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and the Environment
Ministry of Health, Welfare and Sport

The combined EURL-*Salmonella* interlaboratory comparison study for Food and Primary production (2017)

Detection of *Salmonella* in hygiene swabs

RIVM Report 2018-0021

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Colophon

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Synopsis

The combined EURL-*Salmonella* interlaboratory comparison study for food products and primary production stages (2017)

Detection of *Salmonella* in hygiene swabs

In October 2017, a combined EURL-*Salmonella* interlaboratory comparison study on the detection of *Salmonella* in food samples and animal primary production stage was organised. In this study, hygiene swabs were chosen to be the matrix. All the laboratories involved were able to detect *Salmonella* in all the contaminated hygiene swab samples; they were all successful in analysing both the blank control sample and the positive control sample correctly. One laboratory made a mistake reporting the positive control negative for *Salmonella* and was, therefore, scored as having a 'moderate performance'.
Blank hygiene swab samples, not contaminated with *Salmonella,* were correctly analysed as negative by almost all the laboratories. One laboratory found *Salmonella* present in two of the six blank samples and this was scored as a 'poor performance'. In a follow-up study this laboratory obtained good results for all samples.

Participation was obligatory for all EU Member State National Reference Laboratories (NRLs) responsible for the detection of *Salmonella* in food samples, and voluntary for NRLs responsible for the detection of *Salmonella* in primary production stage samples. These latter laboratories had already participated in the compulsory EURL study for the detection of *Salmonella* in primary production samples which was organised in March 2017. A total of 56 NRLs participated in this study: 33 NRLs for *Salmonella* in Food matrices and 23 NRLs for *Salmonella* in Primary Production Stage matrices (PPS). The participants came from all 28 EU Member States (MS), four of the NRLs were based in third European countries and one was based in a non-European country. The EURL-*Salmonella* is situated at the Dutch National Institute for Public Health and the Environment (RIVM). An important task of the EURL-*Salmonella* is to monitor and to improve the performance of the National Reference Laboratories in Europe.

Keywords: *Salmonella*, EURL, NRL, interlaboratory comparison study, *Salmonella* detection method, hygiene swabs

Publiekssamenvatting

Het gecombineerde EURL-*Salmonella*-ringonderzoek Voedsel en Productie dieren (2017)

Detectie van *Salmonella* in oppervlaktebemonsteringsponsjes

In oktober 2017 is het gecombineerde EURL-*Salmonella*-ringonderzoek gehouden om *Salmonella* aan te tonen in sponsjes die gebruikt worden om oppervlakten van te onderzoeken materialen te bemonsteren. De sponsjes zijn gebruikt, omdat ze geschikt zijn als monster voor zowel de NRL's Voedsel, als de NRL's Dieren voor de voedselproductie. Om praktische redenen zijn deze NRL's dit jaar samengevoegd.

Alle deelnemers waren in staat om *Salmonella* in de sponsjes op te sporen. Ook hebben de laboratoria de meegestuurde controlemonsters correct geanalyseerd. Eén laboratorium heeft een fout gemaakt in de rapportage van het controlemonster waarin *Salmonella* was aangetroffen. Hierdoor kreeg dit laboratorium een matige score. Bijna alle laboratoria konden de monsters waar geen *Salmonella* aan was toegevoegd (blanco), als zodanig opsporen. Eén laboratorium vond echter *Salmonella* in twee van de zes blanco monsters en scoorde daardoor een onvoldoende. Dit laboratorium heeft in de herkansing wel alle monsters goed beoordeeld.

Deze kwaliteitstoets is verplicht voor alle Nationale Referentie Laboratoria (NRL's) van de Europese lidstaten die ervoor verantwoordelijk zijn om *Salmonella* in voedsel aan te tonen; het is vrijwillig voor NRL's die *Salmonella* aantonen in de leefomgeving van dieren die voor de voedselproductie worden gehouden. Deze laatste laboratoria hadden in maart 2017 al deelgenomen aan het verplichte EURL-ringonderzoek naar *Salmonella.*

In totaal hebben 56 NRL's deelgenomen: 33 NRL's om *Salmonella* in voedsel aan te tonen en 23 NRL's om *Salmonella* aan te tonen in leefomgeving voor dieren die voor de voedselproductie worden gehouden. De NRL's waren afkomstig uit alle 28 EU lidstaten, vier 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, oppervlaktebemonsteringsponsjes, *Salmonella*-detectiemethode

Contents

Summary

In October 2017 the combined EURL-*Salmonella* interlaboratory comparison study on the detection of *Salmonella* in samples from food origin and primary production stage was organised. Because of recurrence of Avian Influenza caused by migrating birds in autumn and winter it was decided to change the order of the interlaboratory comparison studies on the detection of *Salmonella* in food and in matrices of the primary production stage. In this study, hygiene swabs were chosen as matrix since it was suitable both as food matrix as well as primary production stage matrix. Participation was obligatory for all EU Member State National Reference Laboratories (NRLs) that are responsible for the detection of *Salmonella* in food samples. The study was voluntary for NRLs that are responsible for the detection of *Salmonella* in primary production stage samples. The latter laboratories already participated in the compulsory EURL study for the detection of *Salmonella* in primary production samples organised in March 2017.

A total of 56 NRLs participated in this study: 33 NRLs for *Salmonella* in Food matrices and 23 NRLs for *Salmonella* in Primary Production Stage matrices (PPS). The participants originated from 28 EU-Member States (MS), 4 NRLs from third European countries (EU candidate or potential EU candidate MSs and members of the European Free Trade Association (EFTA)) and one NRL from a non-European country (Israel).

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

Each NRL received twenty blindly coded samples, consisting of twelve hygiene swabs artificially contaminated with background flora and two different levels of *Salmonella* Typhimurium (6x low (5 cfu) and 6x high (107 cfu)), six blank hygiene swabs 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 2 October 2017 the contaminated hygiene swab samples were packed and sent to the NRLs. Upon arrival, the NRLs were asked to store the samples at 5 °C until the start of the analysis.

Method
All laboratories were asked to use ISO 6579-1:2017 and select the appropriate enrichment media in accordance with the samples being considered as food matrix or as PPS matrix.

Results control samples
All laboratories scored well analysing both the procedure control as well as their own positive control sample. One laboratory made a mistake in reporting a negative result for the positive control, while their raw data indicated a positive result. This laboratory (lab code 28, NRL Food) scored a moderate performance.

