National Institute for Public Health
and the Environment
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EU Interlaboratory comparison study food VI (2013)

Detection of *Salmonella* in minced chicken meat

RIVM report 2014-0010

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Colophon

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Abstract

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In 2013, it was shown that 32 out of 35 National Reference Laboratories (NRLs) in the European Union were able to detect high and low levels of *Salmonella* in minced chicken meat. Two laboratories made an initial transcription error when processing the raw data, which led to their performance being rated as 'moderate'. One laboratory continued to underperform during the follow-up study. Despite a significant improvement, this laboratory still had a sensitivity problem in the detection of *Salmonella*. Depending on the method used, the laboratories detected *Salmonella* in 61 to 78% of the contaminated samples. The detection of *Salmonella* in this study was made more difficult because of high levels of "interfering" bacteria in the minced chicken meat. These are some of the conclusions of the Sixth EU Interlaboratory Comparative Study of Food Samples, which was organized by the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*).

Interlaboratory comparative study obligatory for EU Member States
The study was conducted in September 2013, with a follow-up study in January 2014. Participation was obligatory for all EU Member State NRLs that are responsible for the detection of *Salmonella* in food samples. EURL-*Salmonella* is part of the Dutch National Institute for Public Health and the Environment (RIVM).

The laboratories used three internationally accepted analysis methods (RVS, MKTTn and MSRV) to detect the presence of *Salmonella* in minced chicken meat. Each laboratory received a package of minced chicken meat contaminated with two different concentrations of *Salmonella* Infantis, or containing no *Salmonella* at all. The laboratories were required to analyse the samples for the presence of *Salmonella* in accordance with the study protocol. In this study, the RVS and MSRV analysis methods produced significantly better results than the MKTTn method in terms of detecting *Salmonella* in minced chicken meat. This underscores the benefits of using more than one analysis method.

New procedures
Two new procedures were introduced and were positively received. For the first time, a food matrix was artificially contaminated with a diluted culture of *Salmonella* at the EURL-*Salmonella* laboratory. The NRLs were no longer required to combine the *Salmonella* samples. The feasibility of this procedure for subsequent studies will be assessed for each study. Furthermore, the participating laboratories were able to submit their findings via the Internet. This procedure will be optimized and continued.

Keywords: *Salmonella*, EURL, NRL, interlaboratory comparison study, *Salmonella* detection method, chicken meat

Publiekssamenvatting

EU Ringonderzoek voedsel VI (2013)
Detectie van *Salmonella* in kippengehakt

In 2013 waren 32 van de 35 Nationale Referentie Laboratoria (NRL's) in de Europese Unie in staat om hoge en lage concentraties *Salmonella* in kippengehakt aan te tonen. Twee NRL's behaalden een matig resultaat als gevolg van een foutieve verwerking van de ruwe data. Een laboratorium scoorde ook tijdens de herkansing onvoldoende. Ondanks grote verbeteringen had het nog steeds problemen met het aantonen van *Salmonella*. In totaal hebben de laboratoria, afhankelijk van de gebruikte methoden, *Salmonella* aangetoond in 61 tot 78 procent van de besmette monsters. Het aantonen van de *Salmonella* werd in deze studie bemoeilijkt doordat er veel "storende" bacteriën in het kippengehakt zaten. Dit blijkt uit het zesde voedselringonderzoek dat werd georganiseerd door het referentielaboratorium van de Europese Unie voor *Salmonella* (EURL-*Salmonella*).

Ringonderzoek verplicht voor Europese lidstaten
Het onderzoek is in september 2013 gehouden, de herkansing was in januari 2014. Alle NRL's van de Europese lidstaten die verantwoordelijk zijn voor de opsporing van *Salmonella* in voedsel, zijn verplicht om aan het onderzoek deel te nemen. Het EURL-*Salmonella* is gevestigd bij het Nederlandse Rijksinstituut voor Volksgezondheid en Milieu (RIVM).

De laboratoria toonden de *Salmonella*-bacterie in kippengehakt aan met behulp van internationaal erkende analysemethoden (RVS, MKTTn en MSRV). Elk laboratorium kreeg een pakket toegestuurd met kippengehakt dat besmet was met *Salmonella* Infantis in twee verschillende concentraties, of zonder *Salmonella*. De laboratoria dienden de monsters volgens een protocol te onderzoeken op de aanwezigheid van *Salmonella*. De analysemethoden RVS en MSRV bleken in deze studie significant betere resultaten te geven dan MKTTn. Dit bewijst het nut om met meerdere analysemethoden te werken.

Nieuwe werkwijzen
Er zijn twee nieuwe werkwijzen ingevoerd die positief zijn ervaren. Dit keer is voor het eerst het te onderzoeken voedselmateriaal (matrix) op het laboratorium van het EURL-*Salmonella* kunstmatig besmet met een verdunde cultuur van *Salmonella*. De NRL's hoeven hierdoor niet meer zelf de monsters met de *Salmonella* samen te voegen. Per studie wordt bekeken of deze werkwijze haalbaar is. Daarnaast konden de deelnemende laboratoria hun bevindingen via internet aanleveren. Deze werkwijze wordt geoptimaliseerd en voortgezet.

Trefwoorden: *Salmonella,* EURL, NRL, ringonderzoek, kippengehakt, *Salmonella*-detectiemethode

Contents

Summary

In September 2013 the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organized the sixth interlaboratory comparison study on the detection of *Salmonella* in a food matrix: minced chicken meat.
The participants were 35 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*): 30 NRLs from the 28 EU Member States (EU-MS) and 5 NRLs from third countries: candidate EU-MSs or potential EU candidate MSs and member countries of the European Free Trade Associations (EFTA).

The most important objective of the study was to test the performance of the participating laboratories for the detection of *Salmonella* at different contamination levels in a food matrix. For this purpose, minced chicken meat samples of 25 grams that were artificially contaminated with *Salmonella* Infantis (SI) at various contamination levels were analysed. The performance of the laboratories was compared with criteria of good performance. In addition, a comparison was made between the prescribed method (ISO 6579: Anonymous, 2002) and the requested method (Annex D of ISO 6579: Anonymous, 2007). For the prescribed method, the selective enrichment media were Rappaport Vassiliadis Soya broth (RVS) and Mueller Kauffmann Tetrathionate novobiocin broth (MKTTn). For the requested method, the selective enrichment was Modified Semi-solid Rappaport Vassiliadis (MSRV) agar.

The samples consisted of minced chicken meat artificially contaminated with a diluted culture of *Salmonella* Infantis (SI) at a low level (approximately 10 CFU/25 g of meat), at a high level (approximately 100 CFU/25 g of meat) and with no *Salmonella* at all (blank samples). The samples were artificially contaminated at the laboratory of the EURL, which was a new procedure for a food study. Before the start of the study, several experiments were carried out to make sure that the samples were fit for use in an interlaboratory comparison study (e.g. choice of *Salmonella* serovar, stability at different storage temperatures, influence of background flora).

Eighteen individually numbered blind samples with minced chicken meat had to be tested by the participants for the presence or absence of *Salmonella*. These samples consisted of six blank samples, six samples with a low level of SI (inoculum 11 CFU/sample, 5 MPN/sample) and six samples with a high level of SI (inoculum 104 CFU/sample, 55 MPN/sample). Additionally, three control samples had to be tested: two blank control samples (procedure control (BPW) and matrix control sample (minced chicken meat)) and one own (NRL) positive control sample (with *Salmonella*).

The laboratories found *Salmonella* in 61-78% of the (contaminated) samples, depending on the selective enrichment medium used. The accuracy rates for the prescribed selective enrichment media for the detection of *Salmonella* in food, MKTTn and RVS, were 73% and 83% respectively. For the requested method (MSRV), the accuracy rate was 85%.
The number of competitive, interfering bacteria in the minced meat was high in this study and interfered with the detection of *Salmonella* in the low-level contaminated minced chicken meat samples. Due to this fact, a decision was made to slightly adjust the criteria of good performance for the low-level contaminated samples.

A comparison between the different media was made. There was a significantly higher chance of finding *Salmonella* after selective enrichment in RVS or on MSRV for SI contaminated minced chicken meat samples compared to selective enrichment in MKTTn.
Longer incubation (two times 24 h) of MSRV gave 10% more positive results.

For the positive control, the majority of the participants (21 laboratories) used a diluted culture of *Salmonella*. The *Salmonella* serovars used for the positive control sample were *S*. Enteritidis (17) and *S*. Typhimurium (8).

A PCR (real time) method was used by three participants as their own method in addition to the prescribed method. Two participants found the same results with the PCR method as with the bacteriological culture method.

Thirty-two out of 35 laboratories achieved the level of good performance. Two NRLs reported a positive result for a blank sample. However, this turned out to be due to transcription errors and the results of the NRLs were indicated as moderate. One participant (non-EU-MS) showed difficulties with the detection of *Salmonella* in all samples and also found a false positive blank result for the procedure control sample. For this NRL, a follow-up study was organized in January 2014. The laboratory largely improved its performance but still did not reach the desired level. The EC, DG Sanco is informed accordingly.

The samples in this food study, i.e. minced chicken meat artificially contaminated with a diluted culture of *Salmonella* Infantis (SI), mimicked 'real life' routine samples more closely and were easier to use than previously used mixtures of matrix and reference materials.
The use of a web-based test report for reporting the results of the study by the participants was successful. The web-based report was used for the first time in a food detection study.

1 Introduction

An important task of the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*), as laid down in the Commission Regulation EC No 882/2004 (EC, 2004), is the organization of interlaboratory comparison studies to test the performances of the National Reference Laboratories (NRLs) for *Salmonella*. The history of the interlaboratory comparison studies on the detection of *Salmonella*, as organized by EURL-*Salmonella* (formerly called CRL-*Salmonella*) since 1995, is summarized on the EURL-*Salmonella* website (EURL-*Salmonella*, 2014).
The objective of the current study, organized by the EURL for *Salmonella* in September 2013, was to see whether the participating laboratories could detect *Salmonella* at different contamination levels in minced chicken meat. This information is important in order to know whether the examination of samples in the EU Member States (MS) is carried out uniformly and whether comparable results can be obtained by NRLs-*Salmonella*. Additionally, the different methods used for the detection of *Salmonella* in minced chicken meat were compared.

The prescribed method for detection of *Salmonella* in a food matrix is ISO 6579 (Anonymous, 2002). However, as good experiences have been gained with selective enrichment on Modified Semi-solid Rappaport-Vassiliadis (MSRV) for the detection of *Salmonella* spp. in animal faeces (Annex D of ISO 6579: Anonymous, 2007), as well as for the detection of *Salmonella* in food and animal feed samples, participating laboratories were also requested to use MSRV for testing the minced chicken meat.

There were some differences between the set-up of this study and that of earlier interlaboratory comparison studies focused on the detection of *Salmonella* spp. in food or feed samples. For the current study, the (meat) samples were artificially contaminated with a diluted culture of *Salmonella* Infantis (SI) at the laboratory of the EURL- *Salmonella*, while in previous studies the participants had to mix matrix and reference material themselves prior to analyses.

Like in earlier studies, the contamination level of the low-level contaminated samples was close to the detection limit of the method and the level of the high-level samples was approximately 5-10 times above the detection limit. In total, 18 minced chicken meat samples were tested, six samples per contamination level (blank, low level and high level) containing one *Salmonella* serovar (*Salmonella* Infantis). Additionally, three control samples (two blank control samples and one positive control sample) were tested. The number and level of samples tested were in accordance with CEN ISO /TS 22117 (Anonymous, 2010).

