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

# EURL-*Salmonella* 8th interlaboratory comparison study Food 2016

Detection of *Salmonella* in minced chicken meat

RIVM Report 2017-0081
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# Colophon



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# Synopsis

**EURL-*Salmonella* 8th interlaboratory comparison study Food 2016**
Detection of *Salmonella* in minced chicken meat

In 2016, it was shown that all 34 National Reference Laboratories (NRLs), 30 of which are located in the European Union, were able to detect high and low levels of *Salmonella* in minced chicken meat. Three NRLs reported *Salmonella* in one 'blank' minced meat sample. This was probably caused by the fact that another type of *Salmonella* was already present in very low levels in the original meat. We present some conclusions from the 8th EU Interlaboratory Comparison Study of Food Samples, organised by the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*). EURL-*Salmonella* is part of the Dutch National Institute for Public Health and the Environment (RIVM).

The study was conducted in September 2016. Participation was obligatory for all EU Member State NRLs responsible for the detection of *Salmonella* in food samples.

The laboratories used internationally accepted methods to detect the presence of *Salmonella* in minced chicken meat samples and analysed the samples according to the same protocol. Each laboratory received a package with minced chicken meat samples contaminated with two different concentrations of *Salmonella* Stanley or containing no *Salmonella* at all. As in earlier studies, the meat samples were artificially contaminated with a diluted culture of *Salmonella* at the EURL-*Salmonella* laboratory.

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

# Publiekssamenvatting

## EURL-*Salmonella* 8e ringonderzoek Voedsel 2016

Detectie van *Salmonella* in kippengehakt

In 2016 waren alle 34 Nationale Referentie Laboratoria (NRL's), waarvan dertig in de Europese Unie, in staat om verschillende concentraties *Salmonella* in kippengehakt aan te tonen. Drie NRL's rapporteerden dat er *Salmonella* zit in een van hun monsters met 'blanco' gehakt. Zeer waarschijnlijk komt dat doordat er in het oorspronkelijke vlees een ander type *Salmonella* zat, in zeer lage concentraties. Dit blijkt uit het achtste ringonderzoek voor voedsel, dat is georganiseerd door het referentielaboratorium van de Europese Unie voor *Salmonella* (EURL-*Salmonella*). Het EURL-*Salmonella* is gevestigd bij het Nederlandse Rijksinstituut voor Volksgezondheid en Milieu (RIVM).

Het onderzoek is in september 2016 gehouden. 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.

Voor het ringonderzoek gebruiken de laboratoria de internationaal erkende analysemethoden en werken ze volgens hetzelfde protocol. Elk laboratorium kreeg een pakket toegestuurd met kippengehakt dat ofwel besmet was met *Salmonella* Stanley in twee verschillende concentraties, of geen *Salmonella* bevatte. De gehaktmonsters werden zoals in eerdere studies op het laboratorium van het EURL-*Salmonella* kunstmatig besmet met *Salmonella*.

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

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# Summary

In September 2016, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised the eighth interlaboratory comparison study on the detection of *Salmonella* in food samples. The matrix of concern was minced chicken meat.
The participants were 34 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*): 30 NRLs from the 28 EU Member States (EU-MS), 4 NRLs from third countries within Europe (EU candidate MS or potential EU candidate MS, members of the European Free Trade Association (EFTA)) and one NRL from a non-European country.

The most important objective of the study was to test the performance of the participating laboratories for the detection of different concentration levels of *Salmonella* in a food matrix. Each laboratory received minced chicken meat samples (25 grams each) artificially contaminated with *Salmonella* Stanley (SSt) at two different contamination levels for analysis.
The participants were asked to follow ISO/FDIS 6579-1:2015 for sample analysis. This document allows the choice of either Rappaport Vassiliadis Soya broth (RVS) or Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar, with the addition of Mueller Kauffmann Tetrathionate novobiocin broth (MKTTn) for the selective enrichment step.
For results, the participants were asked to report what would have been reported as they would for routine samples, meaning that the indication ‘positive’ (1) or ‘negative’ (0) for each sample (after confirmation) was sufficient (independent to the combination of selective enrichment medium and isolation medium).
The laboratories’ performance was compared to the pre/set criteria for good performance.

The samples consisted of minced chicken meat artificially contaminated with a diluted culture of *Salmonella* Stanley (SSt) at a low level (approximately 15-20 CFU/25 g of meat), at a high level (approximately 50-100 CFU/25 g of meat), or with no *Salmonella* at all (blank samples). The samples were artificially contaminated at the EURL-*Salmonella* laboratory. Before starting the study, several experiments were carried out to make sure that the samples were fit for use in an interlaboratory comparison study: choice of *Salmonella* serovar, stability at different storage temperatures (–20 °C, 5 °C and 10 °C), and influence of background flora. Results from the pre-tests showed that the number of *Salmonella* as well as the amount of background flora in the meat samples were most stable when stored at –20 °C. The pre-tests were performed with minced turkey meat. At the last minute, the choice of the matrix for this study was changed to minced chicken meat the batch of minced turkey meat used was naturally contaminated with *Salmonella*.

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 SSt (inoculum 16 CFU/sample) and six samples with a high level of SSt (inoculum 73 CFU/sample). Additionally, two control

samples had to be tested: one blank control sample (procedure control (BPW)) and one own (NRL) positive control sample (with *Salmonella*).

Thirty-three of the 34 laboratories found *Salmonella* in all (contaminated) samples resulting in a sensitivity rate of 99%.

Nine participants used PCR as an own method: all found the same results as with the bacteriological culture method; most (eight) used a real time PCR.

Most participants (19) performed all three selective enrichment media (MKTTn, MSRV and RVS), 9 laboratories MKTTn and MSRV, and 6 MKTTn and RVS.

For the positive control, the majority of the participants (21 laboratories) used a diluted culture of *Salmonella*. The *Salmonella* serovars most frequently used for the positive control sample were *S*. Enteritidis (14) and *S*. Typhimurium (7). The concentration of the positive control varied between 1 – $10^4$ CFU/sample. For the positive control, it is advisable to use a concentration close to the detection limit of the method and a *Salmonella* serovar not often isolated from routine samples.

Three laboratories found one blank minced chicken meat sample positive for *Salmonella*. This is acceptable, as a 100% guarantee about the negativity of the matrix cannot be given. Independent additional typing showed the presence of *Salmonella* Infantis at all three laboratories. Hence, it is likely that the minced chicken meat was naturally contaminated with *S.*Infantis, albeit at a very low level because all other blank samples tested by NRLs and EURL (>200) were negative.

All laboratories achieved the level of good performance.

# 1 Introduction

An important task of the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*), as laid down in Commission Regulation EC No. 882/2004 (EC, 2004), is the organisation of interlaboratory comparison studies to test the performance of the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*). The history of the interlaboratory comparison studies on the detection of *Salmonella*, as organised by EURL-*Salmonella* (formerly called CRL-*Salmonella*) from 1995, is summarized on the EURL-*Salmonella* website (EURL-*Salmonella*, 2017).

The objective of the current study, organised by EURL-*Salmonella* in October 2016, was to see whether the participating laboratories could detect different contamination levels of *Salmonella* in minced chicken meat. This is important in order to know whether the examination of samples is carried out uniformly in all EU Member States (MS), and whether comparable results are obtained by all NRLs-*Salmonella*.

The participants were asked to follow the procedure for detection of *Salmonella* in official samples using ISO/FDIS 6579-1:2015. In this document, Mueller Kauffmann Tetrathionate novobiocin broth (MKTTn) is prescribed as the first selective enrichment medium; as the second selective enrichment medium either Rappaport Vassiliadis Soya broth (RVS) or Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar can be used.

The set-up of this food study was comparable to the interlaboratory comparison studies that have been organised since 2013 on the detection of *Salmonella* in food: minced chicken meat and whole liquid egg (Kuijpers et al., 2014, Kuijpers and Mooijman, 2016) and animal feed (Kuijpers et al., 2015). For the current study, the (food) samples were artificially contaminated with a diluted culture of *Salmonella* Stanley (SSt) at the EURL-*Salmonella* laboratory.

