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EURL-*Salmonella* Combined Proficiency Test- Interlaboratory Study for Primary Production Stage and Food 2024

Detection of *Salmonella* in fabric swabs

RIVM report 2025-0036
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Colophon

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Synopsis

EURL-*Salmonella* combined Proficiency Test- Interlaboratory Study for Primary Production Stage and Food 2024

Detection of *Salmonella* in fabric swabs

Since 1992, the National Reference Laboratories (NRLs) from the EU Member States have been obliged to take part in an annual quality control, which consists of conducting 'proficiency tests'. The objective of these proficiency tests is to detect *Salmonella* bacteria in samples taken from the living environment of animals, such as stables or the food and food producing environments. In 2024, almost all NRLs from the EU Member States were able to detect *Salmonella* in fabric swab samples. With one exception, all participating laboratories were able to detect both high and low concentrations of *Salmonella.*

One laboratory did not detect *Salmonella* in all four fabric swab samples with low concentrations; however, they proved that they were able to detect *Salmonella* in samples with extra-low levels of *Salmonella*. Therefore, it was not necessary to organise a follow-up study with extra samples. The performance of two laboratories was not evaluated because of the very long transport time of the samples – 25 days – in which *Salmonella* bacteria did not survive. This was the outcome of the proficiency test organised by the European Union Reference laboratory in October 2024.

In total, 68 laboratories took part in this proficiency test. They included the NRLs from the 27 EU Member States, 13 NRLs from other European countries and one NRL from non-European countries. The laboratories used a mandatory, internationally recognised analytical method to detect *Salmonella* in fabric swab samples. Each laboratory package contained samples that had either been artificially contaminated with three different concentrations of *Salmonella* Typhimurium or had not been contaminated at all.
The proficiency test was organised by the European Union Reference Laboratory (EURL) for *Salmonella*, which is located at RIVM. One of the EURL-*Salmonella*s key tasks is to monitor the quality of the *Salmonella* NRLs in Europe.

Keywords: *Salmonella*, EURL, NRL, proficiency test, interlaboratory study, fabric swabs, *Salmonella* detection method

Publiekssamenvatting

EURL-*Salmonella* gecombineerd ringonderzoek – interlaboratorium-studie productiedieren en voedsel 2024

Detectie van *Salmonella* in veegdoekjes

Sinds 1992 zijn de Nationale Referentie Laboratoria (NRL's) van de Europese lidstaten verplicht om elk jaar hun kwaliteit te laten toetsen met zogeheten ringonderzoeken. Het doel van dit ringonderzoek is *Salmonella* bacteriën opsporen in monsters uit de leefomgeving van dieren, zoals stallen en voedsel of voedselproductieomgeving. In 2024 waren bijna alle NRL's uit de EU-lidstaten in staat om *Salmonella* aan te tonen in veegdoekjes. Op één na konden alle deelnemers hoge en lage concentraties *Salmonella* aantonen.

Daarnaast zijn er in de interlaboratoriumstudie ook monsters getest met een extra lage concentratie *Salmonella*. Deze bevatten slechts één *Salmonella* bacterie per veegdoekje. Deze monsters waren vooral bedoeld om de kwaliteit van de methode zelf te testen.

Eén laboratorium had moeite om *Salmonella* aan te tonen in veegdoekjes met een lage concentratie *Salmonella;* dit laboratorium was wel in staat om *Salmonella* aan te tonen in veegdoekjes met een extra lage concentratie *Salmonella*. Een vervolgstudie was daarom niet nodig. De kwaliteit van één van de laboratoria kon niet worden getest omdat de toegestuurde monsters 25 dagen onderweg zijn geweest. Hierdoor kon *Salmonella* afsterven tijdens het transport. Dit blijkt uit het ringonderzoek dat het overkoepelende Europese laboratorium in oktober 2024 organiseerde.

In totaal hebben 68 NRL's aan dit ringonderzoek meegedaan. Dat zijn de NRL's uit de 27 Europese lidstaten en 14 NRL's uit andere landen. De laboratoria gebruikten een verplichte, internationaal erkende analysemethode om *Salmonella* in veegdoekjes aan te tonen. Elk laboratorium kreeg een pakket toegestuurd met 16 veegdoekjes die kunstmatig waren besmet met drie verschillende concentraties *Salmonella* Typhimurium of zonder deze bacterie.

Het ringonderzoek is georganiseerd door het Europese Referentie Laboratorium (EURL) voor *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, interlaboratorium studie, veegdoekjes, *Salmonella*-detectiemethode

Contents

Summary

In October 2024, the European Union Reference Laboratory (EURL) for *Salmonella* combined Proficiency Test (PT) and Interlaboratory study (ILS) on the detection of *Salmonella* in samples from the Primary Production Stage (PPS) and Food was conducted. Participation was mandatory for the National Reference Laboratories (NRLs) for *Salmonella* of all European Union (EU) Member States (MSs) that are responsible for the detection of *Salmonella* in PPS samples and/or in Food samples. A total of 68 NRLs-*Salmonella* participated in this study: 37 NRLs for PPS and 31 NRLs for Food, originating from 37 countries. 54 participants originated from the 27 EU Member States, 13 NRLs from other European countries (EU candidate MSs or potential EU candidate MSs, members of the European Free Trade Association (EFTA) and third countries), and one NRL from a non-European country.

Samples

In this study, the matrix under analysis was fabric swabs, artificially contaminated in the EURL-*Salmonella* laboratory with a diluted culture of *Salmonella* Typhimurium (STm).

Each NRL-*Salmonella* had to analyse the following set of blindly coded samples:

- 3 negative fabric swab samples (no *Salmonella* added);
- 3 fabric swab samples with a high-level of *Salmonella* Typhimurium (STm);
- 4 fabric swab samples with a low-level of *Salmonella* Typhimurium (STm);
- 6 fabric swab samples with an extra-low-level of *Salmonella* Typhimurium (STm).

The samples were prepared at the EURL-*Salmonella* laboratory and stored at 5 °C for approximately one week until the day of dispatch. On Monday 30 September 2024, the fabric swab samples were packed and sent to the NRLs-*Salmonella*. The NRLs were asked to store the samples at 5 °C upon arrival until the start of the analysis. The laboratories were asked to start the analyses on Wednesday 2 October 2024 and no later than Monday 7 October 2024.
The six extra-low-level samples were included in this study to allow the calculation of performance characteristics of the method (ILS part of the study). The results of these samples were not used for evaluation of the performance of the participants (PT part of the study).

Method

All laboratories used the prescribed method EN ISO 6579-1:2017(/A1:2020) to test the samples. Only two participating NRLs were not accredited for this method (lab codes 37 and 38).

Twenty participating NRLs also reported results for a second method. For the majority of the participants, the results of the alternative methods were identical to those obtained using EN ISO 6579-

1:2017(/A1:2020), except for sample B4. Two NRLs (lab codes 41 and 42) did not detect *Salmonella* in this sample when using the alternative method, while *Salmonella* was detected using the bacteriological culture method.

Results

Almost all 68 participants detected *Salmonella* in two or more out of the four fabric swab samples contaminated with a low level of *Salmonella* Typhimurium (4,5 cfu/sample). One laboratory (lab code 61) scored an unsatisfactory performance as this laboratory tested three out of the four low-level samples negative for *Salmonella*. These results do not fall within the criteria for good performance, which permit two negative samples. The sensitivity rate for these samples was 92,3%.

Almost all participants detected *Salmonella* in all three samples contaminated with a high level of *Salmonella* Typhimurium (37,5 cfu/sample). Two NRLs (lab codes 53 and 54) tested two out of the three high-level samples negative for *Salmonella*. This does not fall within the criteria for good performance. The sensitivity rate for these samples was 96,6%.

All participants correctly scored all three negative samples as negative. The specificity rate of the negative samples was 100%.

Overall, the NRLs-*Salmonella* scored well in this Proficiency Test, with an accuracy of 95,9%. One participant (lab code 61) scored an unsatisfactory performance because of problems with detecting *Salmonella* in the low-level samples. However, this participant proved their ability to detect *Salmonella* in low concentrations through their results in the extra-low-level samples. This laboratory tested three out of the six extra-low-level samples, with a concentration of 1,2 cfu *Salmonella* per sample, positive. Taking these results into account, the performance of this participant was amended to moderate performance. The performance of two NRLs scoring two out of three high-level samples negative was not evaluated due to the samples' very long transport time. It is likely that this negatively affected the quality of the samples.

1 Introduction

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

In October 2024, the EURL-*Salmonella* conducted a PT to evaluate whether the NRLs responsible for the detection of *Salmonella* in samples from the Primary Production Stage (PPS) and/or Food could detect *Salmonella* at different contamination levels in fabric swabs. The results from PTs such as this one show whether the examination of samples in the EU Member States (EU-MSs) is carried out uniformly and whether all NRLs-*Salmonella* obtain comparable results.
The method prescribed in this PT for the detection of *Salmonella* spp. is set out in EN ISO 6579-1:2017(/A1:2020).

At the same time of the preparation of this EURL-*Salmonella* PT, a discussion was going on in the ISO subcommittee for microbiology of the food chain (ISO/TC34/SC9), that ISO methods for frequently tested pathogens such as *Salmonella* should be validated for all relevant product categories. EN ISO 6579-1(/A1:2020) is validated for several product categories, but for some product categories, performance characteristics are still missing. In literature, several validation studies were found from which data could be used to calculate the missing performance characteristics. However, performance characteristics were still lacking for the category 'environmental samples from the Food or Feed production', as well as for the use of modified semi-solid Rappaport-Vassiliadis (MSRV) agar as a (second) selective enrichment medium for analysing food, animal feed, or environmental samples.

The choice of the matrix for the EURL-*Salmonella* PT 2024 was fabric swabs, which form part of the category 'environmental samples from the Food or Feed production'. Additionally, it was expected that several NRLs-*Salmonella* for food would use MSRV agar as second selective enrichment medium. It was, therefore, discussed with ISO/TC34/SC9 whether it would be possible to combine this EURL-*Salmonella* PT with a so-called interlaboratory study (ILS) to also generate data for the determination of performance characteristics for this missing product category. However, the set-up for the number and type of samples, as well as the aim of an interlaboratory study differs from that of the EURL-*Salmonella* PTs. In a PT, the performance of the participating laboratories is tested, while in an ILS, the performance of the method is tested. Therefore, a compromise was sought.

