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

EURL-*Salmonella* Proficiency Test Primary Production, 2018

Detection of *Salmonella* in boot socks with chicken faeces

RIVM Report 2019-0028

I.E. Pol-Hofstad | K.A. Mooijman

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

EURL-*Salmonella* Proficiency Test Primary Production, 2018

Detection of *Salmonella* in boot socks with chicken faeces

RIVM Report 2019-0028

Colophon

DOI 10.21945/RIVM-2019-0028

I.E. Pol-Hofstad (author), RIVM
K.A. Mooijman (author), RIVM

Contact:
Irene Pol-Hofstad
Centre for Zoonoses and Environmental Microbiology (Z&O)
Irene.Pol@RIVM.nl

This investigation was performed by order, and for the account, of the European Commission, Directorate-General for Health and Food Safety (DG-SANTE), within the framework of RIVM project number E/114506/18/RO European Union Reference Laboratory for *Salmonella* 2018.

This is a publication of:
National Institute for Public Health and the Environment
P.O. Box1 | 3720 BA Bilthoven
The Netherlands
www.rivm.nl/en

Synopsis

EURL-*Salmonella* Proficiency Test Primary Production, 2018
Detection of *Salmonella* in boot socks with chicken faeces

In October 2018, the EURL-*Salmonella* organised a Proficiency Test on the detection of *Salmonella* in animal production samples. Boot sock samples with chicken faeces were selected as matrix. All but one laboratory were successful in finding *Salmonella* in the contaminated boot sock samples. One laboratory had some problems with the contaminated boot sock samples, scoring the majority of the samples negative for *Salmonella*. This was most likely caused by the inactivation of the *Salmonella* bacteria due to the long transport period and the high temperatures the samples experienced during transport to this laboratory.

Participation was obligatory for all EU Member State National Reference Laboratories (NRLs) responsible for analysing *Salmonella* in animal production samples. In total, 36 NRLs participated in this study: 29 participants originated from 28 EU Member States (MS), six were based in third European countries, 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 in Europe.

Keywords: *Salmonella*, EURL, NRL, Proficiency Test, *Salmonella* detection method, boot socks

Publiekssamenvatting

Het EURL-*Salmonella* ringonderzoek productiedieren (2018)

Detectie van *Salmonella* in overschoentjes met kippenmest

In oktober 2018 organiseerde het EURL-*Salmonella een* ringonderzoek om *Salmonella* aan te tonen in kippenmest die op overschoentjes zit. Alle deelnemers op één na waren hiertoe in staat. Eén laboratorium heeft problemen gehad met de analyse van de monsters en kon in het grootste gedeelte van de monsters geen *Salmonella* aantonen. Dit kwam hoogst waarschijnlijk doordat de bacteriën niet meer in leven waren na de lange transporttijd en de hoge temperaturen waaraan het pakje met monsters is blootgesteld tijdens het transport naar dit laboratorium.

Deze jaarlijkse kwaliteitstoets is verplicht voor alle Nationale Referentie Laboratoria (NRL's) van de Europese lidstaten die ervoor verantwoordelijk zijn *Salmonella* aan te tonen in de leefomgeving van dieren die voor de voedselproductie worden gehouden. In totaal hebben 36 NRL's deelgenomen: 29 NRL's afkomstig uit alle 28 EU-lidstaten, zes NRL's uit andere Europese landen en één 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, overschoentjes, kippenmest, *Salmonella*-detectiemethode

Contents

Summary

In October 2018, the EURL-*Salmonella* Proficiency Test on the detection of *Salmonella* in primary production stage samples was organised. A total of 36 National Reference Laboratories (NRLs) participated in this study: 29 NRLs originating from 28 EU-Member States (MS), six 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. Participation was obligatory for all EU Member State NRLs responsible for the detection of *Salmonella* in primary production stage samples.

In this study, boot socks with chicken faeces from a pathogen free (SPF) farm was used. The boot sock samples with chicken faeces were artificially contaminated with a diluted culture of *Salmonella* Infantis at the EURL laboratory.

Each NRL received twenty blindly coded samples consisting of twelve boot sock samples with chicken faeces artificially contaminated with two different levels of *Salmonella* Infantis (6x low (10 cfu) and 6x high (53 cfu)), six blank boot socks with chicken faeces, and two control samples consisting 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 24 September 2018, the contaminated boot sock samples with chicken faeces 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.

Method
Most laboratories used EN-ISO 6579-1:2017, two laboratories used EN-ISO 6579:2002/Amd.1:2007 (Annex D), and three laboratories used another method.

Results control samples
All laboratories scored well, analysing both the procedure control as well as their own positive control sample.

Results artificially contaminated boot sock samples
All laboratories but two detected *Salmonella* in the boot sock samples with chicken faeces contaminated with a low level of *Salmonella*. Two laboratories (lab codes 1 and 3) found one of the six samples negative for *Salmonella*. This is still well within the criteria for good performance, which permit three negative samples.
Almost all laboratories detected *Salmonella* in all six high level samples. One laboratory (lab code 26) scored one of the six high-level samples negative. This is still within the criteria for good performance which permit one negative sample. The sensitivity score was 99.3% for these samples.
One laboratory (lab code 35) experienced problems with their samples. They found five of the six low-level samples negative for *Salmonella* and one of the six high-level samples negative. This was most likely due to temperature abuse during transport as the parcel arrived at the

laboratory after eight days, and the samples had experienced temperatures of 26 to 28 °C for several days. For that reason, the quality of the samples could not be guaranteed, and the results of this laboratory were not included in the evaluation.
All blank samples were scored correctly negative, resulting in a specificity of 100%.

Overall, the laboratories scored well in this Proficiency Test. The accuracy was 99.5%. Thirty-five laboratories fulfilled the criteria of good performance. The results of one laboratory were not included in the evaluation because of temperature abuse during sample transport.

1 Introduction

An important task of the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*), as laid down in Commission Regulation No 882/2004 (EC, 2004) and its successor 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 (http://www.eurlsalmonella.eu).

In October 2018, the EURL-*Salmonella* organised a PT to evaluate whether the NRLs responsible for the detection of *Salmonella* in Primary Production stage (PPS) samples could detect *Salmonella* at different contamination levels in boot sock samples with chicken faeces. 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* (Kuijpers & Mooijman, 2018; Pol-Hofstad & Mooijman, 2018). For the current study, boot sock samples with chicken faeces were artificially contaminated with a diluted culture of *Salmonella* Infantis (SI) at the EURL-*Salmonella* laboratory.
In total, eighteen boot sock samples had to be tested: six samples per contamination level (blank, low and high concentrations of *Salmonella* Infantis). Additionally, two control samples were tested: one procedure control and one positive control. The sample number and contamination levels were in accordance with CEN-ISO/TS 22117:2010.