As in earlier studies, the contamination level of the low-level samples was close to the detection limit of the method and the level of the high-level samples was approximately 5–10 times that of the detection limit. In total, 18 minced chicken meat samples were tested by each NRL: 6 samples per contamination level (low-level and high-level) containing one *Salmonella* serovar (*Salmonella* Stanley) and 6 with no *Salmonella* (blank). Additionally, two control samples (one blank control sample and one positive control sample) were tested. The number and level of samples tested were in accordance with ISO/TS 22117:2010.

# 2 Participants

| Country | City | Institute / NRL-*Salmonella* |
|---|---|---|
| **Austria** | Graz | Austrian Agency for Health and Food Safety (AGES), Institute for Medical Microbiology and Hygiene (IMED) |
| **Belgium** | Brussels | Institute of Public Health Lab of Food Pathogens (WIV-ISP) |
| **Bulgaria** | Sophia | National Diagnostic Research Veterinary Institute (NDRVMI), National Reference Centre of Food Safety |
| **Croatia** | Zagreb | Croatian Veterinary Institute, Laboratory for Food Microbiology (CVI) |
| **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 (SVI) |
| **Denmark** | Ringsted | Danish Veterinary and Food Administration (DVFA-1), Microbiology Ringsted |
| **Estonia** | Tartu | Estonian Veterinary and Food Laboratory |
| **Finland** | Helsinki | Finnish Food Safety Authority Evira Research Department, Microbiology Unit |
| **France** | Ploufragan | ANSESLaboratoire de Ploufragan-Plouzané, Unité Hygiène et Qualité des Produits Avicoles et Porcins (UHQPAP) |
| **Germany** | Berlin | Federal Institute for Risk Assessment (BfR) |
| **Greece** | Halkis | Veterinary Laboratory of Chalkis, Hellenic Republic, Ministry of Reconstruction of Production, Environment and Energy |
| **Hungary** | Budapest | National Food Chain Safety Office, Food and Feed Safety Directorate |
| **Iceland** | Reykjavik | Matis ohf, Icelandic Food and Biotech R&D |
| **Ireland** | Kildare | Central Veterinary Research Laboratory CVRL/DAFM Backweston, Department of Agriculture, Food and Marine |
| **Israel** | Kiryat Malachi | Southern Laboratory for Poultry Health Laboratory Egg and Poultry |
| **Italy** | Legnaro PD | Istituto Zooprofilattico Sperimentale delle Venezie, OIE |
| **Latvia** | Riga | Institute of Food Safety, Animal Health and Environment, BIOR Animal Disease Diagnostic Laboratory |

| Country | City | Institute / NRL-*Salmonella* |
|---|---|---|
| **Lithuania** | Vilnius | National Food and Veterinary Risk Assessment Institute, Food Microbiology Section |
| **Luxembourg** | Dudelange | Laboratoire National de Santé, Département des Laboratoires officiels d'analyses de contrôle |
| **Malta** | Valletta | Public Health Laboratory (PHL), Microbiology Evans Building |
| **Netherlands** | Bilthoven | National Institute for Public Health and the Environment (RIVM/CIb) Infectious Disease Control, Centre for Zoonoses and Environmental Microbiology (cZ&O) |
| **Netherlands** | Wageningen | Netherlands Food and Consumer Product Safety Authority (nVWA) Consumer and Safety Division, Microbiology |
| **Norway** | Oslo | Norwegian Veterinary Institute, Bacteriology Section |
| **Poland** | Pulawy | National Veterinary Research Institute (NVRI),<br>Department of Hygiene of Food of Animal Origin |
| **Portugal** | Vairao | Instituto Nacional de Investigação Agrária e Veterinária<br>Unidade de Tecnologia e Segurança Alimentar (INIAV) |
| **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, Majadahonda | Centro Nacional de Alimentación (CNA) Agencia Española de Seguridad Alimentaria y Nutricion (AESAN) National Food Centre |
| **Sweden** | Uppsala | National Veterinary Institute (SVA), Department of Bacteriology |
| **Switzerland** | Bern | Institute of veterinary Bacteriology, Vetsuisse Faculty |
| **United Kingdom** | York | Public Health England (PHE) Food Water and Environmental Microbiology FW&E, York Laboratory |
| **United Kingdom** | Belfast | Agri-Food and Bioscience Institute (AFBI) Veterinary Science Division (VSD) Bacteriology |

# 3 Materials and methods

## 3.1 Preparation of artificially contaminated minced meat samples

### *3.1.1 General*

The matrix used in this interlaboratory comparison study was minced chicken meat. Minced chicken meat was obtained from Plukon, Ommel in the Netherlands. For the pre-tests, two minced turkey meat batches (3 kg and 2 kg) from a local butcher were tested. For the interlaboratory comparison study, 18 kg minced chicken meat was used. This last batch arrived at EURL-*Salmonella* on 19 September 2016.

Immediately after receipt of the minced meat, 5 samples (for the pre-test) or 10 samples (for the interlaboratory comparison study) of 25 g each were checked for the absence of *Salmonella* in accordance with ISO/FDIS 6579-1:2015. For this purpose, 225 ml of Buffered Peptone Water (BPW) was added to each of the 25 g samples. After pre-enrichment at 37 (± 1) °C for 16 to 18 hours, selective enrichment was carried out in Rappaport-Vassiliadis Soya broth (RVS) and Mueller Kaufmann Tetrathionate novobiocin broth (MKTTn) and on Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar. The MKTTn and RVS tubes and the suspect growth on MSRV plates were then plated out on Brilliance *Salmonella* Agar (BSA) and confirmed biochemically.

After verifying the absence of *Salmonella*, the minced meat was repacked in portions of 25 g in Whirl-Pak plastic bags, after which the test portions were artificially contaminated with three different levels (Blank, low and high level) of *Salmonella* Stanley (SSt) and stored at –20 °C (see Section3.3.2).

### *3.1.2 Pre-tests for the preparation of minced turkey meat samples*

The minced meat samples were artificially contaminated at the EURL-*Salmonella* laboratory with a diluted culture of *Salmonella*. Some experiments were performed prior to the start of the interlaboratory comparison study, especially in relation to the stability of *Salmonella* in the artificially contaminated minced meat samples when stored at different temperatures.

For the contamination, two different Salmonella serovars were tested: *Salmonella* Typhimurium (STM) from the American Type Culture Collection (ATCC 14028, Manassas, USA) and *Salmonella* Stanley (SST, strain 14.5 EURL *Salmonella*) isolated from chicken and humans respectively. Each strain was inoculated in Buffered Peptone Water (BPW) and incubated at (37 ± 1) °C overnight. Each culture was then diluted in peptone saline solution in order to inoculate the minced meat samples with approximately 10– 15 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 agar plate and incubated at 37 °C for 20-24 hours.

Samples of 25 g minced turkey meat were artificially contaminated with a dilution of a *Salmonella* culture (different levels of SSt and STM).

Additional, control samples were prepared without the addition of *Salmonella* (blank samples).

For the first pre-tests, all (artificially contaminated) minced turkey meat samples were stored at –20 °C, 5 °C and 10 °C for a period of 0, 7, 14 and 21 days. After each storage time at the different temperatures, the artificially contaminated SSt, STM and blank minced turkey meat samples were tested for the presence of *Salmonella* following ISO/FDIS 6579-1:2015, with selective enrichment on MSRV agar and in MKTTn broth. In the 2013 study using minced chicken meat as matrix, the samples were stored at 5 °C which resulted in a high amount of background flora interfering with the detection of *Salmonella*, especially for the low-level contaminated chicken meat samples (Kuijpers et al., 2014). The second pre-test analysed whether storing the samples at –20 °C would prevent the growth of interfering flora. To test the stability of the artificially contaminated samples as well as possible negative effects of repeated freezing and thawing, three batches of contaminated samples were tested for the presence of *Salmonella* after the following storage conditions:

- 3 weeks at –20 °C;
- 2 weeks at –20 °C, followed by 1 week at 5 °C;
- 1 week –20 °C, followed by 1 week at 5 °C, followed by 1 week at –20 °C.