The general design of the EURL-*Salmonella* PTs is based on information described in EN ISO 22117:2019. Examples of EURL-*Salmonella* PTs can be found in the reports of PTs conducted earlier, e.g. see Diddens &

Mooijman, 2023; Pol-Hofstad & Mooijman, 2023 and Pol-Hofstad & Mooijman, 2024. In general:

- There are approximately 30-35 participating NRLs-*Salmonella* (from different organisations);
- Participants originate from 30-35 different European countries;
- Usually, a total of 14 samples has to be tested by each laboratory, consisting of a Food/Feed/PPS matrix:
  - 6 samples artificially contaminated with a low level of *Salmonella* (approximately 10 cfu/sample);
  - 4 samples artificially contaminated with a high level of *Salmonella* (approximately 50 cfu/sample);
  - 4 negative matrix samples (no *Salmonella* added).
- Resulting in a total of 420-490 results: 180-210 results for low level samples and 120-140 results for high level samples and 120-140 results for negative samples.

The design of an ILS is described in EN ISO 17468:2023. For qualitative methods, this protocol includes:

- There are at least 10 valid datasets from at least 10 participants;
- Participants from at least 5 different organisations;
- Participants from at least 2 different countries;
- Maximum of 3 datasets per organisation;
- A total of 24 samples has to be tested by each laboratory, consisting of a Food/Feed/PPS matrix:
  - 8 samples artificially contaminated with a (very) low level of the target organisms (approximately 1 cfu/sample), to obtain fractional recovery (25-75% positive);
  - 8 samples artificially contaminated with a 'high' level of the target organism (approximately 5-10x low level);
  - 8 negative matrix samples (no target organism added).
- In total at least 240 results needed (80 results per level).

The main differences between the designs of the ILS and the EURL-*Salmonella* PT are the number of samples to be tested and the contamination level of the low-level samples. After discussion with ISO/TC34/SC9, a compromise was found for the number and type of samples for a combined PT-ILS.
For the current study, fabric swabs were artificially contaminated with a combination of *Citrobacter freundii* and *Escherichia coli* to mimic background flora in natural samples. In addition, the fabric swab samples were contaminated with a diluted culture of *Salmonella* Typhimurium (STm) at the EURL-*Salmonella* laboratory. In total, 16 (blindly coded) samples had to be tested by each laboratory:

- 3 negative fabric swab samples (no *Salmonella* added);
- 3 fabric swab samples with a high-level of STm;
- 4 fabric swab samples with a low-level of STm;
- 6 fabric swab samples with an extra-low level STm.

For the evaluation of the performance of the laboratories, the results of the negative samples, the high-level samples and the low-level samples were used. To determine the performance characteristics of EN ISO 6579-1, the results of all samples were used.

2 Participants

In Table 2.1 the countries, cities and the institute names of the participating NRLs for detection of *Salmonella* in Primary Production Stage samples (PPS) are displayed. In Table 2.2, this is displayed for the NRLs-*Salmonella* Food.

*Table 2.1 List of participating NRLs-*Salmonella *Primary Production Stage*

Country	City	Institute
Austria	Graz	Austrian Agency for Health and Food Safety (AGES/VEMI)
Belgium	Brussels	Sciensano
Bosnia and Herzegovina	Ilidza	Veterinary Institute - Veterinary Faculty; Laboratory for bacteriology and mycology
Bulgaria	Sofia	National Diagnostic and Research Veterinary Institute (NDRVMI), National Reference Centre of Food Safety
Croatia	Zagreb	Croatian Veterinary Institute, Poultry Centre,
Cyprus	Nicosia	Cyprus Veterinary Services, Laboratory for General Bacteriology and Microbiology
Czech Republic	Praha	State Veterinary Institute
Denmark	Ringsted	Danish Veterinary and Food administration
Estonia	Tartu	National Centre for Laboratory Research and Risk Assessment (LABRIS)
Finland	Kuopio	Finnish Food Authority,
France	Ploufragan	Anses, Laboratoire de Ploufragan-Plouzané, Unité Hygiène et Qualité des Produits Avicoles et Porcins (HQPAP)
Germany	Berlin	German Federal Institute for Risk Assessment (BfR)
Greece	Chalkida	Laboratory and Research Division
Hungary	Budapest	National Food Chain Safety Office, Food and Feed Safety Directorate, Microbiological NRL
Ireland, Republic of	Kildare	Central Veterinary Research Laboratory (CVRL/DAFFM), Laboratories Backweston, Department of Bacteriology
Israel	Kiryat Malachi	Laboratory of the Israel Poultry and Egg Board
Italy	Padova Legnaro	Istituto Zooprofilattico Sperimentale delle Venezie, OIE
Kosovo	Pristina	Food and Veterinary Laboratory
Latvia	Riga	Institute of Food Safety, Animal Health and Environment BIOR, Bacteriology and Parasitology Division

Country	City	Institute
Lithuania	Vilnius	National Food and Veterinary Risk Assessment Institute, Laboratory of Microbiology and Pathology, Bacteriology Group
Luxembourg, Grand-Duchy of	Diddeléng	Laboratoire de Médicine Vétérinaire de l'État, Bactériologie
Malta	Valletta	Malta Public Health Laboratory (PHL), Evans Building
Netherlands, the	Bilthoven	National Institute for Public Health and the Environment (RIVM), Centre for Zoonosis and Environmental Microbiology (Z&O)
Northern Ireland	NRL tasks PPS are carried out by NRL Ireland	
Norway	Ås	Norwegian Veterinary Institute, Section of Microbiology
Northern Ireland	NRL tasks carried out by NRL Ireland (NRL PPS)	
Poland	Pulawy	National Veterinary Research Institute, department of microbiology
Portugal	Vairão	Instituto Nacional de Investigação Agrária e Veterinária, Food Microbiology Laboratory
Republic of Moldova	Chisinau	National Centre for Animal Health, Plant and Food Safety
Romania	Bucharest	Institute for Diagnosis and Animal Health
Serbia	Novi Sad	Scientific Veterinary Institute 'Novi Sad'
Slovak Republic	Dolny Kubin	State Veterinary and Food Institute
Slovenia	Ljubljana	National Veterinary Institute, Veterinary Faculty (UL, NVI)
Spain	Madrid, Algete	Laboratorio Central de Veterinaria
Spain	Lugo	Laboratorio Nacional de Sanidad Vegetal
Sweden	Uppsala	Swedish Veterinary Agency
Switzerland	Zurich	National Reference Centre for Poultry and Rabbit Diseases (NRGK), Institute of Food Safety and Hygiene, University of Zurich
United Kingdom	Addleston	Animal and Plant Health Agency

Table 2.2 List of Participating NRLs-Salmonella Food

Country	City	Institute
Austria	Graz	Austrian Agency for Health and Food Safety (AGES/VEMI)
Belgium	Brussels	Sciensano
Bulgaria	Sophia	National Diagnostic and Research Veterinary Institute (NDRVMI), National Reference Centre of Food Safety
Croatia	Zagreb	Croatian Veterinary Institute, Laboratory for Food Microbiology (CVI)
Cyprus	Nicosia	Cyprus Veterinary Services

Country	City	Institute
Czech Republic	Prague	State Veterinary Institute (SVI)
Denmark	Ringsted	Danish Veterinary and Food Administration, Department of Microbiology
Estonia	Tartu	National Centre for Laboratory Research and Risk Assessment (LABRIS)
Finland	Helsinki	Finnish Food Authority, Laboratory and Research Division
France	Ploufragan	ANSES Laboratoire de Ploufragan-Plouzané, Unité Hygiène et Qualité des Produits Avicoles et Porcins (HQPAP)
Germany	Berlin	German Federal Institute for Risk Assessment (BfR)
Greece	Chalkida	Veterinary Laboratory of Chalkida
Hungary	Budapest	National Food Chain Safety Office, Food Chain Safety Laboratory Directorate, Microbiological NRL
Iceland	Reykjavik	Matís ohf, Food Safety and Analytical services
Italy	Legnaro PD	Istituto Zooprofilattico Sperimentale delle Venezie, OIE
Kosovo	Pristina	Food and Veterinary Laboratory
Latvia	Riga	Institute of Food Safety, Animal Health and Environment, BIOR, Microbiology and Pathology Laboratory
Lithuania	Vilnius	National Food and Veterinary Risk Assessment Institute, Bacteriology Unit
Luxembourg	Dudelange	Laboratoire National de Santé, surveillance alimentaire
Malta	Valletta	Malta Public Health Laboratory (PHL), Evans Building
Netherlands, the	Bilthoven	National Institute for Public Health and the Environment (RIVM), Centre for Zoonoses and Environmental Microbiology (cZ&O)
Netherlands, the	Wageningen	Wageningen Food Safety Research (WFSR)
Norway	Ås	Norwegian Veterinary Institute, Bacteriology Section
Northern Ireland	NRL tasks carried out by NRL Belgium (NRL food)	
Poland	Pulawy	National Veterinary Research Institute (NVRI), Department of Hygiene of Food of Animal Origin
Portugal	Vairão	Instituto Nacional de Investigação Agrária e Veterinária, Food Microbiology
Romania	Bucharest	Hygiene and Veterinary Public Health Institute (IISPV)
Serbia	Novi Sad	Scientific Veterinary Institute "Novi Sad"
Slovak Republic	Dolny Kubin	State Veterinary and Food Institute
Spain	Majadahonda	Centro Nacional de Alimentación
Sweden	Uppsala	Swedish Veterinary Agency

Country	City	Institute
Switzerland	Zürich	Institute for Food Safety and Hygiene, University of Zurich
United Kingdom	London	UK Health Security Agency

3 Materials and Methods

3.1 Preparation of artificially contaminated fabric swab samples

3.1.1 General

The matrix used for this PT was fabric swabs. Fabric swabs are suitable to be used as control samples for the food production area as well as for the (animal) Primary Production Stage (PPS) area. The fabric swabs were artificially contaminated with background flora and a diluted culture of *Salmonella* Typhimurium (STm) at the EURL-*Salmonella* laboratory.

3.1.2 Pre-tests for the preparation of fabric swab samples

Dry fabric swabs were ordered from supplier MLS (order nr OSB4010, Sodibox fabric swabs, 32cm x 40cm). The fabric swabs were moistened by adding 20 ml of peptone saline solution and left at room temperature until totally soaked (approximately 30 minutes). No neutralising buffer was present or added.

The moistened fabric swabs were artificially contaminated with background flora by adding 1 ml of an even mixture of *Escherichia coli* and *Citrobacter Freundii* (approximately 10^6 cfu/sample) and with two different concentrations (approximately 1 and 10 cfu) of *Salmonella* Typhimurium (ATCC 14028).
To test the stability of the samples during transport and storage conditions, the samples were stored at 5 °C and at 10 °C for three weeks. After zero, one, two, and three weeks of storage, six fabric swab samples were tested at each time interval for the presence of *Salmonella* according to EN ISO 6579-1:2017/A1:2020. In addition, one fabric swab sample was tested for the concentration of background flora according to section 3.1.4 (number of *Enterobacteriaceae* and total aerobic count) at the beginning and at the end of the storage period.