2 Participants

Country	City	Institute
Austria	Graz	Austrian Agency for Health and Food Safety (AGES IMED/VEMI)
Belgium	Brussels	Sciensano
Bosnia and Herzegovina	Sarajevo	Veterinary faculty Sarajevo, department Health care of Poultry
Bulgaria	Sofia	National Diagnostic and Research Veterinary Institute (NDRVMI), National Reference Centre of Food Safety
Croatia	Zagreb	Croatian Veterinary Institute, 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, Research and Laboratory Services Department
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) National Veterinary Reference Laboratory for *Salmonella*
Greece	Chalkida	Veterinary Laboratory of Chalkida
Hungary	Budapest	National Food Chain Safety Office, Food and Feed Safety Directorate
Iceland	Reykjavik	Matís ohf, Analysis and Infrastructure
Israel	Kiryat Malachi	Southern Poultry Health Laboratory (Beer Tuvia)
Ireland, Republic of	Kildare	Central Veterinary Research Laboratory (CVRL/DAFFM) Laboratories Backweston, Department of Agriculture, Food and the Marine, Bacteriology
Italy	Padova Legnaro	Istituto Zooprofilattico Sperimentale delle Venezie, OIE
Latvia	Riga	Institute of Food Safety, Animal Health and Environment BIOR Animal Disease Diagnostic Laboratory, Food Safety and Environment

Country	City	Institute
		investigation Laboratory
Lithuania	Vilnius	National Food and Veterinary Risk Assessment Institute, Bacteriology Unit and Food Microbiology Unit
Luxembourg	Dudelange	Laboratoire de Médicine Vétérinaire de l"Etat, Bacteriologie
Macedonia, FYR of	Skopje	Food Institute, Faculty of Veterinary medicine Laboratory for food and feed microbiology
Malta	Valletta	Malta Public Health Laboratory (PHL), Evans Building
Netherlands, the	Bilthoven	National Institute for Public Health and the Environment (RIVM/Cib), Centre for Infectious Diseases Control, Centre for Zoonosis and Environmental Microbiology (Z&O)
Norway	Oslo	Norwegian Veterinary Institute, Section of Microbiology - animals and fish
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
Sweden	Uppsala	National Veterinary Institute
Switzerland	Zurich	National reference Centre for Poultry and Rabbit Disease
United Kingdom	Addlestone	Animal and Plant Health Agency (APHA), Bacteriology Department
	Belfast	Agri-Food and Bioscience Institute (AFBI) Veterinary Sciences Division Bacteriology

3 Materials and methods

3.1 Preparation of artificially contaminated boot sock samples with chicken faeces

3.1.1 *General*

The matrix in this PT was boot socks (Sodibox, Nevez, France) to which chicken faeces from a broiler breeder flock was added. The boot sock samples were artificially contaminated with a diluted culture of *Salmonella* Infantis at the EURL-*Salmonella* laboratory.

3.1.2 *Pre-tests for the preparation of boot sock samples with chicken faeces*

The batch of faeces was collected by the Animal Health Service from a *Salmonella* free broiler breeder flock (GD, Deventer). The batch of faeces (2 kg) for the pre-tests arrived at the EURL on 11 June 2018 and was stored at 5 °C. Immediately on receipt, five samples of 25 g of chicken faeces were taken randomly from the batch and tested for presence of *Salmonella* according to EN-ISO 6579-1:2017.

The boot socks were moisturised by adding 15 ml of peptone saline solution (PFZ) and left at room temperature for one to several hours to allow the fluid to thoroughly moisten the boot socks. Subsequently, 10 grams of chicken faeces was added to the boot socks. Some boot socks were artificially contaminated with different low concentrations (4, 10 and 14 colonies) of a diluted culture of *Salmonella* Infantis (15-A7 from *EURL-Salmonella*'s own collection).

To test the stability of the contaminated boot sock samples with chicken faeces during transport and storage, they were stored at 5 °C and 10 °C for a period up to three weeks. Five samples were tested for presence of *Salmonella* according to EN-ISO 6579-1:2017, and one sample was tested for the concentration of background flora according to EN-ISO 21528-2:2017 and EN-ISO 4833-1:2013 after zero, one, two and three weeks of storage.

3.1.3 *Preparation of boot sock samples with chicken faeces for Proficiency Test*

A large batch (15 kg) of chicken faeces from the same flock as the pre-tests arrived at the EURL-*Salmonella* laboratory on Tuesday 11 September 2018. Five samples each of 25 g were tested for the presence of *Salmonella* according to EN-ISO 6579-1:2017. After testing negative, 10 grams of chicken faeces was added to each pre-moistened boot sock sample (see 3.1.2) and subsequently artificially contaminated with *Salmonella* Infantis by adding no more than 0.5 ml of the appropriate dilution of an overnight culture. Two concentration levels were used: low (5-10 cfu/sample) and high (50-100 cfu/sample). The concentration of the inoculum used to contaminate the boot sock samples was determined by streaking the inoculum on XLD agar plates. Immediately after artificial contamination, the samples were stored at 5 °C until transport to the participating laboratories on Monday 24 September 2018.

3.1.4 *Determination of the level of background flora in boot sock samples with chicken faeces*

To obtain information on the level of background flora in the samples, the number of aerobic bacteria and the number of *Enterobacteriaceae* were determined in the samples of blank boot socks with chicken faeces using EN-ISO 4833-1:2013 and EN-ISO 21528-2:2017, respectively. To each boot sock sample, 225 ml of peptone saline solution was added. After mixing by hand (kneading), serial dilutions were prepared in peptone saline and analysed on PCA (Plate Count Agar) and VRBG (Violet, Red Bile Glucose Agar) to obtain the total number of aerobic bacteria and *Enterobacteriaceae*.

3.1.5 *Determination of the number of* Salmonella *in boot sock samples with chicken faeces by MPN*

The level of contamination of *Salmonella* in the artificially contaminated boot sock samples was determined by using a five-tube most probable number (MPN) technique. For this, ten-fold dilutions of five boot sock 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. From the number of confirmed positive dilutions, the MPN of *Salmonella* in the original sample was calculated using an MPN program in Excel (Jarvis et al., 2010).

3.2 Design of the Proficiency Test

3.2.1 *Number and type of samples*

Each participant received eighteen artificially contaminated boot sock samples with chicken faeces numbered B1 to B18. In addition, the laboratories had to test two control samples (C1 and C2). Table 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 which 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 boot socks moistened with 15 ml of Buffered Peptone Water (BPW) only had to be analysed. The protocol and test report used can be found in Annex I and II respectively.

3.2.2 *Shipment of parcels and temperature recording during shipment*

The twenty coded samples containing the contaminated boot sock samples with chicken faeces, the blank samples, and the control samples were packed in two safety bags. The safety bags 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 on receipt. To monitor exposure to abusive temperatures during shipment and storage, a micro temperature logger was placed in between the samples to record the temperature.

Table 1. Overview of the number and type of samples tested per laboratory in the Proficiency Test.

Contamination level	Boot sock samples + chicken faeces (n=18)
***S.* Infantis low level**	6
***S.* Infantis high level**	6
Blank (BL)	6
	Control samples (n=2)
Blank procedure control (BPW only)	1
Positive control (own control with *Salmonella*)	1

3.3 Methods

The method prescribed for this PT was EN-ISO 6579-1:2017, which consists of a pre-enrichment in Buffered Peptone Water (BPW) and selective enrichment on Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar, followed by plating-out on Xylose Lysine Deoxycholate agar (XLD) and a second medium of choice. Confirmation was 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.

3.4 Statistical analysis of the data

The specificity, sensitivity and accuracy rates were calculated for the artificially contaminated boot sock samples with chicken faeces. For the control samples, only the accuracy rates were calculated. The rates were calculated with the following formulae:

$$\text{Specificity rate: } \frac{\text{Number of negative results}}{\text{Total number of (expected) negative samples}} \times 100\%$$

$$\text{Sensitivity rate: } \frac{\text{Number of positive results}}{\text{Total number of (expected) positive samples}} \times 100\%$$

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

3.5 Criteria for good performance

For the determination of 'good performance', the criteria indicated in Table 2 were used.

Table 2. Criteria for testing good performance in the Proficiency Test.

