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

Combined EURL-*Salmonella* Proficiency Test Primary Production and Food, 2020

Detection of *Salmonella* in hygiene swab samples

RIVM report 2020-0204

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Synopsis

Combined EURL-*Salmonella* Proficiency Test for Primary Production and Food, 2020
Detection of *Salmonella* in hygiene swab samples

The National Reference Laboratories (NRLs) of the European Union were able to detect *Salmonella* in hygiene swab samples in the annual EURL-*Salmonella* Proficiency Test. All laboratories were successful in finding *Salmonella* in high and low concentrations in the contaminated hygiene swab samples. All but two laboratories scored good results. These two laboratories reported the positive control sample as having a negative result. One laboratory made an administrative error in reporting their positive result accidently as a negative one and scored a moderate performance. The other laboratory proved with their raw data that they misinterpreted the purpose of this sample. They considered this sample as a normal sample and added an extra sample as their positive control. This laboratory also scored a moderate performance.

This was the outcome of the Proficiency Test for detection of *Salmonella* in hygiene swab samples organised by the coordinating EURL-*Salmonella* in October 2020.

Since 1992, all NRLs from EU members states are obliged to participate in the annual quality control proficiency tests for *Salmonella*. Every Member State has to appoint a National Reference Laboratory, which is responsible for analysing *Salmonella* in samples taken from the animal primary production stage (PPS). Because of the nature of the samples, the participation of NRLs that are responsible for analysing *Salmonella* in food samples was allowed on voluntary basis. In total, 65 NRLs participated in this study: 37 NRLs PPS and 28 NRLs Food originating from 28 EU Member States (MS), five NRLs were based in third countries in Europe (non-EU members), and one was based in a non-European country.

The EURL-*Salmonella* is located at the Dutch National Institute for Public Health and the Environment (RIVM). An important task of the EURL-*Salmonella* is to monitor and improve the performance of the National Reference Laboratories for *Salmonella* in Europe.

Keywords: *Salmonella*, EURL, NRL, Proficiency Test, Hygiene swab samples, *Salmonella*-detection method

Publiekssamenvatting

Het gecombineerde EURL-*Salmonella* ringonderzoek productiedieren en Voedsel (2020)
Detectie van *Salmonella* in hygiënesponsjes

De Nationale Referentie Laboratoria (NRL's) van de Europese lidstaten waren in 2020 in staat om *Salmonella* aan te tonen in hygiënesponsjes. Alle deelnemers konden hoge en lage concentraties *Salmonella* aantonen. Op twee laboratoria na hebben alle laboratoria een goede score behaald. De twee laboratoria haalden een matige score. Dit blijkt uit het ringonderzoek dat het overkoepelende laboratorium in oktober 2020 organiseerde.

Het is de bedoeling dat de laboratoria aantonen of er *Salmonella* op de sponsjes zit die worden gebruikt om de hygiëne te testen in ruimtes waar dieren worden gefokt. Ze ontvangen daarvoor kant-en-klaar sponsjes met of zonder *Salmonella*. De laboratoria moeten het ook kunnen aantonen op een controlesponsje, waarop zij zelf de *Salmonella* moeten toevoegen. De twee laboratoria met de matige score konden geen *Salmonella* aantonen in het controlesponsje. Een van laboratoria had per ongeluk aangegeven dat er geen *Salmonella* inzat, terwijl het wel was aangetoond. Het andere laboratorium had de bemonstering op het controlesponsje niet volgens de instructies uitgevoerd.

Sinds 1992 zijn de NRL's van de Europese lidstaten verplicht om elk jaar mee te doen aan kwaliteitstoetsen. Dit zijn de zogeheten ringonderzoeken voor *Salmonella*. Elke lidstaat wijst hiervoor een laboratorium aan, het Nationale Referentie Laboratorium. Dit keer mochten ook de laboratoria die verantwoordelijk zijn voor de voedselproductie vrijwillig meedoen aan dit ringonderzoek met sponsjes.

In totaal hebben 65 NRL's aan dit ringonderzoek deelgenomen: 37 NRL's leefomgeving van dieren voor voedselproductie en 28 NRL's voedsel, afkomstig uit 28 EU-lidstaten, vijf NRL's uit andere Europese landen en een NRL uit een niet-Europees land.

Het Europese Referentielaboratorium (EURL) *Salmonella* is gevestigd bij het Nederlandse Rijksinstituut voor Volksgezondheid en Milieu (RIVM). Een belangrijke taak van het EURL-*Salmonella* is toezien op de kwaliteit van de nationale referentielaboratoria voor deze bacterie in Europa.

Kernwoorden: *Salmonella*, EURL, NRL, ringonderzoek, hygiëne sponsjes, *Salmonella*-detectiemethode

Contents

Summary

In October 2020, the combined EURL-*Salmonella* Proficiency Test on the detection of *Salmonella* in food and primary production stage samples was organised. A total of 65 National Reference Laboratories (NRLs) for *Salmonella* participated in this study: 37 NRLs PPS and 28 NRLs Food originating from 28 EU-Member States (MS), including the NRLs from United Kingdom and Northern Ireland, five from third European countries (EU candidate or potential EU candidate MS and members of the European Free Trade Association (EFTA)), and one from a non-European country. Two participants did not report results. Participation was obligatory for all EU Member State NRLs that are responsible for the detection of *Salmonella* in primary production stage samples.

In this study, hygiene swab samples were used that were artificially contaminated with background flora, as well as with a diluted culture of *Salmonella* Typhimurium at the EURL-*Salmonella* laboratory.

Each NRL received sixteen blindly coded samples consisting of 10 hygiene swab samples artificially contaminated with background flora and two different concentrations of *Salmonella* Typhimurium: six low-level contaminated samples (MPN concentration: 3.3 cfu/sample) and four high-level contaminated samples (MPN concentration: 35 cfu/sample). Additionally, four negative hygiene swab samples (no *Salmonella* added) and two control samples had to be analysed. The control samples consisted of a procedure control blank and a control sample to be inoculated by the participants using their own positive control strain. The samples were stored at 5 °C until the day of transport. On Monday, 28 September 2020, the contaminated hygiene swab samples were packed and sent to the NRLs. On arrival, the NRLs were asked to store the samples at 5 °C until the start of the analysis on Monday, 5 October 2020.

Method
Most laboratories used the prescribed method EN ISO 6579-1:2017. The majority (45 laboratories) used this method. There were 12 laboratories that indicated that they were already following the recently published amendment of EN ISO 6579-1 (EN ISO 6579-1:2017/A1:2020).
One laboratory reported that they used only a PCR method.

Results control samples
Of the 65 participants, 63 laboratories scored well, analysing both the procedure control as well as their own positive control sample correctly. Two laboratories reported their positive control accidently as testing negative for *Salmonella*. These laboratories scored a moderate performance (lab codes 63 and 66).

Results for artificially contaminated hygiene swab samples
All laboratories detected *Salmonella* in the hygiene swab samples contaminated with a low level of *Salmonella*. Three laboratories (lab codes 1, 3 and 39) tested one of the six samples as negative for *Salmonella*, another laboratory (lab code 67) tested three of the six samples as negative for *Salmonella*. These results are still within the criteria for good performance, which permit three negative samples. The sensitivity rate was 98.4% for these samples.

Almost all laboratories detected *Salmonella* in all four high-level samples. One laboratory (lab code 3) scored one of the four high-level samples as negative. This is still within the criteria for good performance, which permit one negative sample. The sensitivity rate was 99.6% for these samples.

All negative samples were scored correctly as negative, resulting in a specificity rate of 100%.

