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

EU Interlaboratory comparison study food IV (2010)

Detection of Salmonella in minced meat

RIVM report 330604020/2011

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Colophon

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This investigation has been performed by order and for the account of European Health and Consumer Protection Directorate-General, within the framework of V/330604/10/CS by the European Union Reference Laboratory for *Salmonella*

Abstract

EU Interlaboratory comparison study food IV (2010)
Detection of *Salmonella* in minced meat

Of the 31 National Reference Laboratories (NRLs) in the European Union that participated in a comparison study in 2010, 28 were able to detect both high and low levels of *Salmonella* in minced meat pork and beef. They achieved the desired outcome on the first attempt. Of the remaining three NRLs, one made a transcription error during the transfer of raw data to the test report, leading to a 'moderate' performance. The two remaining NRLs obtained the desired outcome in a follow-up study. Cross-contamination of blank samples with other samples provided for testing or with samples from their own laboratory is the most likely explanation for the initial deviation of their results from the desired outcome.

These are the results of the fourth interlaboratory comparison study on food organised by the European Union Reference Laboratory (EURL) for *Salmonella*. The study was conducted in September 2010, with the follow-up study in January 2011. All NRLs responsible for *Salmonella* detection from all European Member States were required to participate in this study. The EURL for *Salmonella* is part of the Dutch National Institute for Public Health and the Environment (RIVM).

Three different analytical methods for demonstrating the presence of *Salmonella* in minced meat were used during the study. Two of these are internationally prescribed methods (MKTTn and RVS) for the detection of *Salmonella* in food. The third is an internationally prescribed method (MSRV) for the detection of *Salmonella* in veterinary samples and is not obligatory in food testing but was included in the study by request of the EURL. On average, the laboratories found *Salmonella* in 99% of the (contaminated) samples independent of which method was used.

The laboratories were obliged to conduct the study according to the instructions provided. Each laboratory received a package containing minced meat (free of *Salmonella*), 29 gelatin capsules containing powdered milk infected with *Salmonella* Typhimurium at two levels and a number of capsules containing sterile milk powder. The laboratories were instructed to spike the minced meat with the capsules and then to test the samples for the presence of *Salmonella*.

Keywords:
Salmonella; EURL; NRL; interlaboratory comparison study; minced meat; detection methods

Rapport in het kort

EU Ringonderzoek voedsel IV (2010)
Detectie van *Salmonella* in gehakt

In 2010 waren 28 van de 31 Nationale Referentie Laboratoria (NRL's) in de Europese Unie in staat om hoge en lage concentraties van de *Salmonella*-bacterie in gehakt van varken en rund aan te tonen. Zij behaalden direct het gewenste niveau. Eén laboratorium maakte een overschrijffout vanuit de ruwe data naar de rapportage ervan, waardoor hun resultaat als matig werd beoordeeld. Twee NRL's behaalden het gewenste resultaat tijdens een herkansing. De onderprestatie werd waarschijnlijk veroorzaakt doordat blanco monsters met andere monsters van het ringonderzoek of monsters van het eigen laboratorium waren besmet.

Dit blijkt uit het vierde voedselringonderzoek dat het Referentie Laboratorium van de Europese Unie (EURL) voor *Salmonella* heeft georganiseerd. Het onderzoek is in september 2010 gehouden, de herkansing was in januari 2011. Alle NRL's van de Europese lidstaten die ervoor verantwoordelijk zijn *Salmonella* op te sporen, zijn verplicht om aan dit onderzoek deel te nemen. Het EURL-*Salmonella* is gevestigd bij het Nederlandse Rijksinstituut voor Volksgezondheid en Milieu (RIVM).

Tijdens de studie zijn drie analysemethodes gebruikt om de *Salmonella*bacterie in gehakt aan te tonen. Twee daarvan zijn internationaal voorgeschreven methoden voor *Salmonella*-detectie in voedsel (RVS en MKTTn). De derde, de internationaal voorgeschreven methode om *Salmonella* in dierlijke mest aan te tonen, is niet verplicht maar is op verzoek van het EURL uitgevoerd (MSRV). Gemiddeld werd door laboratoria in 99% van de (besmette) monsters *Salmonella* gedetecteerd, ongeacht de methode.