2 Participants

Country	City	Institute / NRL *Salmonella*
Austria	Graz	Austrian Agency for Health and Food Safety (AGES) Institute for Medical Microbiology and Hygiene
Belgium	Brussels	Scientific Institute of Public Health (WIV-ISP)
Bulgaria	Sophia	National Diagnostic Research Veterinary Institute (NDRVMI), National Reference Centre of Food Safety
Croatia	Zagreb	Croatian Veterinary Institute, Lab for Food Microbiology
Cyprus	Nicosia	Ministry of Agriculture, Natural Resources and Environment Veterinary Services Laboratory for the Control of Foods of Animal Origin (LCFAO)
Czech Republic	Prague	State Veterinary Institute
Denmark	Ringsted	Danish Veterinary and Food Administration, Microbiology Ringsted
Estonia	Tartu	Estonian Veterinary and Food Laboratory
Finland	Helsinki	Finnish Food Safety Authority Evira Research Department, Microbiology Unit
France	Ploufragan	Anses Laboratoire de Ploufragan -Plouzané, Unité Hygiène et Qualité des Produits Avicoles et Porcins (HQPAP)
Germany	Berlin	Federal Institute for Risk Assessment (BFR)
Greece	Halkis	Veterinary Laboratory of Chalkis, Hellenic Republic Ministry of Rural Development and Food
Hungary	Budapest	National Food Chain Safety Office, Food and Feed Safety Directorate
Iceland	Reykjavik	Matis, Icelandic Food and Biotech R&D
Ireland	Kildare	Central Veterinary Research Laboratory CVRL/DAFM Backweston, Department of Agriculture, Food and Marine
Italy	Legnaro PD	Istituto Zooprofilattico Sperimentale delle Venezie, OIE
Latvia	Riga	Institute of Food Safety, Animal Health and Environment, BIOR Animal Disease Diagnostic Laboratory
Lithuania	Vilnius	National Food and Veterinary Risk Assessment Institute, Food Microbiology section
Luxembourg	Luxembourg	Laboratoire de Médecine Vétérinaire de l'Etat (LMVE)
Macedonia, FYR of	Skopje	Faculty of Veterinary Medicine, Food Institute Laboratory of Food and Feed Microbiology
Malta	Valletta	Public Health Laboratory (PHL) Microbiology Evans Building
Netherlands, the	Bilthoven	National Institute for Public Health and the Environment (RIVM/CIb) Infectious Disease Control, Centre for Zoonoses and Environmental Microbiology (cZO)
Netherlands, the	Wageningen	Netherlands Food and Consumer Product Safety Authority (nVWA) Consumer and Safety Division, Microbiology
Norway	Oslo	Norwegian Veterinary Institute, Section of Bacteriology

Country	City	Institute
Poland	Pulawy	National Veterinary Research Institute (NVRI) Department of Hygiene of Animal Feeding Stuffs
Portugal	Vairao	Instituto National de Investigação Agrária e Veterinária Unidade de Tecnologia e Segurança Alimentar, (LNIV) Bacteriology Laboratory of the Animal Health Unit in
Romania	Bucharest	Hygiene and Veterinary Public Health Institute (IISPV)
Slovak Republic	Bratislava	State Veterinary and Food Institute
Slovenia	Ljubljana	National Veterinary Institute, Veterinary Faculty (UL)
Spain	Madrid, Majahonda	Centro Nacional de Alimentación (CNA) Agencia Española de Seguridad Alimentaria (AESAN)
Sweden	Uppsala	National Veterinary Institute (SVA), Department of Bacteriology
Switzerland	Bern	Vetsuisse faculty Bern, Institute of Veterinary Bacteriology, Centre for Zoonoses, Bacterial Animal Diseases (ZOBA)
Turkey	Kecioren, Ankara	Etlik Veterinary Control Central Research Institute, Bacteriological Diagnosis Laboratory
United Kingdom	London	Public Health England, Food Water and Environmental Microbiology (FW&E) Microbiology Network London
United Kingdom	Belfast	Agri-Food and Bioscience Institute (AFBI) Veterinary Sciences Division Bacteriology

3 Materials and methods

3.1 Artificial contamination of minced chicken meat samples

3.1.1 Pre-tests for the preparation of minced chicken meat samples

The matrix in this interlaboratory comparison study was minced chicken meat. Because the artificial contamination of food samples with a diluted culture was not used in earlier food studies, some experiments were performed prior to the start of the study. The stability of two different *Salmonella* serovars were tested for the artificial contamination of chicken meat samples at different contamination levels and during storage at different temperatures. For this, the following *Salmonella* serovars were tested: *Salmonella* Typhimurium (STM) ATCC 14028 and *Salmonella* Infantis (SI) isolated from laying hens. The ATTC strain was obtained from the American Type Culture Collection (ATCC, Manassas, USA). The *Salmonella* Infantis strain was used in the 14th Typing study (Strain S3) organized by the EURL in 2009 (Jacobs et al. 2011). Each strain was inoculated in Buffered Peptone Water (BPW) and incubated at (37 ± 1) °C overnight. Next, each culture was diluted in peptone saline solution to be able to inoculate the minced meat samples with approximately
5-10 CFU/sample and 50-100 CFU/sample. For the enumeration of the contamination level (CFU/ml), 0.1 ml of the diluted culture was spread over an XLD plate and incubated at 37 °C for 20-24 hours.
Samples of 25 g of minced chicken meat were artificially contaminated with a dilution of a *Salmonella* culture (different levels of STM or SI). All minced chicken meat samples were stored at – 20 °C, 5 °C and 10 °C for a period of 0, 7, 14 and 21 days. Additionally, some samples were stored at -20 °C for 1 to 4 weeks, followed by storage at 5 °C and 10 °C to test the influence of thawing on the samples. After each storage time at the different temperatures, the artificially contaminated SI, STM and blank minced chicken meat samples were tested for the presence of *Salmonella* following Annex D of ISO 6579 (Anonymous, 2007), with selective enrichment on Modified Semi-solid Rappaport-Vassiliadis (MSRV) and, for some samples, also following ISO 6579 (Anonymous, 2002) with selective enrichment in Rappaport Vassiliadis Soya broth (RVS) and/or Mueller Kauffmann Tetrathionate novobiocin Broth (MKTTn).
To obtain an indication of the amount of the background flora in the samples, the blank minced chicken meat samples were tested for the number of aerobic bacteria and *Enterobacteriaceae*. For this purpose, the ISO procedures for establishing the total number of aerobic bacteria (ISO 4833: Anonymous, 2003) and for analysing the *Enterobacteriaceae* count (ISO 21528-2: Anonymous, 2004) were followed.

3.1.2 Determination of contamination level in minced chicken meat samples by MPN

The level of contamination in the final minced chicken meat samples, as used at the time of the study, was determined by using a five-tube, most probable number (MPN) technique. For this, tenfold dilutions of five minced chicken meat samples of 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 by following Annex D of ISO 6579 (Anonymous, 2007) and ISO 6579 (Anonymous, 2002). From the number of confirmed positive dilutions, the MPN of *Salmonella* in the original sample was calculated by using an MPN software program in Excel, freely available on the Internet (Jarvis et al., 2010).

3.2 Minced chicken meat

3.2.1 General

A batch of 25 kg *Salmonella*-free minced chicken meat was provided by Plukon, Wezep, the Netherlands. The minced chicken meat arrived at EURL-*Salmonella* on 3 September 2013, where it was stored at 5 °C. Ten samples, each 25 g, were checked for the absence of *Salmonella* following ISO 6579 (Anonymous, 2002) and Annex D of ISO 6579 (Anonymous, 2007). For this purpose, the ten 25 g samples were each added to 225 ml of Buffered Peptone Water (BPW). After pre-enrichment at 37 (± 1)°C for 16 - 18 hours, selective enrichment was carried out in Rappaport-Vassiliadis Soya broth (RVS), Mueller Kaufmann Tetrathionate novobiocin broth (MKTTn) and on Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar. Next, the MKTTn and RVS tubes and the suspect growth on MSRV plates were plated-out on Xylose Lysine Deoxycholate agar (XLD) and Brilliance *Salmonella* agar (BSA) and confirmed biochemically.
After checking the absence of *Salmonella*, the minced chicken meat was repacked (on 5 and 6 September 2013) in portions of 25 g in Whirl-pak plastic bags and stored at –20 °C (see 3.3.1).

3.2.2 Total bacterial count in minced chicken meat

The total number of aerobic bacteria in the minced meat was investigated by following ISO 4833 (Anonymous, 2003). A portion of 20 g of the minced chicken meat was homogenized in 180 ml of peptone saline solution in a plastic bag. The content was mixed by using a stomacher (for 60 sec). Next, tenfold dilutions were prepared in peptone saline solution. Two times 1 ml of each dilution was brought into two empty Petri dishes (diameter 9 cm). To each dish, 15 ml of molten Plate Count Agar (PCA) was added. After the PCA was solidified, an additional 5 ml PCA was added to the agar. The plates were incubated at
(30 ± 1) °C for (72 ± 3) hours and the total number of aerobic bacteria was counted after incubation.

3.2.3 Number of Enterobacteriaceae *in minced chicken meat*

In addition to the total number of aerobic bacteria, the *Enterobacteriaceae* count was determined by following ISO 21528-2 (Anonymous, 2004). A portion of 20 g of the minced chicken meat was homogenized in 180 ml of peptone saline solution in a plastic bag. The contents were mixed using a stomacher (for 60 sec). Next, tenfold dilutions were prepared in peptone saline solution. Two times 1 ml of each dilution was brought into two empty Petri dishes (diameter 9 cm). To each dish, 10 ml of molten Violet Red Bile Glucose agar (VRBG) was added. After the VRBG was solidified, an additional 15 ml of VRBG was added to the agar. These plates were incubated at (37 ± 1) °C for (24 ± 2) hours and the number of typical violet-red colonies were counted after incubation. Five typical colonies were tested for the fermentation of glucose and for a negative oxidase reaction. After this confirmation, the number of *Enterobacteriaceae* was calculated.

3.3 Design of the interlaboratory comparison study

3.3.1 Samples: minced chicken meat

Approximately three weeks before the study, a total of 960 minced chicken meat samples were prepared. As a part of this, the following steps were performed:

- labelling of each plastic bag;
- adding 25 g of minced chicken meat to each plastic bag and storing samples at - 20 °C.
- adding approximately 0.1 ml of a diluted culture of *Salmonella* Infantis to a part of the defrosted minced chicken meat sample. The contamination levels aimed at were 10–15 CFU/sample, 50–100 CFU/sample and blank.
- storing samples at – 20 °C until transport on 23 September 2013.

On 23 September 2013 (one week before the study), the minced chicken meat samples were prepared for shipment (see 3.3.2) and sent to the participants by door-to-door courier service. After arriving at the laboratories, the minced chicken meat samples had to be stored at 5 °C until the start of the study.
Further details about the shipping and handling of the samples and the reporting of the test results can be found in the protocol (EURL-*Salmonella*, 2013a), in the Standard Operation Procedure (SOP, EURL-*Salmonella*, 2013b) and in a print-out from the web-based test report (EURL-*Salmonella*, 2013c). The protocol, SOP and test report used during the study can be found on the EURL-*Salmonella* website or can be obtained by corresponding with the author of this report.

Eighteen minced chicken meat samples (numbered B1–B18) and three control samples (numbered C1-C3) had to be tested by each participant. Table 1 gives an overview of the number and type of samples to be tested by the participants.
For the control samples, the laboratories were asked to use their own positive *Salmonella* control, which they normally use when analysing routine samples for the detection of *Salmonella*. In addition to this positive control, blank controls of the BPW and of the matrix had to be analysed.

Table 1. Overview of the number and type of samples tested per laboratory in the interlaboratory comparison study

Contamination level	Test samples with minced chicken meat (n=18)
S. Infantis low level (SI)	6
S. Infantis high level (SI)	6
Blank (BL)	6
	Control samples (n=3)
Own control with *Salmonella*	1
Minced chicken meat	1
BPW	1

3.3.2 Sample packaging for shipment and temperature recording during shipment

To each NRL, 21 plastic bags were sent containing the artificially contaminated chicken meat samples, blank meat samples, or no meat at all (controls). The 21 bags were packed in one plastic safety bag. The safety bag was placed in one large shipping box, together with three frozen (-20 °C) cooling devices. Each shipping box was sent to the participants as 'biological substances category B (UN3373)' using a door-to-door courier service. To monitor exposure to abusive temperatures during shipment and storage, micro temperature loggers were used to record the temperature during

transport. These loggers are tiny units sealed in a stainless steel case 16 mm in diameter and 6 mm deep. Each shipping box contained one logger packed in one of the safety bags. The loggers were programmed by the EURL-*Salmonella* to measure the temperature every hour. Each NRL had to return the temperature recorder to EURL-*Salmonella* on the day the laboratory started the study. At the EURL-*Salmonella,* the loggers were read using a special computer program and all recorded temperatures from the start of the shipment until the start of the study were transferred to an Excel sheet.

3.4 Methods

The NRLs could follow the pre-treatment procedures for the meat samples as they are normally used in daily routine analyses (e.g. pre-warming of BPW, different ways of mixing the samples in BPW).
The prescribed method of this interlaboratory comparison study for detection of *Salmonella* in the meat samples was ISO 6579 (Anonymous, 2002) and the requested (additional) method was Annex D of ISO 6579 (Anonymous, 2007). In addition, the NRLs could use their own method, such as a Polymerase Chain Reaction (PCR) procedure.

