2025	10-6-2025	6 days
06	3-6-2025	3-6-2025	-
07	3-6-2025	3-6-2025	-
08	3-6-2025	10-6-2025	7 days
09	4-6-2025	9-6-2025	5 days
10	3-6-2025	3-6-2025	-
11	3-6-2025	3-6-2025	-
12	3-6-2025	10-6-2025	7 days
13	3-6-2025	3-6-2025	-
14	3-6-2025	3-6-2025	-
15	3-6-2025	4-6-2025	1 day
16	3-6-2025	3-6-2025	-
17	3-6-2025	4-6-2025	1 day
18	4-6-2025	4-6-2025	-
19	4-6-2025	4-6-2025	-
20	3-6-2025	4-6-2025	1 day
21	4-6-2025	4-6-2025	-
22	3-6-2025	3-6-2025	-
23	3-6-2025	4-6-2025	1 day
24	10-6-2025	10-6-2025	-
25	3-6-2025	3-6-2025	-
26	3-6-2025	9-6-2025	6 days
27	3-6-2025	4-6-2025	1 day
28	3-6-2025	3-6-2025	-
29	3-6-2025	5-6-2025	2 days
30	4-6-2025	5-6-2025	1 day
31	3-6-2025	4-6-2025	1 day
32	3-6-2025	4-6-2025	1 day
33	3-6-2025	4-6-2025	1 day
34	9-6-2025	9-6-2025	-
35	3-6-2025	10-6-2025	7 days

- : proficiency test started on the day the samples arrived

Two laboratories (laboratory codes 9 and 21) used three selective enrichment media; MSR/V agar, Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn), and Rappaport-Vassiliadis soya broth (RVS). Two laboratories used MSR/V and MKTTn as selective enrichment media (laboratories 18 and 33), and one laboratory used MSR/V and RVS as selective enrichment media (laboratory code 7). The other thirty laboratories only used MSR/V as the selective enrichment medium.

Table 4.4 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 35) used a longer incubation time than prescribed for the pre-enrichment in BPW. Laboratory 11 reported a deviating concentration of novobiocin, a deviating pH, and an incubation temperature for MSR/V that deviated from what is prescribed in EN ISO 6579-1:2017(/A1:2020). Laboratory 25 also reported a deviating concentration of novobiocin for MSR/V from what is prescribed. Two laboratories (laboratory codes 28 and 33) did not report the pH of MSR/V.

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

Laboratory code	BPW	MSRV		
	incubation (h)	concentration novobiocin (mg/L)	pH	Temperature (°C)
EN ISO 6579-1:2017 (/A1:2020)	18 ± 2 h	10 mg / L	5,1 – 5,4	41,5 °C ± 1 °C
11	17	18 ^a	5,5 ^a	37 ^a
25	20	20 ^a	5,2	41,5
28	19	10	-	41,5
33	20	10	-	41,6
35	24 ^a	10	5,2	41,5

^a : deviations from EN ISO 6579-1:2017(/A1:2020)

- : no information reported

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 4.5. Three laboratories did not report the use of XLD (laboratory codes 11, 17, and 30). Most laboratories used brilliant green agar (BGA) and Rambach as a second isolation medium. Four laboratories used XLD and two additional selective isolation media.

Table 4.5 Second selective solid isolation media used by the participating laboratories

Media	No. of users
ASAP	1
BGA	10
BGA(mod)	3
BPLS	3
BSA	3
BxLH	1
CHROMagar <i>Salmonella</i>	2
Chromogenic <i>Salmonella</i> agar	1
Iris agar	1
Rambach	8
Rapid <i>Salmonella</i> agar	4
<i>Salmonella</i> agar CHROMID	1
<i>Salmonella</i> Differential agar	1

Explanations of the abbreviations used are provided 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.

Twenty-one 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.

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 their positive control samples were *S. Typhimurium* (seven participants), *S. Enteritidis* (seven participants), *S. Nottingham* (five participants), and *S. Infantis* (four participants). Twelve participants used another *Salmonella* serovar. More details are presented in Table 4.7.