De laboratoria moeten de studie volgens voorschrift uitvoeren. Elk laboratorium kreeg daarvoor een pakket toegestuurd met gehakt en 29 gelatinecapsules met melkpoeder dat twee verschillende besmettingsniveaus *Salmonella* Typhimurium bevatte en enkele capsules met steriel melkpoeder. De laboratoria moesten vervolgens het gehakt en de capsules samenvoegen en onderzoeken of er *Salmonella* in aanwezig was.

Trefwoorden:*Salmonella*; EURL; NRL; ringonderzoek; gehakt; detectiemethode

Contents

Summary

In September 2010, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised the fourth interlaboratory comparison study on bacteriological detection of *Salmonella* in a food matrix (mixed minced meat of pork and beef). Participants were 31 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) of the EU-Member States and of countries from the European Free Trade Association (EFTA): Norway, Switzerland and Iceland.

The first and most important objective of the study was to see whether the participating laboratories could detect *Salmonella* at different contamination levels in a food matrix. To do so, minced meat samples of 25 grams each, were analysed in the presence of reference materials (capsules) containing either milk powder artificially contaminated with *Salmonella* (at various contamination levels) or sterile milk powder. A proposal for good performance was made and the performance of the laboratories was compared to this proposal. In addition to the performance testing of the laboratories, a comparison was made between the prescribed methods (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. Optionally, a laboratory could also use other, own media or procedures for the detection of *Salmonella*.

Twenty-nine individually numbered capsules had to be tested by the participants for the presence or absence of *Salmonella*. Twenty-four of the capsules had to be examined in combination with each 25 grams of *Salmonella* negative meat: eight capsules contained approximately 5 colony forming particles (cfp) of *Salmonella* Typhimurium (STM5), eight capsules contained approximately 50 cfp of *S.* Typhimurium (STM50) and eight blank capsules. The other five capsules, to which no meat had to be added, were control samples, comprising three capsules STM5, one capsule STM50 and one blank capsule.

On average, the laboratories found *Salmonella* in 99% of the (contaminated) samples either using the selective enrichment media prescribed for the food method (MKTTn and RVS) or the method for testing veterinary samples (MSRV).

Twenty-eight out of 31 laboratories achieved the level of good performance on the first attempt. One NRL scored a moderate performance because they made an initial transcription error. Two laboratories needed a follow-up study to reach the desired level. The reason for their initially deviating results was most probably cross-contamination.

1 Introduction

An important task of the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*), as laid down in the Commission Regulation (EC, 2004), is the organisation of interlaboratory comparison studies. The history of the interlaboratory comparison studies on the detection of *Salmonella*, as organised by EURL-*Salmonella* since 1995 is summarised in Annex 1. In earlier ringtrials, the detection of *Salmonella* spp. in veterinary, animal feed and food samples was studied. The current study was the fourth study for the detection of *Salmonella* spp. in meat. The organisation of the interlaboratory comparison study on minced meat was discussed with the NRLs for *Salmonella* at the annual EURL-Salmonella workshop in June 2010 (Mooijman, 2010). The first and most important objective of the study, organised by the European Union Reference Laboratory (EURL) for *Salmonella* in September 2010, was to see whether the participating laboratories could detect *Salmonella* at different contamination levels in minced meat. This information is important to know whether the examination of samples in the EU Member States is carried out uniformly and comparable results can be obtained by all National Reference Laboratories for *Salmonella* (NRL-*Salmonella*). The second objective was to compare the different methods for the detection of *Salmonella* in minced meat.

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) but also in food and animal feed samples, participating laboratories were requested also to use MSRV for testing the meat.