The prescribed (and requested) method in summary:

Pre-enrichment in:
- Buffered Peptone Water (BPW)

Selective enrichment in/on:
- Rappaport Vassiliadis Soya broth (RVS);
- Mueller Kaufmann Tetrathionate novobiocin broth (MKTTn);
- Modified Semi-solid Rappaport-Vassiliadis medium (MSRV) (requested);

Plating-out on the following isolation media:
- Xylose Lysine Desoxycholate agar (XLD);
- second plating-out medium of choice;

Confirmation:
- Confirmation by means of appropriate biochemical tests (ISO 6579, Anonymous, 2002) or by reliable, commercially available identification kits and/or serological tests.

3.5 Statistical analysis of the data

The specificity, sensitivity and accuracy rates were calculated for the artificially contaminated minced chicken meat samples. For the control samples, only the accuracy rates were calculated. The rates were calculated according to the following formulae:

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

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

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

Mixed effect logistic regression (Gelman and Hill, 2007) was used for modelling the binary outcomes as a function of a fixed effect part, consisting of the level of contamination (CFU), enrichment media and isolation media, and a random effect part, consisting of the different laboratories. Mutual differences between media and contamination level are shown as odds ratios (OR) stratified by medium. The odds of detecting *Salmonella* is the probability of detecting *Salmonella* divided by the probability of not detecting it. An odds ratio is the ratio of the odds of detecting *Salmonella* in one group to the odds of detecting it in another group and can be interpreted as an effect size. Groups are, for instance, two different media.

A Bayesian approach was adopted to prevent spurious odds ratios, i.e. zero or infinite odds ratios. This was done by putting a uniform prior on the probability of detecting *Salmonella*. As a result, the eventual odds and odds ratios will be 'shrunken' towards one and values equal to zero or infinity are made impossible.

Results were analysed using the statistical software R (R Development Core Team, 2014).

3.6 Good performance

For the determination of good performance, the criteria as indicated in Table 2 were used. For the determination of 'good performance' per laboratory, the results found with all combinations of the prescribed and requested selective enrichment media and isolation media used by the laboratory were taken into account. For example, if a laboratory found 5/6 low-level contaminated samples positive with RVS/XLD, but no positives with any other selective enrichment medium or isolation medium, this was still considered as a good result. The opposite was used for the blank samples. Here also, all combinations of media used per laboratory were taken into account. If, for example, a laboratory found 2/6 blank samples positive with MKTTn/BGA but no positives with the other media, this was still considered a 'no-good' result.
The results will therefore be presented for selective enrichment in RVS, MKTTn or on MSRV in combination with the isolation medium (XLD or non-XLD) that gave the highest number of *Salmonella* isolations (e.g. RVS/x).

Table 2. Criteria for testing good performance in the Food VI study (2013)

Minimum result		
Contamination level	Percentage positive	No. of positive samples/ total no. of samples
Samples Minced chicken meat artificially contaminated		
S. Infantis high level (SI high)	80 %	5/6
S. Infantis low level (SI low)	30 %	2/6
Blank (BL)[1]	20 % at max[1]	1/6 at max[1]
Control samples		
Positive control (Own control with *Salmonella)*	100 %	1 /1
Procedure control (BPW)	0 %	0 /1
Matrix control (Minced chicken meat)	0 %	0 /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% pos.) is considered acceptable.

4 Results

4.1 Artificial contamination of minced chicken meat samples

4.1.1 Pre-tests for the preparation of minced chicken meat samples

Five sets of experiments were performed. For each set of experiments, the stability of *Salmonella* in the minced chicken meat samples was tested during storage of the samples at different temperatures for up to three weeks. During each set of experiments, different variables were tested in different combinations (see Section 3.1.1). Table 3 and Figure 1 show the results of all tested samples.

Table 3. Stability tests of chicken meat artificially contaminated with Salmonella *Typhimurium (STM) and S. Infantis (SI)*

Days of storage	**Storage at -20 ºC**			**Storage at +5 ºC**					**Storage at +10 ºC After storage at -20 ºC**			
	STM40	**STM11**	**SI4**	**STM40**	**STM11**	**SI63[A#]**	**SI14[A#]**	**SI4**	**SI63[A#]**	**SI59[B#]**	**SI14[A#]**	**SI4[B#]**
number of positive samples/number of tested samples												
0	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6		2/2B 6/6A		2/2B 6/6A
7	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	**2/6**
14	6/6	6/6	6/6	6/6	6/6	6/6	6/6	**5/6**				
21	6/6		6/6	6/6				**5/6**				

All samples were analysed by using selective enrichment medium MSRV. Samples indicated with # were also analysed with selective enrichment media RVS and MKTTn, the best score of the media is given.

Indicated are serovars and contamination levels in the minced chicken meat. For example, STM40 indicates *Salmonella* Typhimurium at a level of 40 CFU/25 g of chicken meat.

[A] tested after storage at -20 ºC for 4 weeks

[B] tested before (B) and after (A) storage at -20 ºC for 1 week

The major findings are summarized below:

Samples artificially contaminated with *Salmonella* Typhimurium (STM 10 – 40 CFU) and *Salmonella* Infantis (SI 14 – 63 CFU) were shown to be stable in minced chicken meat samples after 2-3 weeks of storage at –20 ºC and +5 ºC.:

- All six contaminated STM samples at a level of 11 - 40 CFU/25 g minced chicken meat were found positive after 14 days of storage at –20 ºC and +5 ºC.
- All six contaminated SI samples at a level of 14 – 63 CFU/25 g minced chicken meat were found positive after 14 days of storage at –20 ºC and +5 ºC.
- Five out of six SI4 samples were found positive after 2-3 weeks of storage at +5 ºC.
- All six SI4 samples were found positive after 3 weeks of storage at –20 ºC.

All subsequent experiments were performed with *S*. Infantis (SI) only.

To mimic abuse temperatures during transport, the samples were also stored at 10 ºC. Samples artificially contaminated with *Salmonella* Infantis at a level of 14 CFU and higher were shown to be stable in minced chicken meat samples during 1 week of storage at 10 ºC.
Chicken meat samples artificially contaminated with a lower level of *S*. Infantis (SI4) were shown to be less stable during storage at 10 ºC; after one week, only two out of six samples were still found positive for *Salmonella*.

The background flora in the minced chicken meat samples during the different experiments showed a decrease after storage at –20 ^{0}C, while the number of interfering flora increased during storage at higher temperatures (5 ^{0}C and 10 ^{0}C). Storage for one week at 10 ^{0}C showed an increase of approximately log 4 CFU/g in the number of *Enterobacteriacea,* as well as in total number of aerobic bacteria. While storage for three weeks at –20 ^{0}C showed a decrease of approximately one log CFU/g in the number of *Enterobacteriacea,* as well as in the number of aerobic bacteria.

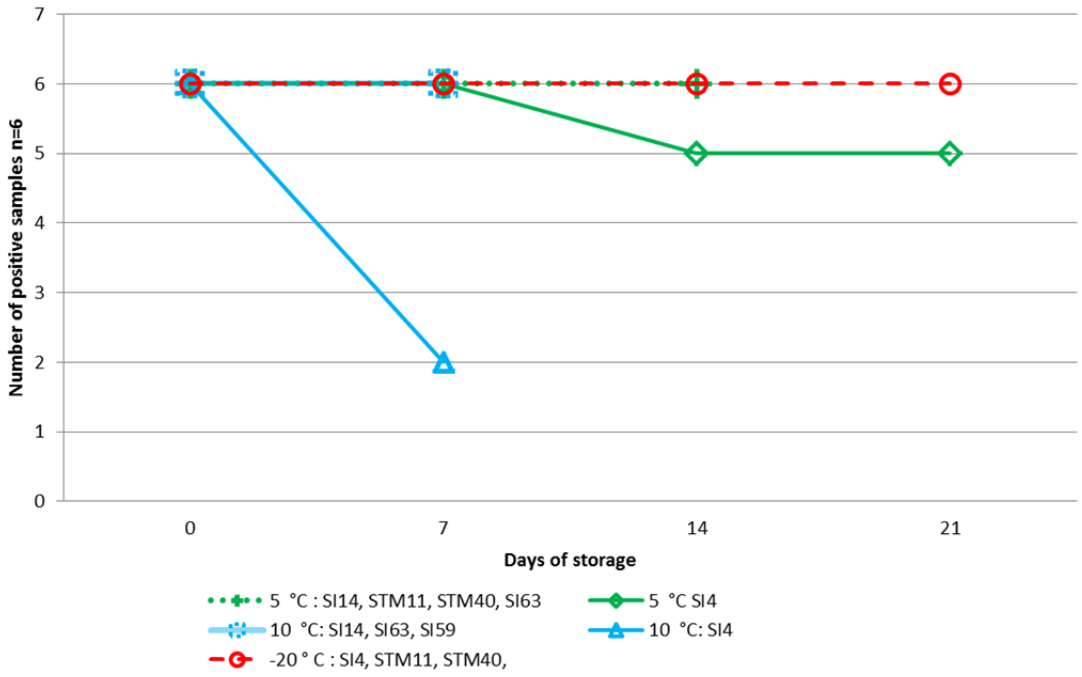

Figure 1. Stability test of minced chicken meat samples artificially contaminated with Salmonella *Typhimurium (STM) or* Salmonella *Infantis (SI)*

From the results of the experiments, a decision was made to use the following samples for the interlaboratory comparison study:

- 25 g of minced chicken meat samples;
- artificially contaminated with a diluted culture of:
  - low-level SI (10–15 CFU/25 g of minced chicken meat)
  - high-level SI (50–100 CFU/25 g of minced chicken meat)
  - blank (0 CFU/25 g of minced chicken meat).

4.1.2 Contamination level of the artificially contaminated minced chicken meat samples

Table 4 shows the contamination levels of the low-level and high-level contaminated minced chicken meat samples. The inoculum level of the diluted SI culture (tested on XLD), as well as the contamination level of the minced chicken meat samples after the inoculation with the diluted culture, were tested. The latter was tested with a five-tube MPN test (see Section 3.1.2). The number of positive minced chicken meat samples for 25 g, 2.5 g and 0.25 g were, respectively, for the low-level SI 5/5, 2/5 and 0/5 and for high-level SI 5/5, 5/5 and 2/5. The calculated MPN/25 g of minced chicken meat is given in Table 4.

Table 4. Number of Salmonella Infantis (SI) in the inoculum and in the minced chicken meat samples

Date of testing	Low-level SI CFU/25 g minced chicken meat	High-level SI CFU/25 g minced chicken meat
12 September 2013 Mean inoculum level	11	104
7 October 2013 Inoculated minced chicken meat stored at 5 °C for one week. MPN (95 % confidence limit)	5 (1.5-16)	55 (16-188)

4.2 Minced chicken meat

The minced chicken meat samples were tested negative for *Salmonella* and stored at -20 °C. On Monday 23 September 2013, the minced chicken meat samples were sent to the NRLs. After receiving them, the NRLs had to store the samples at 5 °C.
The number of aerobic bacteria and the number of *Enterobacteriaceae* were tested twice; firstly, on the day the minced chicken meat arrived at the EURL (5/09/2013) and, secondly, after storage at – 20 °C, followed by 5 °C for one week (7/10/2013). Table 5 summarizes the results, showing that the amount of background flora increased after storage at 5 °C.
A few participants performed additional tests on the contaminating bacteria and identified *Proteus vulgaris* and *Hafnia alvei* in the minced chicken meat.

Table 5. Number of aerobic bacteria and number of Enterobacteriaceae per gram of minced chicken meat

Date	*Enterobacteriaceae* CFU/g	Aerobic bacteria CFU/g
5 September 2013	$1.1*10^6$	$7.1*10^7$
7 October 2013 After storage at 5 °C for 1 week	$1*10^8$	$4.4*10^8$

4.3 Technical data: interlaboratory comparison study

4.3.1 General

Thirty-five NRLs for *Salmonella* participated in this study: 30 NRLs from 28 EU-MS and 5 NRLs from non-EU MSs. The non-EU MSs consisted of EU candidate MSs or potential EU candidate MSs and members of the European Free Trade Association (EFTA). Thirty-two laboratories performed the study on the planned date (week 40 starting on 30/09/2013). Two laboratories (lab codes 21 and 27) performed the study one week earlier. Laboratory 9 had some technical problems at the start of the performance. A second parcel was sent to the laboratory and they started the study on 8 October.