The concentration of *Salmonella* in the positive control samples used by the various participants varied widely. For example, thirteen participants used a concentration between 1–10 cfu/sample and four participants used a concentration of 1 000 cfu/sample or higher (see Table 4.8). Three laboratories did not determine the concentration of *Salmonella* used for their positive control samples.

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

Biochemical	Serological	Serotyping	PCR	MALDI-TOF	Number of participants
X					1
X	X				4
X	X	X			6
X	X	X	X		1
X	X	X		X	1
X	X			X	1
X		X			4
X				X	3
	X				2
	X	X			1
	X		X		1
	X			X	2
		X		X	2
				X	6

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

Salmonella serovar	Number of participants
S. Abaetetuba	1
S. Adabraka	1
S. Agbeni	1
S. Alachua	1
S. Blegdam	1
S. Braenderup	1
S. Enteritidis	7
S. Harleystreet	1
S. Infantis	4
S. Nottingham	5
S. Regent	1
S. Tennessee	1
S. Tranoroa	1
S. Typhimurium	7
S. Weltevreden	1
S. bongori serovar 66:z41:-	1

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

Concentration <i>Salmonella</i> (cfu/sample)	Number of participants
1 - 10	13
11 - 20	3
21 - 120	10
200 - 500	2
≥ 1 000	4
Not Determined	3

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. This way, possible cross-contamination can be detected more easily.

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. Seven laboratories (laboratory codes 5, 7, 10, 12, 22, 26, and 32) reported the use of a matrix in combination with their positive control. These laboratories used the following matrices with their positive controls: animal feed, chicken faeces and pig faeces.

4.4 Artificially contaminated chicken faeces samples

4.4.1 General

Table 4.9 shows the results of the tested chicken faeces samples. It shows that the higher storage temperature of the samples from laboratories 5, 19, and 30, or the technical deviations (see section 4.2.4), did not substantially influence the final results.

4.4.2 Negative samples

Thirty-four laboratories scored all four negative samples correctly: *Salmonella* was not detected. Only laboratory 33 reported a negative sample as positive. The laboratory was contacted by the EURL-*Salmonella* for an explanation. Raw data showed that an administrative error was made in a worksheet as a result of which one negative sample was mistakenly reported as positive. The raw data showed that the result of the negative samples was, in fact, correctly negative for *Salmonella*.

Table 4.9 Number of (artificially contaminated) chicken faeces samples tested positive for *Salmonella* at each laboratory

Laboratory code	Number of samples in which <i>Salmonella</i> is detected		
	Negative n = 4	Low level STm n = 6	High level STm n = 4
1, 9, 25, and 28	0	4	4
24	0	5	3
12, 13, 14, 23, 33 ^a , and 35	0	5	4
All other NRLs- <i>Salmonella</i> (n = 24)	0	6	4
Criteria for good performance	0	≥3	≥3

^a : Laboratory 33 made an administrative error, mistakenly reporting a negative sample as positive for *Salmonella*. The number of samples in which *Salmonella* was detected, as presented in this table, is based on the correct raw data.

4.4.3

Low-level contaminated Salmonella chicken faeces samples

Twenty-four laboratories detected *Salmonella* in all six low-level contaminated chicken faeces samples. Seven laboratories detected *Salmonella* in five out of six low-level contaminated samples, and four laboratories 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 out of the six samples. See Figure 4.6.

4.4.4

High-level contaminated Salmonella chicken faeces samples

All laboratories but one, detected *Salmonella* in all four chicken faeces samples artificially contaminated with a high level of *Salmonella* Typhimurium. Laboratory 24 detected *Salmonella* in three out of four high-level contaminated samples, which is still within the criteria for good performance. The results of all laboratories are shown in Figure 4.7.