The set-up of this study was comparable to earlier interlaboratory comparison studies on the detection of *Salmonella* spp. in veterinary, animal feed and food samples. The contamination level of the low-level capsules was close to the detection limit of the method; the level of the high-level samples approximately five to ten times above the detection limit. Five control samples, comprising different reference materials, had to be tested without the addition of meat. These reference materials consisted of 3 capsules containing approximately 5 cfp of *Salmonella* Typhimurium (STM5), 1 capsule containing approximately 50 cfp of *S*. Typhimurium (STM50) and 1 blank capsule. Twenty-four samples of *Salmonella* negative minced meat (25 grams each) spiked with three different reference materials had to be examined. For the latter samples, the different reference materials consisted of two levels of *Salmonella* Typhimurium (STM5 and STM50) and blank reference materials.

2 Participants

Country	City	Institute
Austria	Graz	Austrian Agency for Health and Food Safety (AGES) Department of food microbiology
Belgium	Brussels	Scientific Institute of Public Health (WIV) Afd. Bacteriology
Bulgaria	Sophia	National Diagnostic and Research Veterinary Institute
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	Esjberg	Danish Veterinary and Food Administration Region West Laboratory
Estonia	Tartu	Estonian Veterinary and Food Laboratory
Finland	Helsinki	Finnish Food Safety Authority Evira Research Department, Microbiology Unit
France	Ploufragan	Anses Laboratoire de Ploufragan, Laboratoire d'Etudes et de Recherches Avicoles, Porcines et Piscicoles Unite HQPAP
Germany	Berlin	Federal Institute for Risk Assessment (BFR) National Reference Laboratory for *Salmonella*
Greece	Halkis	Veterinary Laboratory of Chalkis Hellenic Republic Ministry of Rural Development and Food
Hungary	Budapest	Central Agricultural Office, Food and Feed Safety Directorate Food Microbiological Diagnostic Laboratory
Iceland	Reykjavik	University of Iceland, Keldur Institute for Experimental Pathology
Ireland	Kildare	Central Veterinary Research Laboratory CVRL / DAF Department of Agriculture, Food and Fishery
Italy	Legnaro (PD)	Istituto Zooprofilattico Sperimentale delle Venezie, OIE National Reference Laboratory for *Salmonella*
Latvia	Riga	Institute of Food Safety Animal Health and Environment BIOR Animal Disease Diagnostic Laboratory
Lithuania	Vilnius	National Food and Veterinary Risk Assessment Institute
Luxembourg	Luxembourg	Laboratoire de Médecine Vétérinaire de l'Etat, LMVE
Malta	Valletta	Public Health Laboratory (PHL) Evans Buildings Dept.
Netherlands, the	Bilthoven	National Institute for Public Health and the Environment (RIVM/Cib) Centre for Infectious Diseases Control Laboratory for Zoonoses and Environmental Microbiology (LZO)
Norway	Oslo	National Veterinary Institute, Section of Bacteriology

Country	City	Institute
Poland	Pulawy	National Veterinary Research Institute (NVRI) Department of Hygiene of Food of animal Origin
Portugal	Lisboa	Laboratório Nacional de Investigação Veterinária (LNIV)
Romania	Bucharest	Hygiene and Veterinary Public Health Institute (IISPV)
Slovak Republic	Bratislava	State Veterinary and Food Institute Reference Laboratory for *Salmonella*
Slovenia	Ljubljana	National Veterinary Institute, Veterinary Faculty
Spain	Madrid Majadahonda	Centro Nacional de Alimentacion, Agencia Espanola de Seguridad Alimantaria y Nutricion (AESAN)
Sweden	Uppsala	National Veterinary Institute (SVA), Department of Bacteriology
Switzerland	Berne	Institute of veterinary bacteriology, Vetsuisse National Centre for Zoonoses (ZOBA)
United Kingdom	Southampton	Health Protection Agency HPA Food, Water & Environmental Microbiology Network, Southampton Laboratory
United Kingdom	Belfast	Agri-Food and Bioscience Institute (AFBI) Veterinary Sciences Division Bacteriology

3 Materials and methods

3.1 Reference materials

Two batches of *Salmonella* reference materials were prepared. For this purpose milk, artificially contaminated with a *Salmonella* strain, was spray-dried (In 't Veld et al., 1996). The obtained highly contaminated milk powder (hcmp) was mixed with sterile (γ-irradiated) milk powder (Carnation, Nestlé, the Netherlands) to obtain the desired contamination level. The mixed powder was filled into gelatin capsules resulting in the final reference materials (RMs).