Overall, the laboratories scored well in this Proficiency Test, with an accuracy of 99.2%. Sixty-three laboratories fulfilled the criteria of good performance. The results of two laboratories were scored as a moderate performance due to them incorrectly reporting the results for the positive control sample.

1 Introduction

An important task of the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*), as laid down in Commission Regulation No 625/2017 (EC, 2017), is the organisation of Proficiency Tests (PT) 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*, 2020).

In October 2020, the EURL-*Salmonella* organised a PT to evaluate whether the NRLs that are responsible for the detection of *Salmonella* in samples from the Primary Production stage (PPS) could detect *Salmonella* at different contamination levels in hygiene swab samples. Due to the nature of the samples, NRLs that are responsible for the detection of *Salmonella* in food samples ('Food') were also invited to participate on a voluntary basis. The results from PTs like this show whether the examination of samples in the EU Member States (EU-MS) is carried out uniformly and whether comparable results can be obtained by all NRLs-*Salmonella*.

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

The design of this study was comparable to previous PTs organised by *EURL-Salmonella* (Diddens & Mooijman, 2020; Pol-Hofstad & Mooijman, 2018 and Pol-Hofstad & Mooijman, 2020). For the current study, hygiene swabs were artificially contaminated with a combination of *Escherichia coli* ATCC 11775 and *Citrobacter freundii* ATCC 8090 to mimic background flora in natural samples. In addition, the hygiene swabs were contaminated with a diluted culture of *Salmonella* Typhimurium (STm) at the laboratory of the EURL-*Salmonella*.

In total, 14 hygiene swab samples had to be tested: four hygiene swab samples artificially contaminated with a high level of STm, six hygiene swab samples artificially contaminated with a low level of STm, and four negative hygiene swab samples (no *Salmonella* added). Additionally, two control samples had to be tested: one procedure control and one positive control. The number of samples and the contamination levels were based on information described in EN ISO 22117:2019.

2 Participants

2.1 Participants NRLs PPS

Country	City	Institute
Austria	Graz	AGES / Institute for Medical Microbiology and Hygiene (VEMI)
Belgium	Brussels	Sciensano
Bulgaria	Sofia	National Diagnostic and Research Veterinary Institute (NDRVMI), National Reference Centre of Food Safety
Croatia	Zagreb	Croatian Veterinary Institute, Poultry Centre, Laboratory for General Bacteriology and Microbiology
Cyprus	Nicosia	Cyprus Veterinary Services Pathology, Bacteriology, Parasitology Laboratory
Czech Republic	Praha	State Veterinary Institute
Denmark	Ringsted	Danish Veterinary and Food administration
Estonia	Tartu	Estonian Veterinary and Food Laboratory, Bacteriology-Pathology Department
Finland	Kuopio	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	Federal Institute for Risk Assessment (BfR) Biological Safety Department
Greece	Chalkida	Veterinary Laboratory of Chalkis
Hungary	Budapest	National Food Chain Safety Office, Food and Feed Safety Directorate, Microbiological NRL
Iceland	Reykjavik	Matís ohf, Food Safety and Analytical services
Ireland, Republic of	Kildare	Central Veterinary Research Laboratory (CVRL/DAFFM) Laboratories Backweston, Department of Bacteriology
Israel	Kiryat Malachi	Southern Poultry Health Laboratory (Beer Tuvia)
Italy	Padova Legnaro	Istituto Zooprofilattico Sperimentale delle Venezie, SCS1-Microbiologia generale e sperimentale - Laboratorio di referenza per le *Salmonellosi*

Country	City	Institute
Latvia	Riga	Institute of Food Safety, Animal Health and Environment BIOR Bacteriology and Parasitology Division
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"Etat, Bacteriologie
Malta	Valletta	Malta Public Health Laboratory (PHL), Evans Building
Malta	Marsa	National Veterinary laboratory
Netherlands, the	Bilthoven	National Institute for Public Health and the Environment (RIVM), Centre for Zoonosis and Environmental Microbiology (Z&O)
North Macedonia	Skopje	Food Institute, Faculty of Veterinary Medicine Laboratory for food and feed microbiology
Norway	Oslo	Norwegian Veterinary Institute, Section of Microbiology
Poland	Pulawy	National Veterinary Research Institute, department of microbiology
Portugal	Vairão	Instituto Nacional de Investigação Agrária e Veterinária , Food Microbiology Laboratory
Romania	Bucharest	Institute for Diagnosis and Animal Health
Serbia	Belgrade	NIVS-Scientific Veterinary Institute of Serbia
Slovak Republic	Bratislava	State Veterinary and Food Institute
Slovenia	Ljubljana	National Veterinary Institute, Veterinary Faculty (UL, NVI)
Spain	Madrid Algete	Laboratorio Central de Veterinaria
Spain	Lugo	Fundación Centro Tecnológico Agroalimentario de Lugo, LSA_CETAL
Sweden	Uppsala	National Veterinary Institute
Turkey	Ankara	Veterinary control Central Research Institute
United Kingdom	Addlestone	Animal and Plant Health Agency (APHA), Bacteriology Department
United Kingdom	Belfast	Agri-Food and Biosciences Institute

2.2 Participants NRL Food

Country	City	Institute / NRL-*Salmonella*
Austria	Graz	AGES / Institute for Medical Microbiology and Hygiene (NRC *Salmonella*)
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)
Czech Republic	Prague	State Veterinary Institute (SVI)
Denmark	Ringsted	Danish Veterinary and Food Administration, Department of Microbiology
Estonia	Tartu	Estonian Veterinary and Food Laboratory, Department of Food Microbiology
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	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	Matis ohf, Food Safety and Analytical services
Ireland	Kildare	Central Veterinary Research Laboratory CVRL/DAFM Backweston, Department of Bacteriology
Italy	Legnaro PD	Istituto Zooprofilattico Sperimentale delle Venezie, SCS1-Microbiologia generale e sperimentale - Laboratorio di referenza per le *Salmonellosi*
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),

Country	City	Institute / NRL-*Salmonella*
		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)
North Macedonia	Skopje	Food Institute, Faculty of Veterinary Medicine Laboratory for Food and Feed Microbiology
Norway	Oslo	Norwegian Veterinary Institute, Bacteriology Section
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)
Slovak Republic	Bratislava	State Veterinary and Food Institute
Slovenia	Ljubljana	Institute of Microbiology and Parasitology, Veterinary Faculty (UL, NVI)
Sweden	Uppsala	National Veterinary Institute (SVA), Department of Microbiology
United Kingdom	London	Public Health England (PHE) Food Water and Environmental Microbiology Laboratory – London

3 Materials and Methods

3.1 Preparation of artificially contaminated hygiene swab samples

3.1.1 General

The matrix used for this PT were hygiene swabs ordered from supplier VWR. Hygiene swabs are suitable to be used as control samples for the food production area, as well as control samples for the (animal) primary production stage. The hygiene swabs were artificially contaminated with background flora, consisting of a mixture of two bacteria, and with a diluted culture of *Salmonella* Typhimurium (ATCC 14028) at the EURL-*Salmonella* laboratory.

3.1.2 Pre-tests for the preparation of hygiene swab samples

Hygiene swab samples were ordered from supplier VWR (no: vwrc710-1020; dry sponges size: 7,5 cm by 3,8 cm). The hygiene swabs were pre-moisturised by adding 10 ml of Peptone Saline solution (PS) and left at room temperature until totally soaked (approx. 30 min). The moisturised hygiene swabs were artificially contaminated with background flora by adding 1 ml of an even mixture of *Escherichia coli* (ATCC 11775) and *Citrobacter freundii* (ATCC 8090) (approx. 10^8 cfu/swab). Additionally, the hygiene swabs were contaminated with a low level (approx. 5 cfu) of *Salmonella* Typhimurium (STm).