Contamination level	% Positive	# Pos samples/ total # samples
Boot sock samples with chicken faeces		
***S.* Infantis high-level (SI)**	Min. 80 %	Min. 5/6
***S.* Infantis low-level (SI)**	Min. 50 %	Min. 3/6
Blank (BL)[1]	Max. 20 %[1]	Max. 1/6[1]
Control samples		
Procedure control (boot socks with BPW only)	0 %	0 /1
Positive control (own control with *Salmonella*)	100 %	1 /1

1. All should be negative. However, as no 100% guarantee of the *Salmonella* negativity of the matrix can be given, 1 positive out of 6 blank samples (20% positive) is considered acceptable.

4 Results and discussion

4.1 Preparation of artificially contaminated boot sock samples with chicken faeces

4.1.1 *Pre-tests for the preparation of boot sock samples with chicken faeces*

The study's set-up was based on the study-design used in 2016 by the *EURL-Salmonella* (Pol-Hofstad and Mooijman, 2016). To test if the contaminated boot sock samples were stable during transport and storage, the boot sock samples were contaminated with a high and a low concentration of *Salmonella* Infantis 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 survival of *Salmonella* using EN-ISO 6579:1-2017. Results are presented in Figure 1.

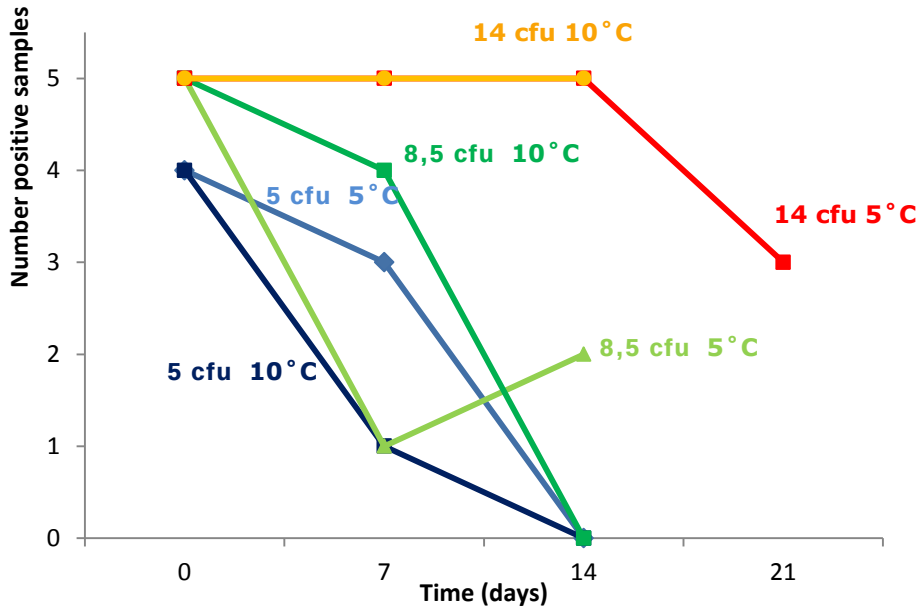


Figure 1. The number of boot sock samples with artificially contaminated chicken faeces that tested positive for Salmonella *after storage for three weeks at 5 °C and two weeks at 10 °C. Different colours indicate different concentrations of* Salmonella *Infantis.*

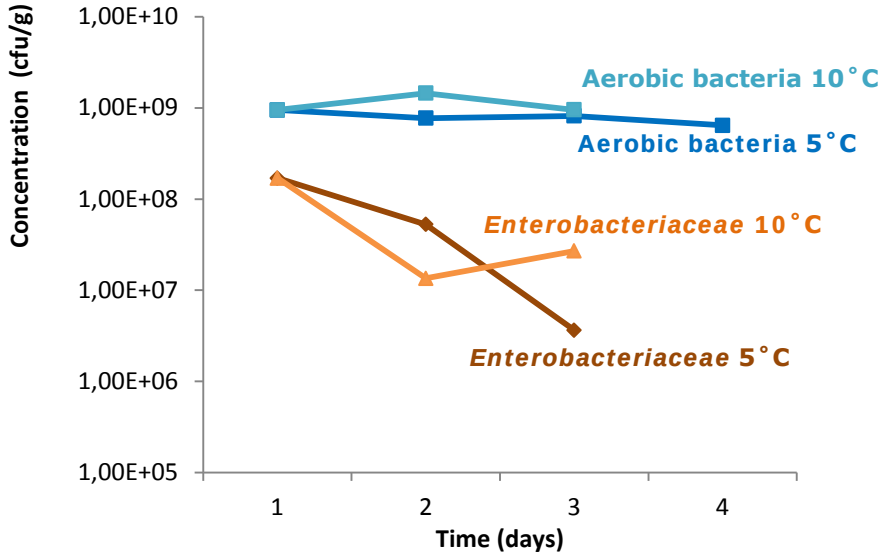


Figure 2. The effect of temperature and storage time on the number of aerobic bacteria and Enterobacteriaceae *in boot sock samples with chicken faeces (dark colour = 5 °C, light colour = 10 °C).*

Figure 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* Infantis. When low contamination levels were used (5 cfu and 8.5 cfu), one to four of the five samples tested negative for *Salmonella* after 1 week of storage. After two weeks, almost all samples were negative. With an increased contamination level (14 cfu), *Salmonella* was still detected in the samples after two weeks of storage at both temperatures. Only two in five samples were found negative after three weeks of storage; this is still acceptable for low contaminated samples.
The effect of storage and temperature on the background flora is shown in Figure 2, little difference can be seen in the number of aerobic bacteria when the samples are stored at 5 °C or at 10 °C. The number of aerobic bacteria remained approximately at the same level (10^9 cfu/g) for up to three weeks. The *Enterobacteriaceae* were more sensitive at either 5 °C or at 10 °C: the number decreased 1 log after two weeks of storage at 10 °C, and 2 log after two weeks of storage at 5 °C. However, sufficient background was left to represent a realistic sample.

4.1.2 *Preparation of boot sock samples with chicken faeces for the Proficiency Test*

Samples for the PT were prepared as described in 3.1.3.

4.1.3 *Background flora in the boot sock samples with chicken faeces*

The concentration of the background flora of the study samples was determined according to EN-ISO 21528-2:2017 and EN-ISO 4833-1:2013 as described in 3.1.4; results are shown in Table 3. The number of *Enterobacteriaceae* varied between 2.8×10^6 cfu/g on the day of preparation (t = 0) to 1.9×10^5 cfu/g after two weeks of storage at 5 °C (t = 14). The number of aerobic bacteria remained constant during the two weeks of storage at approximately 10^8 cfu/g.

Table 3 Number of aerobic bacteria and Enterobacteriaceae *per gram of chicken faeces*

Date of testing	t = 0 days (17 Sept 2018)	t = 14 days (1 Oct 2018)
***Enterobacteriaceae* cfu / g**	$2.8x10^6$	$1.9x10^5$
Aerobic bacteria cfu / g	$4.9x10^8$	$1.2x10^8$

4.1.4 *Number of* Salmonella *in boot sock samples with chicken faeces*

The boot sock samples with chicken faeces were artificially contaminated at the EURL-*Salmonella* laboratory by adding the appropriate volume of a diluted *Salmonella* culture. Table 4 shows the contamination level of the diluted culture of *Salmonella* Infantis used as inoculum to contaminate the boot sock samples with chicken faeces. The low-level samples were inoculated with 10 cfu, while the high-level samples were inoculated with 53 cfu. After inoculation, the samples were stored at 5 °C for almost two weeks until transport to the participants on 1 October 2018. The final contamination level of *Salmonella* in the boot sock samples with chicken faeces was determined by performing a five-tube Most Probable Number (MPN) test in the week of the interlaboratory comparison study. Results show that the concentration of *Salmonella* in the samples was in line with the anticipated concentration, taking into account the expected decrease of *Salmonella* Infantis during storage (see table 4).