3.1.3 Preparation of the fabric swab samples for the combined PT-ILS

Pre-moistened fabric swabs were artificially contaminated with a suspension of background flora, consisting of an even mixture of *E. coli* and *C. freundii* (approximately 10^6 cfu/sample), followed by artificially contamination with four different concentrations of STm:

- 3 negative fabric swab samples (no *Salmonella* added);
- 3 fabric swab samples with a high level of STm: aiming at 50 cfu/test portion;
- 4 fabric swab samples with a low level of STm: aiming at 10 cfu/test portion;
- 6 fabric swab samples with an extra-low level of STm: aiming at 1,5 cfu/test portion;

The concentration of the inoculum used to contaminate the fabric swab samples was confirmed by streaking the inoculum on xylose lysine deoxycholate (XLD) agar plates (or TSA plate in case of background flora). This was done 15-fold for the extra-low and low concentrations. Immediately after artificial contamination, the high-, low-, extra-low-

level and negative samples were stored at 5 °C until transportation to the participating laboratories on Monday 30 September 2024.

3.1.4 Determination of the level of background flora in fabric swab samples

The total number of aerobic bacteria and the number of *Enterobacteriaceae* in the fabric swab samples was assessed by following EN ISO 4833-1:2013 and EN ISO 21528-2:2017, respectively. The fabric swab samples were homogenised in buffered peptone water (BPW), and ten-fold dilutions were analysed on plate count agar (PCA) and violet red bile glucose (VRBG) agar.

3.1.5 Determination of the level of Salmonella *in fabric swab samples by MPN*

The contamination level of *Salmonella* in the artificially contaminated fabric swabs samples was determined using a most probable number (MPN) technique. To test for the concentration of *Salmonella* in the low-level and extra-low-level fabric swab samples, 5 fabric swab samples with double the amount of the *Salmonella* suspension inoculum, 20 fabric swab samples with the normal amount of *Salmonella* suspension inoculum and 5 samples with half the amount of the *Salmonella* suspension inoculum were used, representing 25 gram each (ISO 16140-2:2016/Amd1:2024). For the high-level fabric swab samples, a regular 10-fold dilution of five artificially contaminated swab samples was used, representing, 25 g, 2,5 g and 0,25 g.
The presence of *Salmonella* was determined in each dilution following EN ISO 6579-1:2017. The MPN of *Salmonella* in the original sample was calculated from the number of confirmed positive dilutions, using freely available Excel-Based MPN software (Jarvis et al., 2010).

3.2 Design of the combined Proficiency Test-Interlaboratory study

3.2.1 Number and type of samples

Each participant received 16 artificially contaminated fabric swab samples, numbered B1 to B16. Table 3.1 gives an overview of the number and types of samples tested by the participants. No control samples were included in this study, since accredited laboratories are supposed to have controls in place in their laboratories.

Table 3.1 Overview of the number and types of samples tested per laboratory in the combined PT-ILS study PPS-Food 2024

Strain and contamination level	Number of fabric swab samples (n = 16)
Salmonella Typhimurium high-level	3
Salmonella Typhimurium low-level	4
Salmonella Typhimurium extra-low-level	6
Negative (no *Salmonella* added)	3

3.2.2 Shipments of parcels and temperature recording during shipment

The 16 blindly coded samples, containing the contaminated and the negative fabric swab samples, were packed into a safety bag. These were placed in one large shipping box, together with four frozen (-20 °C) cooling elements. The shipping boxes were sent to the

participants as 'biological substances category B' (UN3373), using a door-to-door courier service. The participants were asked to store the samples at 5 °C upon receipt. To monitor exposure to abusive temperatures during shipment and storage, a micro temperature logger was placed among each batch of samples to record the temperature.

3.3 Methods

In contrast to previous studies, when the start-date was set on the Monday after arrival of the samples, participants were now asked to start the study on Wednesday 2 October 2024 and no later than Monday 7 October 2024. Participants had to store the samples at 5 °C until the start of the analyses. The protocol and result form can be found on the EURL-*Salmonella* website (EURL-*Salmonella* 2024a, 2024b).

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 to detect *Salmonella* in food samples, animal feed samples, environmental samples from the food production area, and samples from the Primary Production Stage. Participants had to select the right procedure when they analysed the samples as PPS or Food samples.
In summary, the procedure described in EN ISO 6579-1:2017(/A1:2020) consists of a pre-enrichment step in buffered peptone water (BPW), followed by a selective enrichment step on modified semi-solid Rappaport-Vassiliadis (MSRV) agar for PPS samples and in Muller-Kauffmann tetrathionate-novobiocin (MKTTn) broth and modified semi-solid Rappaport-Vassiliadis (MSRV) agar and/or Rappaport-Vassiliadis with soya (RVS) broth for Food/Feed samples. Next, the selective enriched cultures had to be plated out on two isolation media: xylose lysine deoxycholate agar (XLD) and a second isolation medium of choice. Suspect samples had to be confirmed by means of appropriate biochemical and serological tests (EN ISO 6579-1:2017 (/A1:2020)) or using reliable (validated) proprietary identification kits.

The participants had to report the final confirmed results by indicating for each 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 were not used to assess the performance of this participant.

3.4 Statistical analysis of the data

From the results for 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:

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

For the ILS part of the study, the performance characteristics of EN ISO 6579-1 were calculated using the information described in EN ISO 17468:2023 for a qualitative method. These performance characteristics consist of specificity, sensitivity for each contamination level, and the level of detection for which 50% of tests give a positive result (LOD_{50}). For the calculation of the LOD_{50} an Excel®-based programme was used, freely available for download at https://standards.iso.org/iso/16140/-2/ed-1/en/amd/1/

3.5 Criteria for good performance

The performance of the NRLs-*Salmonella* was based on the results found using EN ISO 6579-1:2017(/A1:2020) only. For assessing the performance of the participants, the criteria indicated in Table 3.2 were used.

Table 3.2 Criteria for good performance

Contamination level	% positive	# positive samples/ total # samples
STm high-level	≥ 65%	≥ 2/3
STm low-level	≥ 50%	≥ 2/4
STm extra-low-level	not used for PT performance	Results are used for determination performance characteristics EN ISO 6579-1
Negative (no *Salmonella* added)	0%	0/3

4 Results and Discussion

4.1 Preparation of artificially contaminated fabric swab samples

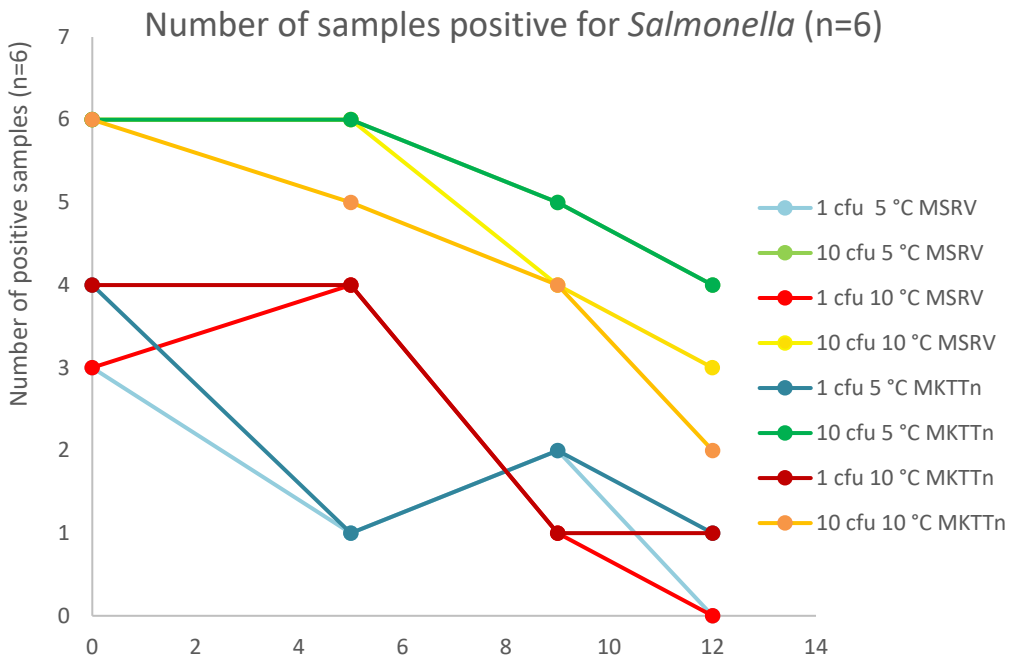
4.1.1 Pre-tests for the preparation of fabric swab samples

The study design was based on the tests performed for the PT PPS organised in 2023 (Pol-Hofstad and Mooijman, 2024) and the combined PT PPS-Food organised in 2022 by the EURL-*Salmonella* (Pol-Hofstad and Mooijman, 2023). The pre-test samples were prepared according to section 3.1.2 and stored at 5 °C and 10 °C to mimic storage and transport conditions for up to three weeks. At day 0 and day 12, the samples were analysed for the presence of *Enterobacteriaceae* and total aerobic count. At day zero, day five, day nine, and day 12, the samples were analysed for the presence of *Salmonella* using EN ISO 6579:1-2017 (see section 3.1.4). The results are presented in Table 4.1 and in Figure 4.1.

Table 4.1 The effect of storage time on the number of total aerobic count and Enterobacteriaceae *in fabric swab samples stored at 5 °C*

Background flora	t = 0 (days)	t = 12 (days)
Aerobic count	4,6 x 10^4 cfu/sample	1,9 x 10^3 cfu/sample
Enterobacteriaceae	3,1 x 10^4 cfu/sample	80 cfu/sample

Figure 4.1 Stability tests of fabric swab samples (n = 6) artificially contaminated with a low and extra-low concentration of Salmonella *Typhimurium*



The total aerobic count decreased by approximately one log during the storage period of almost two weeks (see Table 4.1). However, the concentration remained sufficient to mimic real-life samples. The number of *Enterobacteriaceae* was approximately 10^4 cfu/sample at the start of the experiment but dropped by more than two log after 12 days of storage at 5 °C. The total amount of background flora was still considered sufficiently representative for real-life PPS or Food samples. The intended concentration of fabric swab samples was 1 cfu and 10 cfu *Salmonella* Typhimurium (STm) per sample. The concentrations of the inoculation volumes were determined tenfold using XLD agar plates. The average concentration of STm used for the extra-low-level samples was 0,9 cfu, while for the low-level samples, the concentration STm was 7,9 cfu. The results in Figure 4.1 show that the STm concentration decreased during the twelve-day storage period. When inoculated with 0,9 cfu STm/sample, only four out of the six samples were positive at day zero and during storage at 5 °C, the number of positive samples decreased to one or two after approximately 5 days of storage, while hardly any samples were positive after 12 days of storage. When the fabric swab samples were inoculated with 7,9 cfu STm/sample, more samples tested positive for *Salmonella* after storage. After 12 days of storage at 5 °C, four out of the six samples still tested positive, while two to three samples were still positive when stored at 10 °C. Therefore, it was decided to slightly increase the intended STm concentration to inoculate the PT-ILS samples. For the extra-low-level fabric swab samples, the intended inoculum was 1,5 cfu STm/sample and for the low-level fabric swab samples, the intended inoculum was 10 cfu STm/sample.