Results artificially contaminated hygiene swab samples
All laboratories detected *Salmonella* in the hygiene swab samples contaminated with a high level of *Salmonella*.
In addition, almost all laboratories detected *Salmonella* in all six low level samples. One laboratory (lab code 11, NRL PPS) scored one of the six low level samples negative. This is well within the criteria for good performance, which allows for three negative samples. The sensitivity score was 99,9% for these samples.
The specificity of the study is given by the correctly scored blank samples; this was 99% for this study. Only one laboratory did not score all six blank samples negative (lab code 24, NRL Food). This laboratory reported two of the six blank samples positive for *Salmonella* and scored a poor performance. This laboratory participated in the follow-up study and obtained correct results for all samples.

Overall, the laboratories scored well in this interlaboratory study. The accuracy was 99,7%. Fifty-four laboratories fulfilled the criteria of good performance, one laboratory score moderate performance and one laboratory scored a poor performance.

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), is the organisation of interlaboratory comparison studies to test the performance of the National Reference Laboratories (NRLs) for *Salmonella*. The history of the interlaboratory comparison studies as organised by EURL-*Salmonella* (formerly called CRL-*Salmonella*) from 1995 onwards is summarised on the EURL-*Salmonella* website (http://www.eurlsalomonella.eu).

In October 2017 the EURL-*Salmonella* organised a combined interlaboratory study to test whether the NRLs for *Salmonella* in Food and Primary Production stage (PPS) could detect *Salmonella* at different contamination levels in hygiene swab samples. The results from interlaboratory studies like this show whether the examination of samples in the EU Member States (EU-MS) is being carried out uniformly and whether comparable results can be obtained by all NRLs-*Salmonella*.

Because of yearly outbreaks of Avian Influenza due to migrating birds during autumn and winter, the organisation of the interlaboratory study for detection of *Salmonella* in primary production samples at the beginning of the year, always faced numerous problems. Control measures due to outbreaks of Avian influenza may include prohibition of the transport of poultry faeces. This caused problems in the availability of faeces during the pre-tests in November and December and also when preparing the study samples at the beginning of the year. Therefore the EURL decided to change the order of the interlaboratory comparison studies on the detection of *Salmonella* in food and in samples from the primary production stage. To overcome the transition phase, hygiene swabs were chosen as matrix, since they are suitable both as food matrix samples as well as PPS samples.

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

The study design of this study was comparable to previous interlaboratory comparison studies (Kuijpers & Mooijman, 2016; Pol-Hofstad & Mooijman, 2016; Pol-Hofstad & Mooijman, 2017). For this study, hygiene swab samples were artificially contaminated with a combination of *E.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, eighteen hygiene swab samples had to be tested: six samples per contamination level (blank, low and high concentrations of *Salmonella* Typhimurium). Additionally, two control samples were tested: one procedure control and one positive control. The number and contamination levels of the samples were in accordance with ISO/TS 22117:2010.

2 Participants

2.1 Participants NRL Food

Country	City	Institute / NRL-*Salmonella*
Austria	Graz	Austrian Agency for Health and Food Safety (AGES IMED/VEMI)
Belgium	Brussels	Scientific Institute of Public Health (WIV-ISP)
Bulgaria	Sophia	National Diagnostic and Research Veterinary Institute (NDRVMI), National Reference Centre of Food Safety
Croatia	Zagreb	Croatian Veterinary Institute, Laboratory for Food Microbiology (CVI)
Cyprus	Nicosia	Cyprus Veterinary Services, Laboratory for the Control of foods of animal origin
Czech Republic	Prague	State Veterinary Institute (SVI)
Denmark	Ringsted	Danish Food Administration, Microbiology Ringsted
Finland	Helsinki	Finnish Food Safety Authority Evira Food and Feed Microbiology Laboratory section
France	Ploufragan	ANSES Laboratoire de Ploufragan-Plouzané, Unité Hygiène et Qualité des Produits Avicoles et Porcins (UHQPAP)
Germany	Berlin	Federal Institute for Risk Assessment (BfR)
Greece	Chalkida	Veterinary Laboratory of Chalkida,
Hungary	Budapest	National Food Chain Safety Office, Food and Feed Safety Directorate
Iceland	Reykjavik	Matis ohf, Analysis and Infrastructure
Ireland	Kildare	Central Veterinary Research Laboratory CVRL/DAFM Backweston, Department of Bacteriology and Parasitology Division
Italy	Legnaro PD	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 investigation Laboratory
Lithuania	Vilnius	National Food and Veterinary Risk Assessment Institute, Bacteriology Unit and Food Microbiology Unit
Luxembourg	Dudelange	Laboratoire National de Santé, surveillance alimentaire
Macedonia, FYR of	Skopje	Food Institute, Faculty of Veterinary Medicine Laboratory for food and feed microbiology
Malta	Valletta	Public Health Laboratory (PHL), Environmental Health Evans Building

Country	City	Institute / NRL-*Salmonella*
Netherlands, the	Bilthoven	National Institute for Public Health and the Environment (RIVM/CIb) Infectious Disease Control, Centre for Zoonoses and Environmental Microbiology (cZ&O)
Netherlands, the	Wageningen	Netherlands Food and Consumer Product Safety Authority (NVWA), Consumer and Safety Division, 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)
Spain	Madrid, Majadahonda	Centro Nacional de Alimentación (AECOSAN), Food Microbiology laboratory
Sweden	Uppsala	National Veterinary Institute (SVA), Department of Microbiology
Switzerland	Bern	Institute of veterinary Bacteriology, Vetsuisse Faculty, University of Bern
United Kingdom	London	Public Health England (PHE) Food Water and Environmental Microbiology Laboratory – London
United Kingdom	Belfast	Agri-Food and Bioscience Institute (AFBI) Veterinary Science Division (VSD) Bacteriology

2.2 Participants NRL PPS

Country	City	Institute
Austria	Graz	Austrian Agency for Health and Food Safety (AGES IMED/VEMI)
Belgium	Brussels	Scientific Institute of Public Health (WIV-ISP)
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
Estonia	Tartu	Estonian Veterinary and Food Laboratory
Finland	Kuopio	Finnish Food Safety Authority Evira Research and Laboratory Services Department