To obtain an indication of the amount of background flora in the samples after storage at different temperatures, the blank minced turkey meat samples (without the addition of *Salmonella*) were tested for the number of aerobic bacteria and *Enterobacteriaceae* (see section 3.1.4).

### *3.1.3 Preparation of minced chicken meat samples for the interlaboratory comparison study*

Minced turkey meat was initially designated as the matrix of this study, however, as the batch was naturally contaminated with *Salmonella,* the choice of the matrix was changed to chicken meat at the last minute. Approximately two weeks before the study, a total of 810 minced chicken meat samples were prepared as follows:

- plastic bags were labelled;
- 25 g of minced chicken meat was added to each plastic bag;
- approximately 0.1 ml of a diluted culture of *S*. Stanley (SSt) was added to the meat sample. The desired three contamination levels were: 15–20 CFU/25 g meat, 50–100 CFU/25 g meat, and blank;
- samples were then stored at –20 °C until transport to the NRLs on 26 September 2016.

### *3.1.4 Determination of amount of background flora in minced meat*

The total number of aerobic bacteria and the number of *Enterobacteriaceae* in minced meat were investigated by following ISO 4833:2003 and ISO 21528-2:2004. The minced meat was homogenised in peptone saline solution and tenfold dilutions were analysed on Plate Count Agar (PCA) and on Violet Red Bile Glucose (VRBG) Agar.

### 3.1.5 *Determination of the number of Salmonella 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 using the five-tube, most probable number (MPN) technique. For this purpose, 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 ISO/FDIS 6579-1:2015. From the number of confirmed positive dilutions, the MPN of *Salmonella* in the original sample was calculated using freely available Excel-based MPN software (Jarvis et al., 2010).

## 3.2 Design of the interlaboratory comparison study

### 3.2.1 *Number and type of samples*

On 26 September 2016 (one week before the study), the minced chicken meat samples were prepared for shipment and sent to the participants by door-to-door courier service. After arrival at the laboratories, the meat samples had to be stored at –20 °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*, 2016a) and in (a print-out from) the web-based test report (EURL-*Salmonella*, 2016b).

Eighteen meat samples (numbered B1–B18) and two control samples (numbered C1 and C2) had to be tested by each participating laboratory. Table 1 gives an overview of the number and type of samples tested by each participant.

*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*. Stanley low level (SSt low) | 6 |
| *S*. Stanley high level (SSt high) | 6 |
| Blank (BL) | 6 |
| | **Control samples (n=2)** |
| Blank procedure control (BPW only) | 1 |
| Positive control (own control with *Salmonella*) | 1 |

For the control samples, the laboratories were asked to use their standard positive *Salmonella* control when analysing samples for *Salmonella* detection. In addition, one blank BPW control had to be analysed.

### 3.2.2 *Shipment of parcels and temperature recording during shipment*

Twenty plastic bags were sent to each NRL containing the meat samples artificially contaminated with *Salmonella*, blank minced meat samples and controls (no meat at all). The 20 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 excessive 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 the safety bag. The loggers were programmed by 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 EURL-*Salmonella*, the loggers were read using a computer program and all recorded temperatures from the start of the shipment until the start of the study were transferred to an Excel sheet.

## 3.3 Methods

The NRLs were asked to follow (as much as possible) the procedures used for routine analyses (e.g. pre-warming of BPW, different ways of mixing the samples in BPW). In addition, the NRLs were asked to follow ISO/FDIS 6579-1:2015 for the detection and confirmation of *Salmonella* and the underlying EN ISO documents (e.g. EN ISO 6887 series) for preparation of test samples.
ISO/FDIS 6579-1 is the revised version of ISO 6579:2002 and describes the (final) updated technical steps for the detection of *Salmonella* in food, animal feed and samples from the primary production stage. An important addition is the option to choose between RVS broth and MSRV agar for the selective enrichment of *Salmonella* from food and animal feed samples. Participating laboratories were also offered this choice in the current study, meaning that, in addition to MKTTn broth, either RVS broth or MSRV agar could be used for selective enrichment. The NRLs were also permitted to use all three selective enrichment media. In addition, the NRLs could use their own method, such as a Polymerase Chain Reaction (PCR) procedure.

The method in summary:

- Pre-enrichment in:
  - -Buffered Peptone Water (BPW);
- Selective enrichment in/on:
  - -Mueller Kaufmann Tetrathionate novobiocin broth (MKTTn);
  - -Rappaport Vassiliadis Soya broth (RVS) and/or
  - -Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar;
- Plating-out on the following isolation media:
  - -first plating-out: Xylose Lysine Desoxycholate agar (XLD);
  - -second plating-out (obligatory): medium of choice;
- Confirmation by means of:
  - Appropriate biochemical tests (ISO/FDIS 6579-1:2015) or reliable, commercially available identification kits and/or serological tests.

## 3.4 Statistical analysis of the data

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

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

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

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

## 3.5 Criteria for good performance

For the determination of 'good performance', the criteria indicated in Table 2 were used. For the determination of 'good performance' per laboratory, the results obtained with all combinations of selective enrichment media and isolation media used by the laboratory were taken into account.

*Table 2. Criteria for good performance in the interlaboratory comparison study*

| **Minimum result** | | |
|---|---|---|
| **Contamination level** | **Percentage positive** | **No. of positive samples/total no. of samples** |
| **Samples Minced chicken meat artificially contaminated** | | |
| *S.* Stanley high level (SSt high) | 80% | 5/6 |
| *S.* Stanley low level (SSt low) | 50% | 3/6 |
| Blank (BL)[1] | 20%[1] | 1/6[1] |
| **Control samples** | | |
| Procedure control (BPW only) | 0% | 0/1 |
| Positive control (own control with *Salmonella*) | 100% | 1/1 |

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

# 4 Results and discussion

## 4.1 Preparation of artificially contaminated minced meat samples

### *4.1.1 General*

All the batches of minced meat (chicken and turkey) used in this study were tested for presence of *Salmonella*. One batch of turkey minced meat tested positive and was destroyed before further use.

### *4.1.2 Pre-tests for the preparation of minced turkey meat samples*

The use of matrices that mimic routine samples is considered more appropriate than the use of reference materials in interlaboratory studies. In 2013, EURL-*Salmonella* started with artificial contamination of matrices with a diluted culture of a *Salmonella* serovar, which is more challenging with regards to sample stability than when using reference materials. In earlier EURL-*Salmonella* studies, this method of artificial contamination was successfully used for the detection of *Salmonella* in boot socks and chicken faeces (Kuijpers and Mooijman, 2014 and 2015) and for the detection of *Salmonella* in minced chicken meat (Kuijpers et al. 2014) and chicken feed (Kuijpers et al. 2015).

Each matrix and *Salmonella* serovar combination can behave differently with respect to the survival of *Salmonella* during storage and transport. For that reason, the artificially contaminated minced meat samples were tested for their 'long-term' stability at –20 °C and 5 °C and for their 'short-term' stability at a temperature that could occur during sample transport (10 °C).

Two sets of experiments were performed. During each set of experiments, different variables were tested in different combinations (see section 3.1.2). For both experiments, the minced turkey meat was stored at -20 °C and during this period the meat was tested negative for *Salmonella* and the amount of background flora was investigated (indicated in Figures 1 and 2 as week -1). The meat was defrosted and artificially contaminated with *Salmonella* and stored at the given temperatures for the given periods; this is the start of the pre-test (indicated in Figures 1 and 2 as week 0).
For the stability test, two different batches of minced turkey meat were used (see Tables 3 and 4 and Figures 1 and 2: batches 1 and 2).

Tables 3 and 4 show the results of the experiments performed with all samples after selective enrichment on/in MSRV, MKTTn and RVS, followed by isolation on BSA and XLD. All samples were tested positive for all used media. The storage of the minced turkey meat samples for different times and at different temperatures did not affected the detection of *Salmonella*.