4.1.2 *Preparation of fabric swab samples for the combined PT-ILS study*

The samples for the combined PT-ILS study were prepared as described in section 3.1.3. Fabric swabs were artificially contaminated with background flora and with *S.* Typhimurium (STm) to reach the intended concentration of 1,5 cfu STm/sample, 10 cfu STm/sample, and 50 cfu STm/sample, representing extra-low, low and high levels of contamination in the fabric swab samples.

4.1.3 *Background flora in the fabric swab samples*

The fabric swab samples were artificially contaminated with 1 ml of an even mixture of *C. freundii* and *E. coli* of approximately 10^6 cfu/ml on 24 September 2024. The final concentration of aerobic bacteria and *Enterobacteriaceae* in the samples was determined on Wednesday 2 October 2024, the start date for performing this combined PT-ILS. Results show that the concentration of background flora remained stable at approximately 10^6 cfu per sample during storage at 5 °C (see Table 4.2).

Table 4.2 Number of aerobic bacteria and Enterobacteriaceae per fabric swab sample

Date	Aerobic bacteria (cfu/sample)	*Enterobacteriaceae (cfu/sample)*
2 October 2024[a]	5,0 x 10^6	7,5 x 10^5

a: After storage at 5 °C for 8 days

4.1.4 *Number of* Salmonella *bacteria in the fabric swab samples*

The fabric swab samples were artificially contaminated at the EURL-*Salmonella* laboratory by adding the appropriate volume of a diluted STm culture. Table 4.3 shows the contamination levels of the diluted culture of *Salmonella* used as inoculum to contaminate the fabric swab samples. Following inoculation, the samples were stored at 5 °C until they were dispatched to the participants on Monday 30 September 2024. The final contamination level of *Salmonella* in the fabric swab samples was determined by performing a Most Probable Number (MPN) test on the start date (Wednesday 2 October) of the combined PT-ILS study according to section 3.1.5 (see Table 4.3).

Table 4.3 Number of S. Typhimurium bacteria per fabric swab sample

Date	Extra-low-level STm cfu/sample	Low-level STm cfu/sample	High-level STm cfu/sample
24 September 2024 Inoculation of fabric swab samples	1,5	9,1	39
2 October 2024[a] MPN of contaminated fabric swab samples (95% confidence limit)	1,2 (0,7-1,8)	4,5 (2,1-9)	37,5 (12-112)

a: After storage at 5 °C for 8 days

4.2 Technical data from the combined PT-ILS

4.2.1 *General*

A total of 68 NRLs-*Salmonella* subscribed to this study: 37 NRLs for PPS and 31 NRLs for food, originating from 36 countries. 54 Participants originated from 27 EU-MSs, 13 participants from other European countries (EU candidate or potential EU candidate MSs, members of the EFTA countries, and the United Kingdom), and one participant was based in a non-European country. 68 lab codes were assigned to the participants (1-68 and 75, lab code 20 was not used). All participants reported their results before the deadline of 1 November 2024. Participants received the interim summary with overall results in April 2025 (Pol-Hofstad and Mooijman, 2025).

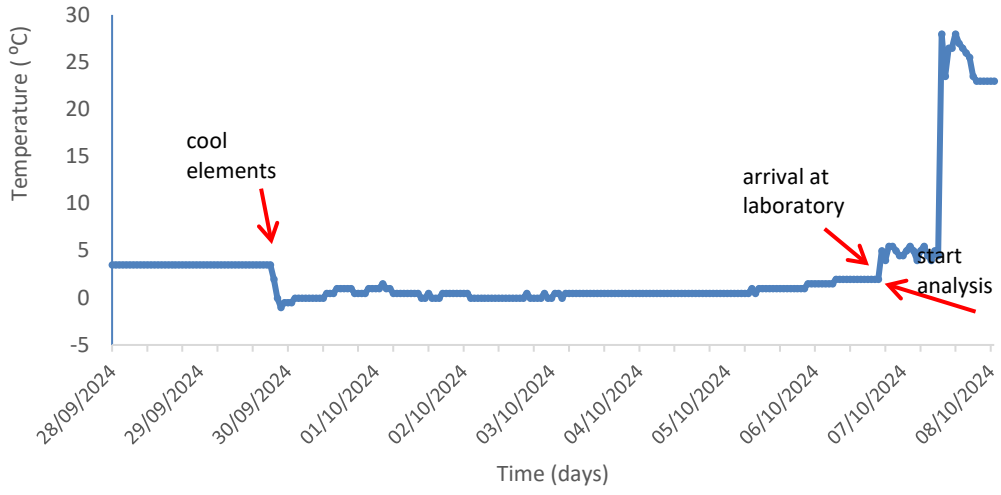
4.2.2 *Accreditation and methods used*

9 participants indicated to be accredited for EN ISO 6579-1:2017 only and 57 participants indicated to be accredited for EN ISO 6579-1:2017 in combination with amendment A1:2020 (EN ISO 6579-1/A1:2020). Six participants were also accredited for other (alternative) methods such as Vidas *Salmonella* UP (SPT), SureTech Real-time PCR, iQcheck *Salmonella* II, MicroSEQ *Salmonella* spp. Two participants (from one laboratory) were not accredited. 57 laboratories used EN ISO 6579-1:2017(/A1:2020) for the detection of *Salmonella* in this study, the other 11 participants used EN ISO 6579-1:2017.

4.2.3 *Transport of samples*

The samples were transported using a door-to-door courier service on Monday 30 September 2024. Two laboratories received the parcel on the day of dispatch. Forty-five parcels were delivered after one day, ten parcels after two days and seven parcels after three days of dispatch. One parcel took seven days to arrive (lab code 7), and another parcel experienced a long delay at the border. This parcel only arrived at the participant (lab codes 53 and 54) after twenty-five days. One parcel was sent 1 week later due to registration mismatch and arrived at its destination after three days (lab code 75).
The samples had to be stored at 5 °C upon arrival at the laboratory. The temperature during transport and storage was registered using a temperature probe. The temperature of the parcels during transport predominantly ranged between -4,5 °C and +4,5 °C, and during storage between 0,5 °C and 8,5 °C. The transport temperature of the parcels arriving late were checked in greater detail. Unfortunately, the temperature probe from the laboratory with lab codes 53 and 54 was lost in the mail, and the temperature profile could not be checked. The temperature profile of lab code 7 is shown in Figure 4.2. The temperature can only stay low for as long as the cooling elements remain frozen. The customs staff were very helpful, keeping the parcel in cold storage until all necessary papers were approved and the parcel was cleared for further transport to the laboratory. Figure 4.2 shows that the temperature during transport was just above zero until the parcel arrived at the laboratory on 7 October 2024. The samples were analysed upon arrival.

Figure 4.2 Temperature profile of the parcel for Laboratory 7



The NRLs-*Salmonella* were requested to start the analyses of the samples on Wednesday 2 October 2024. In case the parcel did not arrive on 2 October, the NRL was advised to start the analyses immediately upon arrival of the parcel at the laboratory, but preferably no later than Monday 7 October 2024. The majority of the participants (n = 43) started the analyses on 2 October 2024. One participant started one day earlier (lab code 51) and nine participants started one day later, on 3 October 2024. Ten laboratories started on 7 October,

three laboratories started on 8 October (lab codes 1, 2, and 75) and two participants started on 25 October 2024 (lab codes 53 and 54).

4.2.4 *Methods*

The prescribed method was EN ISO 6579-1:2017(/A1:2020).This method prescribed MSRV agar to be used for analyses of PPS samples and MKTTn and RVS and/or MSRV for analyses of food samples as selective enrichment media. XLD agar and a second medium of choice have to be used as isolation media. Table 4.4 shows which second plating-out media were used by the participants.

Table 4.4 Second plating-out media used by the NRLs-Salmonella

Media	No. of users
BxLH	1
BSA	5
Chromo	4
BGA	14
ASAP	1
Compass *Salmonella* agar	1
Rapid *Salmonella*	8
RSAL	2
Endo agar	0
BPLS	6
BGA mod	7
Rambach	16
SM2	3
Salmonella differential agar	2
IRIS *Salmonella* (BIOKAR)	1

Explanations of the abbreviations used are given in the list of abbreviations.

Technical details on the deviations from the prescribed method (EN ISO 6579-1:2017(/A1:2020)) are listed in Table 4.5 (grey-shaded cells); six laboratories reported details of deviations. One laboratory (lab code 51) incubated the BPW pre-enrichment for too many hours (24 hours instead of 16-20 hours). Two laboratories used MKTTn with a pH outside the prescribed range of 7,0-8,2 (lab codes 17 and 68). Three laboratories used MKTTn with a lower novobiocin concentration than prescribed (lab codes 32, 65, and 68). One laboratory (lab code 50) used MSRV with a higher novobiocin concentration than prescribed, and one laboratory (lab code 65) did not report the novobiocin concentration at all.
Laboratory 1 identified itself as an NRL PPS but used MKTTn and RSV broth instead of MSRV agar, which is not in accordance with EN ISO 6579-1:2017(/A1:2020).

The last step in the procedure for *Salmonella* detection is the confirmation step. All participating laboratories performed one or several confirmation tests for *Salmonella*. Table 4.6 summarises all reported combinations. The majority of the participants (42) used a biochemical test in combination with other confirmation methods, such as serological testing, serotyping, PCR testing, or MALDI-TOF. Eighteen laboratories used only one confirmation test.

Table 4.5 Reported technical deviations from the prescribed method EN ISO 6579-1:2017(/A1:2020)

Lab code	BPW		RVS		MKTTn			MSRV		
	Incu-bation time	T (°C)	pH	T (°C)	T (°C)	pH	Novo-biocin	T (°C)	pH	Novo-biocin
EN ISO 6579-1	**16–20 h**	**34-38**	**5,0-5,4**	**41,5 +/- 1**	**37 +/- 1**	**7,0–8,2**	**40 mg/l**	**41,5 +/- 1**	**5,1–5,4**	**10 mg/l**
17	18	36	5,2	41,5	36	6,6 *	40 mg/L			
32	19	37	5,2	42	37	7,7	10 mg/L*	42	5,2	10 mg/L
50	18,75	37,1						41,3	5,4	18 mg/L*
51	24	37	5,2	41,5	37	7,7	40 mg/L			
65	18,5	37			37	8,2	10 mg/L*	41,5	5,2	*
68	20	37	5,1	41,5	37	8,65*	20 mg/L*			

* Deviations from EN ISO 6579-1:2017 are indicated in grey

Table 4.6 Number of participants using the various confirmation methods

Number of labs	Bio-chemical	Serological	Serotyping	PCR	Maldi-toff	Other
14	x	x				
6	x		x			
1	x	x	x	x		
8					x	
1	x	x			x	
4			x		x	
11	x	x	x			
2	x				x	
2	x		x		x	
1			x			Chromogen
2	x	x	x		x	
2		x				
4			x			
1	x				x	MS
2	x					
2		x	x			
2						PCR
2		x				PCR
1		x	x		x	

4.3 Control samples

4.3.1 General

Sample bags for control samples are no longer provided for by the EURL-*Salmonella*. Each NRL-*Salmonella* is expected to include (process) control samples according to their own Standard Operating Procedure

and quality system. Table 4.7 presents the *Salmonella* serovars used by the participants for the positive control samples.