To test the stability of the contaminated hygiene swab samples during transport and storage conditions, the pre-test samples were stored at 5 °C and 10 °C for a period of up to three weeks. After zero, one, two and three weeks of storage, five samples were tested at each time interval for the presence of *Salmonella* according to EN ISO 6579-1:2017. In addition, one hygiene swab sample was tested each week for the concentration of background flora according to EN ISO 21528-2:2017.

3.1.3 Preparation of hygiene swab samples for the Proficiency Test

Moisturised hygiene swab samples were artificially contaminated with a suspension of background flora, consisting of an even mixture of *E.coli* and *C. freundii* (approx. 10^8 cfu/ml). Additionally, one-third of the total number of hygiene swabs with background flora was contaminated with a low level (approx. 7 cfu/sample) of STm and one-third with a high level (approx. 45 cfu/sample) of STm by adding 0,1 ml of the appropriate dilution of an overnight culture. One-third was not inoculated with *Salmonella* (negative samples). The concentration of the inoculum used to contaminate the hygiene swabs was confirmed by streaking the inoculum on XLD (Xylose Lysine Deoxycholate) agar plates. Immediately after artificial contamination, the high, low and negative samples were stored at 5 °C until being transported to the participating laboratories on Monday, 28 September 2020.

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

Moisturised hygiene swab samples were artificially contaminated with a mixture of *E. coli* (ATCC 11775) and *C. freundii* (ATCC 8090) to mimic the presence of background flora, aiming for an end concentration of 10^8 cfu/swab. The total number of *Enterobacteriaceae* in hygiene swabs was investigated by following EN ISO 21528-2:2017. The hygiene swab

samples were homogenised (kneaded) in peptone saline solution and 10-fold dilutions were analysed on Violet Red Bile Glucose (VRBG) Agar.

3.1.5 Determination of the number of Salmonella in hygiene swab samples by MPN

The level of contamination with *Salmonella* in the artificially contaminated hygiene swab samples was determined using a five-tube most probable number (MPN) technique. For this, 10-fold dilutions of five artificially contaminated hygiene swab samples at each contamination level were tested, representing 25 g, 2,5 g, and 0,25 g of the original sample. The presence of *Salmonella* was determined in each dilution 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 Proficiency Test

3.2.1 Number and type of samples

Each participant received 14 artificially contaminated hygiene swab samples, numbered B1 to B14. In addition, the laboratories had to test two control samples (C1 and C2). Table 3.1 gives an overview of the number and type of samples tested by the participants.

For the control samples, the laboratories were asked to use their own positive *Salmonella* control strain that they normally use when analysing routine samples for the detection of *Salmonella*. In addition to this positive control (C2), a procedure control (C1) consisting of only Buffered Peptone Water (BPW) had to be analysed. The protocol and test report can be found in Annexes I and on the EURL website (EURL Salmonella 2020a).

Table 3.1 Overview of the number and type of samples tested per laboratory in the Proficiency Test PPS-Food 2020

Contamination level	Hygiene swab samples (n=14)
***S*. Typhimurium low level**	6
***S*. Typhimurium high level**	4
Negative (no *Salmonella* added)	4
	Control samples (n=2)
Blank procedure control (BPW only)	1
Positive control (own control with *Salmonella*)	1

3.2.2 Shipment of parcels and temperature recording during shipment

The 16 coded samples containing the contaminated and the negative hygiene swab samples and the two unopened sterile bags with hygiene swabs for the control samples were packed in two safety bags. These were placed in one large shipping box together with four frozen (-20 °C) cooling devices. The shipping boxes were sent to the participants as 'biological substances category B' (UN3373) via 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 between the samples to record the temperature.

3.3 Methods

The method prescribed for this PT was EN ISO 6579-1:2017. Hygiene swabs can be considered as control samples for the food production area as well as control samples for the (animal) primary production stage. NRLs should use the appropriate method for the chosen matrix approach (food or PPS).
The method starts with pre-enrichment in Buffered Peptone Water (BPW). Next, selective enrichment is carried out in Mueller Kaufmann Tetrathionate novobiocin broth (MKTTn) and in Rappaport Vassiliadis Soya broth (RVS) and/or Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar when considering hygiene swabs as food samples. When the hygiene swabs are considered as primary production stage samples, selective enrichment is carried out on Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar only. Plating-out is carried out on Xylose Lysine Deoxycholate agar (XLD) and a second isolation medium of choice. Confirmation is performed using the appropriate biochemical and serological tests as prescribed in EN ISO 6579-1:2017 or using reliable, validated identification kits. In addition to the EN ISO method, the NRLs were free to use their own method, such as a Polymerase Chain Reaction (PCR) procedure.
Only the results obtained with the prescribed method, EN ISO 6579-1:2017, were used to assess the performance of the participant.

3.4 Statistical analysis of the data

The specificity, sensitivity and accuracy rates were calculated for the artificially contaminated hygiene swab samples. For the control samples, only the accuracy rates were calculated. The rates were calculated with the following formulae:

Specificity rate:

$$\frac{\text{Number of negative results}}{\text{Total number of (expected) negative samples}} \text{ x } 100\%$$

Sensitivity rate:

$$\frac{\text{Number of positive results}}{\text{Total number of (expected) positive samples}} \text{ x } 100\%$$

Accuracy rate:

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

3.5 Criteria for good performance

For the determination of 'good performance', the criteria indicated in Table 3.2 were used.

*Table 3.2 Criteria for testing good performance in the combined EURL-*Salmonella *PT PPS-Food 2020*

Contamination level	% positive	# positive samples/ total # samples
Hygiene swab samples		
***S*. Typhimurium high-level**	Min. 80 %	Min. 3/4
***S*. Typhimurium low-level**	Min. 50 %	Min. 3/6
Negative (no *Salmonella* added)	0 %	0/4
Control samples		
Procedure control (BPW only)	0 %	0 /1
Positive control with *Salmonella*	100 %	1 /1

4 Results and Discussion

4.1 Preparation of artificially contaminated hygiene swab samples

4.1.1 Pre-tests for the preparation of hygiene swab samples

The study design was based on the one used in 2017 by the *EURL-Salmonella* (Pol-Hofstad and Mooijman, 2018). To test whether the hygiene swab samples were stable during transport and storage, the samples were contaminated with a high level of background flora and with a low concentration of approx. 5 cfu of *Salmonella* Typhimurium per hygiene swab sample, as described in 3.1.2.

The pre-test samples were stored at 5 °C to mimic storage conditions and at 10 °C to test the effect of temperature abuse during transport. The pre-test samples were stored for up to three weeks and analysed for the presence of *Salmonella* using EN ISO 6579:1-2017. The results are presented in Figure 4.1.