Table 4 Number of Salmonella *Infantis (SI) in the inoculum and in the inoculated boot sock samples with chicken faeces.*

Date of testing	Low level SI (cfu / sample)	High level SI (cfu / sample)
18 Sept 2018 (Inoculum level diluted culture)	10	53
1 Oct 2018 MPN contaminated chicken faeces (95 % confidence limit)	3.3 (1.1-10.3)	17.3 (6.5-45)

4.2 Technical data Proficiency Test

4.2.1 *General*

A total of 36 NRLs *Salmonella* participated in this study: 29 originated from 28 EU-MS, six from third European countries (EU candidate or potential EU candidate MS and members of the EFTA countries), and one from a non-European country.

4.2.2 *Accreditation*

Almost all laboratories (28) were accredited according to EN-ISO 6579-1:2017, seven laboratories were accredited for EN-ISO 6579: 2002/Amd.1:2007(Annex D), four laboratories were accredited for EN-ISO 6579:2002, and two laboratories did not specify the method for

which they are accredited. Four laboratories were accredited for other methods: two for OIE manual, one for Bax Q7-Quantitative PCR *Salmonella,* and one for NMKL 187:2016.

4.2.3 *Transport of samples*

The samples were transported using a door-to-door courier on Monday 24 September 2018. Twenty-six laboratories received the parcel within one day of dispatch, six participants within two days, and two laboratories within three days. Two parcels took more than a week to arrive due to customs transport problems. One parcel arrived after eight days (lab code 35) and one parcel took ten days (lab code 22) to arrive at its destination. The temperature during transport and storage was recorded using a temperature recorder placed between the samples in the sample bag. The temperature during transport was predominantly between -3 °C and +5 °C. The temperature during transport in the parcel for laboratory 22 remained under 10 °C for 9 days. On the last day, the temperature rose to 16 °C. The temperature of the parcel for laboratory 35 rose to high values of up to 28 °C after 5 days and continued to be exposed to these high temperatures for another 3 days before it reached the laboratory. The participants were asked to store the parcel at 5 °C on arrival in their laboratories. The storage temperature at the receiving laboratories ranged from 0 – 7 °C.

Each laboratory was asked to test the samples using the prescribed method (EN-ISO 6579-1:2017) using MSRV agar as selective enrichment medium and XLD agar plus a second plating-out medium of their own choice for plating out. Table 5 shows which second plating-out media were chosen by the laboratories.

Table 5. Second plating-out media used by the NRLs.

Media	No. of users
BGA^{mod}	6
Rambach	7
BPLS	3
BGA	8
SM(ID)2	2
BxLH	1
ASAP	1
BSA	2
Other	6

Explanations of the abbreviations used are given in the ‘List of abbreviations’.

Technical details on the method which deviated from the prescribed ISO method (EN-ISO 6579-1:2017) are listed in Table 6 (grey-shaded cells); eight laboratories reported details of deviations. Four laboratories (lab codes 11, 15, 20 and 36) incubated the BPW for a longer period than prescribed. The pH of the used BPW was too high in two cases (lab codes 12 and 20). Four laboratories used MSRV with a pH higher than prescribed (lab codes 12, 23, 36, 37). In addition, four laboratories used

MSRV with a deviating concentration of Novobiocin (lab codes 11, 13, 36 and 37). Laboratory 13 replied after enquiries that they were using the right concentration, and reporting 1000 mg/l was a typing error.

Table 6. Reported technical deviations from the prescribed EN-ISO 6579-1:2017.

Lab code	BPW		MSRV	
	Incubation time (h:min)	pH	pH	Novobiocin
EN-ISO 6579-1	16–20 h	6.8–7.2	5.1–5.4	10 mg/l
11	20:30	7	5.2	20
12	19:00	7.3	5.9	10
13	19:20	7	5.3	1000
15	20:30	7	5.2	10
20	20:35	7.3	5.2	10
23	18:00	7.2	5.5	10
36	25:00	7	5.6	5
37	19:40	7.2	5.5	20

Table 7: Number of laboratories using the different confirmation methods.

Number of labs	Biochemical	Serological	Serotyping	PCR	other
5	x				
6	x	x			
1	x	x			x
2	x	x	x		
1	x	x	x	x	
6	x		x		
1	x		x	x	
4	x				x
1		x			x
3			x		
2			x		x
4					x

All participating laboratories performed one or several confirmation tests for *Salmonella*. In Table 7, all reported combinations are summarised. Other methods were: Maldi-tof, API 20E, Rapid 20E, Kligler, and Chromogenic agar method. Twelve laboratories used only one confirmation test; most laboratories used a combination of two or more confirmation methods.

4.3 Control samples

4.3.1 *General*

Two control samples were sent to the laboratories. One was used as a procedure control. The other was used as a positive control to which the laboratories had to add their own positive control strain normally used in their routine analysis for *Salmonella* detection.

Procedure control blank (moistened boot socks)
All laboratories scored good results for this control sample.

Positive control with Salmonella
All laboratories correctly scored their own *Salmonella* positive control sample as positive. The majority of the participants used a diluted culture of *Salmonella* as a positive control (24 laboratories). Others used a lenticule disc (8), a freeze-dried ampoule (2), frozen culture (1), a cryobank (1) with *Salmonella*. The *Salmonella* serovars used for the positive control sample are shown in Table 8. The majority of the NRLs-*Salmonella* use *S*. Enteritidis or *S*. Typhimurium for their positive control samples. But 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 8. Salmonella *serovars used by participants for the positive control samples.*

***Salmonella* serovar**	**Number of users**
S. Enteritidis	15
S. Typhimurium	7
S. Nottingham	6
S. Alachua, S. Blegdam, S. Infantis, S. Bongori, S. Harleystreet, S. Regent, S. Tranaroa, S. Tennessee.	1 (per serovar)

4.3.2 *Correct scores of the control samples*
Table 9 shows the number of correctly analysed control samples for all participants and for the EU-MS only. No differences were found between these two groups. All laboratories showed correct results, resulting in accuracy rates of 100%.

Table 9. Correct scores found with the control samples by all participants and by the laboratories of the EU-MS only (EU).

Control samples		All labs n=36	EU n = 29
Procedure control blank (moistened boot socks) n=1	No. of samples	36	29
	No. of negative samples	36	29
	Specificity in %	**100%**	**100%**
Positive control (own *Salmonella*) n=1	No. of samples	36	29
	No. of positive samples	36	29
	Sensitivity in %	**100%**	**100%**
All control samples n=2	No. of samples	72	58
	No. of correct samples	72	58
	Accuracy in %	**100%**	**100%**

4.4 Artificially contaminated boot sock samples with chicken faeces

4.4.1 *General*

Boot sock samples with chicken faeces artificially contaminated with two different levels of *Salmonella* Infantis, low (approx. 10 cfu) and high (approx. 53 cfu), as well as blank samples, were analysed for the presence of *Salmonella* by the participants. Table 10 shows the overall results found by the participants.

Table 10. Number of positive results found for the artificially contaminated boot sock samples with chicken faeces by each participant.

	Number of positive isolations		
	Blank n=6	SI low n=6	SI high n=6
Criteria good performance	≤1	≥3	≥5
Lab code 26	0	6	5
Lab code 1 and 3	0	5	6
Lab code 35	0	**1**	5
All other NRLs	0	6	6

Bold numbers = result below level of good performance

Blank samples

All laboratories correctly analysed the blank samples negative for *Salmonella*.