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

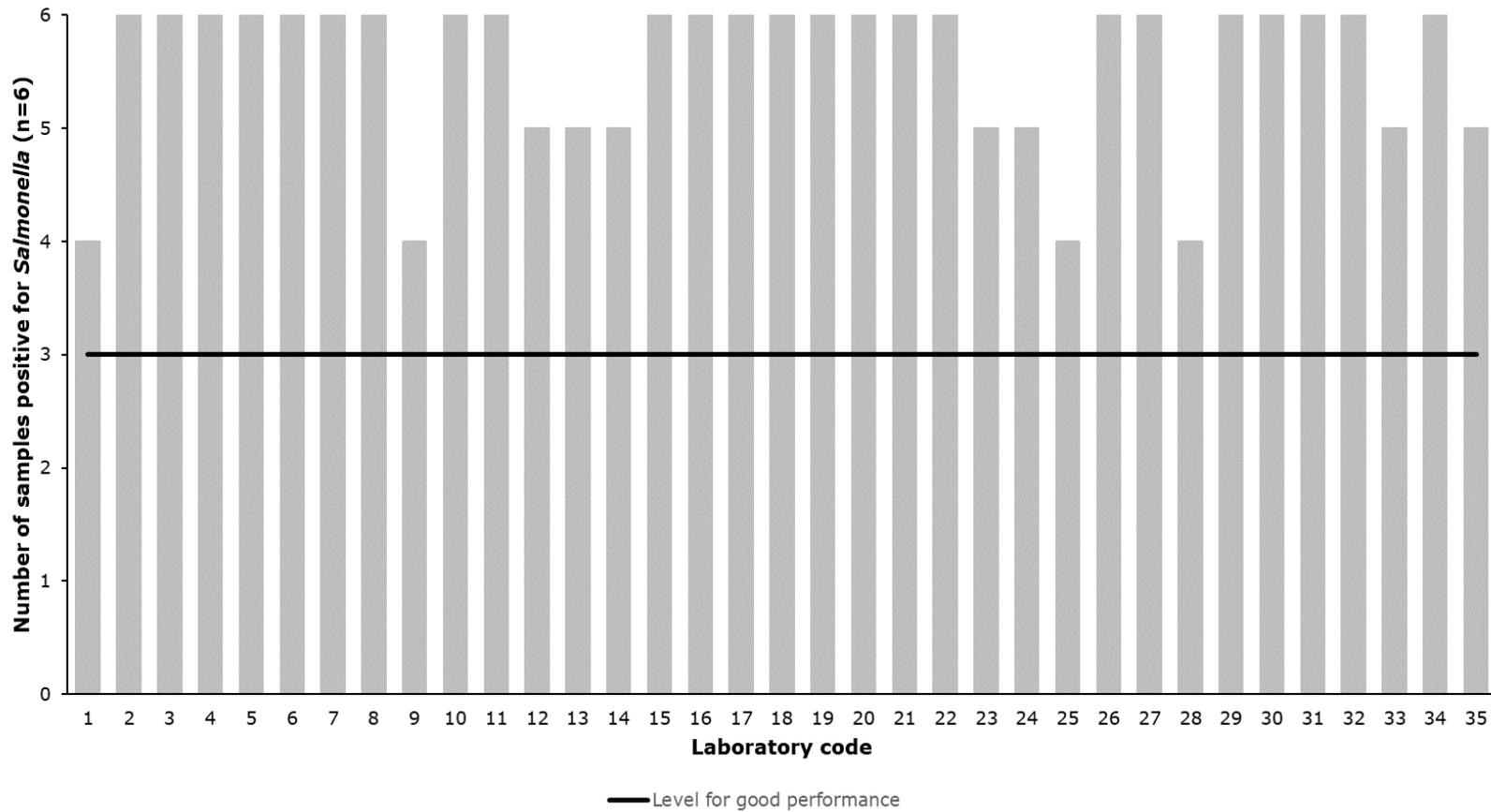
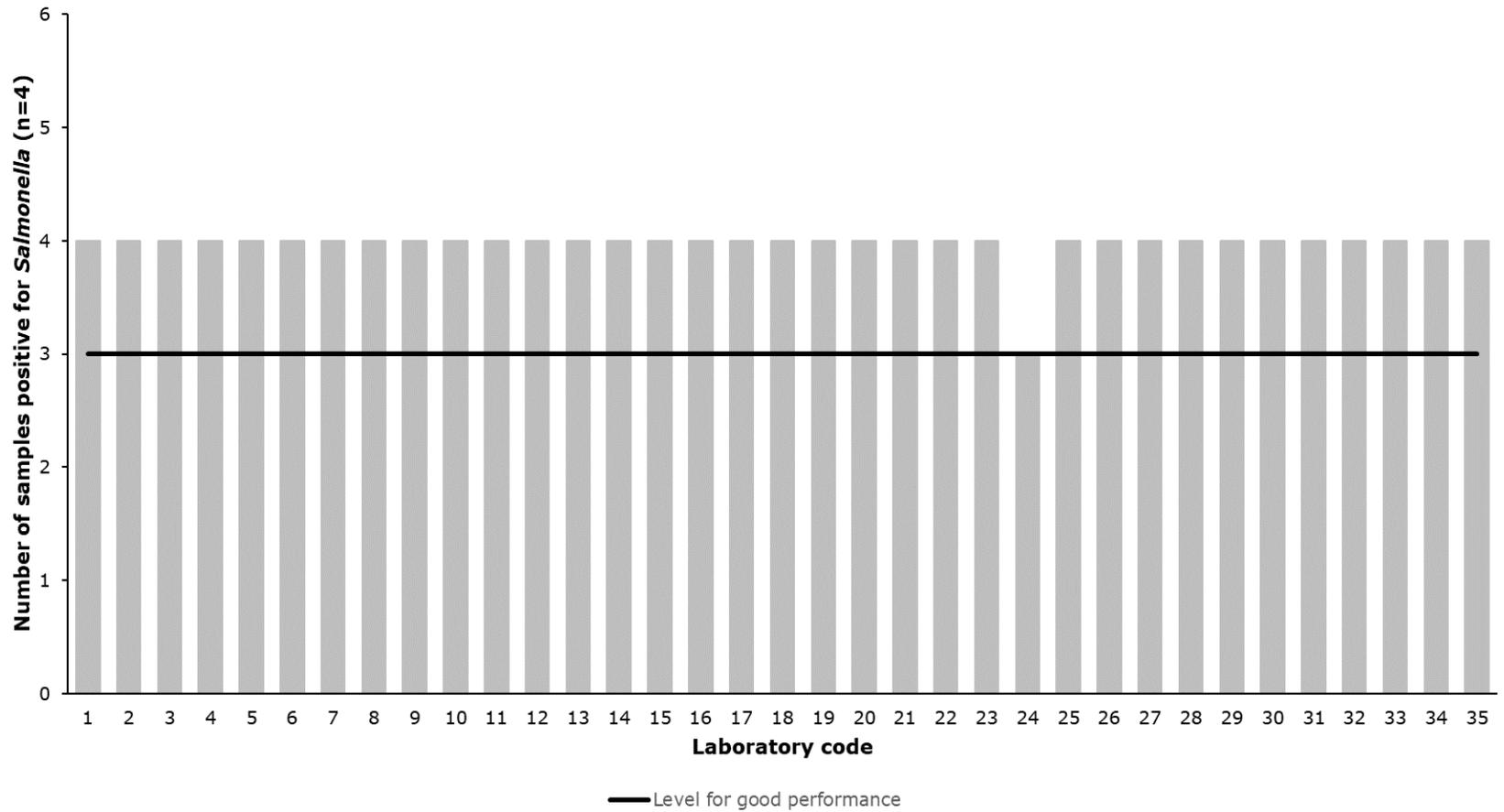