- The target levels of the two batches of RMs were:5 and 50 colony forming particles (cfp) per capsule for *Salmonella* Typhimurium (STM5 and STM50).

Before filling all mixed powders into gelatin capsules, test batches of 60 capsules were prepared of each mixture to determine the mean number of cfp per capsule and the homogeneity of the mixture. The remaining mixed powders were stored at –20 °C. If the test batches fulfilled the pre-set criteria for contamination level and homogeneity, the relevant mixed powders were completely filled into gelatin capsules and stored at –20 °C.
The pre-set criteria were:

- mean contamination levels should lie between target level minus 30% and target level plus 50% (e.g., between 70 and 150 cfp if the target level is 100 cfp);
- for the homogeneity within one batch of capsules the maximum demand for the variation between capsules should be $T_2/(I-1) \leq 2$, where T_2 is a measure for the variation between capsules of one batch (see formula in Annex 2) and I is the number of capsules.

The contamination levels of the capsules were determined following the procedure as described by Schulten et al. (2000). In short, the procedure is as follows:

- reconstitution of each capsule in 5 ml peptone saline solution in a Petri dish at (38.5 ± 1) °C for (45 ± 5) min;
- repair of *Salmonella* by the addition of 5 ml molten double concentrated plate count agar (dPCA) to the reconstituted capsule solution, and after solidification, incubation at (37 ± 1) °C for (4 ± ½) h;
- after incubation, 10 ml of molten double concentrated Violet Red Bile Glucose agar (dVRBG) was added as an over layer and, after solidification, the plates were incubated at (37 ± 1) °C for (20 ± 2) h.

3.2 Minced meat samples

3.2.1 General

A batch of 30 kilograms minced meat (a mixture of 60% pork and 40% beef) arrived at EURL-*Salmonella* on 29 July 2010 as frozen portions of 1 kilogram. The minced meat was obtained from Deli XL, Ede, The Netherlands. The minced meat was repacked in portions of approximately 750 gram and stored at –20 °C. The meat was tested negative for *Salmonella* following the procedure as described in ISO 6579 (Anonymous, 2002) and Annex D of ISO 6579 (Anonymous, 2007). For this purpose, 10 portions of 25 grams were each added to 225 ml Buffered Peptone Water (BPW). After pre-enrichment at (37 ± 1) °C for 16–18 h, selective enrichment was carried out in Rappaport Vassiliadis Soya

(RVS), Mueller Kaufmann Tetrathionate novobiocin (MKTTn) and on Modified Semi-solid Rappaport Vassiliadis (MSRV). Next, the tubes and suspect plates were plated-out on Xylose Lysine Deoxycholate agar (XLD) and Brilliant Green Agar (BGA) and confirmed biochemically. The minced meat was stored at –20 °C until further use.

3.2.2 Total bacterial count in minced meat

The total number of aerobic bacteria was investigated in the minced meat. The procedure of ISO 4833 (Anonymous, 2003) was followed for this purpose. In summary, a portion of 20 grams of meat was homogenised in 180 ml of peptone saline solution in a plastic bag. The content was mixed by using a pulsifier (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) ^{o}C for (72 ± 3) hours and the total number of aerobic bacteria was counted after incubation.

3.2.3 Number of Enterobacteriaceae *in minced meat*

In addition to the total count of aerobic bacteria, the *Enterobacteriaceae* count was determined. The procedure of ISO 21528-2 (Anonymous, 2004) was used for this purpose. In summary, a portion of 20 grams of meat was homogenised in 180 ml of peptone saline solution in a plastic bag. The content was mixed by using a pulsifier (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 VRBG was added to the agar. The plates were incubated at (37 ± 1) ^{o}C for (24 ± 2) hours and the number of typical violet-red colonies was 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: capsules and minced meat

On Monday 20 September 2010 (one week before the study), the reference materials (29 individually numbered capsules) and 750 grams of *Salmonella* negative minced meat were packed with cooling devices as biological substance category B (UN 3373) and send by courier service to each participant. After arrival at the participant laboratory, the capsules had to be stored at –20 ^{o}C and the minced meat had to be stored at +5 ^{o}C until the start of the study. Details about mailing and handling of the samples and reporting of test results can be found in the Protocol (Annex 4) and Standard Operation Procedure (Annex 5). The test report used during the study can be found at the EURL-*Salmonella* web site: http://www.rivm.nl/crlsalmonella/prof_testing/detection_stud/ or can be obtained through the corresponding author of this report.