Low level contaminated Salmonella *Infantis samples*

Almost all laboratories were able to detect *Salmonella* in all six boot sock samples with chicken faeces contaminated with a low inoculum level of approximately 10 cfu *Salmonella* Infantis. Two laboratories (lab codes 1 and 3) reported one of the six samples negative for *Salmonella*. In respect of low-level samples, a negative score for a maximum of three out of six samples is regarded acceptable. Hence these laboratories scored well above the criteria for good performance.

Laboratory 35 scored five out of the six samples negative for *Salmonella*. The parcel for this laboratory had experienced a delay at the border customs and was exposed to very high temperatures for a prolonged period of time (see 4.2.3). It is likely that the high temperature affected the survival of *Salmonella* in the samples, explaining the high level of negative samples found by this laboratory. Since the quality of these samples could not be guaranteed, the performance of laboratory 35 was not evaluated. The results of all participants are shown in *Figure* 3.

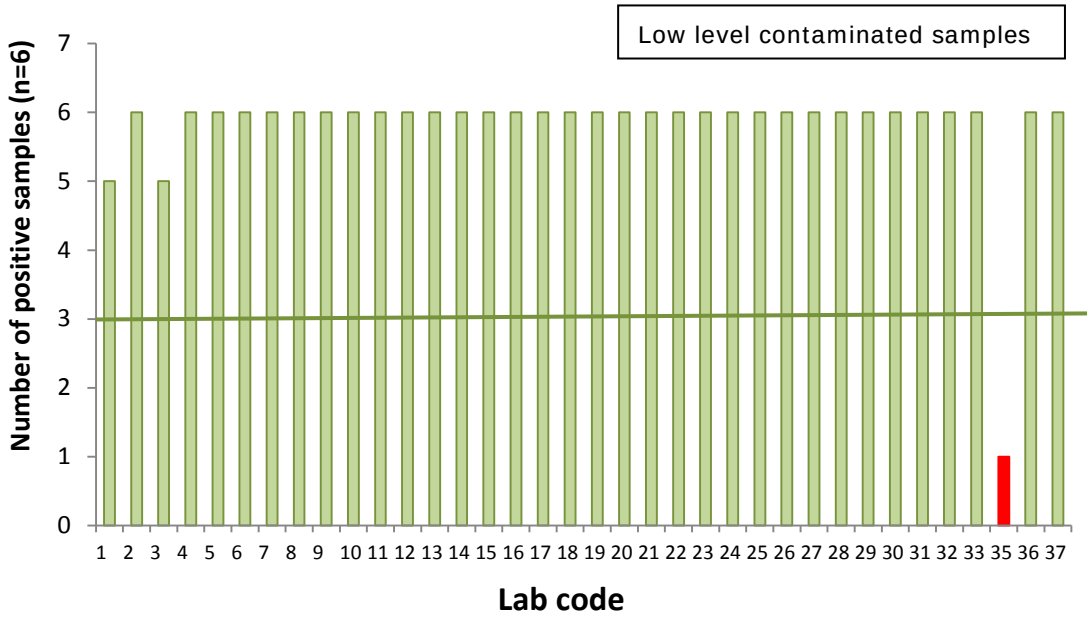


Figure 3. Number of positive Salmonella *isolations per laboratory found in the boot sock samples contaminated with low level* Salmonella *Infantis (n=6).*
= is level of good performance

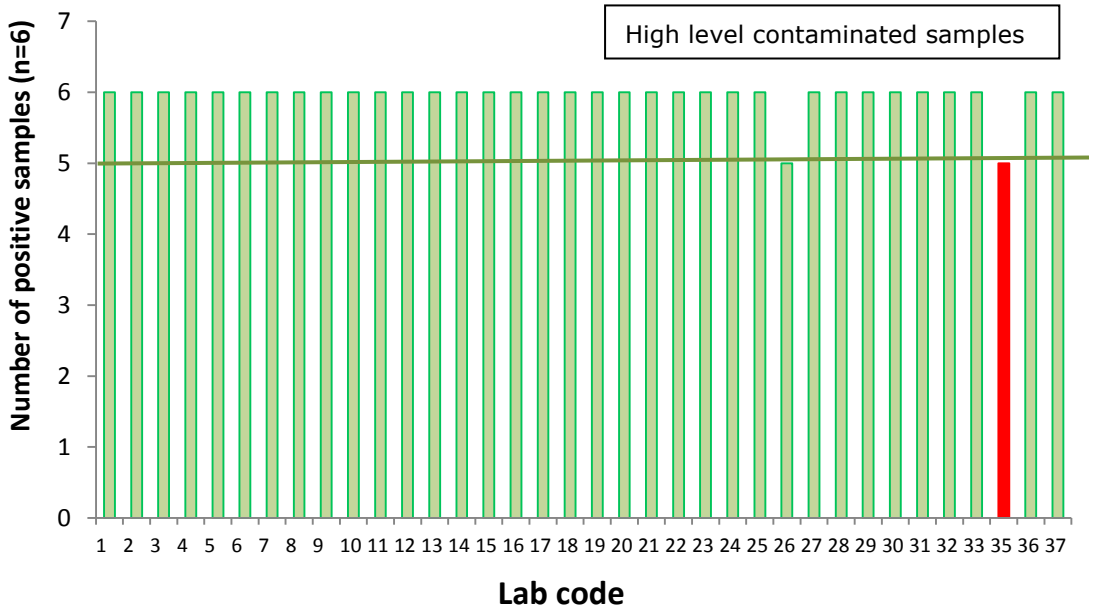


Figure 4. Number of positive Salmonella isolations per laboratory found in the boot sock samples contaminated with high level Salmonella Infantis (n=6).
= is level of good performance

High-level contaminated Salmonella *Infantis samples*
Almost all laboratories were able to detect *Salmonella* in all six samples inoculated with a high inoculum level of approximately 53 cfu of *Salmonella* Infantis. Laboratory 26 found one boot sock sample negative for *Salmonella*. The results are shown in Figure 4.

4.4.2 *Specificity, sensitivity and accuracy rates of the artificially contaminated samples*
Table 11 shows the specificity, sensitivity and accuracy rates for all artificially contaminated boot sock samples with chicken faeces. The calculations were performed on the results of all participants excluding laboratory 35, and on the results of the EU-MS only. Hardly any differences were found between these groups. All participants performed well in this study: the specificity rate (100%) and the sensitivity rates (low level: 99%; high level 99.5%) were very high for the group of participants as a whole.

Table 11. Specificity, sensitivity and accuracy rates found by the participating laboratories with the artificially contaminated boot sock samples with chicken faeces.

Boot sock samples with chicken faeces		All participants n=35	EU-MS n=29
Blank n=6	No. of samples	210	174
	No. of negative samples	210	174
	Specificity in %	**100**	**100**
Low level SI n=6	No. of samples	210	174
	No. of positive samples	208	173
	Sensitivity in %	**99**	**99.4**
High level SI n=6	No. of samples	210	174
	No. of positive samples	209	173
	Sensitivity in %	**99.5**	**99.4**
All boot sock samples with SI	No. of samples	420	348
	No. of positive samples	417	346
	Sensitivity in %	**99.3**	**99.4**
All boot sock samples (pos. and neg.)	No. of samples	630	522
	No. of correct samples	627	520
	Accuracy in %	**99.5**	**99.6**

4.4.3 *PCR (own method)*

This year, six laboratories (lab codes 9, 12, 24, 28, 35, and 37) also performed a PCR method to analyse the boot sock samples with chicken faeces as an additional detection technique (see Table 12). Almost all perform PCR as part of their routine analysis. They all tested the samples by PCR after pre-enrichment in BPW and all used a real-time PCR except laboratory 37 which used an end-time PCR. All laboratories used a validated PCR method.