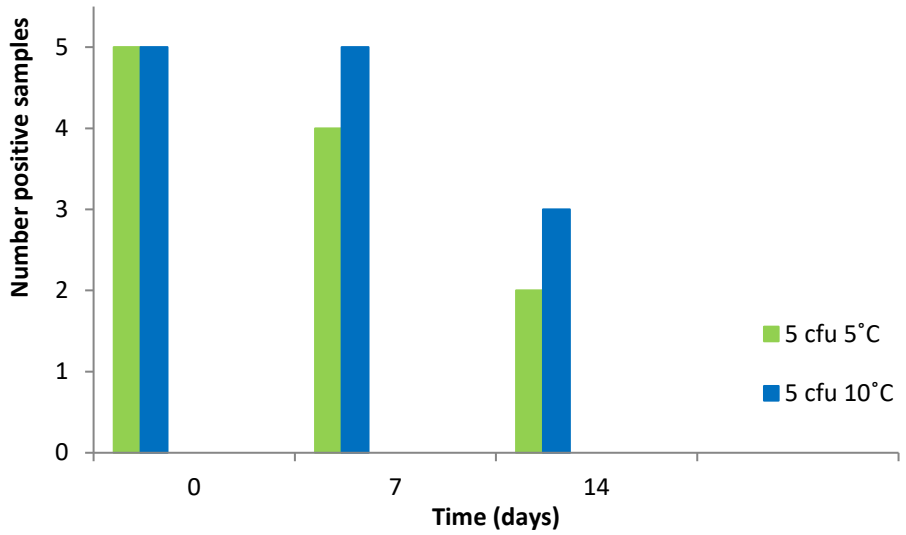

Figure 4.1 Stability tests of hygiene swab samples artificially contaminated with background flora and approx. 5 cfu of Salmonella *Typhimurium, after storage at 5 °C and 10 °C for two weeks*

Figure 4.1 shows that the storage of the pre-test samples at 5 °C or 10 °C for two weeks had a relatively large effect on the survival of *Salmonella* Typhimurium. After one week of storage at both temperatures, almost all samples still tested positive for *Salmonella.* However, after two weeks of storage only two to three samples were found positive. After three weeks of storage, the samples were not further analysed since the remaining number of *Salmonella* present in the samples after two weeks of storage was considered to be too low for use in a PT. For this reason, it was decided that a higher concentration (approx. 8 cfu) of *Salmonella* Typhimurium was to be used to inoculate the PT samples in order to prevent *Salmonella* being absent in the samples at the start of the analysis at the laboratories.

The effect of storage time and of the temperature during storage on the background flora in the pre-test samples is shown in Figure 4.2. As a result of miscalculation, the initial contamination level was too low and a second inoculation with the same background flora was performed to reach the desired background level of approx. 10^8 cfu/ml. Variation is seen in the survival of *Enterobacteriaceae* in the hygiene swab samples during storage at 5 °C and at 10 °C. A decrease of 2 log units was seen after three weeks of storage at 5 °C, while a slight increase in the number of *Enterobacteriaceae* was seen when stored at 10 °C. However, the remaining amount of background flora was sufficient to represent a realistic sample, even when taking into account the worst-case scenario.

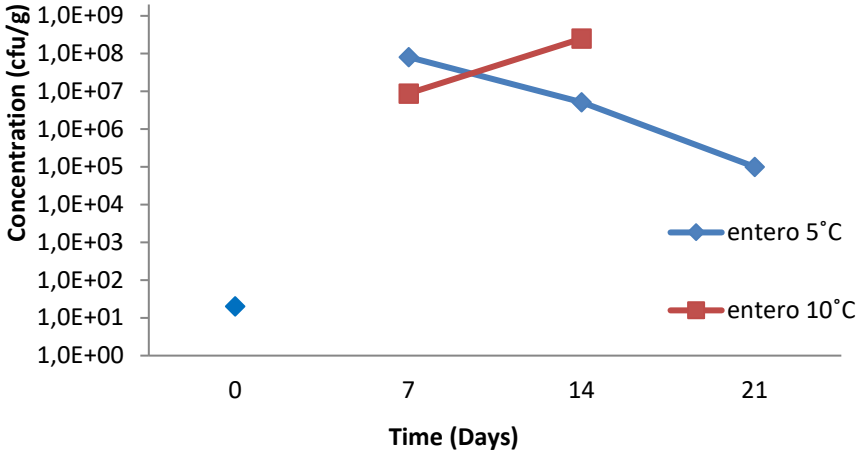

Figure 4.2 The effect of temperature and storage time on the number of Enterobacteriaceae in hygiene swab samples

4.1.2 Preparation of hygiene swab samples for the Proficiency Test

Samples for the PT were prepared as described in 3.1.3. Samples were contaminated with approx. 8 cfu/sample and approx. 50 cfu/sample of *Salmonella* Typhimurium, representing low and high levels of contamination in samples.

4.1.3 Background flora in the hygiene swab samples

The concentration of the background flora in the study samples was determined according to EN ISO 21528-2:2017, as described in 3.1.4. The total amount of background flora in the hygiene samples was 1,2 x 10^8 cfu/swab.

4.1.4 Number of Salmonella in Hygiene swab samples

The hygiene swab samples were artificially contaminated at the EURL-*Salmonella* laboratory by adding the appropriate volume of a diluted *Salmonella* culture. Table 4.1 shows the contamination level of the diluted culture of *Salmonella* Typhimurium used as inoculum to contaminate the hygiene swab samples. The results show that the intended levels of approximately 8 cfu for the low-level samples and 50 cfu for the high-level samples were nearly reached.

Table 4.1 Number of Salmonella *Typhimurium (STm) in the inoculums and in the hygiene swab samples*

Date of testing	Low level STm (cfu)	High level STm (cfu)
23 Sept 2020 (inoculum level diluted culture)	7	47
5 Oct 2020 MPN contaminated hygiene swab samples (95 % confidence limit)	3,3 (1,1-10,4)	35 (11-110)

After inoculation, the samples were stored at 5 °C for one week until being transported to the participants on 28 September 2020. The final contamination level of *Salmonella* in the hygiene swab samples was determined by performing a five-tube Most Probable Number (MPN) test in the week of the PT study (see Table 4.1).

4.2 Technical data for the Proficiency Test

4.2.1 General

A total of 67 NRLs *Salmonella* subscribed to this study: 37 NRLs PPS and 30 NRLs Food originating from 34 countries. The participants originated from 28 EU-MS (including the UK), five NRLs from third European countries (EU candidate or potential EU candidate MS and members of the EFTA countries), and one NRL from a non-European country. In total 65 NRLs *Salmonella* reported their results, two NRLs Food did not return their results.

4.2.2 Accreditation and Methods used

Fifty-two laboratories were accredited according to EN ISO/IEC 17025:2017 for EN ISO 6579-1:2017 and the majority (45 laboratories) used this method for the detection of *Salmonella*. Twenty-one laboratories indicated that they were already accredited for the recently published amendment of EN ISO 6579-1 (EN ISO 6579-1:2017/A1:2020). There were 19 laboratories that used this amendment in this PT. One laboratory reported that it used only a PCR method, but did report results for two methods, including the selective enrichment media MKTTn and RVS.

4.2.3 Transport of samples

The samples were transported using a door-to-door courier service on Monday, 28 September 2020. Two laboratories received the parcel on the same day of dispatch. Forty-two parcels were delivered after one day, 10 parcels after two days, five parcels after three days and three parcels arrived after four days from dispatch. Three parcels arrived very late due to delays at the borders. One of these parcels (lab code 66) arrived after eight days and another parcel (lab code 30) after nine days from dispatch. The third parcel (lab code 54) arrived only after 17 days from dispatch due to serious delays at the border. Parcels 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 was predominantly between -4 °C and 7 °C. The temperature of the parcels arriving late was checked in greater detail. The parcel of laboratory 66 arrived on 5 October 2020 with a temperature

still below 5 °C. The parcel of laboratory 30 was exposed to 'room temperatures' during transport for several days (see Figure 4.3). The temperature of the samples remained at approx. 1 °C for three days. From 30 September 2020, the temperature rose quickly to 10 °C on 1 October and to 18 °C on 2 October 2020. After 4 October, the temperature rose to 24 °C and stayed this high until arrival at the laboratory on 6 October 2020. The parcel of laboratory 54 was also exposed to elevated temperatures (see Figure 4.4). The samples remained cool at 1 °C until 30 September 2020. The temperature increased to 10 °C on 1 October and to 18 °C on 2 October. The temperature remained at 18 °C for three more days until 5 October 2020, after which the temperature dropped to 10 °C for the remaining days until the parcel arrived at the laboratory on 15 October 2020.