Country	City	Institute
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)
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 investigation Laboratory
Lithuania	Vilnius	National Food and Veterinary Risk Assessment Institute, Bacteriology Unit and Food Microbiology Unit
Netherlands, the	Bilthoven	National Institute for Public Health and the Environment (RIVM/Cib), Centre for Infectious Diseases Control, Centre for Zoonoses and Environmental Microbiology (Z&O)
Norway	Oslo	Norwegian Veterinary Institute, Section of Bacteriology
Portugal	Vairão	Instituto Nacional de Investigação Agrária e Veterinária , Food Microbiology
Slovenia	Ljubljana	Institute of Microbiology and Parasitology, Veterinary Faculty (UL, NVI)
Spain	Madrid Algete	Laboratorio Central de Veterinaria, Bacteriology 1
Switzerland	Bern	Institute of veterinary Bacteriology, Vetsuisse Faculty, University of Bern
United Kingdom	Addlestone	Animal and Plant Health Agency (APHA), Bacteriology Department
United Kingdom	Belfast	Agri-Food and Bioscience Institute (AFBI) Veterinary Sciences Division Bacteriology

3 Materials and methods

3.1 Preparation of artificially contaminated hygiene swab samples

3.1.1 General

The matrix in this interlaboratory comparison study was hygiene swabs ordered from WVR. Hygiene swabs are suitable to be used as food matrix as well as primary production stage matrix. The hygiene swabs were artificially contaminated with background flora consisted of a mixture of two bacteria and with a diluted culture of Salmonella at the laboratory of the EURL-Salmonella.

3.1.2 Pre-tests for the preparation of hygiene swab samples

Hygiene swab samples were ordered from VWR (no: vwrc710-1020; dry sponges size: 7.5 cm by 3.8 cm). Different bacteria were tested for suitability as background flora by testing interference with *Salmonella* confirmation tests. Two strains of *Enterobacter cloacae* from own culture collection (WR3 and M578), *Escherichia coli* (ATCC 11775), *Pseudomonas aeruginosa* (ATCC 27857), *Klebsiella pneumoniae* (ATCC 13883) and *Citrobacter freundii* (ATCC 8090) were selected for suitability tests.
The hygiene swabs were moisturised by adding 10 ml of Buffered Peptone Water (BPW) and left until totally soaked. The moisturised hygiene swabs were artificially contaminated with a suitable combination of background flora (10^6 cfu/swab) and with a high or a low concentration of a diluted culture of *Salmonella* Typhimurium (ATCC 14028). To test the stability of the contaminated hygiene swab samples during transport and storage, they were stored at 5 °C and 10 °C for a period up to thirteen weeks. Five samples were tested for the presence of *Salmonella* according to ISO 6579-1:2017 and one sample was tested for the concentration of background flora according to ISO 21528-2:2004 after zero, one, two, three, seven, ten and thirteen weeks of storage.

3.1.3 Preparation of hygiene swab samples for interlaboratory comparison study

Moisturised hygiene swabs were artificially contaminated with a suspension of background flora (consisting of a mixture of *E.coli* (ATCC 11775) and *Citrobacter freundii* (ATCC 8090) approx. 10^6 cfu/ml) and with *Salmonella* Typhimurium by adding 0.1 ml of the appropriate dilution of an overnight culture. Two *Salmonella* concentration levels were used; low (5 cfu/sample) and high (107 cfu/sample). The concentration of the inoculum used to contaminate the hygiene swab was confirmed by plating the relevant dilution on XLD (Xylose Lysine Deoxycholate) agar plates. Immediately after artificial contamination, the samples were stored at 5 °C until transport to the participating laboratories on Monday, 2 October 2017.

3.1.4 Determination of amount of background flora in hygiene swabs

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

10^6 cfu/swab. The total number of *Enterobacteriaceae* in hygiene swabs was investigated by following ISO 21528-2:2004. The hygiene swab samples were homogenised (kneaded) in peptone saline solution and ten-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 in the artificially contaminated hygiene swab samples was determined by using a five-tube most probable number (MPN) technique. For this, ten-fold dilutions of five hygiene swab samples at each contamination level were tested representing 10 g, 1 g and 0.1 g of the original sample. The presence of *Salmonella* was determined in each dilution by following ISO 6579-1:2017. The MPN of *Salmonella* in the original sample was calculated from the number of confirmed positive dilutions, using an MPN program in Excel (Jarvis, Wilrich & Wilrich, 2010).

3.2 Design of the interlaboratory comparison study

3.2.1 Number and type of samples

Each participant received eighteen artificially contaminated hygiene swab samples that were numbered B1 to B18. 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 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 Buffered Peptone Water (BPW) only had to be analysed. The protocol and test report used during the study can be found on the EURL-*Salmonella* website or can be obtained from the author of this report (EURL-*Salmonella* 2017a; 2017b).

3.2.2 Shipment of parcels and temperature recording during shipment

The twenty coded samples containing the contaminated hygiene swab samples, the blank samples and the control samples were placed 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 3.1. Overview of the number and type of samples tested per laboratory in the interlaboratory comparison study.

Contamination level	Test samples hygiene swabs (n=18)
***S.* Typhimurium low level (STM low)**	6
***S.* Typhimurium high level (STM high)**	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 interlaboratory comparison study was ISO 6579-1:2017. Hygiene swabs can be considered both as a food matrix and as a primary production sample. NRLs should use the appropriate method for the chosen matrix approach (food or PPS). The method starts with a pre-enrichment in Buffered Peptone Water (BPW). Selective enrichment is carried out on Mueller Kaufmann Tetrathionate novobiocin broth (MKTTn); 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 medium of choice. Confirmation is performed using the appropriate biochemical and serological tests as prescribed in ISO 6579-1:2017 or using reliable, validated identification kits. Laboratories were noted that the hygiene swabs were moisturised with 10 ml BPW and that adding 90 ml of BPW would result in the primary dilution. In addition to the 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 hygiene swab samples. For the control samples, only the accuracy rates were calculated. The rates were calculated according to 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 3.2 were used. Due to the nature of the matrix used in this study, the criteria for blank samples were altered. For sterile hygiene swabs (blank samples) no positive samples are allowed.

Table 3.2. Criteria for good performance in the interlaboratory comparison study.

Minimum result		
Contamination level	**Percentage positive**	**No. of positive samples/ total no. of samples**
Samples Hygiene swabs artificially contaminated		
***S.* Typhimurium high level (STM high)**	Min. 80%	Min. 5/6
***S.* Typhimurium low level (STM low)**	Min. 50%	Min. 3/6
Blank (BL)	0%	0/6
Control samples		
Procedure control (BPW only)	0%	0/1
Positive control (own control with *Salmonella*)	100%	1/1

3.6 Follow-up study

For the follow-up study an additional set of hygiene swab samples was prepared according to paragraph 3.1.3. Samples were stored at 5 °C at the EURL laboratory until transportation. The sample set consisted of ten sample coded B1-B10, four high contaminated samples and six blank samples. In addition, two control samples (C1 and C2) were added.