The majority of NRLs found identical results with their PCR method and the bacteriological culture method. Two laboratories (lab codes 12 and 35) found different results. Laboratory 12 found two low level samples negative for *Salmonella* with the PCR method but positive with the bacteriological culture method. Both samples were found positive when retested with PCR. No explanation was found for the initial negative results. Laboratory 35 found two blank samples positive and two low-level samples positive with the PCR method in contrast to the results obtained with the bacteriological culture method. In addition, they found two high level samples negative for *Salmonella* using the PCR method. However, because of temperature abuse during transport of this particular parcel, the quality of the samples could not be guaranteed, and their results were not further evaluated.

*Table 12. Details of Polymerase Chain Reaction (PCR) procedures used by NRLs-*Salmonella *as own method during the Proficiency Test.*

Lab code	PCR method	Validated (by)	Commer-cially available	Routinely used number of test / 2016	DNA extraction after enrichment	Reference
9	Real Time	National	N	268	BPW	DIN 101352013/ 00.00.98
12	Real Time	AFNOR	Y	1329	BPW	REF4403870
24	Real Time	National	N	144	BPW	Malorny et al. 2004
28	Real Time	NF validation AOAC-RI	Y	-	BPW	ISO 16140
35	Real Time	National	N	4000	BPW	ISO 6579:2002/ Amd 1 2007. Annex D
37	End-Time	Nordval	Y	7500	BPW	Nordval certificate #030

4.5 Performance of the NRLs

4.5.1 *General*

All laboratories were able to detect *Salmonella* in high and low concentrations in boot sock samples with chicken faeces. Of the 36 laboratories, 35 fulfilled the criteria of good performance. One laboratory had problems with the contaminated boot sock samples, scoring five of the six low-level samples negative for *Salmonella* and one of the six high-level samples negative. This was most likely caused by the high temperature experienced during transport, which negatively affected the concentration of *Salmonella* in the boot sock samples with chicken faeces. Due to the poor temperature conditions in the parcel during the seven days of transport, the quality of the samples could not be guaranteed and therefore the results of this laboratory could not be evaluated.

5 Conclusions

All NRLs for *Salmonella* were able to detect high and low levels of *Salmonella* in boot sock samples with chicken faeces.

Thirty-five NRLs scored a ‘good performance’.

Results of laboratory 35 were not evaluated because the quality of the samples could not be guaranteed due to temperature abuse during transport.

The accuracy, specificity and sensitivity rates of the control samples were all 100%.

The sensitivity rate of the boot sock samples with chicken faeces artificially contaminated with a low level of *S*. Infantis was 99%.

The sensitivity rate of the boot sock samples with chicken faeces artificially contaminated with a high level of *S*. Infantis was 99.5%.

The accuracy rate of the NRLs in detecting *Salmonella* in the artificially contaminated boot sock samples with chicken faeces was 99.3%.

Six participants used a PCR technique in addition to the prescribed bacteriological culture method. Four laboratories reported identical results for both methods. One laboratory found two low level samples negative for *Salmonella* in contrast to their positive results using the bacteriological culture method. Laboratory 35 was excluded from the evaluation due to temperature abuse of the parcel during transport.

List of abbreviations

AFNOR	Association Française de Normalisation
AOAC	Association of Official Analytical Chemists
ASAP	AES *Salmonella* Agar Plate
BGA	Brilliant Green Agar
BGA (mod)	Brilliant Green Agar (modified)
BL	Blank (no colony-forming units)
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
EC	European Commission
EFTA	European Free Trade Association
EU	European Union
EURL	European Union Reference Laboratory
ISO	International Organization for Standardization
MPN	Most Probable Number
MS	Member State
MSRV	Modified Semi-solid Rappaport-Vassiliadis
NRL	National Reference Laboratory
PCA	Plate Count Agar
PCR	Polymerase Chain Reaction
PPS	Primary Production Stage
PT	Proficiency Test
RIVM	Rijksinstituut voor Volksgezondheid en het Milieu (National Institute for Public Health and the Environment)
RS	Rapid *Salmonella*
SI	*Salmonella* Infantis
SM (ID)2	*Salmonella* Detection and Identification-2
SPF	Specific Pathogen Free
VRBG	Violet Red Bile Glucose
XLD	Xylose Lysine Deoxycholate
Z&O	Zoonoses and Environmental Microbiology

References

EC 2004. Commission Regulation (EC) No. 882/2004 of the European Parliament and of the Council of 29 April 2004 on the official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. *Official Journal of the European Union* L 165 of 30 April. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2004R0882:20060525:EN:PDF (access date December 2016).

EC 2017. Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products, amending Regulations (EC) No 999/2001, (EC) No 396/2005, (EC) No 1069/2009, (EC) No 1107/2009, (EU) No 1151/2012, (EU) No 652/2014, (EU) 2016/429 and (EU) 2016/2031 of the European Parliament and of the Council, Council Regulations (EC) No 1/2005 and (EC) No 1099/2009 and Council Directives 98/58/EC, 1999/74/EC, 2007/43/EC, 2008/119/EC and 2008/120/EC, and repealing Regulations (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council, Council Directives 89/608/EEC, 89/662/EEC, 90/425/EEC, 91/496/EEC, 96/23/EC, 96/93/EC and 97/78/EC and Council Decision 92/438/EEC (Official Controls Regulation)Text with EEA relevance. *Journal of the European Union* L 95 of 7 April. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L:2017:095:TOC (access date 22 February 2019).

EURL-*Salmonella* (2017c). *History of EURL-Salmonella interlaboratory comparison studies on the detection of Salmonella.* https://www.eurlsalmonella.eu/sites/default/files/2018-06/History%20of%20EURL%20detection%20version%20September%202017.pdf (access date February 2019).

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

EN-ISO 6579:2002. *Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Salmonella spp.* International Organization for Standardization, Geneva, Switzerland.

EN-ISO 6579:2002/Amd.1:2007. *Amendment 1: Annex D: Detection of Salmonella spp. in animal faeces and in environmental samples from the primary production stage.* International Organization for Standardization, Geneva, Switzerland.

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

EN-ISO/IEC 17025:2005. *General requirements for the competence of testing and calibration laboratories*. International Organization for Standardization, Geneva, Switzerland.

EN-ISO 16140. *Microbiology of the food chain - Method validation - Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method.* International Organization for Standardization, Geneva, Switzerland.

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

EN-ISO/TS 22117:2010. *Microbiology of food & animal feeding stuffs - Specific requirements & guidance for Proficiency Testing (PT) by interlaboratory comparison.* International Organization for Standardization, Geneva, Switzerland.

Jarvis B., C. Wilrich & P.-T. Wilrich (2010). Reconsideration of the derivation of most probable numbers, their standard deviations, confidence bounds and rarity values, *Journal of Applied Microbiology*, 109:1660–7. Link to MPN calculation programme: http://www.wiwiss.fu-berlin.de/fachbereich/vwl/iso/ehemalige/wilrich/index.html (access date December 2018).

Kuijpers A.F.A & K.A. Mooijman (2018). *EURL-Salmonella 4th interlaboratory comparison study Animal Feed 2018*; Detection of *Salmonella* in chicken feed. RIVM report 2018-0023, Bilthoven, the Netherlands. https://www.rivm.nl/bibliotheek/rapporten/2018-0023.pdf (access date February 2019).