Figures 1 and 2 show the amount of background flora for the first and second stability test (batches 1 and 2), respectively.
In the first stability test, the amount of background flora in the minced meat remained relatively stable after storage at –20 °C for 3 weeks (see Figures 1 and 2). When stored at 5 °C and 10 °C, the number of aerobic

bacteria and *Enterobacteriaceae* in the minced meat increased from $10^7$ to $10^9$–$10^{10}$ CFU/g and from $10^2$ to $10^6$–$10^7$ CFU/g respectively after 3 weeks (see Figure 1).
In the second stability test, the minced meat was stored alternately at –20 °C and 5 °C for different time periods (see Table 4). This test also showed that the number of aerobic bacteria and *Enterobacteriaceae* remained stable when the meat was stored at –20 °C only, and increased when the meat was alternately also stored at 5 °C (see Figure 2).

*Table 3. Stability tests of minced turkey meat samples artificially contaminated with low level of Salmonella Typhimurium (STM) and Salmonella Stanley (SSt)*

| **Weeks of storage** | **Temperature of storage** Artificially contaminated minced turkey meat (Batch 1) | | | | | |
|---|---|---|---|---|---|---|
| | **–20 °C** | | **5 °C** | | **10 °C** | |
| | STM12 | SSt 6 | STM12 | SSt 6 | STM12 | SSt 6 |
| | number of positive samples (n=4) MSRV/MKTTn/RVS & BSA/XLD | | | | | |
| 0 | 4 | 4 | 4 | 4 | 4 | 4 |
| 1 week | 4 | 4 | 4 | 4 | 4 | 4 |
| 2 weeks | 4 | 4 | 4 | 4 | 4 | 4 |
| 3 weeks | 4 | 4 | 4 | 4 | 4 | 4 |

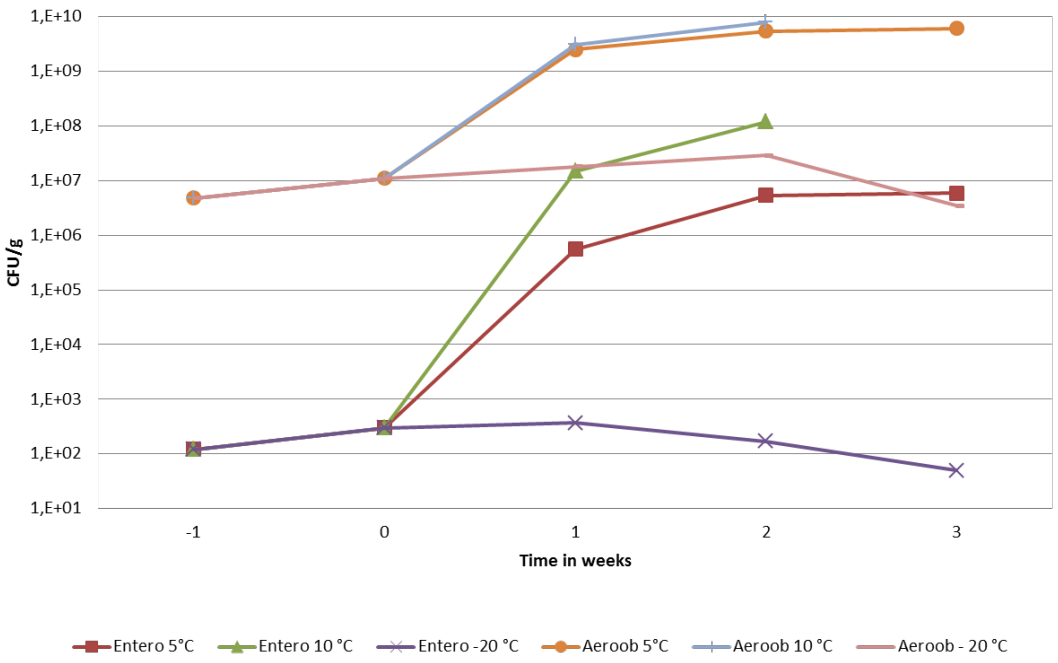



*Figure 1 Number of aerobic bacteria and number of Enterobacteriaceae per gram of minced turkey meat (CFU/g) after storage at –20 °C, 5 °C and 10 °C.*

*Table 4. Stability tests of minced turkey meat samples artificially contaminated with low and high level Salmonella Stanley (SSt)*

| **3 weeks of storage** | **Artificially contaminated minced turkey meat** (Batch 2) | |
|---|---|---|
| | **SSt12** | **SSt42** |
| | number of positive samples (n=6) MSRV/MKTTn/RVS & BSA/XLD | |
| 3 weeks –20 °C | 6 | 6 |
| 2 weeks –20 °C, 1 week 5 °C | 6 | 6 |
| 1 week –20 °C, 1 week 5 °C, 1 week –20 °C | 6 | 6 |

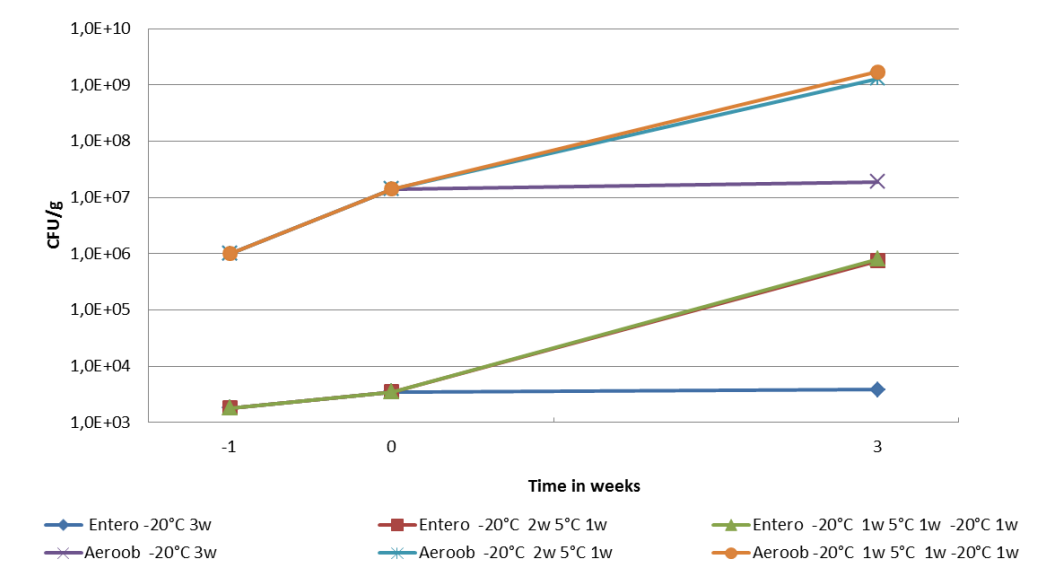



*Figure 2. Number of aerobic bacteria and number of Enterobacteriaceae per gram of minced turkey meat (CFU/g) after storage alternately at –20 °C and 5 °C.*

The major findings of both stability tests are:

- Minced turkey meat samples artificially contaminated with a low level of *Salmonella* Stanley (6 CFU/25 g) or *Salmonella* Typhimurium (12 CFU/25g) were stable after storage at –20 °C, 5 °C and 10 °C for three weeks.
- Minced turkey meat samples artificially contaminated with *Salmonella* Stanley at a level of 12 and 42 CFU/25 g were shown to be stable when stored alternately at –20 °C and 5 °C for 3 weeks.

### 4.1.3 *Preparation of minced chicken meat samples for interlaboratory comparison study*

All pre-tests were performed with minced turkey meat as this was planned as the matrix of choice for the interlaboratory comparison study. However, as the batch of turkey meat tested positive for *Salmonella* after the initial control, a new batch of minced meat was needed. Minced chicken meat was then chosen as the turkey meat was no longer available. The results of the pre-tests with turkey meat were considered

to be representative for minced chicken meat, so the following samples for the interlaboratory comparison study were used:

- for each participant, 18 x 25 g of minced chicken meat (following ISO/TS 22117:2010);
- each sample individually inoculated with a diluted culture of *Salmonella*:
  - low-level *S*. Stanley (SSt) with aimed level 15–20 CFU/25 g minced chicken meat;
  - high-level *S*. Stanley (SSt) with aimed level 50-100 CFU/25 g minced chicken meat;
  - blank: 0 CFU/25 g minced chicken meat.