4.3.2 Accreditation/certification

Thirty-three laboratories are accredited for their quality system according to ISO/IEC 17025 (Anonymous, 2005) and two EU-MS laboratories (9 and 12) are still in the process of accreditation. Thirty-one laboratories are accredited for ISO 6579 (detection of *Salmonella* in food and animal feeding stuffs), 27 of them are also accredited for Annex D of ISO 6579. In addition to ISO 6579, five laboratories mentioned another accredited method (e.g. VIDAS or a national method for detection of *Salmonella*). Laboratory 3 (non-EU-MS) is accredited only for the detection of *Salmonella* in animal faeces and veterinary samples by using MSRV (Annex D of ISO 6579). Laboratory 11 (non-EU-MS) is accredited for the detection of *Salmonella* by using RVS and MSRV.

4.3.3 Transport of samples

Twenty-six participants received the samples within one day after dispatch and seven participants within two days. Two parcels, both sent to non-EU-MSs, were delayed. The parcel for laboratory 23 was held for two days at the customs office and the parcel for laboratory 11 was kept for one day at the airport. For two participants, the parcels were transported to an NRL in a neighbouring country (door-to-door). These parcels were picked up by the relevant NRL the day after arriving at the first NRL and required some extra hours of transport.
The majority of the NRLs returned the temperature recorders to the EURL-*Salmonella* at the time they started the study, as requested. Two participants returned the temperature recorder immediately after arrival of the samples at their institute (as in earlier studies). Three temperature loggers were broken. For the majority of the parcels, the temperature did not exceed 0 °C during transport. During storage at the NRL, the temperature was between 0 °C and 5 °C. The exceptions were the laboratories 11, 23, 26 and 28, where the samples were stored between 5 °C and 11 °C.

4.3.4 Media

Each laboratory was asked to test the samples using the prescribed method (ISO 6579) and the requested method (Annex D of ISO 6579). Thirty-three laboratories used the selective enrichment media RVS, MKTTn and MSRV in combination with XLD and a second plating-out medium of their own choice. Two laboratories (9 and 23) did use the prescribed selective enrichment media RVS and MKTTn, but did not use or did not report the results of the requested method MSRV. Although laboratory 9 tested the samples with MSRV, they used a batch of MSRV which had exceeded the maximum allowable storage time. The laboratory did not report the results, as it was not possible to obtain correct readings from the MSRV-plates.

Table 6 provides information on the pH of the media, the concentration of Novobiocin in MKTTn and MSRV, and on the incubation times. The table lists only the reported deviations from the prescriptions.

Four laboratories (2, 5, 24 and 26) reported a longer incubation time for the pre-enrichment in BPW. Four laboratories (2, 10, 11 and 22) reported a pH of 7.3 instead of the prescribed maximum pH of 7.2 for BPW.
Laboratory 31 used RVS at a pH of 6.9 instead of the prescribed maximum pH of 5.4.
Four laboratories (11, 14, 17 and 28) used MKTTn at a pH of 6.8-7.5 instead of the prescribed of 7.8-8.2. Six laboratories (11, 14, 17, 21, 24, and 32) used MKTTn with a lower concentration of novobiocin than the prescribed 0.04 g/L.
Four laboratories (2, 5, 7 and 9) used MSRV with a higher concentration of novobiocin than the prescribed 0.01 g/L and laboratory 22 used a lower concentration than the prescribed 0.01 g/L. Laboratory 23 used MSRV without novobiocin. Three laboratories (11, 12 and 16) reported a higher pH (5.5–5.6) for the MSRV than the prescribed maximum pH of 5.4. Laboratory 24 reported an incubation temperature of 37 °C for MSRV instead of the prescribed temperature of 40.5-42.5 °C.
Laboratories 9, 16, 23 and 33 did not report the pH of any of the used media.

Table 6. 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
Prescribed ISO 6579 or ISO 6579 annex D	16-20 h	6.8-7.2	5.0-5.4	7.8-8.2	40 mg/L	5.1-5.4	10 mg/L
2	**25 :42**	**7.3**	5.4	7.9	40	5.2	**20**
5	**20 :45**	7.0	5.2	8.0	40	5.2	**20**
7	18 :30	7	5.2	7.9	40	5.3	**20**
9	18 :07	-	-	-	39	5.2	**20**
10	20 :00	**7.3**	5.2	8.0	39	5.3	10
11	19:40	**7.3**	5.4	**7.5**	**0**	**5.6**	10
12	21:00	7.1	5.4	7.9	40	5.5	10
14	20:00	7.2	5.2	**7.3**	**10**	5.2	10
16	19:00	7.2	5.3	**-**	40	**5.6**	10
17	19:45	7.2	5.2	**6.8**	**4**	5.2	10
21	18:00	7.2	5.2	8	**10**	5.2	10
22	19:30	**7.3**	5.2	8.0	0.040	5.2	**0.05**
23	18:00	7	5.3	8	40	**-**	**-**
24*	**24:05**	7.1	5.1	8.1	**4**	5.1	10
26	**21:00**	7	5.2	8.2	40	5.2	10
28	18:10	7.0	5.3	**7.3**	39	5.2	10
31	18:20	7.08	**6.9**	8.2	40	5.3	10
32	19:00	7.09	5.4	7.8	**10**	5.4	10
33	17:00	**-**	**-**	**-**	39	**-**	10

Bold numbers/ grey cells =Deviating from ISO 6579 and/or from ISO 6579 Annex D

\- =No information

* MSRV incubation at 37 °C instead of at the prescribed 40.5-42.5 °C

A second plating-out medium of choice was obligatory. Table 7 shows the second isolation media used by the participants. Most laboratories used BGA (Anonymous, 1993) or a Chromogenic medium (e.g. Rambach) as a second plating-out medium.

Table 7. Second plating-out media used by participants

Media	Number of users	Lab code
BGA^{mod} (ISO 6579, 1993)	7	3, 6, 15, 16, 24, 32, 35
Rambach (Merck)	7	1, 7, 8, 14, 18, 25, 28
BGA	6	2, 11, 19, 20, 26, 30
SM(ID)2 (Biomerieux)	4	5, 17, 29, 33
BSA (=OSCM)	3	4, 13, 27
RS (Bio-rad)	3	12, 31, 34
Chromo *Salmonella* (BIOGERM)	1	9
Salmonella Chromogenic Medium II (Oxoid)	1	10
BPLS (Merck)	1	21
BGA Base w/Phosphates (Merck)	1	22
MAC (Oxoid)	1	23

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

The use of an extra non-selective plating agar between the 'isolation' and 'confirmation' steps was optional. A total of 32 laboratories performed this extra step (e.g. by using Nutrient agar; Anonymous, 2002).

All participating laboratories performed one or several confirmation tests for *Salmonella, see* Tables 8 and 9. Four laboratories (5, 8, 21 and 35) performed serological tests only and seven laboratories (2, 9, 10, 17, 19, 23 and 31) performed only a biochemical test. Two laboratories (10 and 13) used the Maldi-Toff test and three (14, 19 and 21) a PCR method for confirmation.

Table 8. Biochemical and other confirmation tests of Salmonella used by the NRLs

Lab code	TSI	UA	LDC	Gal	VP	Indole	Kit	Other
1	+	+	+	+	-	+		semi-solid glucose agar
2, 12, 15	-	-	-	-	-	-	API20E	
3, 16,	+	+	+	-	-	-		
4	+	+	+	+	+	+		Citochrome Oxidase, ONPG
5, 8, 35	-	-	-	-	-	-		
6, 20, 23, 25, 34	+	+	+	+	+	+		
7	+	-	-	-	-	-	VITEK 2 GN	
9	+	-	-	-	-	+	BBL	Oxidase
10	-	-	-	-	-	-		MALDI TOF MS
11	+	-	+	-	-	-		
13	+	+	+	-	-	-	Enterotest	MALDI TOF MS
14, 19	+	+	+	+	+	+		PCR
17	-	-	-	-	-	-	RapidID32E	
18	+	+	+	+	+	+	BD BBLCRYSTAL	
21	-	-	-	-	-	-		PCR
22, 27, 32	+	+	+	-	-	+		
24, 28	+	+	+	-	-	-	API20E	
26	+	-	-	-	-	-	MICROGEN GN-ID-A	
29	-	-	-	-	-	+	BBL	
30	+	+	+	+	-	+		
31	-	-	-	-	-	-	API ID 32 E	
32	+	+	+	-	-	+		MAC
33	-	-	-	-	-	-	Microbact GNB 12A	

Table 9. Serological confirmation tests of Salmonella used by the NRLs

	Serological			
	O antigens	**H antigens**	**Vi antigens**	**Other**
4, 6	+	+	+	-
1, 5, 7, 8, 13, 14, 15, 16, 20, 22, 25, 28, 32, 34	+	+	-	-
11	+	+	-	Wellcolex
12	+	-	+	-
3, 6, 18, 21, 27, 30, 33, 35	+	-	-	-
24	-		+	-
29	-	-	-	A-I Vi and A-S Vi
2, 9, 10, 17, 19, 23, 31	-	-	-	-

- = Not done / not mentioned.

4.4 Control samples

4.4.1 General

Table 10 gives the results of all control samples. The results given in the table are the highest number of positive isolations found with all combinations of selective

enrichment media and isolation media per laboratory. Annex 1 gives more details on the results per selective enrichment medium (RVS, MKTTn and MSRV) in combination with the isolation media used per laboratory.
Thirty-three laboratories scored all three control samples correctly with at least one of the used media.

Table 10. Total number of positive results from the control samples per laboratory

Lab code	The highest number of positive isolations found with any used medium combination		
	Own control with *Salmonella* n=1	BPW n=1	Minced chicken meat n=1
Good performance	**1**	**0**	**0**
1-8, 10-22, 24-35	1	0	0
9, 23	1	**1**	0

Bold number = deviating result.
Grey cell = result below level of good performance.

Positive control with Salmonella
Thirty-two laboratories scored good results with their own *Salmonella* positive control sample and detected *Salmonella* with all used media. The laboratories 9, 13 and 21 could not detect *Salmonella* in some of the used selective enrichment media inoculated from the pre-enriched culture in BPW. Laboratory 9 found only one positive result after selective enrichment in MKTTn and isolation on XLD; all other used media combinations gave negative results. Laboratory 13 could not detect *Salmonella* after selective enrichment on MSRV in combination with isolation on BSA, but scored the same sample as positive with all other used media combinations. Laboratory 21 could not detect *Salmonella* after selective enrichment in MKTTn, but scored the same sample correctly positive when using RVS and MSRV.

For the positive control samples, the majority of the participants used a diluted culture of *Salmonella* (21 laboratories). Others used a lenticule disc (5), a Freeze-dried ampoule (4), capsule (1), cryovial (1), kwik-stik (1) or a culti-loop (1) with *Salmonella.* Table 11 shows the *Salmonella* serovars used for the positive control samples. Most often, *Salmonella* Enteritidis (17) and *Salmonella* Typhimurium (8) were used. The concentration of *Salmonella* in the positive control samples, used by the different participants, varied between 6 and 10^9 CFU/sample.

Table 11. *Salmonella* serovars used by the participants for the positive control samples

Salmonella serovar	Number of users
S. Enteritidis	17
S. Typhimurium	8
S. Nottingham	3
S. Goldcoast*, S.* Infantis *S.* Bongori, *S.* Harleystreet, *S.* Alachua, *S.* Abony, *S.* Blegdam	1

Procedure control Blank (only BPW)
Thirty-three laboratories correctly analysed the one procedure control sample (no matrix, only BPW) correctly as negative for *Salmonella*. The laboratories 9 and 23 reported this sample to be positive for *Salmonella*.

Matrix control Blank (minced chicken meat)
All laboratories correctly analysed the one minced chicken meat control sample (25 g of matrix) as negative for *Salmonella,* irrespective of the media used.

The results were compared with the definition of 'good performance' (see Section 3.6). Laboratories 9 and 23 did not fulfil these criteria for the control samples, as they scored the procedure control as a false positive.