Five control capsules had to be tested without meat (numbered C1–C5). Twenty-four capsules (numbered 1–24) were each tested in combination with 25 grams of minced meat (negative for *Salmonella*). Table 1 shows the types, the number of capsules and meat samples to be tested.

Table 1 Overview of the types and the number of capsules tested per laboratory in the interlaboratory comparison study

Capsules	Control capsules (n=5) No matrix added	Test samples (n=24) with 25 g *Salmonella* negative minced meat
S. Typhimurium 5 (STM5)	3	8
S. Typhimurium 50 (STM50)	1	8
Blank	1	8

3.3.2 Sample packaging and temperature recording during shipment

The capsules and the minced meat were packed in two plastic containers firmly closed with screw caps (biopacks). Both biopacks were placed in one large shipping box, together with four frozen (–20 °C) cooling devices. Each shipping box was sent as biological substances category B (UN3373) by door-to-door courier service. For the control of exposure to abusive temperatures during shipment and storage, so-called micro temperature loggers were used to record the temperature during transport. These loggers are tiny sealed units in a 16 mm diameter and 6 mm deep stainless steel case. Each shipping box contained one logger, packed in the biopack with capsules. The loggers were programmed by the EURL-*Salmonella* to measure the temperature every hour. Each NRL had to return the temperature recorder immediately after receipt of the parcel to the EURL. At the EURL-*Salmonella* the loggers were read by means of the computer and all data from the start of the shipment until the arrival at the National Reference Laboratories were transferred to an Excel graphic, which shows all recorded temperatures.

3.4 Methods

The prescribed method of this interlaboratory comparison study was ISO 6579 (Anonymous, 2002) and the requested (additional) method was Annex D of ISO 6579 (Anonymous, 2007). Additional to the prescribed methods, the NRLs were also allowed to use their own methods. This could be different medium combinations and/or investigation of the samples with alternative methods, like Polymerase Chain Reaction (PCR)-based methods.

In summary:

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

Selective enrichment in/on:
- Rappaport Vassiliadis Soya broth (RVS) (prescribed);
- Mueller Kaufmann Tetrathionate novobiocin broth (MKTTn) (prescribed);
- Modified semi-solid Rappaport Vassiliadis agar (MSRV) (requested);
- own selective enrichment medium (optional).

Plating-out on:
- Xylose Lysine Desoxycholate agar (XLD) (prescribed);
- second plating-out medium for choice (obligatory);
- own plating-out medium (optional).

Confirmation of identity:

- Confirmation by means of appropriate biochemical tests or by reliable, commercially available identification kits and serological tests. Follow the instructions of ISO 6579.

3.5 Statistical analysis of the data

The specificity, sensitivity and accuracy rates were calculated for the control samples and the artificially contaminated samples with minced meat (negative for *Salmonella* spp.). The specificity, sensitivity and accuracy 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 was used for modelling the binary outcomes as a function of a fixed effect part, consisting of the capsules, enrichment media and isolation media and a random effect part, consisting in the different laboratories. Mutual differences between media and capsules are shown as odds ratios stratified by medium. A Bayesian approach was adopted to prevent spurious odds ratios. This was done by putting vague prior information on the odds ratios: OR = 1 with a 95% confidence interval of 0.1 - 10 (Greenland, 2006).

An odds ratio can be interpreted as an effect size and is the ratio of the odds of detecting *Salmonella* in one group to the odds of detecting it in another group. Groups are, for instance, two different media. Results were analysed using the statistical software R (R Development Core Team, 2011). The lme4 package was used for the mixed effect logistic regression (Bates and Maechler, 2011).