After contamination of the meat with SSt, the samples had to be stored at –20 °C by both the EURL and the NRLs.

#### 4.1.4 *Background flora in minced chicken meat*

The number of aerobic bacteria and the number of *Enterobacteriaceae* were tested at the EURL-*Salmonella* laboratory on the date of the interlaboratory comparison study. Table 5 shows the results of these tests after storage for 3 days at +5 °C followed by 10 days at –20 °C.

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

| **Date** | ***Enterobacteriaceae* CFU / g** | **Aerobic bacteria CFU / g** |
|---|---|---|
| 3 October 2016 storage for 3 days at +5 °C followed by 10 days at –20 °C | $4*10^4$ | $2*10^6$ |

#### 4.1.5 *Number of Salmonella in minced chicken meat samples*

Table 6 shows the contamination levels of the minced meat samples contaminated with SSt at low and high levels. The inoculum level of the diluted SSt culture (tested on XLD), as well as the contamination level of the minced chicken meat samples after inoculation with the diluted culture, were tested. The latter was tested using a five-tube MPN test (see Section 3.1.5). The number of positive minced chicken meat samples tested on 21 September for 25 g, 2.5 g and 0.25 g were: low-level SSt 5/5, 5/5 and 1/5; high-level SSt 5/5, 5/5 and 2/5 respectively. The calculated MPN/25 g of meat is given in Table 6.

After storage and transport, the contamination level in the samples with a low level of contamination was approximately 35 MPN/25 g (with a 95% confidence interval of 11–110 MPN/25 g) on the day of the study, which was somewhat higher than expected (inoculum was 16 CFU/25 g).

The amount of background flora in the minced chicken meat samples used for the interlaboratory study was comparable to the levels in the minced turkey meat samples used for the pre-tests. The amount of background flora was much lower compared to the minced chicken meat samples used in the 2013 study (Kuijpers et al., 2014). The samples used in 2013 were stored for one week at 5 °C after storage at –20 °C, which resulted in a 4-log higher number of *Enterobacteriacea* ($10^8$ CFU/g *Enterobacteriaceae* and aerobic bacteria).

Storing the artificially contaminated minced chicken meat at –20 °C was successful, showing it to be applicable in interlaboratory comparison studies.

*Table 6. Number of Salmonella Stanley (SSt) in the inoculum and in the contaminated minced chicken samples*

| **Date of testing** | **Low-level SSt CFU / 25 g Minced chicken meat** (95% confidence limit) | **High-level SSt CFU / 25 g Minced chicken meat** (95% confidence limit) |
|---|---|---|
| 21 September 2016 (inoculum of meat) | 16 | 73 |
| 3 October 2016 MPN of meat, inoculated with SSt (95 % confidence limit) after storage for 10 days at –20 °C | 35<br><br>(11–110) | 55<br><br>(16–188) |

## 4.2 Technical data interlaboratory comparison study

### *4.2.1 General*

Thirty-four NRLs-*Salmonella* participated in this study: 30 NRLs from the 28 EU Member States (MS) and 4 NRLs from non-EU countries. The non-EU countries consisted of EU candidate MS or potential EU candidate MS, members of the European Free Trade Association (EFTA) and a non-European country.

All laboratories performed the study on the planned date (week 40, starting on 3 October 2016).

### *4.2.2 Accreditation*

All laboratories were accredited for their quality system according to ISO/IEC 17025:2005. According to EC regulations 882/2004 (EC, 2004) and 2076/2005 (EC, 2005), each NRL has to be accredited in its relevant work field. Thirty-two laboratories were accredited for ISO 6579 (detection of *Salmonella* in food and animal feeding stuffs); 28 were also accredited for Annex D of ISO 6579:2002. Laboratory 3 (non-EU-MS) was only accredited for the detection of *Salmonella* in samples from the primary production stage by using MSRV (Annex D of ISO 6579:2007). Laboratory 23 (non-EU-MS) was accredited for Annex D of ISO 6579:2007 and a method using only RVS broth for selective enrichment.

### *4.2.3 Transport of samples*

Twenty-seven participants received the samples within one day of dispatch and six participants within two days. The parcel for laboratory 3 (non-EU-MS) was retained by customs and arrived after three days. As requested, all NRLs returned the temperature recorders to the EURL-*Salmonella* at the time they started the study.

To stabilise the level of *Salmonella* Stanley 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 for the majority of the parcels was at least -1 °C during transport. 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 the highest temperatures (lab codes 8, 14 and 33) scored all samples correctly.

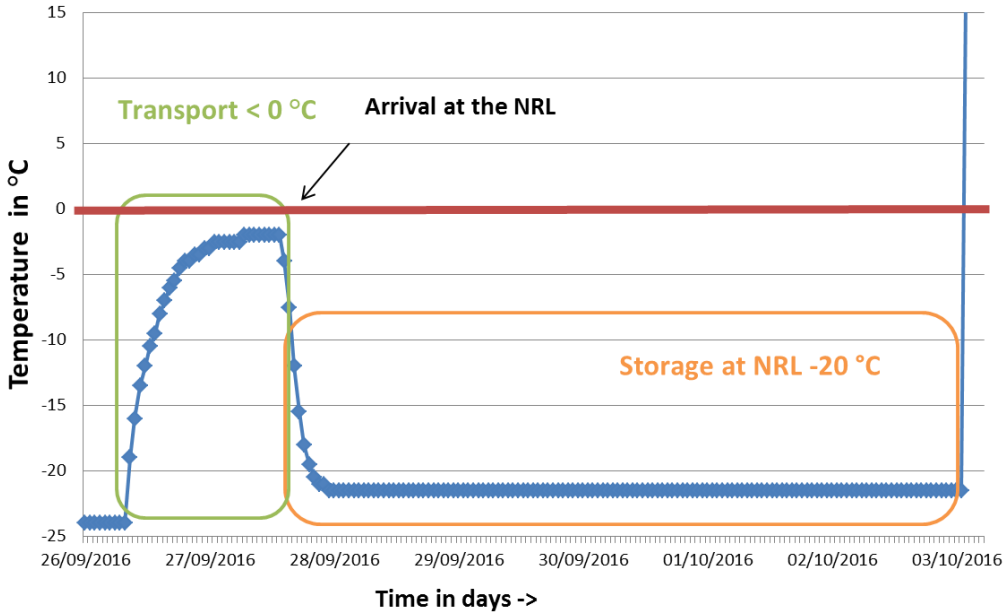



*Figure 3. Record of the temperature of a parcel during transport and storage at a laboratory (lab code 1)*

The minced meat samples had to be stored at –20 °C after receipt at the participating laboratory. The temperature was generally between –15 °C and –28 °C. Exceptions were laboratories 26 and 32 where the samples were stored at –7 °C and 1 °C respectively. According to the information of the temperature recorder of laboratory 6 showed that the sample was stored at room temperature. It is probable that this recorder was separated from the samples after arrival at the laboratory. An example of the temperature record during transport and storage at a laboratory (lab code 1) is shown in Figure 3.

### *4.2.4 Media*

Each laboratory was asked to follow the final draft version of the International Standard ISO (FDIS) 6579-1:2015.
As requested, all laboratories used MKTTn as a selective enrichment medium. In the 2015 food study (Kuijpers and Mooijman, 2016) participants were able to choose between RVS and MSRV for the first time. In the current study, nineteen participants used all three selective enrichment media (MKTTn, RVS and MSRV) while in the 2015 study, 27 NRLs used all three media. Six laboratories (4, 6, 16, 27, 29 and 32) only used RVS in combination with MKTTn and nine laboratories (2, 3, 8, 11, 15, 18, 21, 28 and 33) only used MSRV in combination with MKTTn.