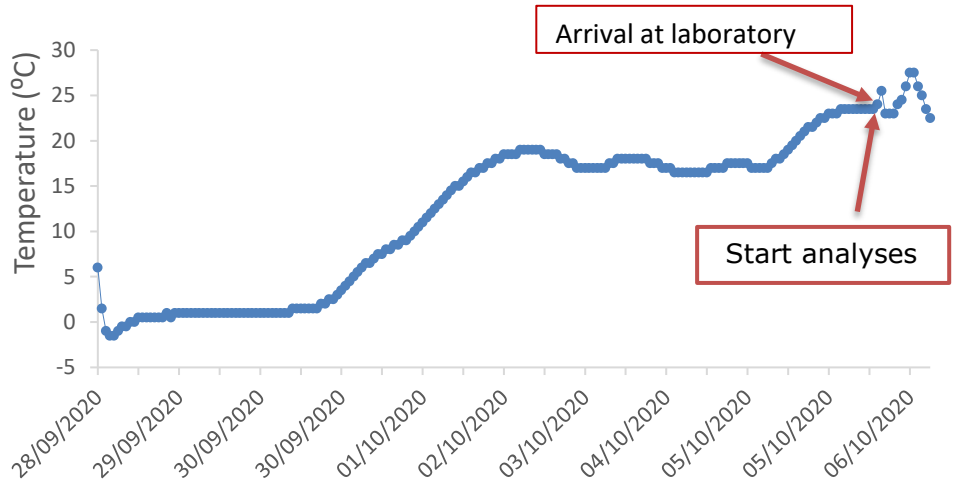

Figure 4.3 Temperature profile of parcel 30

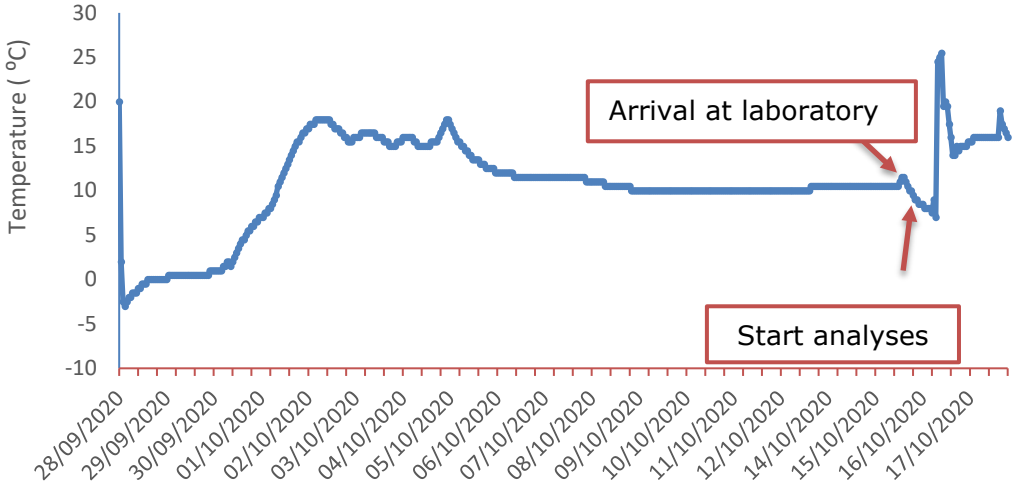

Figure 4.4 Temperature profile of parcel 54

The participants were asked to store the parcel at 5 °C upon arrival at their laboratories. The storage temperature at the receiving laboratories ranged from 0 – 10 °C. Most laboratories started the analyses on 5 October 2020. However, five laboratories started the analysis one day later because of national Holidays on 5 October. Laboratory 54 started the analyses the day after the arrival of the parcel on 15 October. One laboratory (lab code 37) started already on the day the parcel arrived (29 September 2020).

4.2.4 Methods

The prescribed method was EN ISO 6579-1:2017, for which MKTTn, RVS and/or MSRV agar had to be used as selective enrichment medium and XLD agar and a second medium, free of choice, for plating out. Table 4.2 shows which second plating-out media were chosen by the participants.

Table 4.2 Second plating-out media used by the NRLs

Media	No. of users
ASAP	1
BGA	12
BGA mod	12
BPLS	6
BSA	2
BxLH	1
Sm (ID)2	2
Rambach	14
Chromo *Salmonella*	3
RAPID'*Salmonella*	5
BSA Oxoid	1
Hectoen enteric agar	1
RSAL	3
Salmonella compass agar	1
SM2	1

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

Technical details on the method that deviated from the prescribed EN ISO method (EN ISO 6579-1:2017) are listed in Table 4.3 (grey-shaded cells); 20 laboratories reported the details of deviations. Two laboratories (lab codes 17 and 63) incubated their BPW solution for too many hours. One laboratory (lab code 42) used RVS with a pH that was slightly too high. Three laboratories (lab codes 18, 27 and 46) incubated their MKTTn at 41,5 ^{0}C instead of the prescribed 37 ^{0}C. Three laboratories (lab codes 4, 38 and 54) used MKTTn with a pH outside the prescribed pH range of 7,0-8,2. And five laboratories (lab codes 21, 27, 42, 45 and 66) used MKTTn with a novobiocin concentration lower than 40 mg/l.
One laboratory did not report the novobiocin concentration at all (lab code 38). One laboratory (lab code 67) incubated MSRV agar at 37 ^{0}C instead of 41,5 ^{0}C. Three laboratories (lab codes 12, 13 and 54) used MSRV with a pH level higher than prescribed. In addition, seven laboratories (lab codes 33, 39, 42, 45, 50, 51 and 67) used MSRV with a concentration of Novobiocin that was higher than the prescribed level of 10 mg/l.

Table 4.3 Reported technical deviations from the prescribed EN ISO 6579-1:2017

Lab code	BPW		RVS	MKTTn			MSRV		
	Incubation time	T (°C)	pH	T (°C)	pH	Novo-biocin	T (°C)	pH	Novo-biocin
EN ISO 6579-1	16–20 h	37	5,0–5,4	37	7,0–8,2	40 mg/l	41,5	5,1–5,4	10 mg/l
4	18:00	37	5,4	37	6,8	40 mg/l			
12	20:00	37					41,5	5,6	10 mg/L
13	20:00	37		37	7,8	40 mg/L	41,5	56	10 mg/L
17	22:00	37	5,31	37,2	7,16	40 mg/L			
18	19:00	37		41,5	8,1	40 mg/L	41,5	5,3	10 mg/L
21	18:00	37		37	8	10 mg/L	42	5,2	10 mg/L
27	19:00	37,1	5,3	41,5	7,8	0 mg/L			
33	20:00	36,9					41,5	5,3	20 mg/l
38	18:00	36	5,3	36	6,8	mg/L			
39	18:00	37	5,2	37	8	40 mg/L	41,5	5,2	50 mg/L
42	18:00	37	5,46	37	8,01	10 mg/L	41,5	5,43	20 mg/L
45	19:00	37	5,1	37	8,1	10 mg/L	41,5	5,2	20 mg/L
46	19:46	37,1		41,8	8,15	40 mg/L	41,8	5,34	10 mg/L
50	20:00	37					41,5	5,1	16 mg/L
51	20:00	37		37	8,1	40 mg/L	41,5	5,1	16 mg/L
54	20:00	37		37	8,61	40 mg/L	41,5	5,54	10 mg/L
63	24:00	37	5,2	37	8	mg/L 40			
66	18:00	37		37	7,55	10 mg/L	41,5	5,4	10 mg/L
67	18:00	37		37	8	40 mg/L	37	5,4	20 mg/L

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

All participating laboratories performed one or several confirmation tests for *Salmonella*. Table 4.4 summarises all reported combinations. Fifty laboratories performed a biochemical test. Fourteen laboratories used only one confirmation test. The majority of participants used a combination of two or more confirmation methods, namely a biochemical test in combination with a serological test, serotyping or a PCR test. Other methods used were: Malditof and Chromogenic agar method.