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

NMKL 187, 2016. *Salmonella*. Detection in foods, animal faeces and environmental materials from primary animal production using MSRV (2016).

Pol-Hofstad, I.E. & K.A. Mooijman (2016). *The 19th EU Interlaboratory comparison study in primary production (2016);* Detection of *Salmonella* in chicken faeces adhering to boot socks. RIVM report 2016-0044, Bilthoven, the Netherlands. https://www.rivm.nl/bibliotheek/rapporten/2016-0044.html (access date April 2018).

Pol-Hofstad, I.E. & K.A. Mooijman (2018). *The combined EURL-Salmonella Interlaboratory comparison study for Food and Primary production (2017);* Detection of *Salmonella* in hygiene swabs. RIVM report 2018-0021, Bilthoven, the Netherlands. https://www.rivm.nl/bibliotheek/rapporten/2018-0021.pdf (access date February 2019).

Annex I

PROTOCOL
INTERLABORATORY COMPARISON STUDY ON DETECTION OF *SALMONELLA* spp. IN SAMPLES FROM PRIMARY PRODUCTION STAGE
Organised by EURL-*Salmonella*, 2018

Introduction

This protocol describes the procedures for the interlaboratory comparison study on the detection of *Salmonella* spp. samples from the primary production stage amongst the National Reference Laboratories (NRLs) for *Salmonella* in the EU. The samples consist of boot socks contaminated with chicken faeces. *Salmonella* is added to the bootsocks at the laboratory of the EURL-*Salmonella*.
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 their 'own' PCR method on the samples, if this is (routinely) used in their laboratories.

Objective

The main objective of the interlaboratory comparison study is to evaluate the performance of the NRLs for *Salmonella* for their ability to detect *Salmonella* spp. at different contamination levels in samples from the primary production stage.

Outline of the study

Each participant will receive one box containing two large plastic safety bags, packed with cooling elements. The plastic safety bags contain 20 numbered plastic bags, consisting of:

- 18 samples of chicken faeces adhering to bootsocks artificially contaminated with different levels of a *Salmonella* serovar (coded B1-B18);
- 2 samples of (moisturised) bootsocks, to be used for the control samples, being only BPW (coded C1), and the (own) positive control of the participating laboratory (coded C2).

Upon arrival: immediately store all the samples at 5°C (± 3 °C) until the day of analyses (1 October 2018).

One safety bag will also contain the small electronic temperature recorder to measure the temperature during transport to the laboratory and storage of the samples at the laboratory. The recorder is packed in

a plastic bag 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 (1 October 2018)**. 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 27 September 2018 (this is 3 working days after the day of mailing).

The performance of the study will start in week 40 (starting on Monday 1 October 2017).

The documents necessary for performing the study are:

- Protocol Interlaboratory comparison study on the detection of *Salmonella* spp. in samples from primary production stage 2018 (this document);
- Instructions for the web based test report: EU Interlaboratory comparison study on the detection of *Salmonella* spp. in samples from primary production stage, chicken faeces adhering to bootsocks 2018;
- 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.

The media to be used for this study will not be supplied by the EURL

All data have to be reported through an electronic result form. This year, the EURL *Salmonella* has changed software for this electronic reporting form to Form desk. 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 **26 October 2018** (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* interlaboratory comparison study on the detection of *Salmonella* spp. in samples from the primary production stage (2018)

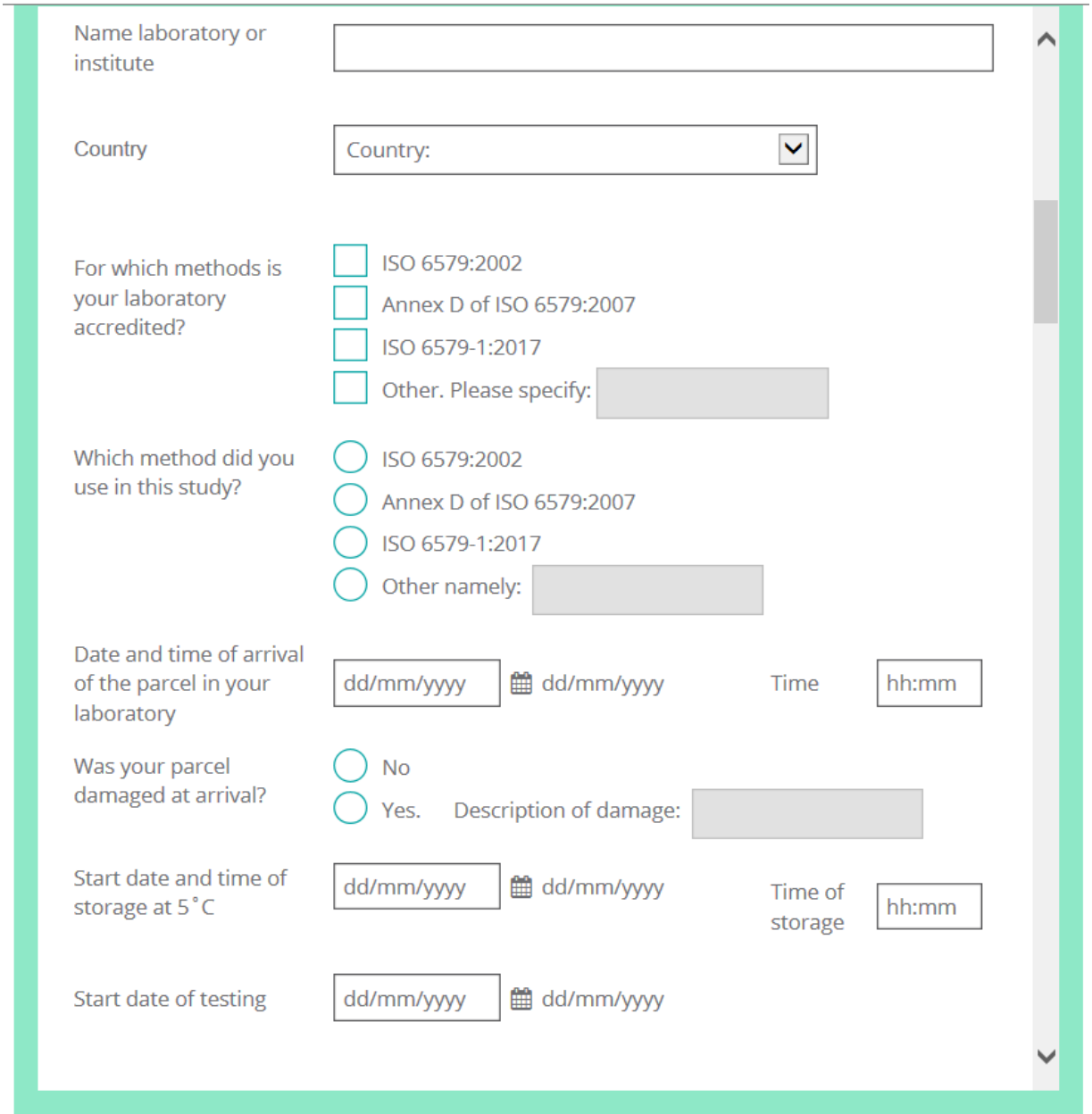
Week (2018)	Dates	Subject
37	In week of 10 September	Mailing of the protocol, lab code, and the questions of the web based test report to the NRLs by email.
38	In week of 17 September	Sending the link for the electronic result form to the participants by email
39	24 September	Mailing of the parcels to the NRLs as Biological Substance Cat. B (UN3373) by DHL courier service Preparation of the media by the NRLs
40	1 October	Preformance of the study
43	Before 26 October	Deadline for completing the electronic submission of results: **26 october** (23:59) After this deadline the electronic submission form will be closed.