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

In practice, hygiene swabs are used to sample for instance food production areas or stables. All micro-organisms present on those surfaces will be taken up by the hygiene swabs. To mimic these practical conditions, background flora was added in addition to *Salmonella* Typhimurium to the sterile hygiene swabs. To test which organisms did not interfere with *Salmonella* confirmation and were therefore suitable as background flora, six different organisms were tested for typical growth characteristics on a number of selective media (see 3.1.2). Results are shown in Table 4.1.

Table 4.1. Growth characteristics of micro-organisms on MSRV, BSA, XLD and BGA agar plates.

	MSRV	**BSA**	**XLD**	**BGA**
***S.* Typhimurium**	**Clear Hallow**	**Black / green col.**	**Black (pink) colonies**	**Pink col. (red hallow)**
E. coli	No growth	White col.	Yellow col.	Green col.
K. pneumonia	No growth	Blue col.	Yellow col.	Green col.
E. cloacae WR3	No growth	Blue/green col.	Yellow col.	Green col.
E. cloacae M578	No growth	Light green col.	Yellow col.	Green col.
P. aeruginosa	No growth	Pink col.	Small pink colonies	**Pink col.**
C. freundii	No growth	White col.	Yellow col.	Green col.

Table 4.1 shows that none of the tested organisms show the same growth characteristics as *Salmonella* Typhimurium on the tested agar plates, indicating that these organisms would not create difficulties in *Salmonella* confirmation. A mixture of *E.coli* and *C. freundii* was chosen to serve as background flora in the study samples.
To test if the contaminated hygiene swab samples were stable during transport and storage, hygiene swabs were contaminated with a high amount of background flora (approx. 10^6 cfu/swab) and a high and low concentration of *Salmonella* Typhimurium as described in 3.1.2. Transport to and storage conditions at the receiving laboratories were mimicked by storing the samples at 5 °C and 10 °C for a number of weeks. Results can be seen in Table 4.2. In February 2017 the first tests with hygiene swabs as matrix were performed. Results show that the number of positive *Salmonella* samples was not affected by storage for up to three weeks at both 5 °C and 10 °C. In addition, the background flora level was also stable over the three weeks storage period. Pre-tests were repeated in August with lower contamination levels of *Salmonella*

Typhimurium, 6 cfu per swab sample and different selective enrichment media. *Salmonella* could still be detected in most of the samples at both storage temperatures. After a storage period of two weeks at 5 °C, one of the five samples was found negative when using MSRV, while all samples were positive when using RVS and MKTTn. After three weeks of storage all three enrichment media scored one of the five samples negative. The background flora remained stable over the storage period at 5 °C while it fluctuated somewhat when stored at 10 °C.

Table 4.2. Stability tests of hygiene swab samples artificially contaminated with background flora (n=1) and Salmonella Typhimurium at high and low levels (number of positive samples per total of 5 samples per level).

Date		Concentration	Temp (°C)	Time (weeks)			
				0	1	2	3
Feb	**Background flora**	(cfu)	5°C	$8.0x10^6$	$7.2x10^6$	$3.4x10^6$	$7.5x10^5$
			10°C		$7.3x10^7$	$9.7x10^7$	-
	STM MSRV	Low (14 cfu)	5°C	5/5	5/5	5/5	5/5
			10°C		5/5	5/5	-
	STM MSRV	High (76 cfu)	5°C	5/5	5/5	5/5	5/5
			10°C		5/5	5/5	-
Aug	**Background flora**	(cfu)	5°C	$8.7x10^6$	$6.6x10^6$	$3.9x10^6$	-
			10°C		$1.6x10^8$	$9.8x10^7$	-
	STM MSRV	Low (6 cfu)	5°C	5/5	5/5	4/5	4/5
			10°C		5/5	5/5	-
	STM RVS/MKTTn	Low (6 cfu)	5°C	5/5	5/5	5/5	4/5
			10°C		5/5	5/5	
Date		**Concentration**	**Temp (°C)**	**Time (weeks)**			
				0	**7**	**10**	**13**
July	**Background flora**	(cfu)	5°C	$6.8x10^6$	$4.7x10^4$	$2.0x10^3$	$5.5x10^5$
	STM MSRV	Low (11 cfu)	5°C	5/5	6/7	5/7	6/7
	STM RVS/MKTTn	Low (11 cfu)	5°C	5/5	6/7	5/7	6/7

Pre-test with even more prolonged storage periods for up to thirteen weeks showed that hygiene swab sample can be prepared a long period before the start of the study without an important reduction in positive samples. *Salmonella* proved to be very stable; only one or two samples of seven samples were found negative after seven-thirteen weeks of storage. This is still acceptable for low contaminated samples. The

background flora was more susceptible to prolonged storage, although the concentration was fluctuating strongly and counts are based on only one sample result.

4.1.2 Preparation of hygiene swab samples for interlaboratory comparison study

Samples for the interlaboratory comparison study were prepared as described in paragraph 3.1.3.

4.1.3 Background flora in the hygiene swab samples

The concentration of the background flora of the study samples was determined according to ISO 21528-2:2004 as described in 3.1.4. Results are shown in Table 4.3. The amount of background flora added to the hygiene swab samples during preparation ranged from 7.7 x 10^5 to 7.3 x 10^7 cfu/swab. After thirteen days of storage at 5 °C, the number of *Enterobacteriaceae* ranged from 1.4 x 10^4 - 7.1 x 10^6 cfu/swab was found.

Table 4.3. Number of Salmonella and Enterobacteriaceae in hygiene swab samples.

Samples	26 sept 2017		9 Oct 2017 (after storage at 5 °C)		
	Inoculum concentration		Conc. STM MPN (MSRV)	Conc. STM MPN (MKTTn)	Entero (cfu/swab)
	STM	Entero (cfu/swab)			
Blank		7.7 x 10^5			7.1 x 10^6
Low	5 cfu	1.3 x 10^6	7 (2.3-22)	7 (2.3-22)	1.4 x 10^4
High	107 cfu	7.3 x 10^7	92 (28-300)	92 (28-300)	4.7 x 10^6

4.1.4 Number of Salmonella *in hygiene swab samples*

The hygiene swab samples were artificially contaminated at the laboratory of the EURL-*Salmonella* laboratory by adding the appropriate volume of a diluted *Salmonella* culture. Table 4.3 shows the contamination level of the diluted culture of *Salmonella* Typhimurium used as inoculum to contaminate the hygiene swabs. The low level samples were inoculated with 5 cfu, while the high level samples were inoculated with 107 cfu. After inoculation, the samples were stored at 5 °C for almost one week until transport to the participants on 2 October 2017. 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 interlaboratory comparison study. Results show that the concentration of *Salmonella* in the samples was in line with the anticipated concentration (see table 4.3).