Table 4.4 Number of laboratories using the different confirmation methods

Number of labs	Bio-chemical	Sero-logical	Sero-typing	PCR	Other
4	x				
9	x	x			
4	x				Malditof
1	x			x	Malditof
4	x	x			Malditof
4	x	x		x	
5	x	x	x		
1	x	x	x	x	
16	x		x		
2	x				Chromogenic agar
5					Malditof
1				x	
1		x			Malditof
2		x	x		
4			x		
2			x		Malditof

4.3 Control samples

4.3.1 General

Two unopened sterile bags with hygiene swabs for the control samples were sent to the laboratories. One was used for the procedure control (C1). The other was used for the positive control to which the laboratories had to add their own positive control strain (C2) normally used in their routine analysis for *Salmonella* detection.

Procedure control (BPW only)

All laboratories analysed the procedure control correctly as being negative for *Salmonella* and scored good results for this control sample.

Positive control with Salmonella

All but two laboratories correctly scored their own *Salmonella* positive control sample as positive. Laboratories 63 and 66 reported this sample as negative for *Salmonella*. Laboratory 66 explained their result as a misunderstanding about the purpose of sample C2 as functioning as a positive control. They analysed C2 as a normal sample and used another own sample as their positive control as proven by their raw data. This laboratory scored a moderate performance.

Laboratory 63 explained that they had made an administrative error in reporting C2 as negative, while their raw data proved it to be positive for *Salmonella*. This laboratory scored a moderate performance as well.

The *Salmonella* serovars used for the positive control sample are shown in Table 4.5. The majority of the NRLs-*Salmonella* use *S*. Enteritidis or *S*. Typhimurium for their positive control samples. However, the use of a less common *Salmonella* serovar in routine samples may be advisable in order to make the detection of possible cross-contamination easier.

Table 4.5 Salmonella *serovars used by participants for the positive control samples*

***Salmonella* serovar**	**Number of users**
S. Enteritidis	17
S. Typhimurium	19
S. Nottingham	8
S. Abaetetuba	4
S. Alachua, *S*. Blegdam, *S*. Bongori, *S.* Harleystreet, *S.* Tranaroa, *S*. Poona, *S*. Infantis, *S.* adabraka	2 (per serovar)
S. Tennessee	1

4.3.2 *Correct scores of the control samples*

Table 4.6 shows the number of correctly analysed control samples for all participants and for the EU-MS only. The data have been corrected for the administrative error and for the mistake in interpreting the purpose of the C2 sample. No differences were found between these two groups. All laboratories showed correct results, resulting in accuracy rates of 100%.

Table 4.6 Correct scores found with the control samples by all participants and by the laboratories of the EU NRLS PPS and NRLS Food only

Control samples		**All labs n = 65**	**EU NRLs PPS n = 30**	**EU NRLs Food n =26**
Procedure control n=1	No. of samples	65	30	26
	No. of negative samples	65	30	26
	Specificity in %	**100%**	**100%**	**100%**
Positive control (own *Salmonella*) n=1	No. of samples	65	30	26
	No. of positive samples	65	30	26
	Sensitivity in %	**100%**	**100%**	**100%**
All control samples n=2	No. of samples	130	60	60
	No. of correct samples	130	60	60
	Accuracy in %	**100%**	**100%**	**100%**

Note: 'EU NRLs' included NRLs from United kingdom and Northern Ireland.

4.4 Artificially contaminated hygiene swab samples

4.4.1 General

Hygiene swab samples artificially contaminated with background flora and two different concentrations of *Salmonella* Typhimurium, low (MPN concentration 3,3 cfu/sample) and high (MPN concentration 37 cfu/sample), as well as negative samples, were analysed for the presence of *Salmonella* by the participants. Table 4.7 shows the overall results found by the participants.

Table 4.7 Number of positive results found with the artificially contaminated hygiene swab samples at each laboratory

	Number of positive isolations		
	Negative n=4	**STm low n=6**	**STm high n=4**
Criteria good performance	0	≥3	≥3
Lab code 1 NRL PPS	0	5	4
Lab code 3 NRL PPS	0	5	3
Lab code 39 NRL PPS	0	5	4
Lab code 67 NRL Food	0	3	4
All other NRLs	0	6	4

Negative hygiene swab samples

All laboratories correctly analysed the negative samples as being negative for *Salmonella*.

Hygiene swab samples contaminated with a low level of Salmonella *Typhimurium*

The majority of the participating laboratories were able to detect *Salmonella* in all six hygiene swab samples that were contaminated with a low inoculum level of approximately 3 cfu *S.* Typhimurium. Three laboratories (lab codes 1, 3 and 39) reported one of the six samples as negative for *Salmonella*. Lab code 67 reported three of the six hygiene swab samples with a low level of contamination as negative for *Salmonella*. In respect of low-level samples, a negative score for a maximum of three out of six samples is regarded as acceptable, hence these laboratories met the criteria for a good performance score. The results of all participants are shown in Figure 4.5.

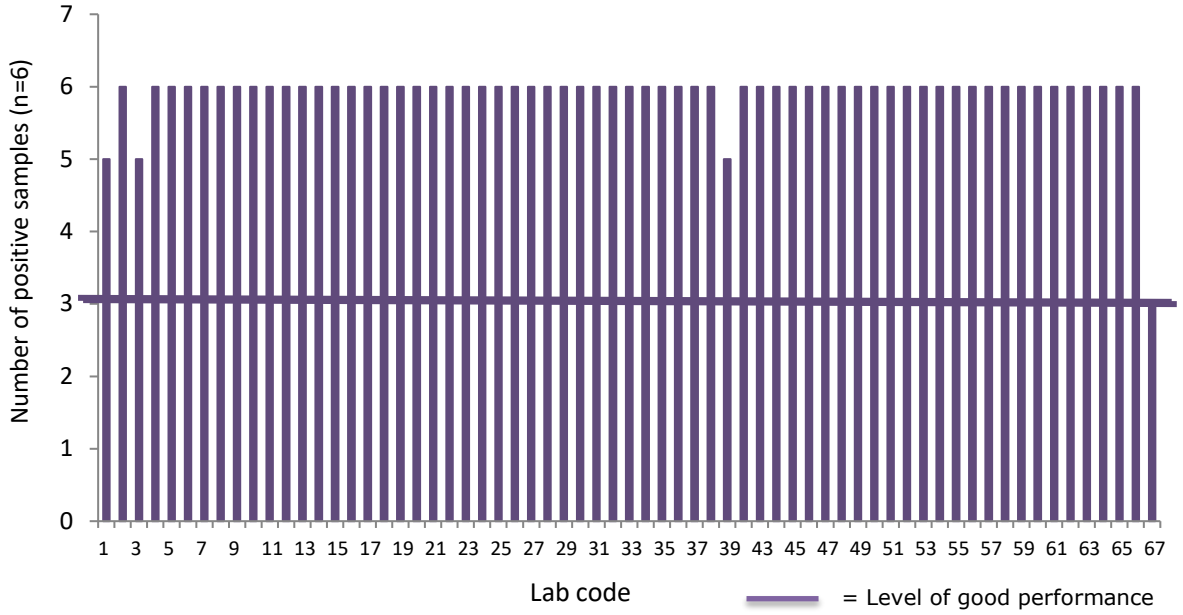

Figure 4.5 Number of positive Salmonella *isolations per laboratory found in the hygiene swab samples contaminated with a low level of* Salmonella *Typhimurium (n=6)*