3.6 Good performance

The criteria used for testing good performance in this study are given in Table 2. For determining good performance per laboratory, all combinations of selective enrichment media and isolation media used by the laboratory were taken into account. For example, if a laboratory found for the STM5 capsules with matrix 5/8 positive with RVS/XLD but no positives with MKTTn or any other selective enrichment or isolation medium, this was still considered a good result. For the blank capsules, all combinations of media used per laboratory were also taken into account. If, for example a laboratory found 2/8 blank capsules positive with MKTTn/BGA but no positives with the other media, this was still considered a 'no-good' result.

Table 2 Criteria for testing good performance in the Food-IV study (2010)

Control samples (capsules, no matrix)	Minimum result	
	Percentage positive	No. of positive samples / total No. of samples
STM50	100%	1/1
STM5	60%	2/3
Blank control capsules	0%	0/1

Samples (capsules with matrix)	Minimum result	
	Percentage positive	No. of positive samples / total No. of samples
Blank[1]	15% at max[1]	1/8 at max[1]
STM50	80%	6/8
STM5	50%	4/8

1: All should be negative. However, as no 100% guarantees about the *Salmonella* negativity of the matrix can be given, 1 positive out of 8 blank samples (15% pos.) will still be considered as acceptable.

4 Results

4.1 Reference materials

Table 3 describes the level of contamination and the homogeneity of the final batches of capsules. The table gives the enumerated minimum and maximum levels within each batch of capsules. The final batches were tested twice: firstly immediately after preparing the batch and secondly, at the time of the interlaboratory comparison study. At the first and second date of testing the mean contamination level of all batches fulfilled the pre-set-criteria as stated in section 3.1. After receipt, the NRLs had to store the capsules at -20 °C. Laboratory 2 stored their parcel with reference material at 5 °C overnight.

Table 3 Level of contamination and homogeneity of STM capsules

	STM5	STM50
Final batch; Test 1		
Date testing capsules	07-04-2010	06-05-2010
Number of capsules tested	50	50
Mean cfp per capsule	6	60
Min-max cfp per capsule	1-14	46-91
T_2 / (I-1)	1.38	1.04
Final batch; Test 2		
Date testing capsules	29-09-2010	29-09-2010
Number of capsules tested	25	25
Mean cfp per capsule	6	55
Min-max cfp per capsule	3-10	46-64
T_2 / (I-1)	0.59	0.75

cfp = colony forming particles; min-max = enumerated minimum and maximum cfp; formula T_2 see Annex 2; I is number of capsules; Demand for homogeneity $T_2/(I-1) \leq 2$

4.2 Minced meat samples

The minced meat was tested negative for *Salmonella* and subsequently stored at –20 °C. On Monday 20 September 2010, the minced meat was mailed to the NRLs. After receipt, the NRLs had to store the meat at 5 °C.
The number of aerobic bacteria and the number of *Enterobacteriaceae* were tested three times; firstly at the day the meat arrived at the EURL (3/8/2010), secondly, after the meat was stored for one week at 5 °C and thirdly, close to the planned date (27/9/2010) of the interlaboratory comparison study. Table 4 shows the results.

Thirty laboratories performed the study in week 39, starting on 27 September 2010. One laboratory (lab code 9) performed the study immediately after arrival of the parcel at the institute at 21 September.

Table 4 Number of aerobic bacteria and the number of Enterobacteriaceae *per gram of minced meat (stored at –20 °C)*

Date	Enterobacteriaceae cfp/g	Aerobic bacteria cfp/g
3 Augustus 2010 after 1 day at 5 °C	<100	$1.6*10^4$
23 Augustus 2010 after 1 week at 5 °C	$1.8*10^2$	$3.1*10^6$
27 September 2010 after 1 week at 5 °C	$3.2*10^2$	$9.5*10^6$

4.3 Technical data interlaboratory comparison study

4.3.1 General

In this study, 31 NRLs participated: 28 NRLs from 27 EU-Member States and three NRLs from countries of the European Free Trade Association.