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

***Salmonella* serovars**	**No. of participants**
S. Enteritidis	16
S. Typhimurium	16
S. Nottingham	9
S. Abaetetuba	4
S. Infantis	4
S. Agbeni	2
S. Blegdam	2
S. bongori serovar. 66:z41:-	2
S. Harleystreet	2
S. Senftenberg	2
S. Tranaroa (*Salmonella enterica* subsp. *salamae*)	2
S. Alachua, *S.* Weltevreden, *S.* Poona, *S.* Regent, *S.* Braenderup, *S.* Tennessee, *S.* Ohio	1 (per serovar)

The majority of the NRLs-*Salmonella* used *S*. Enteritidis or *S*. Typhimurium for their positive control samples.
The concentration of *Salmonella* in the positive control samples used by the participants ranged between 1 cfu and a very high concentration of 10^8 cfu per sample (see Table 4.8). Three participants did not determine the concentration of *Salmonella* in the control samples. A positive control sample for a detection method should demonstrate that media are capable of supporting growth of the target organism in low numbers. To obtain information on the sensitivity of a method, the concentrations 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, any 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. Only 11 laboratories performed the positive control in the presence of a matrix.

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

Concentration *Salmonella* (cfu/sample)	**Number of participants**
1-10	23
11-20	2
21-120	23
121-1000	4
1001-10^8	11
not determined	3
>LOD	2

4.4 Artificially contaminated fabric swab samples

4.4.1 General

The participants analysed all fabric swab samples artificially contaminated with three different concentrations of STm (extra-low: 1,2 cfu/sample; low: 4,5 cfu/sample and high: 37,5 cfu/sample), as well as the negative samples, for the presence of *Salmonella*. Only the results of the negative, low- and high-level samples were used to determine the performance of the NRLs-*Salmonella* (PT part of the study). The results of all samples were used to determine the performance characteristics of the method (ILS part of the study). Table 4.9 shows the overall results of the PT samples found by the participants. In Appendix 1, the results per sample and per laboratory are given.

Table 4.9 Number of fabric swab samples tested positive for Salmonella *by each participant for the PT part of the combined PT-ILS study*

	Number of positive samples		
	Negative n = 3	**STm Low n = 4**	**STm High n = 3**
Criteria Good Performance	**0**	**≥2**	**≥2**
Lab codes 53 and 54	0	2	1*
Lab code 61	0	1*	3
Lab codes 19 and 75	0	2	3
Lab codes 3, 4, and 51	0	4	2
10 participants	0	3	3
All other participants (n = 50)	0	4	3

* Grey cells = result below level of good performance

4.4.2 Negative fabric swab samples

Alle NRLs correctly scored the negative fabric swab samples negative for *Salmonella*. The results for all participants are shown in Figures 4.2 and 4.3.

Figure 4.2 Number of positive Salmonella *isolations per NRL PPS laboratory found in the fabric swab samples not contaminated with* Salmonella *Typhimurium*

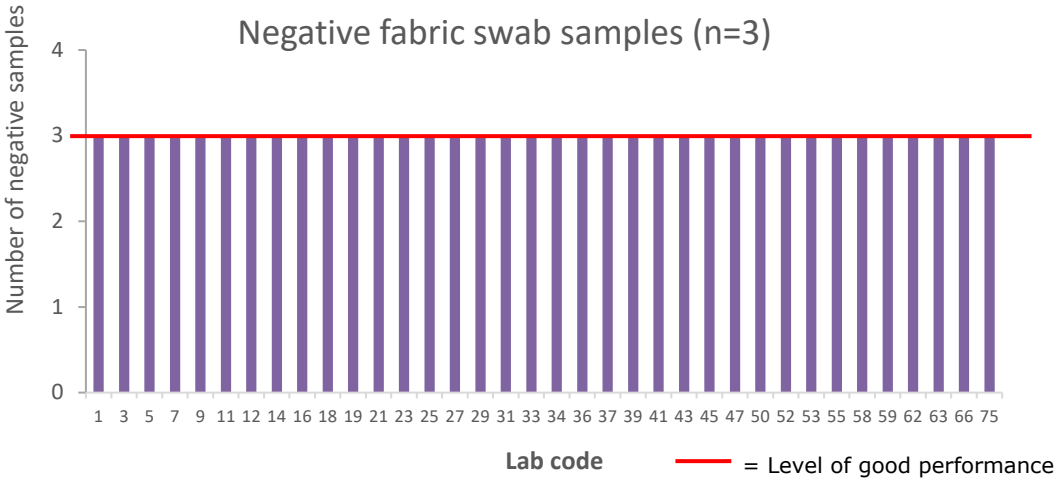
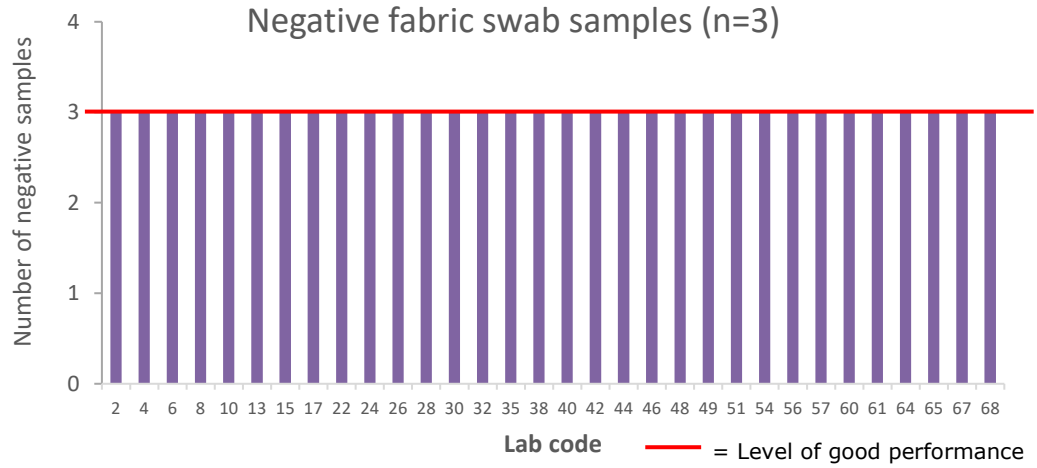


Figure 4.3 Number of positive Salmonella *isolations per NRL food laboratory found in the fabric swab samples not contaminated with* Salmonella *Typhimurium*



4.4.3 *Fabric swab samples contaminated with a low level of* Salmonella *Typhimurium*

Most of the participating laboratories were able to detect *Salmonella* in all four fabric swab samples that were contaminated with a low inoculum level of 4,5 cfu *S.* Typhimurium per fabric swab sample. Ten laboratories reported one out of the four samples as negative for *Salmonella*. Four laboratories (lab codes 19, 53, 54, and 75) reported two out of the four samples as negative for *Salmonella*. And one laboratory (lab code 61) reported three samples as negative for *Salmonella*. With respect to these low-level samples, a negative score for at most two out of four samples was regarded as acceptable. Therefore, laboratory 61 did not meet the criteria for good performance. The results for all participants are shown in Figures 4.4 and 4.5.

Figure 4.4 Number of positive Salmonella *isolations per NRL PPS laboratory found in the fabric swab samples contaminated with a low concentration of* Salmonella *Typhimurium (4,5 cfu/sample)*

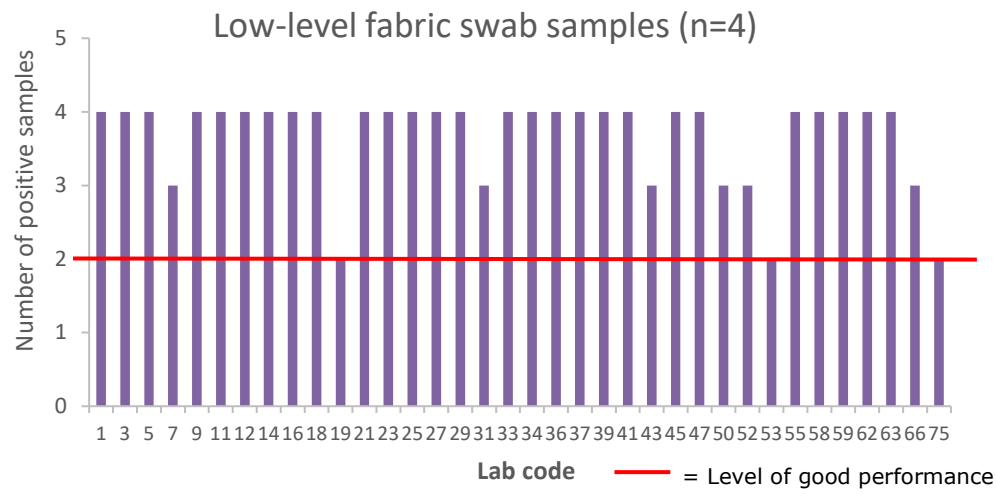
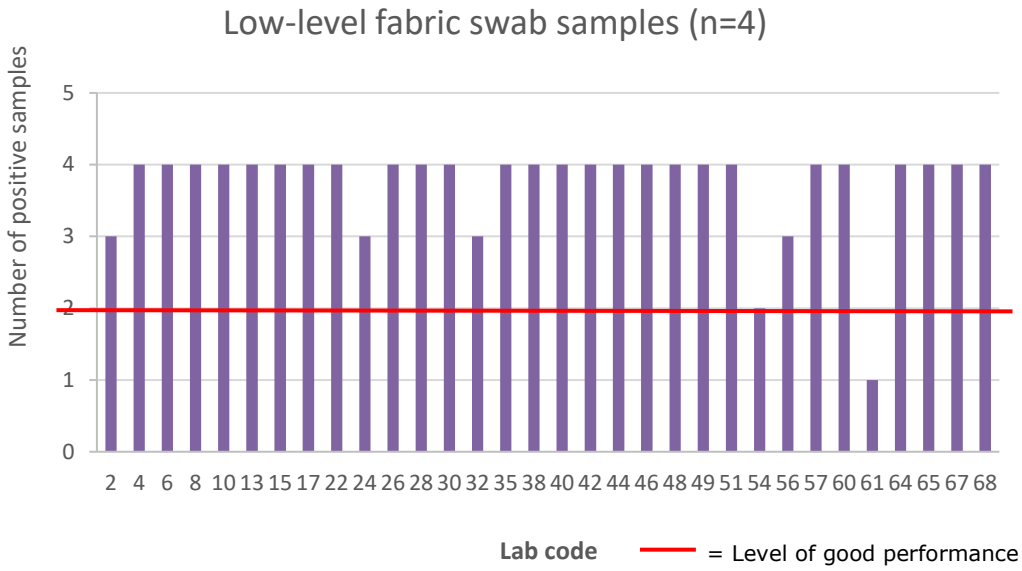


Figure 4.5 Number of positive Salmonella *isolations per NRL food laboratory found in the fabric swab samples contaminated with a low concentration of* Salmonella *Typhimurium (4,5 cfu/sample)*



4.4.4 *Fabric swab samples contaminated with a high level of* Salmonella *Typhimurium*

Almost all participating laboratories were able to detect *Salmonella* in all three fabric swab samples that were contaminated with a high inoculum level of 37,5 cfu *S*. Typhimurium per sample. Three laboratories reported one out of the three samples as negative for *Salmonella* (lab codes 3, 4, and 51). Two laboratories (lab codes 53 and 54) reported two out of the three samples as negative for *Salmonella*. With respect to these high-level samples, a negative score for at maximum one out of three samples was regarded as acceptable. Therefore, laboratories 53 and 54 did not meet the criteria for good performance. The results for all participants are shown in Figures 4.6 and 4.7.