4.2 Technical data interlaboratory comparison study

4.2.1 General

A total of 56 NRLs participated in this study: 33 NRLs for *Salmonella* in food matrices and 23 NRLs for *Salmonella* in Primary Production matrices (PPS). The participants originated from 28 EU-Member States (MS), 4 NRLs from third European countries (EU candidate or potential EU candidate MSs and members of the EFTA) and one NRL from a non-European country (Israel).

4.2.2 Accreditation

All laboratories were accredited according to ISO/IEC 17025:2005. 29 laboratories were accredited for ISO 6579:2002, 25 laboratories were accredited for Annex D of ISO 6579:2007 and 22 laboratories were accredited for ISO 6579-1:2017, 2 laboratories were accredited for NMKL 71:1999. Most laboratories used ISO 6579-1:2017 to analyse the hygiene swab samples, although 21 of these were not yet accredited for this method.

4.2.3 Transport of samples

The samples were transported using a door-to-door courier on Monday 2 October 2017. Forty-four laboratories received the parcels within one day of dispatch and eleven participants within two days. One laboratory received the parcels after three days. Participants were asked to store the parcel at 5 °C on arrival in their laboratories. 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 -5 °C and +6 °C. The storage temperature at the receiving laboratories ranged from 0 – 10 °C.

4.2.4 Media

Each laboratory was asked to test the samples using the prescribed method (ISO 6579-1:2017) using RVS, MKTTn and/or MSRV agar depending on the approach of the hygiene swab samples as food or primary production matrix. As selective enrichment medium, XLD agar plus a second plating-out medium of their own choice had to be used. Table 4.4 shows which second plating-out media were chosen by the laboratories.

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

Media	No. of users
BGA^{mod}	11
Rambach	12
BPLS	7
BGA	8
RS	6
SM(ID)2	3
BxLH	0
ASAP	1
BSA	4
Other	4

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

Technical details on the method which deviated from the prescribed ISO method (ISO 6579-1:2017) are listed in Table 4.5 (grey-shaded cells). There were 29 laboratories reporting details of deviations. Four laboratories (lab codes 12, 27, 29 and 35) incubated the BPW for a longer period than prescribed. Two laboratories did not provide any information on the incubation period of BPW (lab codes 43 and 47). The pH of the used BPW was too high in five cases (lab codes 10, 22, 23, 25 and 42) and one laboratory (lab code 53) did not report any information on the pH. Most deviations were made in the novobiocin concentration of MKTTn. sixteen laboratories (lab codes 3, 4, 5, 12, 17, 19, 22, 36, 38, 41, 43, 46, 47, 49, 54, and 57) used MKTTn with a deviating novobiocin concentration ranging from 0-39 mg/l. In addition, three laboratories (lab codes 3, 31 and 52) used MKTTn with a deviating pH. Also the novobiocine concentration of MSRV was not correct in five cases (lab codes 2, 4, 5, 30 and 43). In addition three laboratories used MSRV with a deviating pH (lab codes 9, 10 and 54). One laboratory (lab code 53) did not report any of the requested media details.
In comparison with previous studies this is a rather large number of deviations.

Table 4.5. Reported technical deviations from the prescribed/requested procedures.

Lab code	BPW		RVS	MKTTn		MSRV	
	Incubation time (h:min)	pH	pH	pH	Novo-biocin	pH	Novo-biocin
ISO 6579-1	16–20 h	6.8–7.2	5.0–5.4	7.0–8.2	40 mg/l	5.1–5.4	10 mg/l
2	18:00	7.2				5.2	**1**
3	18:00	7.2	5.3	**6.6**	**4**		
4	20:00	7.1	5.3	8	**0,04**	5.2	**0,05**
5	20:00	7.1	5.3	8	**0,04**	5.2	**0,05**
9	18:00	7.1	5.3	7.8	40	**5.5**	10
10	20:00	**7.3**		7.7	40	**5.5**	10
12	**20:25**	7.0	5.1	7.0	**39**		
17	18:00	7.2	5.2	8	**10**		
19	17:00	7		8	**10**	5.3	10
22	18:10	**7.3**	5.3	7.8	**0**		
23	18:10	**7.3**				5	10
25	18:30	**7.3**				5.0	10
27	**21:35**	7.0				5.2	10
29	**20:30**	7.1		7.9	40/1	5.3	10
30	20:00	7.2	5.4	8.0	40	5.3	**20**
31	18:15	7	5.2	**6.6**	40		
35	**21:00**	7.2	5.2	8	40	5.3	10
36	18:25	7.2		8.1	**20mg**	5.3	10
38	19:30	7.1	5.2	7.5	**39**	5.3	10
41	17:55	7.0	5	7.9	**10**	5.1	10
42	20:00	**7.3**	5.3	7.7	40		
43	**??**	6.9	5.0	8.1	**5mg/l**	5.3	**1000**
46	17:00	7.1	5.2	8	**4**	5.2	10
47	**??**	7.0	5.2	8.0	**4**	5.2	10
49	19:00	7.1		8.1	**10 mg/L**	5.4	10 mg/L
52	19:40	6.9	5.3	**8.3**	40		
53	18:30	-		**-**	40	**-**	10
54	20:00	7.0	5.4	8.0	**10**	**5.48**	10
57	18:00	6.9		7.9	**39**	5.4	10

Grey cells = Deviating from ISO 6579-1:2017
- = No information supplied

All participating laboratories performed one or several confirmation tests for *Salmonella*. In Table 4.6 all reported combinations are summarised. Other methods were specified as Maldi-tof, VIDAS, microbact Identification Galeries or Chromogenic agar method. There were ten laboratories that used only one confirmation test. Most laboratories used a combination of two or more confirmation methods.

Table 4.6: Number of laboratories using the different confirmation methods.

Number of labs	Biochemical	Serological	Serotyping	other	PCR
3			X		
3	X				
1	X				x
12	X		X		
4	X		X		x
7	X	X			
4	X	X			X
5	X	X	X		
1	X	X	X		X
1	X	X		X	
2	X	X		X	X
2	X	X	X	X	
1	X		X	X	
2	X			X	
1			X	X	
4				X	
1				X	X
2			X	X	

4.3 Control samples

4.3.1 General

Two control samples were sent to the laboratories. One was used as a procedure control (BPW only). 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 the detection of *Salmonella*.