Table 12. Correct scores found with the control samples by all laboratories ('All') and by the laboratories of the EU member states ('EU')

Control Samples		RVS/X		MKTTn/X		MSRV/X*	
	Laboratories	All n=35	EU n=30	All n=35	EU n=30	All n=33	EU n=29
Positive control (Own *Salmonella*) n=1	No. of samples	35	30	35	30	33	29
	No. of positive samples	34	29	34	29	33	29
	Correct score in %	**97**	**97**	**97**	**97**	**100**	**100**
Procedure control Blank (BPW) n=1	No. of samples	35	30	35	30	33	29
	No. of negative samples	33	29	33	29	33	29
	Correct score in %	**94**	**97**	**94**	**97**	**100**	**100**
Matrix control Blank Blank minced chicken meat n=1	No. of samples	35	30	35	30	33	29
	No. of positive samples	35	30	35	30	33	29
	Correct score in %	**100**	**100**	**100**	**100**	**100**	**100**
All control samples	No. of samples	105	90	105	90	99	87
	No. of correct samples	102	88	102	88	99	87
	Accuracy in %	**97**	**98**	**97**	**98**	**100**	**100**

X = isolation medium with the highest number of positives

*Results without Laboratory 9 (EU-MS) and 23 (non EU-MS): they did not use MSRV

4.4.2 *Correct scores of the control samples*

Table 12 shows the correct scores found with the control samples for the different selective enrichment media RVS, MKTTn and MSRV in combination with the isolation medium that gave the highest number of positives. The calculations were performed on the results of all participants and on the results of only the EU-MS. Only minor differences were found between these groups.
The laboratories scored an excellent result for the control samples, with accuracy rates varying between 97 % and 100 %.

4.5 Results for minced chicken meat samples artificially contaminated with *Salmonella*

4.5.1 *Results per level of* Salmonella *and per laboratory*

General
Table 13 shows the results of the minced chicken meat samples artificially contaminated with *Salmonella* Infantis. The results given in this table are the highest number of positive isolations found with the different selective enrichment media (RVS, MKTTn and MSRV) in combination with 'the best' isolation medium. Annex 2 gives more details on the results per selective enrichment medium RVS, MKTTn and MSRV in combination with the used isolation media per laboratory. Not all media combinations gave the same results.

Blank samples
Twenty-nine laboratories correctly scored all six blank minced chicken meat samples as negative for *Salmonella* with all used media. Four laboratories (8, 17, 24, and 34) found one blank sample of the six to be positive for *Salmonella* with one selective enrichment medium, while they found the same sample correctly to be negative with another selective enrichment medium inoculated from the same BPW. Two laboratories (25 and 26) found one and two blank samples, respectively, to be positive for *Salmonella* with all used media.
All blanks should be tested negative. However, as no 100 % guarantee for the *Salmonella* negative status of the minced chicken meat could be given, one positive in the six blank samples (80 % negative) will still be considered as acceptable.

High-level contaminated Salmonella *Infantis samples*
Thirty-two laboratories detected *Salmonella* in all six samples, containing *Salmonella* Infantis at an inoculum level of approximately 55 CFU/25 g of minced chicken meat with at least one of the used selective enrichment media. Two laboratories (2 and 8) could not detect *Salmonella* in one of the six high-level contaminated samples. Laboratory 23 found only one positive sample after selective enrichment in MKTTn in combination with isolation on XLD. All other samples tested negative for *Salmonella* with all media combinations.

Low-level contaminated Salmonella *Infantis samples*
Only eight laboratories detected *Salmonella* in all six samples, containing *Salmonella* Infantis at an inoculum level of approximately 5 CFU/25 g of minced chicken meat with at least one of the used media. On average, the participants found four of the six low level contaminated samples to test positive for *Salmonella,* but mostly only with one or two of the used selective enrichment media. Three laboratories (2, 8 and 10) scored at the minimum level of good performance with two of the six samples testing positive for *Salmonella*.
Laboratory 23 could not detect *Salmonella* in the six low-level contaminated samples with any of the used media (RVS and MKTTn).

Table 13. Number of positive results found with the artificially contaminated minced chicken meat samples (25g) per laboratory

Lab code	The highest number of positive isolations found with selective enrichment medium (RVS, MKTTn or MSRV) in combination with 'the best' isolation medium		
	Blank n=6	SI Low n=6	SI High n=6
Good performance	**≤1**	**≥2**	**≥5**
1	0	6	6
2	0	**2**	**5**
3	0	6	6
4	0	**4**	6
5	0	**5**	6
6	0	**3**	6
7	0	6	6
8	**1**	**2**	**5**
9	0	**3**	6
10	0	**2**	6
11	0	**3**	6
12	0	6	6
13	0	**4**	6
14	0	6	6
15	0	**3**	6
16	0	**3**	6
17	**1**	**5**	6
18	0	**5**	6
19	0	**4**	6
20	0	**5**	6
21	0	**4**	6
22	0	**3**	6
23	0	**0**	**1**
24	**1**	6	6
25	**1**	**3**	6
26	**2**	**4**	6
27	0	**5**	6
28	0	**5**	6
29	0	**5**	6
30	0	**5**	6
31	0	**3**	6
32	0	6	6
33	0	6	6
34	**1**	**5**	6
35	0	6	6

Bold number = deviating result. Grey cell = result below level of good performance.

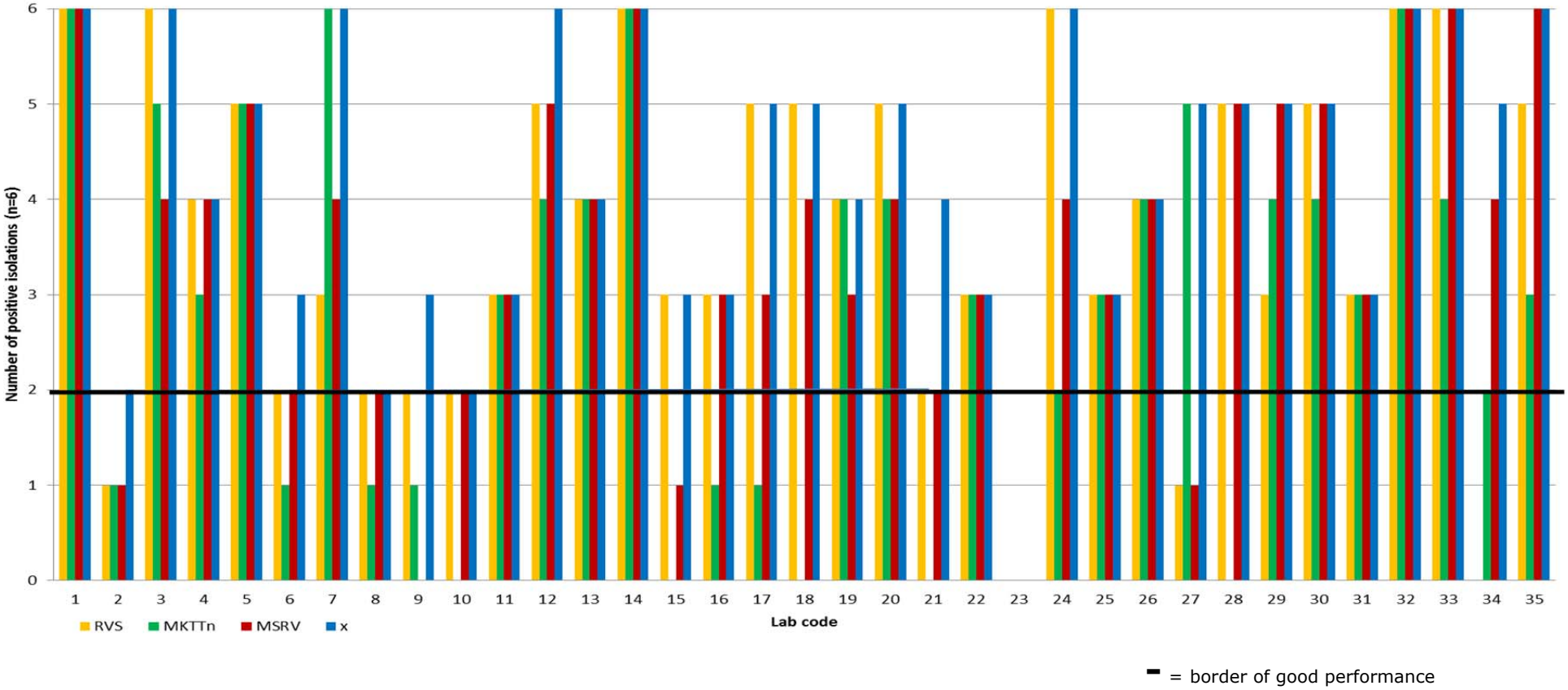

Figure 2. Results per laboratory found with the minced chicken meat samples artificially contaminated with SI low (n=6) after selective enrichment in RVS, MKTTn and on MSRV, followed by isolation on the 'best' selective plating agar and all possible combinations of media giving the highest number of positive results (x).

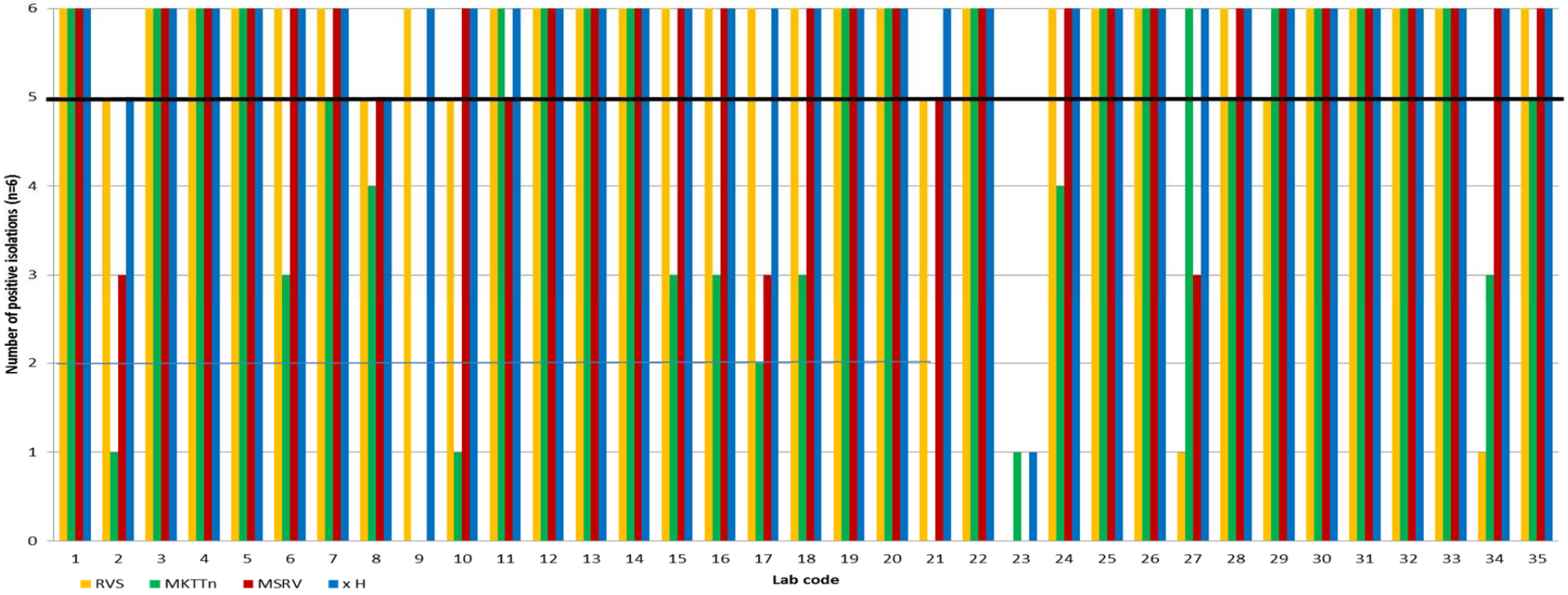

Figure 3. Results per laboratory found with the minced chicken meat samples artificially contaminated with SI high (n=6) after selective enrichment in RVS, MKTTn and on MSRV, followed by isolation on the 'best' selective plating agar and all possible combinations of media giving the highest number of positive results (x).

The results of the artificially contaminated minced chicken meat samples were compared with the definition of 'good performance' (see Section 3.6) and 33 laboratories fulfilled these criteria.
Two laboratories (2 and 8) scored at the minimum level of good performance.
Laboratory 21 could not detect *Salmonella* in any of the samples using the prescribed method MKTTn. However, for the prescribed method RVS and the requested method MSRV, they scored within the lines of good performance.
Two laboratories scored below the level of good performance.
Laboratory 23 showed that it had problems with the detection of *Salmonella* in both low-level and high-level contaminated minced chicken meat samples with all used media.
Laboratory 26 reported that two blank minced chicken meat samples tested positive for *Salmonella* with all used media.

4.5.2 Results per selective enrichment medium, per level of contamination and per laboratory

Figures 2 and 3 show the number of positive isolations per level of artificially contaminated minced chicken meat sample and per laboratory after pre-enrichment in BPW and selective enrichment in RVS, MKTTn and on MSRV, followed by isolation on selective plating agar. Furthermore, all possible combinations of media giving the highest number of positive results (x) are given. The selective enrichment medium and/or isolation medium which gave the highest number of positives varied per laboratory.