Figure 4.6 Number of positive Salmonella *isolations per NRL PPS laboratory found in the fabric swab samples contaminated with a low concentration of* Salmonella *Typhimurium (37,5 cfu/sample)*

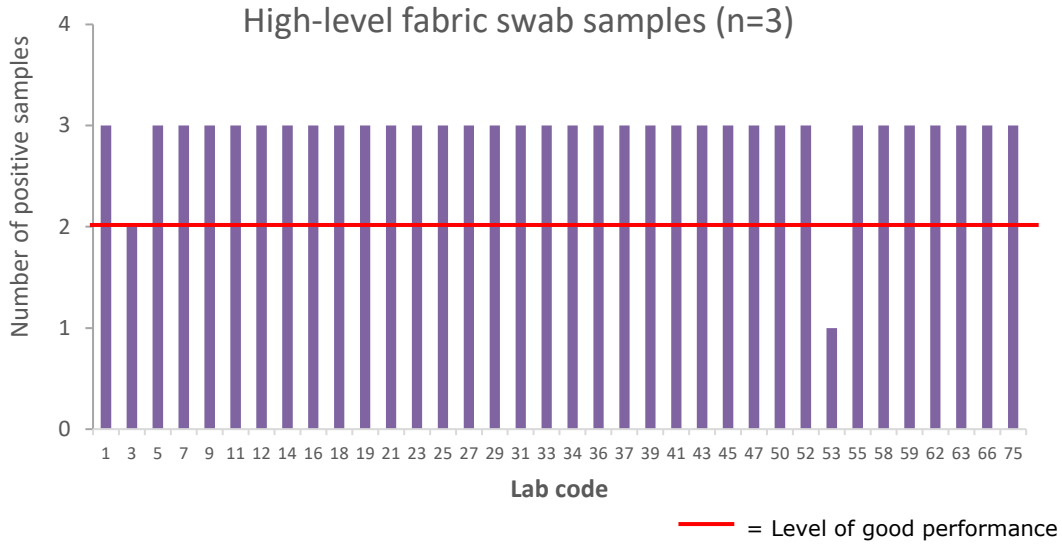
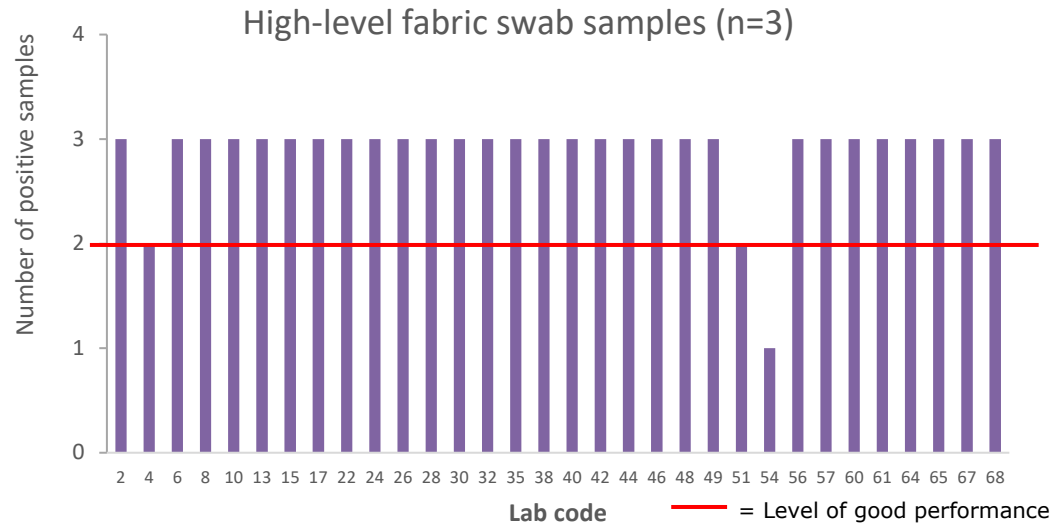


Figure 4.7 Number of positive Salmonella *isolations per NRL food laboratory found in the fabric swab samples contaminated with a high concentration of* Salmonella *Typhimurium (37,5 cfu/sample)*



4.4.5 *Fabric swab samples contaminated with an extra-low level of* Salmonella *Typhimurium*

The extra-low-level fabric swab samples contained only approximately 1 cfu STm/sample. The results of these samples were not used to determine the performance of the laboratories but were only intended to determine the performance of the method, EN ISO 6579-1. Still, all participants also analysed these samples as part of the blind set of samples within this PT-ILS. Despite the very low contamination level, 67 out of the 68 participants detected *Salmonella* in at least one out of the six extra-low-level samples. The number of positive samples varied per laboratory; an overview is given in Table 4.10.

For the calculation of LOD_{50}, it is prescribed to include samples at a 'fractional positive level' in the ILS. This means that 25% to 75% of the samples at a specified level should test positive for the target strain. In the case of the extra-low-level fabric swab samples tested in this PT-ILS, this fractional positive level was well achieved, with a total of 53,4% of the 408 samples tested positive by the 68 participants.

Table 4.10 Overview of number of laboratories testing different numbers of extra-low-level fabric swab samples positive for Salmonella *(n=6)*

	Number of extra-low-level fabric swab samples tested positive for *Salmonella*						
	0	**1**	**2**	**3**	**4**	**5**	**6**
Number of laboratories	1	7	10	23	15	10	2

4.4.6 Specificity, sensitivity and accuracy rates of the artificially contaminated samples (PT part)

Table 4.11 shows the specificity, sensitivity and accuracy rates for the artificially contaminated fabric swab samples of the PT part of the study. The calculations were performed on the results for all participants, on those for EU MSs only, and on those for the groups of NRLs PPS and NRLs Food. All participants but three performed well in this study: the sensitivity rates (low-level: 92,3%; high-level 96,6%) were very high. Hardly any differences were found between the different groups of participants as shown in Table 4.11. The specificity of the negative samples was 100%. The accuracy calculated for all the fabric swab samples in the PT part of the study was 95,9%.

Table 4.11 Specificity, sensitivity and accuracy rates found by the participating laboratories (all participants and EU-MSs only) for the fabric swab samples in the PT part of the combined PT-ILS

Fabric swab samples		**All participants n=68**	**EU MSs only n=60**	**NRL PPS n=36**	**NRL Food n=32**
Negative samples n=4	No. of samples	204	180	108	96
	No. of negative samples	204	180	108	96
	Specificity in %	**100**	**100**	**100**	**100**
Low-level STm n=4	No. of samples	272	240	144	128
	No. of positive samples	251	224	132	119
	Sensitivity in %	**92,3**	**93,3**	**91,7**	**93**
High-level STm n=3	No. of samples	204	180	108	96
	No. of positive samples	197	174	105	92
	Sensitivity in %	**96,6**	**96,7**	**97,2**	**95,8**
All fabric swab samples with STm n=7	No. of samples	476	420	252	224
	No. of positive samples	448	398	237	211
	Sensitivity in %	**94,1**	**94,8**	**94,0**	**94,2**
All fabric swab samples (pos. and neg.) n=11	No. of samples	680	600	360	320
	No. of correct samples	652	578	345	307
	Accuracy in %	**95,9**	**96,3**	**95,8**	**95,9**

4.5 Second detection method

In the current PT, 22 laboratories also used a second method to analyse the fabric swab samples. An overview of the methods used per laboratory can be found in Table 4.12. The majority of laboratories used a PCR method, while two laboratories used VIDAS as a second method. Not all methods were validated or routinely used by the participants. Almost all NRLs found identical results with their second method when testing the negative, low- and high-level fabric swab samples compared to the prescribed bacteriological culture method. Only laboratories 41 and 42 reported sample B4 with a low concentration of STm as negative using the alternative method and as positive using the bacteriological culture method.