Figure 4.7 Number of chicken faeces samples artificially contaminated with a high level of Salmonella Typhimurium (n=4) that tested positive for Salmonella, per laboratory



4.4.5 Specificity, sensitivity, and accuracy rates of the (artificially contaminated) chicken faeces samples

Table 4.10 shows the specificity, sensitivity, and accuracy rates of the chicken faeces samples tested in this PT. The calculations were performed on the results from all participating laboratories and on those from the participants from EU Member States only.

Table 4.10 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 for the (artificially contaminated) chicken faeces samples*

Chicken faeces samples		All n = 35	EU MS n = 27
Negative (n = 4)	No. of samples	140	108
	No. of negative samples	140	108
	Specificity	100%	100%
Low level of STm (n = 6)	No. of samples	210	162
	No. of positive samples	195	153
	Sensitivity	93%	94%
High level of STm (n = 4)	No. of samples	140	108
	No. of positive samples	139	108
	Sensitivity	99%	100%
All chicken faeces samples artificially contaminated with <i>Salmonella</i>	No. of samples	350	270
	No. of positive samples	334	261
	Sensitivity	95%	97%
All chicken faeces samples	No. of samples	490	378
	No. of correct samples	474	369
	Accuracy	97%	98%

* Laboratory 33 made an administrative error, mistakenly reporting a negative sample as positive for *Salmonella*. The specificity and accuracy in this table were calculated using the correct raw data.

4.5 Second detection method

Five participants also used a second detection method for analysing the samples, but the results of this second method were not used to assess their performance. An overview of the methods used per laboratory is presented in Table 4.11.

Three laboratories used a real-time PCR as an additional method, one laboratory used a PCR, and one laboratory did not specify the method used. 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 34 tested three out of four high-level contaminated chicken faeces samples positive for *Salmonella* with the second detection method, whereas they tested four out of the four high-level contaminated chicken faeces samples positive with EN ISO 6579-1:2017(/A1:2020).

Table 4.11 Details of the second detection methods used by participants during the proficiency test on detection of *Salmonella* in chicken faeces samples

Laboratory code	Second detection method	Validated	Validated by	Reference	Number of tests/year, when routinely used
16	Real-time PCR	Yes	§64 of the National Food and Feed Code	Malorny et al. (2004) AEM 70:7046-7052	180
18	Real-time PCR	Yes	In-house	MA-VP-15	N/A
24	PCR	No	N/A	N/A	N/A
29	Not specified	Yes	National Accreditation Board	LA. 01.139	N/A
34	Real-time PCR	Yes	National Accreditation Board	EN ISO 22119:2011	1090

N/A: Not Applicable

4.6 Performance of the NRLs-*Salmonella*

Within 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).

Thirty-four laboratories fulfilled the criteria for good performance. Laboratory 33 initially scored an unsatisfactory performance, because they reported to have detected *Salmonella* in a negative sample. Raw data showed that an administrative error was made in a worksheet as a result of which one negative sample was mistakenly reported as positive for *Salmonella*. The raw data showed that the result of the negative samples was, in fact, correctly negative for *Salmonella*. For that reason, the performance of laboratory 33 was adjusted from unsatisfactory to moderate.

5 Conclusion

Thirty-four NRLs-*Salmonella* fulfilled the criteria for good performance in the EURL-*Salmonella* proficiency test for the detection of *Salmonella* in chicken faeces samples. Laboratory 33 scored a moderate performance. The NRLs-*Salmonella* originated from 27 EU Member States and 8 third countries (EU candidate countries, members of the European Free Trade Association (EFTA), the United Kingdom, and one non-European country).

The specificity rate of the negative chicken faeces samples was 100%.

Based on the results of all laboratories, the sensitivity rate of the chicken faeces samples artificially contaminated with *Salmonella* Typhimurium was 95%.

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

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List of acronyms

AEM	Applied and Environmental Microbiology
ASAP	AES <i>Salmonella</i> 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 <i>Salmonella</i> agar
BxLH	brilliant green xylose lysine sulphonamide
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
PPS	primary production stage
PT	proficiency test
RIVM	Rijksinstituut voor Volksgezondheid en het Milieu (National Institute for Public Health and the Environment)
RVS	Rappaport-Vassiliadis soya broth
spp.	species
STm	<i>Salmonella</i> Typhimurium
VRBG	violet red bile glucose agar
XLD	xylose lysine deoxycholate agar

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