The results found with the artificially minced chicken meat samples were compared with the proposed definition of 'good performance' (see Section 3.6). In Figures 2 and 3, the border of good performance is indicated by a black horizontal line.

Table 14 presents the percentages of positive isolations after 24 hours of incubation in RVS, MKTTn and MSRV and after an additional 24 hours of incubation on MSRV. The majority of the laboratories used BGA(modified) as the second plating-out medium (see Table 7).
An extra incubation time of 24 h for MSRV gave, on average, 10% more positive results. For low-level SI contaminated samples, the percentages of positive results were 49% after 24 h and 60% after 48 h of incubation on MSRV. For the high level of contaminated SI samples this was respectively 84% and 93%.

Table 14. Mean percentages of positive results for the detection of Salmonella in the artificially contaminated minced chicken meat samples after selective enrichment in RVS, MKTTn and on MSRV incubated for 24 hours, and for a total of 48 hours on MSRV, followed by isolation on different plating out media

Plating out medium	**Selective enrichment medium**		
	RVS	**MKTTn**	**MSRV**
	24h	24h	24 / 48 h
Best score XLD or other isolation media	75%	61%	78%
XLD	71%	57%	67 / 77%
Other isolation media (most often BGA)	74%	54%	66 / 76%

Tables 15 and 16 show the differences between selective enrichment media and isolation media per contamination level as odds ratios (OR). In addition, the 95% confidence intervals and p-values are given.

In Table 15, the odds of finding a positive isolation with the different plating-out media are compared, given a selective enrichment medium. For instance, the odds of finding *Salmonella* in the low-level contaminated SI samples after selective enrichment in MKTTn is a factor of 1.28 higher when XLD is used as isolation medium, compared to an isolation medium other than XLD. In general, if RVS is used as selective enrichment medium, the Odds Ratios (ORs) are smaller than the ORs for MKTTn and MSRV. In other words, when MKTTn or MSRV is used for selective enrichment, it is easier to detect *Salmonella* if XLD is used compared to other isolation media. No significant differences were found for the different selective enrichment media after plating out on XLD or on another isolation media.

Table 15. Number of positive isolations found with XLD compared with the number of positive isolations found with other isolation media, given a selective enrichment medium.
Samples: minced chicken meat, artificially contaminated with Salmonella Infantis

Selective enrichment medium	Compared isolation media	CFU	Odds Ratios	95% lower	95% upper	p-value*
RVS	XLD compared with media other than XLD	Low	0.79	0.5	1.24	0.310
		High	0.9	0.49	1.66	0.740
		Low & High	0.84	0.57	1.23	0.390
MKTTn	XLD compared with media other than XLD	Low	1.28	0.81	2.02	0.300
		High	1.16	0.72	1.87	0.540
		Low & High	1.22	0.88	1.68	0.240
MSRV	XLD compared with media other than XLD	Low	1.02	0.64	1.62	0.930
		High	1.25	0.58	2.73	0.550
		Low & High	1.13	0.72	1.77	0.580
All selective enrichment media	XLD compared with media other than XLD	Low	1.01	0.78	1.32	0.940
		High	1.09	0.76	1.57	0.630
		Low & High	1.05	0.84	1.31	0.660

* significant difference in case $p < 0.05$.

The interpretation of Table 16 is similar to that of Table 15, except that selective enrichment media are mutually compared, given XLD as isolation medium.
For instance, the odds of finding *Salmonella* from all SI samples after selective enrichment in RVS is a factor of 2.58 higher, compared to MKTTn. When RVS is used as selective enrichment medium compared to MSRV, the odds become smaller (factor of 0.7). However, this difference is not significant. In general, if RVS or MSRV is used as selective enrichment medium, the chance of finding *Salmonella* is larger than when MKTTn is used. These differences are significant.

Table 16. Number of positive isolations found with a selective enrichment medium compared with the number of positive isolations found with another selective enrichment medium, given that the isolation is on XLD
Samples: minced chicken meat artificially contaminated with Salmonella Infantis

Compared selective enrichment media	Isolation medium	CFU	Odds Ratios	95% lower	95% upper	p-value*
RVS compared with MKTTn	XLD	Low	1.91	1.21	3.03	0.010
		High	3.48	2.03	6.06	0.000
		Low & High	2.58	1.81	3.69	0.000
RVS compared with MSRV	XLD	Low	0.88	0.56	1.39	0.590
		High	0.58	0.28	1.18	0.140
		Low & High	0.72	0.46	1.09	0.120
MKTTn compared with MSRV	XLD	Low	0.46	0.29	0.72	0.000
		High	0.17	0.08	0.31	0.000
		Low & High	0.28	0.18	0.41	0.000

* Significant difference in case $p < 0.05$.

Figure 4 shows the performance of each laboratory as odds ratios compared to the mean of all laboratories for the artificially contaminated samples. In this calculation, the blank samples are not used. The mean (OR = 1) is defined as the odds of detecting *Salmonella* based on the fixed effects only (SI low or high, enrichment medium and isolation medium). Laboratories below the mean (OR < 1) have a lower probability of detecting *Salmonella*. The laboratories 2, 9, 10, 21, 27 and 34 scored a lower number of positive results but, still scored within the lines of good performance. However, these laboratories still may have a sensitivity problem with one of their media. For example, the laboratories 10 and 21 scored a lower number of positive results with one of the selective-enrichment media, but with another selective enrichment medium they scored better results.
Eight laboratories (1, 3, 7, 12, 14, 32, 33 and 35) scored all samples correctly with at least one of the used media, but only three of them scored all samples correctly for all used media. Figure 4 shows the highest scores for those three laboratories (1, 14 and 32).

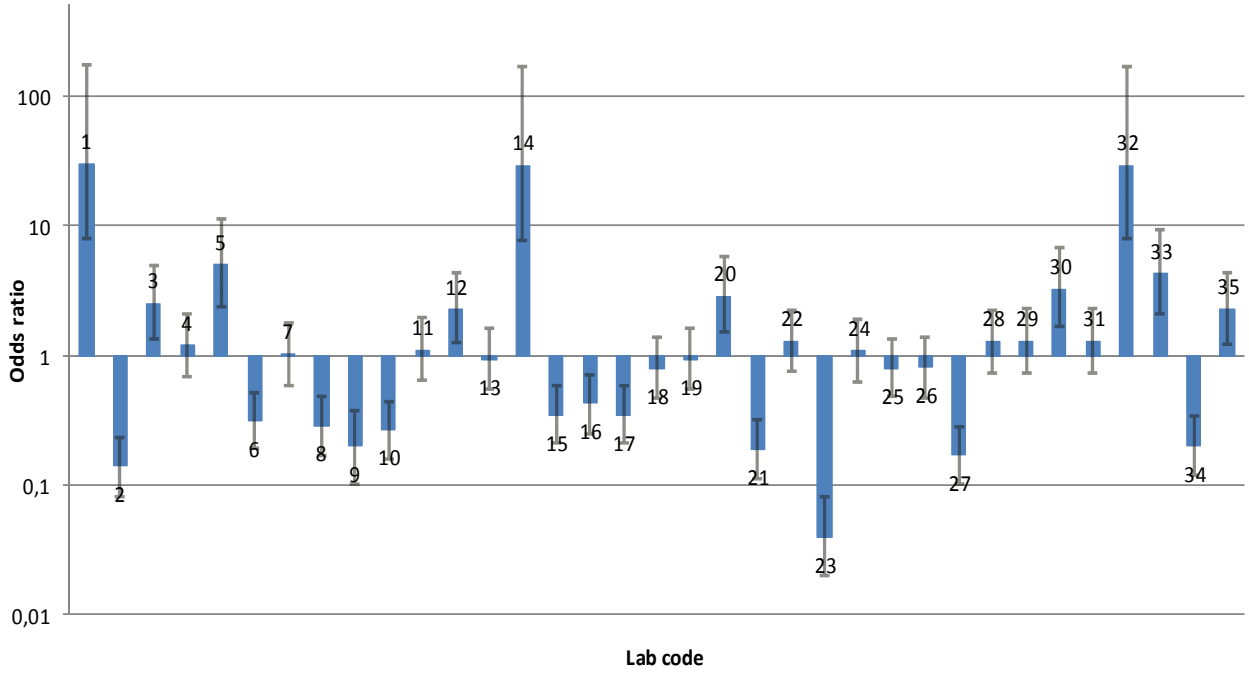

Figure 4 Performance of each laboratory compared to the mean of all laboratories for the artificially contaminated minced chicken meat samples (without blanks)

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

Table 17 shows the specificity, sensitivity and accuracy rates for all levels of artificially contaminated minced chicken meat samples. This table gives the results for the different selective enrichment media (RVS, MKTTn and MSRV) and isolation on selective plating agar showing the highest number of positives (x). The calculations were performed on the results of all participants and on the results of the participants of the EU-MS only. Only minor differences were found between these groups. The specificity rates were comparable for the different selective enrichment media: 97-98%. The lowest sensitivity rate (47%) was found with the low-level contaminated meat samples with selective enrichment in MKTTn. The highest sensitivity rate was found with selective enrichment on MSRV. The accuracy rates were comparable for the selective enrichment media RVS and MSRV (83-85%), but were lower for MKTTn (73-75%).

Table 17. Specificity, sensitivity and accuracy rates found by the participating laboratories with the artificially contaminated minced chicken meat samples after selective enrichment in RVS, MKTTn and on MSRV and on an isolation medium with the highest number of positives

Minced chicken meat samples		RVS/X		MKTTn/X*		MSRV/X**	
	Laboratories	All n=35	EU n=30	All n=35	EU n=30	All n=33	EU n=29
Blank (n=6)	No. of samples	210	180	210	180	198	174
	No. of negative samples	205	176	206	176	193	170
	Specificity in %	**98**	**98**	**98**	**98**	**97**	**98**
SI low (n=6)	No. of samples	210	180	210	180	198	174
	No. of positive samples	128	115	99	90	123	112
	Sensitivity in %	**61**	**64**	**47**	**50**	**62**	**64**
SI high (n=6)	No. of samples	210	180	210	180	198	174
	No. of positive samples	189	167	157	139	186	164
	Sensitivity in %	**90**	**93**	**75**	**77**	**94**	**94**
All samples with *Salmonella*	No. of samples	420	360	420	360	396	348
	No. of positive samples	317	282	256	229	309	276
	Sensitivity in %	**75**	**78**	**61**	**64**	**78**	**79**
All samples	No. of samples	630	540	630	540	594	522
	No. of correct samples	522	458	462	405	502	446
	Accuracy in %	**83**	**85**	**73**	**75**	**85**	**85**

X =isolation medium with the highest number of positives

* =results without Laboratory 9 (EU-MS) and 23 (non-EU-MS): they did not use MSRV

4.6 PCR (own method)

Three laboratories (14, 19 and 21) applied a real time PCR method as an additional detection technique. Two laboratories (19 and 21) used a validated PCR method routinely. Table 18 gives further details of the PCR techniques used.

Table 18. Details of Polymerase Chain Reaction procedures used as own method during the interlaboratory comparison study

Lab code	Real-time PCR	Validated	Commercially available	Number of tests/year	DNA extraction after enrichment in	Reference
14	+	-	+ (Roche)	-	RVS	
19	+	+ intra laboratory	-	43	BPW	
21	+	+	-	89	MSRV	Malorny, 2004

Table 19. Number of positive results found for the artificially contaminated minced chicken meat samples by using a PCR technique and the bacteriological culture technique

	Lab 14		Lab 19		Lab 21	
	BAC (RVS)	PCR	BAC	PCR	BAC (MSRV)	PCR
SI low (n=6)	6 (6)	6	**4**	**4**	**4 (2)**	**3**
SI high (n=6)	6 (6)	6	6	6	**6** (5)	**5**
Blank (n=6)	0 (0)	0	0	0	0 (0)	0

BAC = bacteriological culture results (best score of selective enrichment in RVS, MKTTn and in MSRV)
Bold numbers = unexpected results
Grey cells = different results found with the PCR method in comparison with the bacteriological culture technique (BAC)

Table 19 gives the results of both the PCR method and the bacteriological culture technique (BAC). Two laboratories (14 and 19) found the same results with the PCR method as with the bacteriological culture method. Overall, laboratory 21 found more positive results with the bacteriological culture technique (BAC), compared to the PCR method. This laboratory performed the PCR after selective enrichment on MSRV (see Table 18) and it would have been expected that the results found with the bacteriological culture method on MSRV and with the PCR would be the same. However, strangely enough, one more sample was found to be positive using the PCR technique compared to the bacteriological culture method on MSRV.