*Table 4.12 Details on the second detection methods used by NRLs-*Salmonella *in the combined PT-ILS PPS-Food 2024*

Lab	Second detection method	Validated (by)	Reference	Routinely #/year
7	Real-Time PCR	National Laboratory Accreditation Authority	EN ISO 22119:2011	1100
10	Thermo Scientific SureTect *Salmonella* Species real-time PCR Assay	Thermo Fischer Scientific	AOAC 051303, AFFNOR UNI 03/07-11/13	1600
13	PCR	No		-
15	Automated PCR System	AFNOR and AOAC	AFNOR: QUA 18/03-11/02/ AOAC: Certificate nº 100201	-
16	qPCR	AFNOR	BRD 07/06 - 07/04	75
17	Real-time PCR	AFNOR	BRD 07/06-07/04	2000
19	VIDAS	National Accreditation Board (IMAB)	AFNOR Bio - 12/10-09/02	1900
23	In-house PCR (Josefsen et al 2007 primers)	LABRIS; in-house validation	not applicable	-
24	Real-Time PCR	in house	MA-VP-15	163
27	Rapid *Salmonella*, MicroSEQ *Salmonella* spp	AFNOR	ABI 29/02-09/10	50
28	*Salmonella* Rapid, MicroSEQ *Salmonella* spp	AFNOR	ABI 29/02-09/10	100
31	Real-Time PCR	§64 of the national Food and Feed Code	Malorny et al.(2004) AEM 70:7046-7052	227

Lab	Second detection method	Validated (by)	Reference	Routinely #/year
32	Real-Time PCR	§64 of the national Food and Feed Code	Malorny et al.(2004) AEM 70:7046-7052	227
41	Real-Time PCR	NordVal	055	-
42	Real-Time PCR	NordVal	055	-
48	VIDAS SLM TEST	AFNOR	BIO 12/1-04/94	-
53	PCR detection invA gene	No	-	-
54	PCR - detection invA gene	No	-	-
61	PCR Rapidfinder	UKAS	N/A	0
75	qPCR	No	-	-

4.6 Performance of the NRLs-*Salmonella* (PT part)

Almost all laboratories were able to detect *Salmonella* in high- and low-level concentrations in the fabric swab samples. Out of the 68 laboratories, three laboratories did not fulfil the criteria for good performance in this study.
Participants with lab codes 53 and 54 (two NRLs from the same laboratory) experienced problems detecting *Salmonella* in the low- and high-level fabric swab samples. However, the parcel with the samples for this laboratory was held at the border for almost three weeks. The parcel arrived only at its destination after 25 days following dispatch. The temperature of the samples during this period could not be checked since the temperature button was lost in the mail. Still, the quality of the samples and the survival of *Salmonella* in the samples could not be guaranteed because of the long duration of the transport at unknown temperatures. Therefore, the performance of lab codes 53 and 54 could not be evaluated in this PT.
Laboratory 61 had difficulties in detecting *Salmonella* in the low-level samples. However, the results for this laboratory analysing the extra-low-level fabric swab sample can be considered as a follow up study, giving this laboratory the opportunity to show their ability to detect *Salmonella* in (very) low concentrations. This laboratory showed good results with these extra-low-level samples, by testing three out of the six samples positive for *Salmonella*. Therefore, it was not considered necessary to organise a follow-up study for this laboratory.

4.7 Performance of the method EN ISO 6579-1 (ILS part)

The performance characteristics still lacking for EN ISO 6579-1 are the ones for the product category 'environmental samples from the Food or Feed production'. For that purpose, only the results for the 31 NRLs-*Salmonella* for Food/Feed shall be taken into account. In addition, it is also possible to determine the performance characteristics for the category 'Primary Production Stage' (PPS), by only using the results for the 37 NRLs-*Salmonella* for PPS.

For the determination of the performance characteristics of the method, it was important to use only results for laboratories that followed the

protocol and EN ISO 6579-1 strictly. For that reason, datasets from 10 laboratories out of the 31 NRLs-*Salmonella* for Food/Feed could not be used for further analyses. A summary of the (small) technical deviations resulting in exclusion of datasets is given below:

- Problems with transport of the samples (>2 days and/or at > 5 °C);
- Start of the analyses later than 03-10-2024 (although the laboratories were requested to start the analyses on 02-10-2024, one additional day for the start of analyses was still considered acceptable);
- Deviating composition of one or more culture media;
- Deviating pH of one or more culture media;
- Deviating incubation temperature for one or more of the culture steps;
- Deviating incubation times for one or more of the culture steps.

After exclusion of these deviating results, a total of 21 (out of 31) valid datasets remained available for calculations of the performance characteristics for the category 'environmental samples from the Food or Feed production'. For the category 'Primary Production Stage', a total of 24 (out of 37) valid datasets remained available.
The raw data of the technically valid data per product category are summarised in Appendix 2.
The performance characteristics calculated from the technically valid data sets are summarised in Table 4.13 (category environmental samples from the Food or Feed production) and in Table 4.14 (category Primary Production Stage).

Table 4.13 Performance characteristics of EN ISO 6579-1:2017 for category environmental samples from the Food or Feed production and item fabric swabs

Parameter	**Negative**	**Extra-low contamination level**	**Low contamination level**	**High contamination level**
		1,2 cfu/test portion	4,5 cfu/test portion	37,5 cfu/test portion
Number of participants	31	31	31	31
Number of samples per participant	3	6	4	3
Number of participants[a] retained after evaluation of data	21	21	21	21
Number of samples retained after evaluation of the data	63	126	84	63
Test portion size	fabric swab	fabric swab	fabric swab	fabric swab
Specificity, in %	100	-	-	-
Sensitivity, in %	-	60	98	98
LOD_{50} (95 % confidence interval), in cfu/test portion	-	1,1 (0,9 to 1,4)		

[a] from 21 different countries
NOTE Strain used for inoculation: S. Typhimurium (ATCC 14028).

Table 4.14 Performance characteristics of EN ISO 6579-1:2017 for category Primary Production Stage and item fabric swabs

Parameter	**Negative**	**Extra-low contamination level**	**Low contamination level**	**High contamination level**
		1,2 cfu/test portion	4,5 cfu/test portion	37,5 cfu/test portion
Number of participants	37	37	37	37
Number of samples per participant	3	6	4	3
Number of participants[a] retained after evaluation of data	24	24	24	24
Number of samples retained after evaluation of the data	72	144	96	72
Test portion size	fabric swab	fabric swab	fabric swab	fabric swab
Specificity, in %	100	-	-	-
Sensitivity, in %	-	58	98	100
LOD_{50} (95% confidence interval), in cfu/test portion	-	0,9 (0,7 to 1,1)		

[a] from 23 different countries
NOTE Strain used for inoculation: S. Typhimurium (ATCC 14028).

5 Conclusions

Sixty-five laboratories fulfilled the criteria of good performance in the combined EURL-*Salmonella* PT–ILS for NRLs PPS and NRLs Food for the detection of *Salmonella* in fabric swab samples.

One laboratory (lab code 61) scored an unsatisfactory performance. This laboratory tested three out of the four low-level samples negative for *Salmonella*. The results for this laboratory regarding the extra-low-level samples was used as a follow-up study, and it showed the ability of this laboratory to detect *Salmonella* at very low levels; therefore the performance of this laboratory was changed into moderate performance.

Two participants (lab codes 53 and 54, two NRLs from same laboratory) experienced problems detecting *Salmonella* in the low- and high-level fabric swab samples. However, the parcel with the samples for this laboratory only arrived at its destination after 25 days following dispatch. As the quality of the samples and the survival of *Salmonella* in the samples could not be guaranteed due to this long transport time, the performance of these participants could not be evaluated in this PT.

The specificity rate of the negative fabric swab samples amounted to 100%

The sensitivity rate of the fabric swab samples artificially contaminated with a low level of STm amounted to 92,3%.

The sensitivity rate of the fabric swab samples artificially contaminated with a high level of STm amounted to 96,6%.

The accuracy rate of all matrix samples amounted to 95,9%

Twenty-two participants used a second method in addition to the prescribed bacteriological culture method. Almost all laboratories reported identical results for both methods. Only two laboratories reported one sample negative using the PCR method which was positive for *Salmonella* using the bacteriological culture method.

Despite the very low contamination level of the extra-low-level fabric swab samples (approximately 1 cfu STm/sample), 67 out of the 68 participants were detecting *Salmonella* in at least one of these six samples. This resulted in a fractional positive level of 53,4%, which fulfilled the requirements to calculate the LOD_{50} of EN ISO 6579-1.
In total, 21 valid data sets for the category environmental samples from the Food or Feed production and 24 valid data sets for the category Primary Production Stage were used for the calculation of the LOD_{50} values. The LOD_{50} for the category environmental samples from the Food or Feed production was calculated to be 1,1 (0,9-1,4).
The LOD_{50} for the category Primary Production Stage was calculated to be 0,9 (0,7-1,1).

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List of abbreviations

AFNOR	Association Française de Normalisation
ASAP	AES *Salmonella* agar plate
ATCC	American Type Culture Collection
BGA	Brilliant green agar
BGA (mod)	Brilliant green agar (modified)
BPLS	Brilliant green phenol-red lactose sucrose
BPW	Buffered peptone water
BSA	Brilliance *Salmonella* agar
BxLH	Brilliant green, xylose, lysine, sulphonamide
cfu	Colony-forming units
HaDEA	European Health and Digital Executive Agency
EFTA	European Free Trade Association
EN	European Standard
EU	European Union
EURL	European Union Reference Laboratory
ILS	Interlaboratory study
ISO	International Organization for Standardization
ISO/TC34/SC9	International Organization for Standardization, Technical Committee 34 on Food Products, Subcommittee 9 – Microbiology of the food chain
LOD_{50}	Level of detection for which 50% of tests give a positive result
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization – Time Of Flight
MKTTn	Muller-Kauffmann tetrathionate-novobiocin
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 Milieu (National Institute for Public Health and the Environment)
RSAL	Rapid'*Salmonella*
RVS	Rappaport-Vassiliadis Soya
SM (ID)2	*Salmonella* detection and identification-2
STm	*Salmonella* Typhimurium
TSA	Tryptone soya agar
VRBG	Violet red bile glucose
XLD	Xylose lysine deoxycholate

Appendix 1 Results per sample and per laboratory of all participants in the combined PT-ILS PPS-Food 2024

Samples No	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16
Level	N	EL	EL	L	L	H	L	EL	EL	N	EL	EL	N	H	L	H
Lab code																
1	ND	D	ND	D	D	D	D	ND	ND	ND	ND	ND	ND	D	D	D
2	ND	ND	D	D	D	D	ND*	D	D	ND	D	D	ND	D	D	D
3	ND	D	D	D	D	D	D	D	D	ND	ND	ND	ND	D	D	ND*
4	ND	D	D	D	D	D	D	D	D	ND	ND	ND	ND	D	D	ND*
5	ND	ND	ND	D	D	D	D	D	D	ND	ND	D	ND	D	D	D
6	ND	ND	ND	D	D	D	D	D	D	ND	ND	D	ND	D	D	D
7	ND	ND	ND	D	ND*	D	D	ND	ND	ND	ND	D	ND	D	D	D
8	ND	ND	ND	D	D	D	D	D	ND	ND	D	D	ND	D	D	D
9	ND	D	D	D	D	D	D	D	D	ND	D	D	ND	D	D	D
10	ND	ND	D	D	D	D	D	D	D	ND	D	D	ND	D	D	D
11	ND	D	D	D	D	D	D	D	ND	ND	D	ND	ND	D	D	D
12	ND	D	D	D	D	D	D	ND	ND	ND	D	D	ND	D	D	D
13	ND	ND	ND	D	D	D	D	D	D	ND	ND	D	ND	D	D	D
14	ND	ND	D	D	D	D	D	D	ND	ND	D	D	ND	D	D	D
15	ND	ND	D	D	D	D	D	D	ND	ND	D	D	ND	D	D	D
16	ND	D	ND	D	D	D	D	ND	D	ND	ND	ND	ND	D	D	D
17	ND	D	ND	D	D	D	D	D	ND	ND	ND	D	ND	D	D	D
18	ND	D	ND	D	D	D	D	ND	D	ND	ND	D	ND	D	D	D
19	ND	D	ND	D	ND*	D	ND*	ND	ND	ND	ND	D	ND	D	D	D
21	ND	D	D	D	D	D	D	ND	D	ND	ND	D	ND	D	D	D
22	ND	D	D	D	D	D	D	ND	D	ND	ND	D	ND	D	D	D
23	ND	ND	ND	D	D	D	D	D	ND	ND	D	D	ND	D	D	D
24	ND	D	ND	ND*	D	D	D	D	ND	ND	ND	D	ND	D	D	D
25	ND	D	D	D	D	D	D	D	ND	ND	ND	ND	ND	D	D	D
26	ND	D	ND	D	D	D	D	D	ND	ND	ND	D	ND	D	D	D