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

Irene Pol-Hofstad
E-mail : Irene.Pol@rivm.nl
Tel. number: + 31 30 274 7057
RIVM / Z&O (internal Pb 63) EURL- *Salmonella*
P.O. Box 1, 3720 BA Bilthoven, The Netherlands
http://www.eurlsalmonella.eu/

Annex II Reporting form PT on detection of *Salmonella* in samples from the primary production stage


EURL Salmonella
EU Interlaboratory comparison study on detection of
Salmonella in samples from primary production
organised by EURL-Salmonella
Chicken faeces adhering to boot socks, October 2018
Laboratory information
Laboratory code
Name contact person
E-mail address contact person



Name laboratory or institute

Country

Country:

For which methods is your laboratory accredited?

- ISO 6579:2002
- Annex D of ISO 6579:2007
- ISO 6579-1:2017
- Other. Please specify:

Which method did you use in this study?

- ISO 6579:2002
- Annex D of ISO 6579:2007
- ISO 6579-1:2017
- Other namely:

Date and time of arrival of the parcel in your laboratory

dd/mm/yyyy dd/mm/yyyy Time hh:mm

Was your parcel damaged at arrival?

- No
- Yes. Description of damage:

Start date and time of storage at 5˚C

dd/mm/yyyy dd/mm/yyyy Time of storage hh:mm

Start date of testing

dd/mm/yyyy dd/mm/yyyy

Positive control sample

What kind of positive control did you use?

- [ ] Capsule
- [ ] Culti loops
- [ ] Culture
- [ ] Freeze dried (ampoule)
- [ ] Lenticule disc
- [ ] Other

What was the concentration of the *Salmonella* control sample?

cfu/sample

What was the volume of BPW used for the pre-enrichment?

ml BPW

Which *Salmonella* serovar did you use in the control samples?

- ○ Enteritidis
- ○ Typhimurium
- ○ Goldcoast
- ○ Nottingham
- ○ Panama
- ○ Poona
- ○ Tranaroa (Salmonella enterica subsp. salamae)
- ○ Other

Pre-enrichment - Buffered Peptone Water (BPW)

Name manufacturer BPW

Code number BPW

pH at the day of use

Start date and time of incubation BPW — dd/mm/yyyy — dd/mm/yyyy — Time — hh:mm

Temperature incubator BPW (˚C)

End date and time of incubation BPW — dd/mm/yyyy — dd/mm/yyyy — Time — hh:mm

Selective enrichment - Modified Semi solid Rappaport medium (MSRV)

Name manufacturer MSRV

Code number MSRV

Concentration novobiocin per 1L medium — mg/L

Temperature incubation MSRV — ˚C

pH at the day of use

Isolation - Xylose Lysine Desoxycholate medium (XLD)

Name manufacturer XLD

Code number XLD

Temperature incubation XLD — ˚C

Isolation - second Isolation medium (perscribed)

Medium information second isolation medium

Used incubation Temperature °C

Name manufacturer

Code number

Confirmaton - Non-selective medium

Did you streak colonies on Non-selective medium before starting confirmaton?

- ○ No
- ☑ Yes Please specify:

Confirmation of *Salmonella* suspected colonies

What media/test did you use for confirmation?

- ☐ Biochemical
- ☐ Serological
- ☐ Serotyping
- ☐ PCR
- ☐ Other Specify:

Detection by PCR (optional)

Did you use PCR in this study?
- (●) Yes
- ( ) No

What kind of PCR did you use?
- ( ) Real time PCR
- ( ) Other PCR. Specify:

Is that PCR commercially available?
- (●) Yes
- ( ) No

Name of PCR

Batch number

Name of manufacturer

Is the PCR validated?
- (●) Yes
- ( ) No

For which matrices is PCR validated?

By which organisation is the PCR is validated:

Reference number of literature reference PCR

Do you use PCR routinely?
- (●) Yes
- ( ) No

How many samples did you test for *Salmonella* using this PCR in 2017?

When did you start with the extraction /detection?
- ( ) Before pre-enrichment in BPW
- ( ) After pre-enrichment in BPW
- ( ) After selective enrichment on MSRV
- ( ) Other. Please Specify:

Table 1: Confirmed results for *Salmonella* after selective enrichment on MSRV
Samples B1 - B18 & C1 - C2

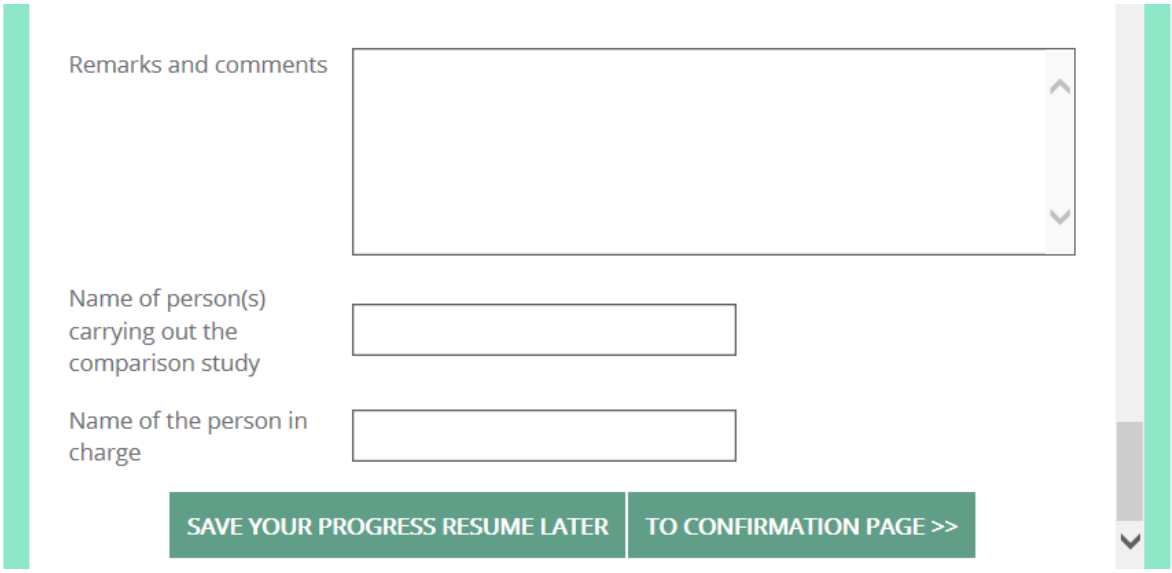
Indicate:
1 for positive confirmed result for *Salmonella*
0 for negative (confirmed) results for *Salmonella*

B1	
B2	
B3	
B4	
B5	
B6	
B7	
B8	
B9	
B10	
B11	
B11	
B12	
B13	
B14	
B15	
B16	
B17	
B18	
C1	
C2	

Table 2: Results of *Salmonella* detection using PCR Samples B1 - B18 & C1 - C2

Indicate:
1 for positive PCR result for *Salmonella*
0 for negative PCR results for *Salmonella*

B1	
B2	
B3	
B4	
B5	
B6	
B7	
B8	
B9	
B10	
B11	
B12	
B13	
B14	
B15	
B16	
B17	
B18	
C1	
C2	


Remarks and comments
Name of person(s) carrying out the comparison study
Name of the person in charge
SAVE YOUR PROGRESS RESUME LATER
TO CONFIRMATION PAGE >>

RIVM
Committed to *health and sustainability* -