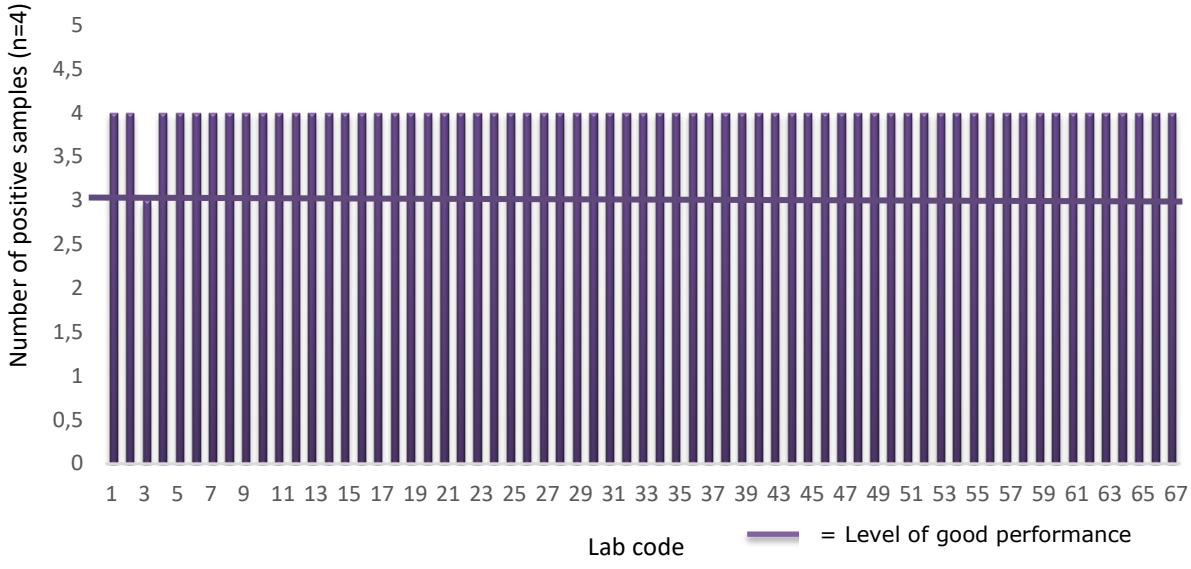

Figure 4.6 Number of positive Salmonella *isolations per laboratory found in the hygiene swab samples contaminated with a high level of* Salmonella *Typhimurium (n=4)*

Hygiene swab samples contaminated with a high level of Salmonella *Typhimurium*
The majority of the participating laboratories were able to detect *Salmonella* in all four samples inoculated with a high concentration of *S.* Typhimurium. Laboratory 3 found one high-level sample to be negative for *Salmonella*. This is still in accordance with the criteria for good performance. The results are shown in Figure 4.6.

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

Table 4.8 shows the specificity, sensitivity and accuracy rates for all artificially contaminated hygiene swab samples. The calculations were performed on the results of all participants and on the results of the EU NRLs PPS and EU NRLs food separately. All participants performed well in this study: the specificity rate (100%), the sensitivity rates (low level: 98,5%; high level 99,6%) and the accuracy rate (99,2%) were very high. Hardly any differences were found between all participants and the EU NRLs PPS or EU NRLs Food as shown in Table 4.8.

Table 4.8 Specificity, sensitivity and accuracy rates found by the participating laboratories (all participants and EU-MS only) with the artificially contaminated Hygiene swab samples

Hygiene swab samples		All participants n=65	EU NRLs PPS n=30	EU NRLs Food n = 26
Negative samples n=4	No. of samples	260	120	104
	No. of negative samples	260	120	104
	Specificity in %	**100**	**100**	**100**
Low level STm n=6	No. of samples	390	180	156
	No. of positive samples	384	178	153
	Sensitivity in %	**98,5**	**98,9**	**98,1**
High level STm n=4	No. of samples	260	120	104
	No. of positive samples	259	119	104
	Sensitivity in %	**99,6**	**99,2**	**100**
All hygiene swab samples with STm n=10	No. of samples	650	300	260
	No. of positive samples	643	297	257
	Sensitivity in %	**98,9**	**99,0**	**98,8**
All hygiene swab samples (pos. and neg.) n=14	No. of samples	910	420	364
	No. of correct samples	903	417	361
	Accuracy in %	**99,2**	**99,3**	**99,2**

Note: 'EU NRLs' included NRLs from the United Kingdom and Northern Ireland.

4.4.3 *Second detection method*

This year, 20 laboratories also used a second method to analyse the hygiene swab samples. An overview of the methods used per laboratory can be found in Table 4.9. All laboratories used a PCR method as a second method. Only two laboratories (lab codes 10 and 11) used a non-validated PCR method. Thirteen laboratories used this second method routinely for sample analysis.

The majority of NRLs found identical results with their second method compared to the prescribed bacteriological culture method. One laboratory (lab code 17) found one low-level sample to be negative for *Salmonella* using their second method, but positive using the bacteriological culture method. Laboratory 67 tested three low-level samples as negative for *Salmonella* using their second method, as they did using the bacteriological culture method.

*Table 4.9 Details on the second detection method used by NRLs-*Salmonella *during the Proficiency Test PPS-Food 2020*

Lab code	Second detection method	Validated (by)	Reference	Routinely # per year
4	qPCR	AFNOR	AFNOR BRD 07/06-07/04	No
10	Kasturi,K.N., Drgon, T.(2017) Real-time PCR Method Detection *Salmonella* spp. Environmental Samples			No
11	Kasturi,K.N., Drgon, T.(2017) Real-time PCR Method Detection *Salmonella* spp. Environmental Samples			No
12	BAX System, standard PCR assay for *Salmonella* (commercial end time PCR-system).	Nordval	Nordval certificate #030	2521
13	BAX System, standard PCR assay for *Salmonella* (commercial end time system).	Nordval	Nordval certificate #030	2521
14	real-time PCR	in house	Josefsen et al. (2007), Malorny et al. (2004)	70
15	real-time PCR	VFL	in-house	70
17	SureTect Real-time PCR	Thermo Fisher Scientific	AOAC 051303, AFFNOR UNI 03/07-11/13	2500
20	PCR	§64 of the national Food and Feed Code	Malorny et al.(2004) AEM 70:7046-7052	No
21	PCR	§64 of the national Food and Feed Code	Malorny et al.(2004) AEM 70:7046-7052	No
24	PCR - iQ-Check *Salmonella* II Kit (Bio-Rad)	AFNOR, AOAC, NORDVAL	BRD 07/06-07/04; 010803; 038	1
25	PCR - iQ-Check *Salmonella* II Kit (Bio-Rad)	AFNOR, AOAC, NORDVAL	BRD 07/06-07/04; 010803; 038	1
30	Real Time PCR 7500	National Laboratory Accreditation Authority	ISO 6579: 1 - 2017	3579