Procedure control blank (BPW only)

All laboratories scored good results for this control samples.

Positive control with Salmonella

As positive control, the majority of the participants used a diluted culture of *Salmonella* (36 laboratories). Others used a lenticule disc (10), a cultiloop (4), a freeze-dried ampoule (2), frozen culture (2), a vitroid or a capsule (1) with *Salmonella*. The *Salmonella* serovars used for the positive control sample are shown in Table 4.7.

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

***Salmonella* serovar**	**Number of users**
S. Enteritidis	20
S. Typhimurium	13
S. Nottingham	8
S. Alachua, S. Blegdam, S. Infantis, S. Bongori, S. Harleystreet	2 (per serovar)
S. Dublin, *S.* Tranaroa, *S.* Zanzibar, *S.* Panama, *S.* Tennessee,	1 (per serovar)

4.3.2 *Correct scores of the control samples*

Table 4.8 shows the number of correctly analysed control samples for all participants, NRLs Food and NRLs PPS. No differences were found between these groups. All laboratories found 100% correct results, with accuracy rates of 100%.

One laboratory made a reporting error by reporting a negative result for the sample intended as positive control (lab code 28). This laboratory could show in their raw data that they obtained a positive result for the positive control sample, but by mistake reported this sample as negative. For this reporting error, laboratory 28 received a moderate performance.

Table 4.8. Correct scores found for the control samples by all participants and by the separate groups of NRLs Food and NRLs PPS.

Control samples		All labs n=56	NRL-Food n=33	NRL-PPS n = 23
Procedure control blank (BPW) n=1	No. of samples	56	33	23
	No. of negative samples	56	33	23
	Specificity in %	**100%**	**100%**	**100%**
Positive control (own *Salmonella*) n=1	No. of samples	56	33	23
	No. of positive samples	56	33	23
	Sensitivity in %	**100%**	**100%**	**100%**
All control samples n=2	No. of samples	112	66	46
	No. of correct samples	112	66	46
	Accuracy in %	**100%**	**100%**	**100%**

4.4 Artificially contaminated hygiene swab samples

4.4.1 *General*

Hygiene swab samples artificially contaminated with two different levels of *Salmonella* Typhimurium, low (approx. 5 cfu) and high (approx. 107 cfu), as well as blank samples, were analysed for the presence of *Salmonella* by the participants. Table 4.9 shows the overall results obtained by the participants.

Table 4.9. Number of positive results found for the artificially contaminated hygiene swab samples at each laboratory.

	Number of positive isolations		
	Blank n=6	STM low n=6	STM high n=6
Criteria good performance	0	≥3	≥5
Lab code 24	**2**	6	6
Lab code 11	0	5	6
All other NRLs	0	6	6

Bold numbers = result below level of good performance

Blank samples
All but one laboratory correctly analysed the blank samples negative for *Salmonella*. Laboratory 24 found two of the six blank samples positive for *Salmonella* and scored a 'poor performance' as a result.

Low-level contaminated Salmonella *Typhimurium samples*
Almost all laboratories were able to detect *Salmonella* in all six hygiene swab samples contaminated with a low inoculum level of approximately 5 cfu. Only one laboratory (lab code 11, NRL PPS) reported one of the six samples negative for *Salmonella*. In respect of low level samples, it is acceptable to score a maximum of three out of six samples as negative, so this laboratory scored well above the criteria for good performance. The results are shown in Figures 4.1 and 4.2.

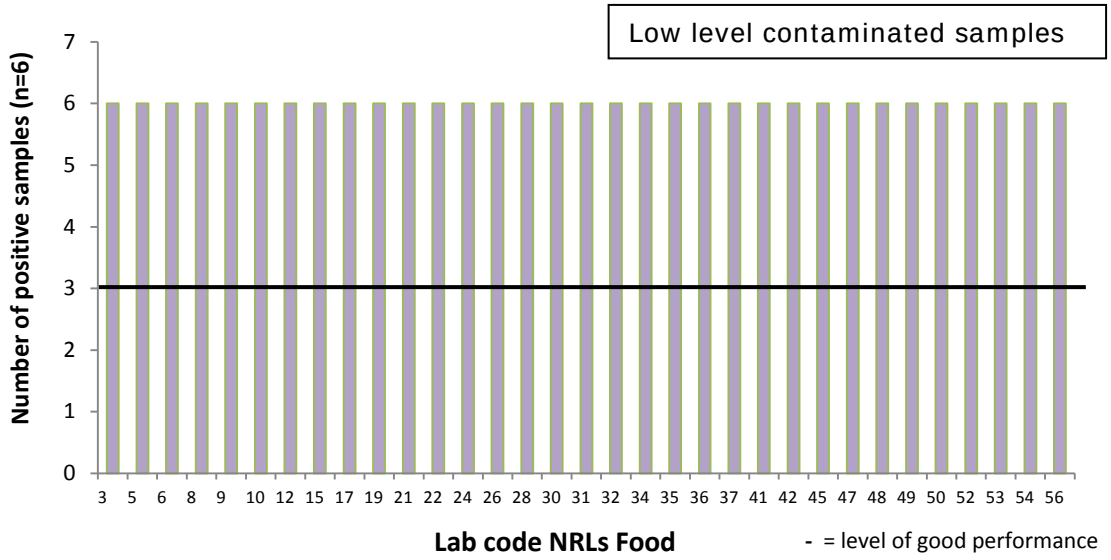

Figure 4.1. Number of hygiene swab samples artificially contaminated with a low level of Salmonella Typhimurium (n=6) that tested positive per laboratory for NRLs Food.