Table 7 shows the reported pH, concentration of Novobiocin, incubation times and temperatures that deviated from the prescribed method (grey

cells). The table lists only those NRLs that reported deviations from the method.

*Table 7. Reported technical deviations from the prescribed procedures*

| Lab code | BPW | | RVS | MKTTn | | MSRV | |
|---|---|---|---|---|---|---|---|
| | Incubation time (h:min) | pH | pH | pH | Novo-biocin | pH | Novo-biocin |
| ISO/FDIS 6579-1 | 16–20 h | 6.8–7.2 | 5.0–5.4 | Complete 7.0-8.2 * | 40 mg/l | 5.1–5.4 | 10 mg/l |
| | | | | | | | |
| 2 | 18:30 | 7.3 | NO | 7.6 | 0 | 5.2 | 10 |
| 4 | 18:20 | - | - | - | - | NO | NO |
| 6 | 18:30 | 7.2 | 5.3 | 6.6 | 4 | NO | NO |
| 9 | 19:10 | 7 | 5.2 | 6.7 | 40 | 5.2 | 10 |
| 13 | 18:58 | 6.9 | 5.1 | 8.1 | 40 | 5.2 | 20 |
| 14 | 20:00 | 7.1 | 5.1 | 8.2 | 0.04 | 5.2 | 0.05 |
| 17 | 22:00 | 7.1 | 5.6 | 8.2 | 40 | 5.3 | 10 |
| 18 | 20:00 | 7 | NO | 8 | 40 | 5.2 | 20 |
| 19 | 20:35 | 7.3 | 5.2 | 7.9 | 40 | 5.5 | 10 |
| 20 | 21:00 | 7.2 | 5.2 | 8 | 10 | 5.3 | 10 |
| 21 | 20:20 | 7 | NO | 8.1 | 40 | 5.6 | 10 |
| 22 | 18:00 | 7 | 5.2 | 8.0 | 40 | 5.2 | 10 |
| 24 | 20:00 | 7 | 5.2 | 7.8 | 40 | 5.3 | 20 |
| 27 | 20:15 | 7.0 | 5.1 | 7.8 | 0.04 | NO | NO |
| 28 | 17:55 | 7.0 | NO | 7.8 | 40 | 5.6 | 10 |
| 29 | 22:15 | 7 | 5.2 | 7.1 | 40 | NO | NO |
| 31 | 20:00 | 7.1 | 5.4 | 8.2 | 10 | 5.4 | 10 |
| 33 | 19:00 | 7.3 | NO | 7.7 | 10 | 5.2 | 10 |

Grey cells = Deviating from ISO/FDIS 6579-1
\- = No information
NO = Did not use this selective enrichment medium (MKTTn or MSRV or RVS)
* = According to ISO/FDIS 6579-1 The pH of the Base medium should be 7.8-8.2 while, complete MKTTn medium should no longer be used if, after storage, the pH is ≤ 7.

Six laboratories reported a longer incubation time for the pre-enrichment in BPW. Three laboratories reported a pH of 7.3 instead of the prescribed maximum pH of 7.2 for BPW.
According to ISO/FDIS 6579-1:2015, the pH of the base medium of MKTTn should be 7.8-8.2. In addition, it indicates that the complete medium should no longer be used if, after storage, the pH is <7. Two laboratories reported the use of MKTTn with a pH below 7. Six laboratories used MKTTn with a lower concentration of Novobiocin than the prescribed 40 mg/L, and one laboratory used MKTTn without the addition of Novobiocin.
One laboratory reported a deviating pH of RVS.
Three laboratories used MSRV with a higher concentration of Novobiocin than the prescribed 10 mg/l, and one laboratory used a lower

concentration of Novobiocin. Three laboratories reported a deviating pH for MSRV.
Laboratory 4 did neither report the pH nor the concentration of Novobiocin for the batches of BPW, MKTTn and RVS.

These deviations did not seem to affect the results as, with the exception of one, all samples were scored correctly (see section 4.4.1).

A second plating-out medium of choice was obligatory. Table 8 shows the second isolation media used by the participants. Most laboratories used BGA (ISO 6579:1993) or a Chromogenic medium (e.g. Rambach) as a second plating-out medium.

*Table 8. Second plating-out media used by the NRLs*

| Media | No. of users |
|---|---|
| BGA$^{mod}$ (ISO 6579, 1993) | 8 |
| Rambach (Merck) | 6 |
| BPLS (Merck, Biolife) | 5 |
| RS (Bio-rad) | 5 |
| BGA (Oxoid, Difco, CONDA) | 3 |
| BSA (Oxoid) | 2 |
| SM(ID)2 (Biomerieux, Biotrading) | 2 |
| ASAP (Oxoid) | 1 |
| Compass *S* (Biokar) | 1 |
| Chromo S (Biogerm) | 1 |

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 30 laboratories performed this extra step (e.g. by using Nutrient agar; ISO 6579:2002). All participating laboratories performed one or several confirmation tests for *Salmonella.*
Twenty-eight laboratories performed a serological test in addition to the other confirmation tests. One laboratory (18) only performed a serological test.

Twenty-eight laboratories performed a biochemical test; four of them did not use any serological test, but two used a PCR method instead. Three laboratories used the MALDI-TOF test in addition to other confirmation tests. Laboratory 17 (non-EU) only performed MALDI-TOF to confirm *Salmonella*. Eleven laboratories used a biochemical kit (e.g. API, VITEK, BBL, Microgen).
Nine participants used a PCR method to confirm *Salmonella* in addition to biochemical and/or serological tests.

## 4.3 Control samples

### *4.3.1 General*

All laboratories scored both control samples (positive and blank) correctly.

**Procedure control blank (BPW only)**
All laboratories analysed the one procedure control sample (no matrix, only BPW) correctly negative for *Salmonella*.

*Table 9. Salmonella serovars used by participants for the positive control samples*

| Salmonella serovar | Number of users |
|---|---|
| *S*. Enteritidis | 14 |
| *S*. Typhimurium | 7 |
| *S*. Nottingham | 5 |
| *S*. Blegdam, *S*. Abaetetuba, *S*. Senftenberg, *S*. Dublin, *S*. bongori, *S*. Tennessee, *S*. Harleystreet, *S*. Alachua | 1 (per serovar) |

**Positive control with *Salmonella***
All laboratories obtained good results with their own *Salmonella* positive control sample and detected *Salmonella*.
For the positive control samples, the majority of the participants (20 laboratories) used a diluted culture of *Salmonella*. Others used a lenticule disc (7), a freeze-dried ampoule (4), a culti loop (1), a kwik-stik (1) or Vitroid disc (1) with *Salmonella.* Table 9 shows the *Salmonella* serovars used for the positive control samples. Participants were asked to use the positive control sample(s) routinely used in their laboratory. *Salmonella* Enteritidis (16) and *Salmonella* Typhimurium (8) were the most frequently used serovars for this purpose. The concentration of *Salmonella* in the positive control samples used by the different participants varied between 1 and $10^4$ CFU/sample.
A positive control sample should demonstrate that media are capable of supporting the growth of a range of organisms in low numbers. To gain insights into the sensitivity of a method, the concentration of a positive control sample should be just above the detection limit of the method. The majority of the participants used a much higher concentration. Furthermore, it may be advisable to use a rarely isolated serovar from the routine samples analysed in the laboratory. In this way, possible cross-contamination can be detected more easily.

The results were compared with the definition of ‘good performance’ (see Section 3.6). All laboratories fulfilled these criteria for the control samples.

### *4.3.2 Correct scores of the control samples*

Table 10 shows the number of correct scores found with the control samples for the different selective enrichment media in combination with the isolation media. The calculations were performed on the results of all participants and separately on the results of the EU-MS. No differences were found between these two groups. All laboratories obtained correct results for the control samples, with accuracy rates of 100%.