Lab code	Second detection method	Validated (by)	Reference	Routinely # per year
32	Real Time PCR	AFNOR	BRD_07/06-07/04	2500
36	BAX Q 7	AFNOR	QUA-18/3-11/02	No
38	Real-Time-PCR	AFNOR	BRD 07/06-07/04	2000
51	Real Time PCR from BIORAD, Kit iQ-Check *Salmonella* II.	AFNOR	AFNOR BRD 07/06 - 07/04	1012
52	Real Time PCR	NF validation: AOAC-RI	ISO16140	No
64	PCR	Afnor	ABI 29/02-09/10	54
67	qPCR	In house	R180001 and R18053	10000

4.5 Performance of the NRLs

4.5.1 General

All laboratories were able to detect *Salmonella* in high and low concentrations in hygiene swab samples. Out of the 65 laboratories, 63 fulfilled the criteria for good performance. Two laboratories (lab codes 63 and 66) reported their positive control as negative for *Salmonella*. Laboratory 66, using their raw data, proved that they misunderstood the purpose of sample C2. It was not considered as a positive control but as a normal sample and was therefore analysed as such and found to be negative. An extra sample was added as a positive control and found to be positive for *Salmonella*. This laboratory scored a moderate performance. Laboratory 63 made an administrative error and accidently reported the positive control as negative. Using their raw data, this laboratory proved that they found the positive control to test positive for *Salmonella*. For this reason, this laboratory also scored a moderate performance in this study.
No follow-up study was deemed necessary, as only administrative errors were made.

5 Conclusions

All NRLs for *Salmonella* were able to detect high and low levels of *Salmonella* in hygiene swab samples.

Sixty-three NRLs scored a 'good performance'. One laboratory (lab code 66) scored a moderate performance because they did not understand that the purpose of sample C2 was to serve as the positive control. One laboratory (lab code 63) scored a moderate performance for making an administrative error and accidently reporting the positive control as negative for *Salmonella*.

The specificity, sensitivity and accuracy rates of the control samples were 100%, 98,5% and 98,5% respectively.

The sensitivity rate of all laboratories that tested the hygiene swab samples that were artificially contaminated with a low level of *S*. Typhimurium was 98,5%.

The sensitivity rate of all laboratories that tested the hygiene swab samples that were artificially contaminated with a high level of *S*. Typhimurium was 99,6%.

The accuracy rate of all laboratories for the detection of *Salmonella* in the artificially contaminated hygiene swab samples was 99,2%.

Twenty participants used a second method in addition to the prescribed bacteriological culture method. All laboratories but one reported identical results for both methods. One laboratory (lab code 17) found one low-level sample to be negative for *Salmonella,* in contrast to the positive result found for the same sample when the bacteriological culture method was used.

List of abbreviations

AOAC	Association of Official Analytical Chemists
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
DG-SANTE	Directorate-General for Health and Consumer Protection
EFTA	European Free Trade Association
EN	European Standard
EU	European Union
EURL	European Union Reference Laboratory
ISO	International Organization for Standardization
MKTTn	Mueller Kauffmann Tetrathionate with novobiocin (broth)
MPN	Most Probable Number
MS	Member State
MSRV	Modified Semi-solid Rappaport-Vassiliadis
NRL	National Reference Laboratory
PCR	Polymerase Chain Reaction
PPS	Primary Production Stage
PS	Peptone saline solution
PT	Proficiency Test
RIVM	Rijksinstituut voor Volksgezondheid en het Milieu (National Institute for Public Health and the Environment)
RSAL	unknown abbreviation
RVS	Rappaport Vassiliadis with soya (broth)
SM (ID)2	*Salmonella* Detection and Identification-2
STm	*Salmonella* Typhimurium
UK	United Kingdom
VRBG	Violet Red Bile Glucose
XLD	Xylose Lysine Deoxycholate

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Annex I. Protocol to EURL *Salmonella* PT PPS-Food 2020

PROTOCOL

EURL-*Salmonella* Combined Proficiency test PPS-Food 2020
Detection of *Salmonella* in hygiene swabs

Introduction
This protocol describes the procedures for the combined Proficiency Test (PT) PPS-Food 2020 on the detection of *Salmonella* spp. in hygiene swabs amongst the National Reference Laboratories (NRLs) for *Salmonella* in the EU. The samples consist of hygiene swabs contaminated with background flora and different concentrations of a *Salmonella* serovar.

Note that the samples are transported with cooling packs and need to be stored at 5°C upon arrival.
The prescribed method is EN ISO 6579-1:2017 (Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp.). Additionally, laboratories (who are interested) can also perform a second detection method to analyse the sample if this is (routinely) used in their laboratories. Only the results obtained with EN ISO 6579-1:2017 are used to assess the performance of the NRL. Please report relevant details of the method(s) used in the result form.

Objective
The main objective of the Proficiency Test is to evaluate the performance of the NRLs for *Salmonella* for their ability to detect *Salmonella* spp. at different contamination levels in chicken faeces.

Outline of the study
Each participant will receive one box containing 2 large plastic safety bags, packed with cooling elements. The plastic safety bags contain 16 numbered plastic bags, consisting of:

- 14 samples with hygiene swabs artificially contaminated with background flora and different levels of a *Salmonella* serovar (coded B1-B14);
- 2 (empty) sample bags, to be used for the control samples, being only BPW (coded C1), and the (own) positive control of the participating laboratory (coded C2).
- 1 sample bag containing a small electronic temperature recorder (coded with lab code)

Upon arrival: all samples have to be stored at 5°C (± 3 °C) until the day of analyses (5 October 2020).

The sample bag containing the small electronic temperature recorder will measure the temperature during transport to the laboratory and storage of the samples at the laboratory. The sample bag with the

recorder are coded with your lab code. **You are urgently requested to return this complete plastic bag with recorder and lab code to the EURL-*Salmonella*, at the day your laboratory starts the study (5 October 2020)**. For this purpose a return envelope with a preprinted address label of the EURL-*Salmonella* is included.

Each box will be sent as biological substance category B (UN3373) by door-to-door (for non-EU-MS sometimes door-to-airport) courier service DHL. Please contact EURL-*Salmonella* when the parcel has not arrived at your laboratory by 1 October 2020 (this is 3 working days after the day of mailing).

The performance of the study will start on Monday 5 October 2020.

The documents necessary for performing the study are:

- Protocol EURL *Salmonella* combined Proficiency test PPS-Food 2020. Detection of *Salmonella* spp. in hygiene swabs (this document);
- Short guidance on electronic submission of data in the result form for the EURL Salmonella Proficiency Test on the detection of *Salmonella* spp. in hygiene swabs;
- EN ISO 6579-1:2017. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp.

All data have to be reported through the result form. The link, which will also become available on the EURL-*Salmonella* website will be sent by email to the participants. Submission of data has to be finalised on 31 **October 2020** (23:59 h CET) at the latest. **Mind that the electronic result form is no longer accessible after this deadline!** In case you foresee problems with the deadline, please contact us beforehand. The EURL will prepare a summary report soon after the study to inform all NRLs on the overall results.

Timetable

EURL- *Salmonella* Proficiency Test Primary Production Stage – Food 2020
Detection of *Salmonella* in hygiene swabs

Week	Date	Subject
27	Week of 29 June	E-mailing of the link to the registration form for the Proficiency Test. Please **register by 30 august** at the latest.
39	Week of 21 September	E-mailing the link for the result form to the participants. E-mailing of the protocol and instructions for the result form to the NRLs. Preparation of media by the NRLs.
40	Week of 28 September	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
41	Monday 5 October	Performance of the Proficiency Test.
44	31 October 2020	Deadline for completing the result form: 31 October 2020 (23:59h CET). After this deadline the result form will be closed.

If you have questions or remarks about this Proficiency Test, or in case of problems,
please contact:

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