4.7 Performance of the NRLs

4.7.1 General

Thirty-two NRLs fulfilled the criteria of good performance and three laboratories scored below these criteria. For the determination of good performance, the results of all media were taken into account. Some laboratories did not score well with one medium, but overall still scored a 'good performance'.
Laboratory 21 could not detect *Salmonella* in any of the samples (including the positive control sample) with the prescribed method MKTTn. However, for the prescribed method RVS and the requested method MSRV they scored within the lines of good performance.
Three laboratories (9, 13 and 21) could not detect *Salmonella* in their positive control sample with some of the used selective enrichment media inoculated from the same pre-enriched culture in BPW.
Four laboratories (8, 17, 24, and 34) found one blank meat sample (out of the six) positive for *Salmonella* with one selective enrichment medium, while they found the same sample correctly negative with another selective enrichment medium inoculated from the same pre-enrichment culture in BPW. This was still considered as good performance.

The three deviating laboratories (9, 23 and 26) were contacted by the EURL-*Salmonella* in November 2013 and asked for possible explanations for their deviating results.

Laboratory 9 reported one blank procedure control sample (only BPW) detected as positive on all media used (RVS and MKTTn). The laboratory indicated that they mixed up the two empty bags to be used for their (own) positive control and the blank procedure control. Additionally, they made a transcription error by reporting the results found with MKTTn as positive while this was in fact tested negative. The laboratory also reported that they routinely perform an extra control on reported data carried out by a second person, but that the two authorised persons were not available at the time of the study. After providing the raw data, it was decided that no further actions were considered necessary for this laboratory and their results were indicated as being a 'moderate performance'.

Laboratory 26 reported two false positive results with the blank minced chicken meat samples with all media used. The laboratory indicated that they had made a transcription error, which was verified by their raw data. The laboratory tabulated all raw data before transferring them into the web-based test report. In this process, a mistake was made with one sample. For normal routine samples, they do not take this extra step. Still, one blank minced chicken meat sample was found to be a false positive, but this was still considered as acceptable. Hence, no further actions were considered necessary for this laboratory and their results were indicated as being a 'moderate performance'.

Laboratory 23 showed difficulties with the detection of *Salmonella* in both low-level and high-level contaminated samples of minced chicken meat. Furthermore, they found a false positive result with the procedure control sample (only BPW). They could not find any possible explanation for the many deviating results. A follow-up study was organized by the EURL-*Salmonella* in January 2014.

4.7.2 Follow-up study

The set-up of the follow-up study was the same as the one used for the full interlaboratory comparison study organized in September 2013.

Before the start of the follow-up study, the minced chicken meat samples, stored at -20 °C since September 2013, were tested for the amount of background flora. On 13 January 2014, the number of aerobic bacteria ($4.8 * 10^8$ CFU/g) and the number of *Enterobacteriaceae* ($4.8 * 10^7$ CFU/g) in the minced chicken meat were tested after it was stored at -20°C for approximately 4 months and placed at 5°C for one week. These numbers were comparable to the numbers found in the minced chicken meat used in the full study (see Table 5). A duplo set of the samples used for this follow-up study was tested by the EURL-*Salmonella* for the presence of *Salmonella* and the samples were scored correctly on all selective enrichment media used (RVS, MKTTn and MSRV).
After the follow-up study, a five-tube MPN test was performed on the samples. The number of positive minced chicken meat samples for 25 g, 2.5 g and 0.25 g were, respectively, for the low-level SI 4/5, 1/5 and 0/5 (2 MPN/sample) and for high-level SI 5/5, 5/5 and 2/5 (55 MPN/sample). The calculated MPN/sample was comparable to the results found in the minced chicken meat samples in the full study (see Table 5).

On Monday 27 January 2014, one parcel with 21 samples in one plastic safety bag was sent to laboratory 24 containing: 3 control samples (numbered C1 – C3), 18 contaminated minced chicken meat samples (numbered B1 – B18) and one temperature recorder.
The performance of the follow-up study started in week 5 (February 2014). The laboratory had to follow the same SOP, protocol and web-based test report as had been used in the study of September 2013 (EURL-*Salmonella*, 2013a, 2013b and 2013c).

During the follow-up study, laboratory 23 used some different or additional media, compared to those used in the full study of September 2013. The deviations are summarized in the scheme below.

	September 2013	January 2014
Selective enrichment		
MSRV (product code)	not used	CM0910 with Novobiocin 15.8mg/500mL
MKTTn (product code)	CM1048 with Novobiocin 40 mg/L	CM0343 with Novobiocin 82 g/L
Isolation media		
First medium XLD (product code)	XLT4 CM1061 used instead of prescribed XLD	CM0469
Second medium		
Name and product code	MacConkey CM0007	BGA mod CM0329
Positive control culture	*S*. Enteritidis 100 CFU/225ml BPW	*S*. Infantis 0.1 ml/225ml BPW

Oxoid is the supplier of the product codes CM

Laboratory 23 scored the three control samples correctly, as well as all high-level contaminated and blank samples with minced chicken meat. Unfortunately, they still showed a sensitivity problem with the low-level contaminated minced chicken meat samples, as the laboratory found only one out of six of these samples as positive. The same results were found with all three selective enrichment media (RVS, MKTTn and MSRV). The laboratory improved considerably, but still scored under the level of good performance (see Section 3.6). This was reported accordingly to EC, DG Sanco in April 2014.

5 Discussion

Artificial contamination of samples with a diluted culture
After many years of using reference materials (capsule or lenticule discs) to artificially contaminate the matrix in the interlaboratory comparison studies of the EURL-*Salmonella*, it was decided to change to the artificial contamination of the samples with a diluted culture at the laboratory of the EURL. The main reason for this change was to better mimic 'real life' routine samples and to enable easier handling of the study-samples for the participants.

The first EURL-*Salmonella* study in which this method of artificial contamination was used successfully was the study conducted for detection of *Salmonella* in boot socks of 2013 (Kuijpers and Mooijman, 2014). As each matrix and *Salmonella* serovar combination may behave differently, the samples of the current study were tested for their 'long-term' stability at storage temperatures (- 20 ºC and 5 ºC) and for 'short-term' stability at temperatures which may occur during the transport of the samples. Experiences from earlier studies had shown that, in general, the transport time of the parcels to the NRLs is 1–2 days at temperatures that remain below 10 ºC most of the time. Only occasionally, the temperature of a parcel during transport may be at ≥15 ºC for a few hours. As the number of *Salmonella* in the minced chicken meat slowly decreases during storage, it was decided to inoculate the low-level contamination samples with 10-15 CFU of a diluted culture of *Salmonella* Infantis. After storage and transport, this resulted in a contamination level close to the detection limit (5 MPN/25 g with CI of 1.5-16 MPN/25g) on the day of the study.

Transport of the samples
To stabilize the level of *Salmonella* Infantis *in the samples* during transport, the materials were packed with frozen cooling elements and transported by courier service. The information provided by the temperature recorders included in the parcels showed that the temperature in the parcels remained below 5 ºC for most of the transport time. It can therefore be assumed that transport did not negatively affect the mean contamination level of the samples. This was confirmed by the fact that the laboratories with the longest transport time and/or in combination with the highest temperatures (lab code 11, 26 and 28) scored close to the mean of all participants.

Accreditation of laboratories
According to EC regulations 882/2004 (EC, 2004) and 2076/2005 (EC, 2005), each NRL should have been accredited in their relevant field before 31st December 2009. Thirty-three laboratories were accredited. Two participants (EU-MSs, lab codes 9 and 12) were still in the process of accreditation, which is relatively late.

Performance of the laboratories
For the evaluation of the laboratories in terms of 'good performance', the best combination of selective enrichment medium (RVS, MKTTn or MSRV) and isolation medium was taken into account (being the combination with the highest number of positive isolations).
In comparison with earlier food studies (Kuijpers et al. 2011 and 2012a), a lower number of participants scored all samples correctly with at least one of the media used. A possible explanation for this is that the number of competitive,

interfering bacteria (*Enterobacteriacea*) in the minced chicken meat was high and interfered with the detection of *Salmonella* in the low-level contaminated chicken meat samples. As a result, it was decided to slightly adjust the criteria of good performance for the low-level, artificially contaminated minced chicken meat (at least 30 % of the samples as positive, instead of at least 50%). Because of this study's high level of difficulty, it was decided not only to slightly adjust the criteria of good performance, but also to take into account the data of both the prescribed media (RVS and MKTTn) and the requested (MSRV) medium in order to determine the performance of each participant.

Three laboratories (lab codes 9, 23 and 26) scored an 'underperformance'.
The problem of the laboratories 9 and 26 concerned a mistake made in numbering the control samples, as well as a mistake in reporting. In the case of reporting the results of routine samples, a transcription error may result in unwanted situations, such as 'incorrectly non-compliance' of a food product. The results of laboratories 9 and 26 were therefore indicated as 'moderate performance'. A follow-up study was considered unnecessary for those laboratories.
Laboratory 23 participated in a follow-up study. Unfortunately, they still showed a sensitivity problem and scored under the level of good performance, although they improved their performance considerably. This laboratory participated for the first time in an EURL-*Salmonella* detection study. A likely explanation (among others) for the problems of this laboratory may be their inexperience with the type of study and the samples used in this study. In earlier studies, it has been observed that laboratories participating for the first time often show an 'underperformance' level, but improve during the course of the studies (Kuijpers and Mooijman 2012b).

Six laboratories (8, 17, 24, 25, 26 and 34) scored a positive result for *Salmonella* in one blank sample, four of them with one selective enrichment medium, while they correctly found the same sample to be negative with another selective enrichment medium inoculated from the same pre-enriched culture in BPW. This was still considered acceptable because no 100% guarantee of the *Salmonella* negativity of the matrix could be given. An explanation for the false positive samples may be cross-contamination or misinterpretation of the results. The high number of background flora in the matrix may have caused problems with reading the isolation media. In combination with a limited confirmation, the *Enterobacteriaceae* present in the matrix may have been misinterpreted as *Salmonella.*

The performance of each laboratory compared with the mean of all laboratories for the artificially contaminated minced chicken meat samples (Figure 4) is an indication of the performance of a laboratory in general (the blanks are not included in this comparison). A laboratory can show a performance under the mean of all laboratories but still score a 'good performance'. This lower score can be caused by a low performance of one of the selective enrichment media. For the determination of good performance, the results of the 'best' media are taken into account; while for the analysis as presented in Figure 4, the results of all media combinations are presented.

According to the criteria used, 32 laboratories scored 'good performance', two laboratories scored 'moderate performance' and one laboratory scored 'under-performance'.

Specificity, sensitivity and accuracy rates
The calculations were performed on the results of all participants and on the results of only the EU-MS. Minor differences (if any) were found between these groups.
The majority of the blank meat samples tested negative, resulting in a specificity rate close to 100%. For the artificially contaminated minced chicken meat samples, it was easier to detect *Salmonella* with RVS and MSRV than it was with MKTTn. This was shown in the sensitivity rates. Especially for low-level contaminated samples, the level was shown to be close to the detection limit of MKTTn, resulting in a sensitivity rate of 47%.
The sensitivity rates are influenced not only by the contamination level of the target organism, but also by the level of interfering background flora. For the current meat samples, the growth of *Salmonella* was more negatively influenced by the background flora after selective enrichment in MKTTn than it was after selective enrichment in RVS or on MSRV. Low sensitivity rates for MKTTn were also observed in the food studies conducted in 2006 (87%) and 2007 (68%), with minced beef used as a matrix, in which the number of interfering background flora (*Enterobacteriaceae*) was close to the current study (Kuijpers et al., 2007 and 2008). In the food study conducted in 2009 (Kuijpers et al., 2010), minced chicken meat was also used as a matrix, but in this study the background flora was much lower, resulting in higher sensitivity rates of approximately 95-98% for RVS and MSRV as well as for MKTTn.

Positive control samples
The participants were asked to use the positive control sample(s) routinely used in their laboratory. *S*. Enteritidis and *S*. Typhimurium were the most frequently used serovars and the concentration varied between 8 – 10^6 CFU/sample. A positive control sample should demonstrate that media are capable of supporting growth of a range of low numbers of organisms. To have an idea concerning the sensitivity of a method, the concentration of a positive control sample should be just above the detection limit of this method. The majority of the participants used a much higher concentration. Furthermore, it may be advisable to use a serovar rarely isolated from the routine samples analysed in the laboratory. In this way possible cross contamination can be detected more easily.
Two laboratories (9 and 21) scored false negative results for their positive *Salmonella* control samples with one of the media used. The results of these controls are a possible indication of problems with the relevant media because both laboratories also scored lower positive results for the same media with the minced chicken meat samples contaminated with *Salmonella*.