Samples No	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16
Level	N	EL	EL	L	L	H	L	EL	EL	N	EL	EL	N	H	L	H
Lab code																
27	ND	ND	D	D	D	D	D	D	ND	ND	D	D	ND	D	D	D
28	ND	D	D	D	D	D	D	D	D	ND	ND	D	ND	D	D	D
29	ND	ND	ND	D	D	D	D	D	D	ND	ND	D	ND	D	D	D
30	ND	ND	ND	D	D	D	D	D	D	ND	ND	D	ND	D	D	D
31	ND	ND	D	ND*	D	D	D	ND	ND	ND	ND	D	ND	D	D	D
32	ND	ND	D	ND*	D	D	D	ND	ND	ND	ND	D	ND	D	D	D
33	ND	D	ND	D	D	D	D	D	ND	ND	ND	D	ND	D	D	D
34	ND	ND	ND	D	D	D	D	ND	D	ND	D	ND	ND	D	D	D
35	ND	ND	ND	D	D	D	D	ND	D	ND	D	ND	ND	D	D	D
36	ND	ND	D	D	D	D	D	D	ND	ND	D	ND	ND	D	D	D
37	ND	D	ND	D	D	D	D	D	D	ND	D	D	ND	D	D	D
38	ND	D	ND	D	D	D	D	D	D	ND	D	D	ND	D	D	D
39	ND	D	D	D	D	D	D	ND	ND	ND	D	ND	ND	D	D	D
40	ND	D	D	D	D	D	D	D	D	ND	D	D	ND	D	D	D
41	ND	D	ND	D	D	D	D	D	D	ND	D	ND	ND	D	D	D
42	ND	D	ND	D	D	D	D	D	D	ND	D	ND	ND	D	D	D
43	ND	D	D	D	D	D	ND*	ND	D	ND	ND	D	ND	D	D	D
44	ND	D	D	D	D	D	D	D	D	ND	ND	ND	ND	D	D	D
45	ND	ND	ND	D	D	D	D	ND	D	ND	D	D	ND	D	D	D
46	ND	D	ND	D	D	D	D	D	ND	ND	ND	D	ND	D	D	D
47	ND	ND	ND	D	D	D	D	D	ND	ND	D	ND	ND	D	D	D
48	ND	ND	ND	D	D	D	D	ND	ND	ND	D	D	ND	D	D	D
49	ND	ND	D	D	D	D	D	D	D	ND	D	D	ND	D	D	D
50	ND	ND	D	D	D	D	D	ND	ND	ND	D	D	ND	D	ND*	D
51	ND	ND	D	D	D	ND*	D	D	D	ND	D	D	ND	D	D	D
52	ND	D	ND	D	D	D	D	ND	D	ND	D	D	ND	D	ND*	D
53	ND	ND	ND	D	ND*	ND*	ND*	ND	ND	ND	D	ND	ND	D	D	ND*
54	ND	ND	ND	D	ND*	ND*	ND*	ND	ND	ND	D	ND	ND	D	D	ND*
55	ND	D	D	D	D	D	D	ND	D	ND	ND	D	ND	D	D	D
56	ND	ND	ND	D	D	D	D	D	ND	ND	D	ND	ND	D	ND*	D

Samples No	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16
Level	N	EL	EL	L	L	H	L	EL	EL	N	EL	EL	N	H	L	H
Lab code																
57	ND	D	D	D	D	D	D	ND	ND	ND	D	ND	ND	D	D	D
58	ND	D	D	D	D	D	D	ND	ND	ND	D	ND	ND	D	D	D
59	ND	D	ND	D	D	D	D	ND	D	ND	ND	D	ND	D	D	D
60	ND	D	ND	D	D	D	D	ND	D	ND	ND	D	ND	D	D	D
61	ND	D	D	D	ND*	D	ND*	ND	ND	ND	D	ND	ND	D	ND*	D
62	ND	D	ND	D	D	D	D	D	D	ND	D	D	ND	D	D	D
63	ND	ND	ND	D	D	D	D	ND	ND	ND	ND	D	ND	D	D	D
64	ND	ND	ND	D	D	D	D	ND	ND	ND	ND	D	ND	D	D	D
65	ND	ND	D	D	D	D	D	ND	ND	ND	ND	ND	ND	D	D	D
66	ND	D	D	D	D	D	ND*	D	D	ND	D	ND	ND	D	D	D
67	ND	D	D	D	D	D	D	D	D	ND	D	ND	ND	D	D	D
68	ND	ND	ND	D	D	D	D	D	ND	ND	ND	D	ND	D	D	D
75	ND	ND	ND	D	D	D	D	D	ND	ND	ND	D	ND	D	D	D

N = Negative
EL = Extra-Low level of *S.* Typhimurium (MPN result 1,2 cfu/sample)
L = Low level of *S.* Typhimurium (MPN result 4,5 cfu/sample)
H = High Level of *S.* Typhimurium (MPN result 37,5 cfu/sample)
D = *Salmonella* detected
ND = *Salmonella* not detected
* = Deviating from expected result

Appendix 2 Raw data from the technically valid data sets for calculation of performance characteristics of EN ISO 6579-1

Table A2.1 Raw data from technically valid data sets for calculation of performance characteristics of category environmental samples of the Food or Feed production

Samples No	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16
Level	N	EL	EL	L	L	H	L	EL	EL	N	EL	EL	N	H	L	H
Lab code																
4	ND	D	D	D	D	D	D	D	D	ND	ND	ND	ND	D	D	ND
6	ND	ND	ND	D	D	D	D	D	D	ND	ND	D	ND	D	D	D
8	ND	ND	ND	D	D	D	D	D	ND	ND	D	D	ND	D	D	D
10	ND	ND	D	D	D	D	D	D	D	ND	D	D	ND	D	D	D
13	ND	ND	ND	D	D	D	D	D	D	ND	ND	D	ND	D	D	D
15	ND	ND	D	D	D	D	D	D	ND	ND	D	D	ND	D	D	D
24	ND	D	ND	ND	D	D	D	D	ND	ND	ND	D	ND	D	D	D
26	ND	D	ND	D	D	D	D	D	ND	ND	ND	D	ND	D	D	D
28	ND	D	D	D	D	D	D	D	D	ND	ND	D	ND	D	D	D
30	ND	ND	ND	D	D	D	D	D	D	ND	ND	D	ND	D	D	D
35	ND	ND	ND	D	D	D	D	ND	D	ND	D	ND	ND	D	D	D
38	ND	D	ND	D	D	D	D	D	D	ND	D	D	ND	D	D	D
40	ND	D	D	D	D	D	D	D	D	ND	D	D	ND	D	D	D
42	ND	D	ND	D	D	D	D	D	D	ND	D	ND	ND	D	D	D
44	ND	D	D	D	D	D	D	D	D	ND	ND	ND	ND	D	D	D
46	ND	D	ND	D	D	D	D	D	ND	ND	ND	D	ND	D	D	D
48	ND	ND	ND	D	D	D	D	ND	ND	ND	D	D	ND	D	D	D
49	ND	ND	D	D	D	D	D	D	D	ND	D	D	ND	D	D	D
56	ND	ND	ND	D	D	D	D	D	ND	ND	D	ND	ND	D	ND	D
64	ND	ND	ND	D	D	D	D	ND	ND	ND	ND	D	ND	D	D	D
67	ND	D	D	D	D	D	D	D	D	ND	D	ND	ND	D	D	D

Table A2.2 Raw data from technically valid data sets for calculation of performance characteristics of category Primary Production Stage

Samples No	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16
Level	N	EL	EL	L	L	H	L	EL	EL	N	EL	EL	N	H	L	H
Lab code																
5	ND	ND	ND	D	D	D	D	D	D	ND	ND	D	ND	D	D	D
9	ND	D	D	D	D	D	D	D	D	ND	D	D	ND	D	D	D
11	ND	D	D	D	D	D	D	D	ND	ND	D	ND	ND	D	D	D
12	ND	D	D	D	D	D	D	ND	ND	ND	D	D	ND	D	D	D
14	ND	ND	D	D	D	D	D	D	ND	ND	D	D	ND	D	D	D
16	ND	D	ND	D	D	D	D	ND	D	ND	ND	ND	ND	D	D	D
18	ND	D	ND	D	D	D	D	ND	D	ND	ND	D	ND	D	D	D
23	ND	ND	ND	D	D	D	D	D	ND	ND	D	D	ND	D	D	D
25	ND	D	D	D	D	D	D	D	ND	ND	ND	ND	ND	D	D	D
27	ND	ND	D	D	D	D	D	D	ND	ND	D	D	ND	D	D	D
29	ND	ND	ND	D	D	D	D	D	D	ND	ND	D	ND	D	D	D
33	ND	D	ND	D	D	D	D	D	ND	ND	ND	D	ND	D	D	D
34	ND	ND	ND	D	D	D	D	ND	D	ND	D	ND	ND	D	D	D
36	ND	ND	D	D	D	D	D	D	ND	ND	D	ND	ND	D	D	D
37	ND	D	ND	D	D	D	D	D	D	ND	D	D	ND	D	D	D
39	ND	D	D	D	D	D	D	ND	ND	ND	D	ND	ND	D	D	D
41	ND	D	ND	D	D	D	D	D	D	ND	D	ND	ND	D	D	D
43	ND	D	D	D	D	D	ND	ND	D	ND	ND	D	ND	D	D	D
47	ND	ND	ND	D	D	D	D	D	ND	ND	D	ND	ND	D	D	D
55	ND	D	D	D	D	D	D	ND	D	ND	ND	D	ND	D	D	D
58	ND	D	D	D	D	D	D	ND	ND	ND	D	ND	ND	D	D	D
62	ND	D	ND	D	D	D	D	D	D	ND	D	D	ND	D	D	D
63	ND	ND	ND	D	D	D	D	ND	ND	ND	ND	D	ND	D	D	D
66	ND	D	D	D	D	D	ND	D	D	ND	D	ND	ND	D	D	D

N = Negative
EL = Extra-Low level of S. Typhimurium (MPN result 1,2 cfu/sample)
L = Low level of S. Typhimurium (MPN result 4,5 cfu/sample)
H = High Level of S. Typhimurium (MPN result 37,5 cfu/sample)
D = Salmonella detected
ND = Salmonella not detected

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