4.3.2 Accreditation/certification

All laboratories indicated to be accredited according ISO/IEC 17025 (Anonymous, 2005).
Nineteen laboratories were accredited for ISO 6579 and annex D of ISO 6579 for different matrices. Eight laboratories (lab codes 9, 10, 14, 20, 21, 23, 24, and 30) were accredited for food and feeding stuffs (ISO 6579) but not for animal faeces and veterinary samples (annex D of ISO 6579). One laboratory (lab code 16) was not accredited for the selective enrichment medium RVS which is prescribed in ISO 6579 for food and feeding stuffs. One laboratory (lab code 19) was only accredited for annex D of ISO 6579. Two laboratories (lab code 2 and 4) were not accredited for ISO 6579 or Annex D of ISO 6579 but to another method for food matrices; laboratory 2 is planning to be come accredited for ISO 6579 in 2011. According to EC Regulation (EC, 2004), each NRL should have been accredited for their relevant work field before 31 December 2009 (EC, 2005).

4.3.3 Transport of samples

Table 5 presents an overview of the transport times and the temperatures during transport of the parcels. The temperature recorders were returned immediately after receipt to EURL-*Salmonella* by all NRLs. The majority of the laboratories received the materials within 1–2 days. However, the parcel of two laboratories of non-EU-MS (lab code 2 and 6) were delayed for 7 and 8 days respectively. Additional these parcels were exposed to higher (transport) temperatures for a longer period of time (1-4 days > 5 ^{o}C). If these latter parcels are not taken into account, the average transport time was 38 hours. For most of the parcels the transport temperature did not exceed 5 ^{o}C. Three parcels were exposed only very shortly (1 hour) to temperatures higher than 5 ^{o}C (lab codes 19, 23 and 31). The parcels sent to laboratories 3 and 30 were exposed somewhat longer (respectively 16 and 14 hours) to temperatures up to 10 ^{o}C and two hours to temperatures above 10 ^{o}C.

Table 5 Overview of the transport times and the temperatures during shipment of the parcels to the NRLs.

	Time in hours					
Lab code	**Total Transport time [1]**	**– 20 °C - 0 °C**	**0 °C - 5 °C**	**5 °C - 10 °C**	**>10 °C**	**Additional Storage[2]**
1	26	8	18			
2	167	7	54	78	28	
3	51	9	24	16	2	
4	25	11	14			20 hours at 0 °C
5	24	8	15	1		24 hours at 20 °C
6	192	9	159	4	20	
7	24	7	17			
8	2	2				
9	24	9	15			
10	24	14	10			
11	47	14	33			
12	22	14	8			
13	27	11	16			
14	24	14	10			
15	25	15	10			
16	24	6	18			21 hours at 5 °C
17	22	6	16			
18	49	7	42			
19	27	20	6	1		
20	23	10	13			
21	23	11	12			
22	23	14	9			
23	26	9	16		1	
24	21	14	7			
25	23	14	9			
26	25	14	11			
27	25	8	17			
28	25	7	18			
29	25	8	17			
30	98	9	73	14	2	
31	24	8	15		1	
Average	38					
Average EU[3]	35					

1 = Transport time according to the courier
2 = Storage time of the samples at the institute before arriving at the laboratory (NRL)
3 = Average transport time to the EU Member-States

Table 6 Media combinations used per laboratory.