*Table 10. Correct scores found with the control samples by all laboratories ('All') and by the laboratories of the EU Member States ('EU') only.*

| **Control Samples** | | **MKTTn and RVS or/and MSRV XLD or 2nd plate** | |
|---|---|---|---|
| | Laboratories | All n=34 | EU n=30 |
| Procedure control blank (BPW) n=1 | No. of samples | 34 | 30 |
| | No. of positive samples | 34 | 30 |
| | **Correct score in %** | **100** | **100** |
| Positive control (own *Salmonella*) n=1 | No. of samples | 34 | 30 |
| | No. of negative samples | 34 | 30 |
| | **Correct score in %** | **100** | **100** |
| All control samples | No. of samples | 68 | 60 |
| | No. of correct samples | 68 | 60 |
| | **Accuracy in %** | **100** | **100** |

## 4.4 Artificially contaminated minced chicken meat samples

### *4.4.1 General*

Table 11 shows the results of the minced chicken meat samples artificially contaminated with *Salmonella* Stanley. The results show the highest number of positive isolations found with the different selective enrichment media (MKTTn and RVS or/and MSRV), in combination with the 'best' isolation medium.

**Blank samples**

Thirty-one laboratories correctly scored all six blank minced chicken meat samples as negative for *Salmonella*. Three laboratories (lab codes 10, 19 and 23) found one blank sample out of six positive for *Salmonella.* All blank samples should have tested negative. However, because no 100% guarantee of the *Salmonella*-negative status of the meat could be given, one positive out of six blank samples (80% negative) is considered acceptable; finding more than one blank sample positive is unlikely. To gain extra additional information on the matrix used, the three laboratories (10, 19 and 23) were requested for information about their positive blank meat sample, e.g. serotype of the isolated *Salmonella* and possible other deviations. All three laboratories isolated *Salmonella* Infantis from the blank meat sample. The laboratories did not have any *S*. Infantis isolated in that period in their laboratory and used another serovar as their positive control. It seems unlikely that the S. Infantis had its origin in a laboratory contamination of one of the NRLs. A possible clarification for the three positive blank meat samples is natural contamination of the chicken meat with *S*. Infantis but at a very low level, as all other blanks (214 samples) were tested negative by the NRLs and the EURL.

**Low-level contaminated *Salmonella* Stanley samples**

All laboratories detected *Salmonella* in all six samples that contained *Salmonella* Stanley at an inoculum level of approximately 16 CFU/25 g minced chicken meat.

*Table 11. Number of positive results found with the artificially contaminated minced chicken meat samples (25g) at each laboratory*

| Lab code | Number of positive isolations | | |
|---|---|---|---|
| | Blank n=6 | SSt low n=6 | SSt high n=6 |
| Good performance | ≤1 | ≥3 | ≥5 |
| 10 | **1** | 6 | **5** |
| 19, 23 | **1** | 6 | 6 |
| All other NRLs | 0 | 6 | 6 |

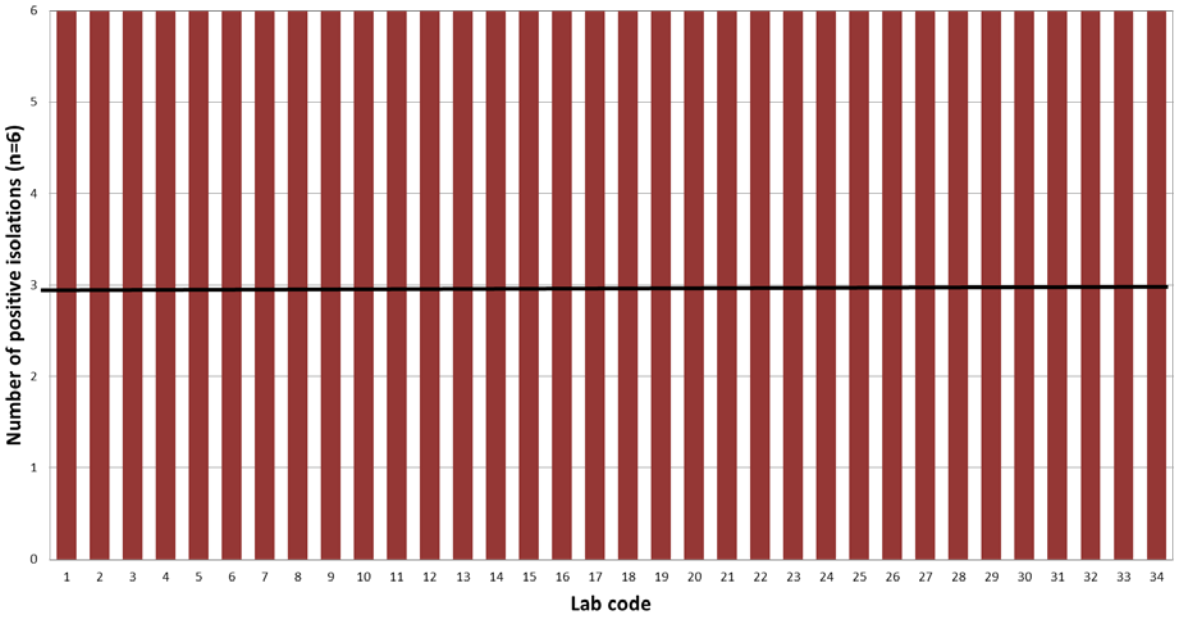
Bold numbers= Deviating results but within the criteria of good performance

**High-level contaminated *Salmonella* Stanley samples**

Thirty-three laboratories detected *Salmonella* in all six samples contaminated with *Salmonella* Stanley at an inoculum level of approximately 73 CFU/25 g minced chicken meat.
One laboratory (lab code 10) could not detect *Salmonella* in one of the six high level contaminated samples. This negative result may have been caused by misinterpretation of the results or exchange with the false positive blank sample. Not finding a sample contaminated at such a high level of 73 CFU/25 g is unlikely.

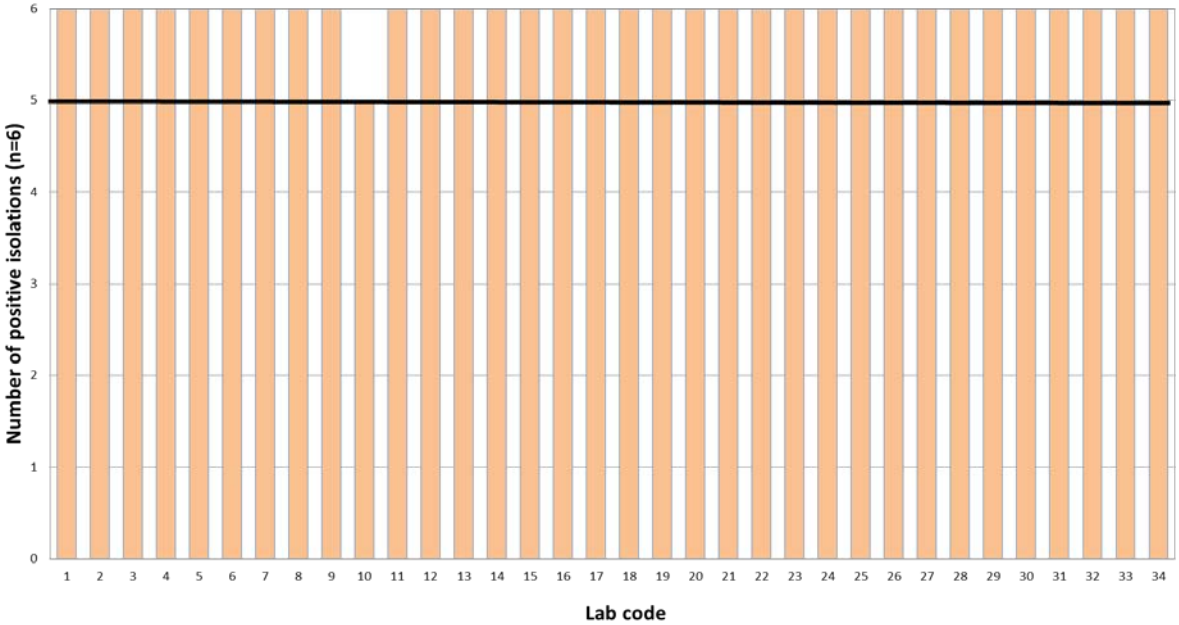
Figures 4 and 5 show the number of positive isolations for each level of artificially contaminated meat sample, and for each laboratory after pre-enrichment in BPW, followed by all combinations of selective enrichment media (MKTTn and RVS and/or MSRV) and plating-out media (XLD and own choice), giving the highest number of positive results. The border of good performance is indicated by the horizontal black line.