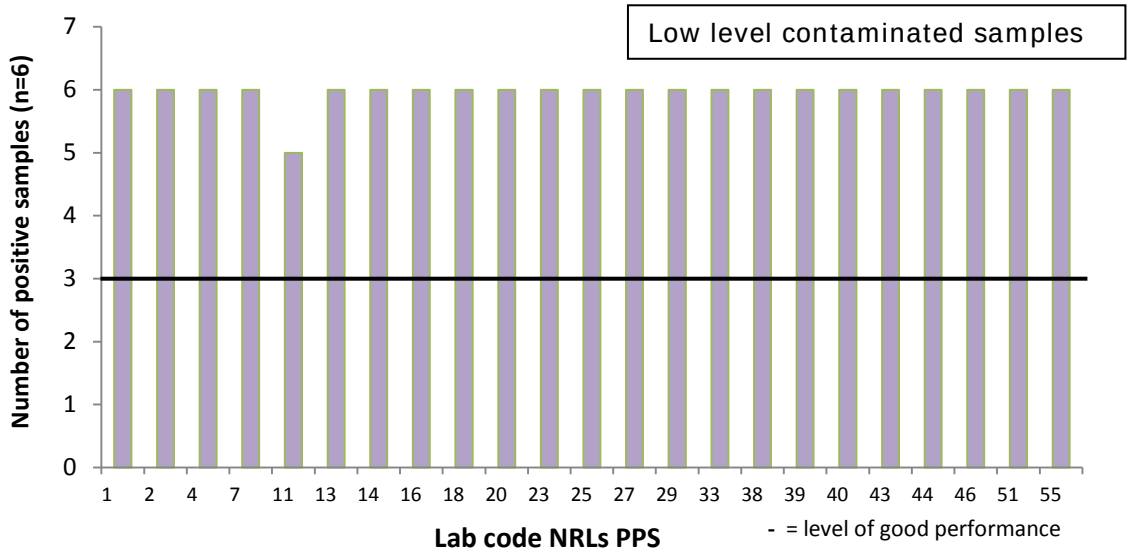

Figure 4.2. Number of hygiene swab samples artificially contaminated with a low level of Salmonella Typhimurium (n=6) that tested positive per laboratory for NRLs PPS.

High-level contaminated Salmonella Typhimurium samples
All laboratories were able to detect *Salmonella* in all six samples inoculated with approximately 107 cfu. The results are shown in Figures 4.3 and 4.4.

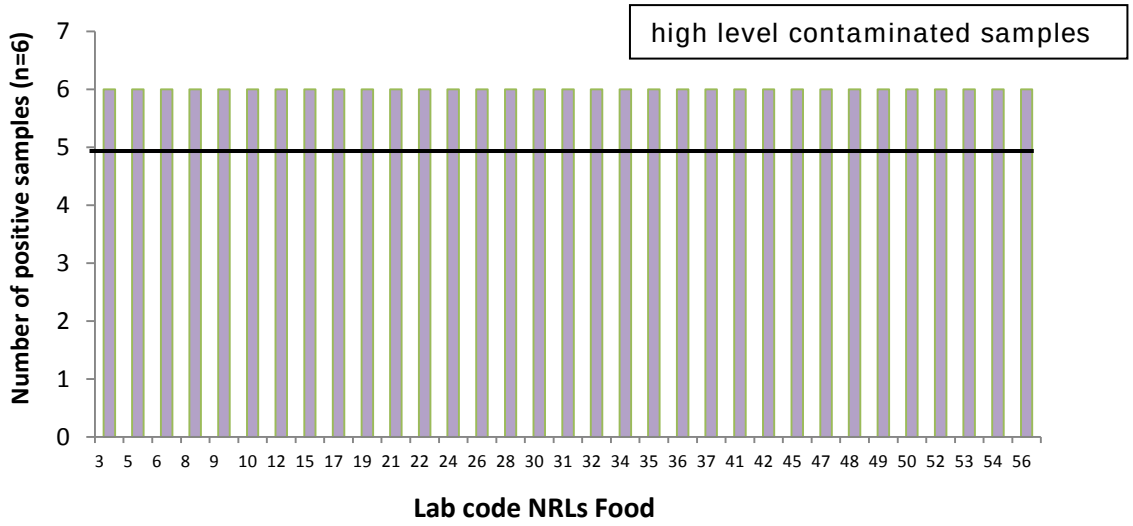

Figure 4.3. Number of hygiene swab samples artificially contaminated with a high level of Salmonella Typhimurium (n=6) that tested positive per laboratory for NRLs Food.

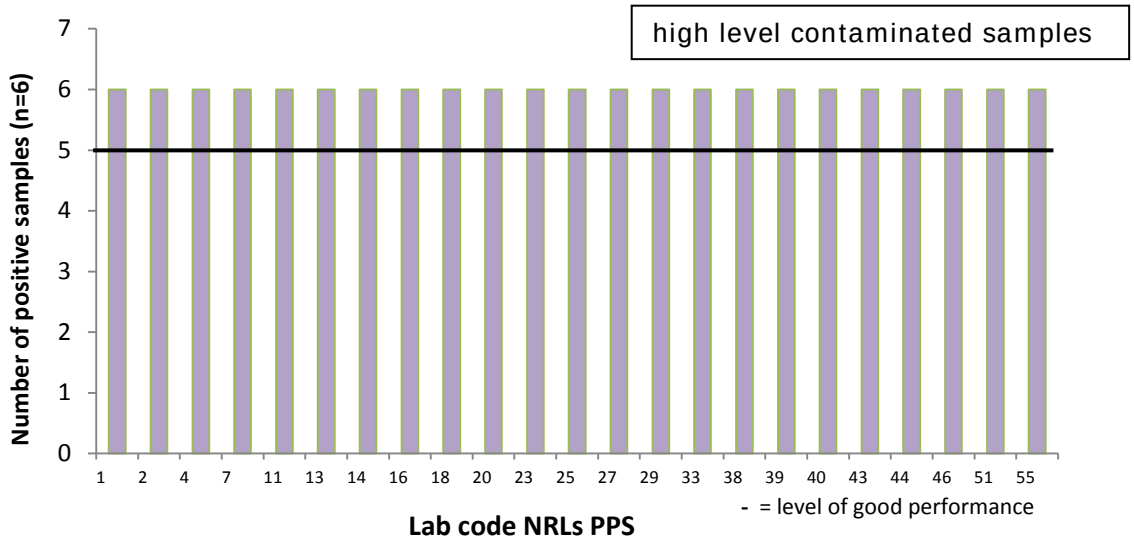

Figure 4.4. Number of hygiene swab samples artificially contaminated with a high level of Salmonella Typhimurium (n=6) that tested positive per laboratory for NRLs PPS.

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

Table 4.10 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 separate groups of NRLs Food and NRLs PPS. Only minor differences were found between these groups. All participants performed very well in this study: the specificity rate (99%) and the sensitivity rates (low

level: 99,7%; high level 100%) were very high for the group of participants as a whole.

Table 4.10. Specificity, sensitivity and accuracy rates found by the participating laboratories with the artificially contaminated hygiene swab samples.