Media and incubation
Some laboratories showed an 'underperformance' for one of the selective enrichment media used, while they correctly scored all samples positive with another selective enrichment medium inoculated from the same pre-enriched culture. This may indicate a sensitivity problem with a selective enrichment medium. However, it may also indicate that one selective enrichment medium is more suited to detect *Salmonella* from samples with high amounts of background flora (RVS and MSRV) than another selective enrichment medium (such as MKTTn). For MKTTn, it was seen that the contaminating bacteria (*Proteus vulgaris* and *Hafnia alvei*) in the matrix overgrew the *Salmonella* colonies on the isolation media used after selective enrichment medium.
During the study, (small) deviations in the prescription of the media (e.g. in pH or concentration of novobiocin) or incubation time have been reported. The influence of these deviations on the results is not always clear. For instance,

laboratories 17 and 21 reported a lower pH and a lower concentration of novobiocin in MKTTn, respectively, than prescribed and scored a lower number of positive meat samples. But whether these low scores were caused by those deviations of MKTTn is hard to trace.

Fifteen participants (43%) scored additional samples as being positive after the extra 24 hours of incubation on MSRV. The increase in the number of positive results after 48 hours of incubation of the selective enrichment on MSRV was 9-11%. An additional 24 hours of incubation of MSRV seems to be more important when the number of interfering background flora (*Enterobacteriaceae*) is high and the contamination level of *Salmonella* is low. In an earlier food study, in which the number of *Enterobacteriacea* in the matrix was low (difference of log 6 CFU/g), MSRV gave only 4% more positive samples after the additional 24 hours of incubation of MSRV (Kuijpers and Mooijman, 2012a).

PCR
Three laboratories used a PCR technique in addition to the prescribed method. Two of them found the same results as they had using the bacteriological culture technique (BAC), while one laboratory found more results that were negative using their PCR method than they did using BAC.
In comparison with former EURL-*Salmonella* studies for the detection of *Salmonella* in food samples, a decrease was seen in the number of NRLs using a PCR technique as their own method. In earlier studies, nine or six laboratories used a PCR technique in addition to the prescribed method, while in the current study only three laboratories performed PCR (Kuijpers et al., 2011 & 2012a). On the other hand, in the studies for detection of *Salmonella* in samples from the primary production stage, an increase was seen in the number of laboratories using PCR (Kuijpers and Mooijman, 2014). However, from this information no conclusions can be drawn concerning the use of PCR techniques in the NRLs, since the use of an own method is voluntary and not prescribed in the interlaboratory comparison studies.

Evaluation of this study
The artificial contamination of a food matrix with a diluted culture at the laboratory of the EURL-*Salmonella* was successful. The samples were easier to handle for the participants and mimicked 'real life' samples more closely than samples used in earlier food studies organized by the EURL-*Salmonella*. Although the preparation of this kind of sample is more complicated for the EURL, the advantages for the participants are significant. For future studies, this method of contaminating samples will therefore be the first choice.
The amount of background flora in the current samples was very high and interfered with the detection of *Salmonella,* especially in the low-level contaminated chicken meat samples. For the next food studies, a shorter storage time at 5 ^{0}C will be considered to prevent the growth of interfering flora.
Reporting of the results via the Internet was used for the first time in a food detection study and was well received by the participants. Furthermore, the data were easier to analyse for the EURL. The use of web-based test reports will therefore be continued in future studies.

6 Conclusions

- Thirty-two out of 35 NRLs for *Salmonella* scored a good performance for the detection of *Salmonella* in high-level and low-level contaminated minced chicken meat samples. Two laboratories scored a 'moderate performance'. One laboratory scored an 'underperformance' in the full study and in the follow-up study.
- The accuracy rates for the control samples after selective enrichment in RVS and MKTTn was 97% and on MSRV it was 100%.
- The specificity rates of the blank minced chicken meat samples of the EU-MS was 98%.
- The sensitivity rates of minced chicken meat samples that were artificially contaminated with low-level *S*. Infantis was 61% - 64% after selective enrichment in RVS and on MSRV and was 47%- 50% for MKTTn.
- The sensitivity rates of the minced chicken meat samples that were artificially contaminated with high-level *S*. Infantis were approximately 30% higher than the rates of the low-level contaminated samples.
- The accuracy rate of the artificially contaminated minced chicken meat samples of the NRLs from the EU-MS was 85% after selective enrichment in RVS and on MSRV and was 75% after selective enrichment in MKTTn.
- Selective enrichment with MSRV and RVS gave a significantly higher chance of finding *Salmonella* Infantis in minced chicken meat in comparison with MKTTn.
- 48 hours of incubation of the selective enrichment medium MSRV overall showed 10% more positive results than 24 hours of incubation.
- Matrix samples that were artificially contaminated with a diluted culture mimicked 'real life' routine samples more closely and were easier to use by the participants than the previously used mixtures of matrix and reference materials.
- The use of a web-based test report by the participants for reporting the results was successful. No (major) problems were indicated by the participants using this method of reporting, which was used for the first time in a food detection study. Furthermore, digital reporting lowers the risk of transcription errors during the analysis of the results.

List of abbreviations

ATCC	American Type Culture Collection
BAC	Bacteriological Culture technique
BGA(mod)	Brilliant Green Agar (modified)
BPLS	Brilliant Green Phenol-red Lactose Sucrose
BPW	Buffered Peptone Water
BSA	Brilliance *Salmonella* Agar (OSCM)
CFU	Colony-Forming Units
EC	European Commission
EFTA	European Free Trade Association
EU	European Union
EURL	European Union Reference Laboratory
Gal	Galactosidase
ISO	International Organization for Standardization
LDC	Lysine Decarboxylase
MAC	MacConkey Agar
MKTTn	Mueller Kauffmann Tetrathionate novobiocin Broth
MPN	Most Probable Number
MS	Member State
MSRV	Modified Semi-solid Rappaport-Vassiliadis
NRL	National Reference Laboratory
OR	Odds Ratio
PCA	Plate Count Agar
PCR	Polymerase Chain Reaction
RIVM	Rijksinstituut voor Volksgezondheid en het Milieu (National Institute for Public Health and the Environment)
RS	Rapid *Salmonella*
RVS	Rappaport Vassiliadis Soya broth
SI	*Salmonella*Infantis
SM (ID)2	*Salmonella* Detection and Identification-2
SOP	Standard Operating Procedure
STM	*Salmonella* Typhimurium
TSI	Triple Sugar Iron agar
UA	Urea Agar
VP	Voges-Proskauer
VRBG	Violet Red Bile Glucose agar
XLD	Xylose Lysine Deoxycholate agar
XLT4	Xylose Lysine Tergitol 4 agar

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Annex 1 Number of positive results of the control samples per laboratory, per selective enrichment medium and per isolation medium

Lab code	RVS XLD/2nd *			MKTTn XLD/2nd *			MSRV XLD/2nd *		
	Positive Control Own n=1	Procedure Control BPW n=1	Matrix control meat n=1	Positive Control Own n=1	Procedure Control BPW n=1	Matrix control meat n=1	Positive Control Own n=1	Procedure Control BPW n=1	Matrix control meat n=1
Good Performance	**1**	**0**	**0**	**1**	**0**	**0**	**1**	**0**	**0**
1	1	0	0	1	0	0	1	0	0
2	1	0	0	1	0	0	1	0	0
3	1	0	0	1	0	0	1	0	0
4	1	0	0	1	0	0	1	0	0
5	1	0	0	1	0	0	1	0	0
6	1	0	0	1	0	0	1	0	0
7	1	0	0	1	0	0	1	0	0
8	1	0	0	1	0	0	1	0	0
9	**0**	1	0	1**/0**	**1**	0	-	-	-
10	1	0	0	1	0	0	1	0	0
11	1	0	0	1	0	0	1	0	0
12	1	0	0	1	0	0	1	0	0
13	1	0	0	1	0	0	1**/0**	0	0
14	1	0	0	1	0	0	1	0	0
15	1	0	0	1	0	0	1	0	0
16	1	0	0	1	0	0	1	0	0
17	1	0	0	1	0	0	1	0	0
18	1	0	0	1	0	0	1	0	0
19	1	0	0	1	0	0	1	0	0
20	1	0	0	1	0	0	1	0	0
21	1	0	0	**0**	0	0	1	0	0
22	1	0	0	1	0	0	1	0	0
23	1	1	0	1	1	0	-	-	-
24	1	0	0	1	0	0	1	0	0
25	1	0	0	1	0	0	1	0	0
26	1	0	0	1	0	0	1	0	0
27	1	0	0	1	0	0	1	0	0
28	1	0	0	1	0	0	1	0	0
29	1	0	0	1	0	0	1	0	0
30	1	0	0	1	0	0	1	0	0
31	1	0	0	1	0	0	1	0	0
32	1	0	0	1	0	0	1	0	0
33	1	0	0	1	0	0	1	0	0
34	1	0	0	1	0	0	1	0	0
35	1	0	0	1	0	0	1	0	0

* =When only one figure is given, both isolation media gave the same result.

\- = not performed

bold numbers = deviating results

grey cells = results are below the criteria of good performance

Annex 2 Number of positive results for the artificially contaminated minced chicken meat samples per laboratory, per selective enrichment medium and per isolation medium

Lab code	RVS XLD/2nd *			MKTTn XLD/2nd *			MSRV XLD/2nd *		
	Blanc	SI Low	SI High	Blanc	SI Low	SI High	Blanc	SI Low	SI High
Good Performance	≤ **1**	≥ **2**	≥ **5**	≤ **1**	≥ **2**	≥ **5**	≤ **1**	≥ **2**	≥ **5**
1	0	6	6	0	6	6	0	6	6
2	0	**1**	**5/4**	0	**1/0**	**1**	0	**1**	**3**
3	0	**5**/6	6	0	**4/2**	6	0	**4**	6
4	0	**4**	6	0	**1/2**	**4**/6	0	**4**	6
5	0	**5**	6	0	**5**	6	0	**5**	6
6	0	**1/2**	**5/4**	0	**1**	**3/1**	0	**2**	6
7	0	**2/3**	**4**/6	0	5/5	**4/5**	0	**4/2**	**5**/6
8	0/**1**	**2**	**5**	0	**1**	**1/4**	0/**1**	**2**	**5**
9	0	**2/1**	6	0	**1/0**	**0**	-	**-**	**-**
10	0	**2**	**5**	0	**0**	**1**	0	**2**	6
11	0	**3**	6	0	**3**	6	0	**3**	**5**
12	0	**5/4**	6/**5**	0	**4/3**	6/**5**	0	**5**	6
13	0	**4**	6	0	**4**	6	0	**3/1**	**5/1**
14	0	6	6	0	6	6	0	6	6
15	0	**2/3**	6	0	**0**	**3/2**	0	**1**	6
16	0	**2/3**	6	0	**1/0**	**3/0**	0	**3**	6
17	0	**5**	6	0	**1**	**2/1**	**1**	**3/1**	**3**
18	0	**5**	6	0	**0**	**3**	0	**4**	6
19	0	**4/3**	6	0	**4/0**	6/**3**	0	**3**	6
20	0	**5**	6	0	**4**	6	0	**4**	6
21	0	**2**	**5/4**	0	**0**	**0**	0	**2**	**5**
22	0	**3**	6	0	**3**	6	0	**3**	6
23	0	**0**	**0**	0	**0**	**1/0**	**-**	**-**	**-**
24	0	6	6	**1**	**2/1**	**4/3**	0	**3/4**	6
25	**1**	**3**	6	**1**	**3**	6	**1**	**3**	6
26	**2**	**4**	6	**2**	**4**	6	**2**	**4**	6
27	0	**0/1**	**0/1**	0	**1/4**	**4**/6	0	**1**	**3**
28	0	**5**	6	0	**0**	**5**	0	**5**	6
29	0	**1/3**	**5/3**	0	**4**	6	0	**5**	6
30	0	**5**	6	0	**4/3**	6	0	**5**	6
31	0	**3**	6	0	**3**	6	0	**3**	6
32	0	6	6	0	6	6	0	6	6
33	0	**3**/6	6	0	**4**	6	0	6	6
34	0/**1**	**0**	**0/1**	0	**2/1**	**2/3**	0	**4**	6
35	0	**4/5**	6	0	**3**	**5/4**	0	6	6

* =When only one figure is given, both isolation media gave the same result.

\- =not performed

bold numbers =deviating results

grey cells =results are below the criteria of good performance

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