The results of the artificially contaminated meat samples were compared to the definition of ‘good performance’ (see section 3.5) and all laboratories fulfilled these criteria.




- = border of good performance

*Figure 4. Number of minced chicken meat samples artificially contaminated with a low level of Salmonella Stanley (n=6) that tested positive per laboratory. Results include all combinations of selective enrichment media (MKTTn and RVS and/or MSRV) and plating-out media (XLD and 2nd plate), giving the highest number of positive results.*




- = border of good performance

*Figure 5. Number of minced chicken meat samples artificially contaminated with a high level of Salmonella Stanley (n=6) that tested positive per laboratory. Results include all combinations of selective enrichment media (MKTTn and RVS and/or MSRV) and plating-out media (XLD and 2nd plate), giving the highest number of positive results.*

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

Table 12 shows the specificity, sensitivity and accuracy rates for all three levels of artificially contaminated minced chicken meat samples. This table gives the results for all possible combinations of selective enrichment media and isolation media, giving the highest number of positive results. The calculations were performed on the results of all participants and on separately for the results of the EU-MS participants only. No differences were found between these two groups. For both groups, the specificity and accuracy rates were 99%, and the sensitivity rate for low-level contaminated meat samples was 100%.

*Table 12. Specificity, sensitivity and accuracy rates found by the participating laboratories with the artificially contaminated minced chicken meat samples.*

| **Meat Samples** | | **MKTTn and RVS and/or MSRV XLD and 2nd plate** | |
|---|---|---|---|
| | Laboratories | All n=34 | EU n=30 |
| Blank | No. of samples | 204 | 180 |
| (n=6) | No. of negative samples | 201 | 179 |
| | **Specificity in %** | **99** | **99** |
| SSt low | No. of samples | 204 | 180 |
| (n=6) | No. of positive samples | 204 | 180 |
| | **Sensitivity in %** | **100** | **100** |
| SSt high | No. of samples | 204 | 180 |
| (n=6) | No. of positive samples | 203 | 179 |
| | **Sensitivity in %** | **99** | **99** |
| All samples with *Salmonella* | No. of samples | 408 | 360 |
| | No. of positive samples | 407 | 360 |
| | **Sensitivity in %** | **99** | **100** |
| All samples | No. of samples | 612 | 540 |
| | No. of correct samples | 608 | 539 |
| | **Accuracy in %** | **99** | **99** |

## 4.5 PCR (own method)

Nine laboratories applied a PCR method in addition to the prescribed culture method. Table 13 gives further details of the PCR techniques used.

All laboratories found the same results when using the PCR method and the bacteriological culture method. This indicates that the PCR methods were well suited for the detection of *Salmonella* in minced chicken meat samples.
Almost all participants used a validated real time PCR with DNA isolation from BPW. Three laboratories used a commercially available PCR method.

*Table 13. Details of Polymerase Chain Reaction procedures used by NRLs-Salmonella as own method during the interlaboratory comparison study*

| Lab code | PCR method | Validated (by) | Commer-cially available | Routinely used number of tests/year | DNA extraction after pre-enrichment in | Reference |
|---|---|---|---|---|---|---|
| 3 | Real-time | Löfström et al. (2010 & 2012) | - | 700 | BPW | Malorny et al. (2004) |
| 8 | Real-time | intra laboratory | - | 42 | BPW | |
| 13 | Real-time | intra laboratory | - | >1000 | BPW | |
| 16 | Real-time | AFNOR and others | + | 200 | BPW | |
| 20 | Real-time | ISO 16140 | - | - | BPW | |
| 22 | Real-time | NO | - | 100 | BPW | Daum LT et al.(2002) |
| 27 | Real-time | AFNOR AOAC | + | - | BPW | |
| 30 | BAX system Q7 | AFNOR | + | 632 | BPW | |
| 33 | Real-time | | - | 52 | BPW | Malorny et al. (2004) |

## 4.6 Performance of the NRLs

For the evaluation of the performance of the laboratories, all combinations of selective enrichment media (MKTTn and RVS and/or MSRV) and isolation media were taken into account.

Three laboratories reported a positive result for a blank sample. All blanks should test negative. However, because a 100% guarantee of the *Salmonella*-negative status of the minced chicken meat could not be given, one positive of the six blank samples (80% negative) can be considered acceptable. A possible clarification for the three positive blank meat samples is natural contamination of the chicken meat at a very low level with *Salmonella* Infantis. All other 211 tested blank samples tested negative for *Salmonella* spp. (201 by the NRLs and 10 by the EURL prior to the interlaboratory study).

According to the criteria used, all laboratories achieved ‘good performance’.

# 5 Conclusions

All 34 NRLs-*Salmonella* achieved good performance with the detection of *Salmonella* in both high-level and low-level contaminated minced chicken meat samples.

High rates of specificity, sensitivity and accuracy were found for the detection of *Salmonella* in artificially contaminated minced chicken meat samples (blank, low-level and high-level): 99–100%.

The accuracy rate for the control samples after selective enrichment in MKTTn and RVS and/or MSRV was 100%.

Some participants may consider the optimisation of the positive control sample used in their routine analysis with respect to the choice of *Salmonella* serovar and/or contamination level.

PCR as a laboratory's own method gave the same results as the bacteriological culture technique.

The storage at –20 °C of artificial contaminated minced meat with a diluted culture of *Salmonella* was successful, showing it to be applicable for interlaboratory comparison studies.

The acceptance of one of six positive blank samples in the criteria for good performance (as we cannot give a 100% guarantee on the negativity of the matrix), was clearly applicable in this study. Three laboratories found one blank sample positive for *Salmonella* Infantis. A possible clarification is natural contamination of the meat but at a very low level, as all other tested blank samples by the NRLs and the EURL tested negative.

# List of abbreviations

| | |
|---|---|
| AFNOR | Association Française de Normalisation (French Standardization Association) |
| AOAC | Association of Analytical Communities |
| ASAP | AES *Salmonella* Agar Plate |
| ATCC | American Type Culture Collection |
| BGA(mod) | Brilliant Green Agar (modified) |
| BL | blank (no colony-forming units) |
| BPLS | brilliant green phenol-red lactose sucrose |
| BPW | Buffered Peptone Water |
| BSA | Brilliance *Salmonella* Agar |
| CFU | colony-forming units |
| DG-SANTE | Directorate-General for Health and Consumer Protection |
| EC | European Commission |
| EFTA | European Free Trade Association |
| EU | European Union |
| EURL | European Union Reference Laboratory |
| FDIS | Final Draft International Standard (ISO) |
| ISO | International Organization for Standardization |
| MKTTn | Mueller-Kauffmann Tetrathionate-Novobiocin broth |
| MPN | most probable number |
| MS | Member State |
| MSRV | Modified Semi-solid Rappaport-Vassiliadis |
| NRL | National Reference Laboratory |
| PCA | Plate Count Agar |
| PCR | Polymerase Chain Reaction |
| RIVM | Rijksinstituut voor Volksgezondheid en Milieu (National Institute for Public Health and the Environment) |
| RS | Rapid *Salmonella* |
| RVS | Rappaport Vassiliadis Soya broth |
| SM (ID)2 | *Salmonella* Detection and Identification-2 |
| SOP | Standard Operating Procedure |
| SSt | *Salmonella* Stanley |
| STM | *Salmonella* Typhimurium |
| VRBG | Violet Red Bile Glucose agar |
| XLD | Xylose Lysine Deoxycholate (agar) |

# References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