Hygiene swab samples		Total labs n = 56	NRLs-Food n = 33	NRLs-PPS n = 23
Blank n=6	No. of samples No. of negative samples **Specificity in %**	336 334 **99%**	198 196 **98.9%**	138 138 **100%**
Low level (STM) n=6	No. of samples No. of positive samples **Sensitivity in %**	336 335 **99.7%**	198 198 **100%**	138 137 **99.3%**
High level (STM) n=6	No. of samples No. of positive samples **Sensitivity in %**	336 336 **100%**	198 198 **100%**	138 138 **100%**
All swab samples with STM	No. of samples No. of positive samples **Sensitivity in %**	672 671 **99.9%**	396 396 **100%**	276 275 **99.6%**
All swab samples (positive and negative)	No. of samples No. of correct samples **Accuracy in %**	1008 1005 **99.7%**	594 592 **99.7%**	414 413 **99.8%**

4.5 PCR (own method)

This year thirteen laboratories (lab codes 16, 17, 25, 26, 27, 30, 31, 33, 34, 35, 37, 48 and 56)) also performed a PCR method on the hygiene swab samples as an additional detection technique (see Table 4.11). Most laboratories tested the samples after pre-enrichment in BPW. Laboratories 16 and 17 started the DNA extraction before pre-enrichment in BPW. All laboratories used a real-time PCR except laboratory 30, which used a commercially available BAX system Q7. 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 33 and 34) found one high level samples negative but also one blank sample positive, in contrast to their correct results obtained with the bacteriological culture method.

Table 4.11. Details of Polymerase Chain Reaction (PCR) procedures used by NRLs-Salmonella as own method during the interlaboratory comparison study.

Lab code	PCR method	Validated (by)	Commercially available	Routinely used number of test/2016	DNA extraction after enrichment in	Reference
16	Real Time	National	N	78	-	
17	Real Time	National	N	78	-	
25	Real Time	Löfström 2012	N	1000	PBW	Malorny et al. 2004
26	Real Time	AFNOR	Y	9800	PBW	
27	Real Time	AFNOR	Y		PBW	
30	BAX system Q7	AFNOR	Y	632	PBW	
31	Real Time	AFNOR	Y	2400	PBW	
33	Real Time	AFNOR	Y	1041	PBW	
34	Real Time	AFNOR	Y	1041	PBW	
35	Real Time	ISO 16140	N		PBW	
37	Real Time	AFNOR and others	Y	249	PBW	
48	Real Time	National	N	> 10000	PBW	
56	Real Time	AFNOR	Y		PBW	

4.6 Performance of the NRLs

4.6.1 General

All laboratories were able to detect *Salmonella* in high and low concentrations in hygiene swab samples. Fifty-four of the 56 laboratories fulfilled the criteria of good performance. One laboratory scored a 'moderate performance' for making an error in reporting of the results of the positive control sample (lab code 28). And one laboratory (lab code 24) scored a 'poor performance' for falsely detecting *Salmonella* in two blank hygiene swab samples. This latter laboratory performed an extensive internal investigation to explain these deviating results. The *Salmonella* in the blank samples was the same serovar as used in the positive hygiene swab samples. Cross contamination in the laboratory was the most likely cause of the false positive blank samples. This laboratory participated in the follow-up study.

4.6.2 Follow-up study

The setup of the follow-up study was similar to the main study. The nature of the samples was based on the false positive blank samples found by laboratory 24. In total, this study contained twelve samples: two control hygiene swab samples (C1 and C2) and ten hygiene swab samples consisting of six blank samples (containing only background flora; 9.6×10^6 cfu/swab) and four artificially contaminated swab

samples inoculated with a high level of *Salmonella* Typhimurium (49 cfu/swab) to test for cross-contamination. A duplicate set of samples was tested by the EURL- S*almonella* for the presence of *Salmonella*.
Samples were prepared according to paragraph 3.6 and stored at 5 °C until transportation on Tuesday 3 April to Laboratory 24 as described in 3.2.2. Laboratory 24 was asked to store the samples at 5 °C until the day of analyses on Monday 9 April 2018.
The results of the follow-up study showed a good performance, all samples were analysed correctly. Most plausible explanation for the false positive blank results in the main study was cross contamination and the laboratory showed that implemented measures to avoid cross-contamination were successful.

5 Conclusions

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

Fifty-four NRLs scored a ‘good performance’ and one laboratory scored a ‘moderate performance’ due to a reporting mistake in the positive control samples. One laboratory scored a ‘poor performance’ for falsely detecting *Salmonella* in two of the six blank hygiene swab samples.

There were no differences in performances between the group of NRLs Food and NRLs PPS.

The accuracy, specificity and sensitivity rates of the NRLs with respect to the control samples after selective enrichment were all 100%.

The sensitivity rate for the hygiene swab samples artificially contaminated with a low level of *S*. Typhimurium was 99.7%.

The sensitivity rate for the hygiene swab samples artificially contaminated with a high level of *S*. Typhimurium was 100%.

The accuracy rate of the NRLs in detecting *Salmonella* in the artificially contaminated hygiene swab samples was 99.7%.

The majority of the NRLs-*Salmonella* use *S.* Enteritidis or *S*. Typhimurium for their positive control samples. But the use of a *Salmonella* serovar that is more rare in routine samples may be advisable in order to make the detection of possible cross contamination easier.

Thirteen participants used a PCR technique in addition to the prescribed classical method. Eleven laboratories reported identical results for both methods. Two laboratories found one high level sample negative for *Salmonella* and one blank sample positive for *Salmonella*.

In the follow-up study, all hygiene swab samples were analysed correctly.

List of abbreviations

AFNOR	Association Française de Normalisation (French Standardization Association)
ASAP	AES Salmonella Agar Plate
ATCC	American Type Culture Collection
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
MKTTn	Mueller-Kauffmann Tetrathionate-Novobiocin broth
MPN	Most Probable Number
MS	Member State
MSRV	Modified Semi-solid Rappaport-Vassiliadis
NRL	National Reference Laboratory
PCR	Polymerase Chain Reaction
RIVM	Rijksinstituut voor Volksgezondheid en het Milieu (National Institute for Public Health and the Environment)
PPS	Primary Production Stage
RS	Rapid *Salmonella*
RVS	Rappaport Vassiliadis Soya broth
STM	*Salmonella* Typhimurium
SM (ID)2	*Salmonella* Detection and Identification-2
VRBG	Violet Red Bile Glucose
XLD	Xylose Lysine Deoxycholate
Z&O	Zoonoses and Environmental Microbiology

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