Lab code	Selective Enrichment media	Plating-out media
1	RVS MKTTn MSRV	XLD Rambach
2	RVS MSRV	XLD BSA
3	RVS MKTTn MSRV	XLD BPLS= BGA^{mod} Rambach
4	RVS MKTTn MSRV	XLD Blaskal BSA
5	RVS MKTTn MSRV	XLD Rambach
6	RVS MKTTn MSRV	XLD BGA^{mod}
7	RV MKTTn MSRV	XLD BPLSA
8	RVS MKTTn MSRV	XLD BGA^{mod}
9	RV MKTTn MSRV	XLD Compass
10	RVS MKTTn MSRV	XLD Rambach
11	RVS MKTTn MSRV	XLD Rambach
12	RVS MKTTn MSRV	XLD BGA^{mod}
13	RVS MKTTn MSRV	XLD SM2
14	RVS MKTTn MSRV	XLD BGA^{mod} RS
15	RV MKTTn MSRV	XLD BGA^{mod}
16	RVS MKTTn MSRV	XLD SM2
17	RVS MKTTn MSRV	XLD BGA^{mod}
18	RVS MKTTn MSRV	XLD Rambach
19	RVS MKTTn MSRV	XLD XLT4 BGA*
20	RVS MKTTn MSRV	XLD RS
21	RVS MKTTn MSRV	XLD BGA^{mod}
22	RVS MKTTn MSRV	XLD BGA^{mod}
23	RVS MKTTn MSRV	XLD BGA^{mod}
24	RVS MKTTn MSRV	XLD SM2
25	RVS MKTTn MSRV	XLD Rambach
26	RV MKTTn MSRV	XLD Brilliance
27	RVS MKTTn MSRV	XLD SM2
28	RVS MKTTn MSRV	XLD BGA^{mod}
29	RVS MKTTn MSRV	XLD BGA
30	RVS MKTTn MSRV	XLD BGA^{mod}
31	RVS MKTTn MSRV	XLD BPLS=BGA^{mod}

Explanations of the abbreviations are given in the 'List of abbreviations'
Compositions of the media not described in ISO 6579 are given in Annex 3
* lab 19 used BGA only in combination with MSRV

For three NRLs (lab code 4, 5 and 16), the time of transport recorded on the test report did not correspond with the time reported by the courier. Presumably the parcel arrived at the time reported by the courier at the institute but due to internal logistics, the parcel arrived later at the laboratory of the NRL. The delay was between 20 and 24 hours and the parcels were stored respectively at 0 °C, 20 °C and 5 °C.

4.3.4 Media

Each laboratory was asked to test the samples with the prescribed (ISO 6579) and the requested (Annex D of ISO 6579) methods. Thirty laboratories used the selective enrichment media RV(S), MKTTn and MSRV with the plating-out medium XLD and a second plating-out medium of own choice. Table 6 shows the media used per laboratory. Laboratory 2 did not use the prescribed medium MKTTn. Four laboratories (lab codes 7, 9, 15 and 26) used the RV instead of the prescribed RVS. Four NRLs (lab codes 3, 4, 14 and 19) used a third plating-out medium. Details on the media which are not described in ISO 6579 are given in Annex 3.
Tables 7-13 give information on the composition of the media that were prescribed and 'requested' and on the incubation temperatures. These tables only indicate the laboratories that reported deviations. Four laboratories (2, 19, 20 and 21) reported small deviations in the dissolving time or temperature of the capsules. The laboratories 6, 20, 23 and 30 incubated the pre-enrichment medium BPW longer than described. Laboratories 13, 26 and 27 did not mention the pH for most of the used media. One laboratory (lab code 26) incubated the selective enrichment medium MKTTn at a deviating temperature of 41.5 °C.

A second plating-out medium for choice was obligatory. Thirteen laboratories used BGA modified (ISO 6579, 1993) or BPLS as a second plating-out medium. Seven laboratories used Rambach, four laboratories SM2 agar, three laboratories Brilliance (BSA) and two laboratories Rapid *Salmonella* (RS). The following media were used only by one laboratory: BGA, XLT4, BPLSA, Blaskal and Compass.

The use of an extra plating agar between the 'isolation' and the 'confirmation' steps was optional. A total of 16 laboratories performed this extra culture step on many different media (e.g., Nutrient agar: ISO 6579, 2002).
Most of the laboratories used both biochemical and serological tests for confirmation of *Salmonella.* Laboratory 7 only used PCR for the confirmation of *Salmonella*. Tables 14 and 15 summarise the used confirmation media and serological tests.

All participating laboratories performed confirmation tests for *Salmonella*: most of them biochemical, serological or both. Six laboratories (lab codes 8, 10, 13, 14, 26 and 28) only used a biochemical test and one laboratory (lab code 7) performed only a PCR. The Tables 14 and 15 summarises the confirmation media and tests.

Table 7 Incubation time and temperature of BPW.

	Prewarming BPW		Dissolving capsules in BPW		Pre-enrichment in BPW	
Lab code	**Time**	**Incubation temperature in °C (min-max)**	**Time (min)**	**Incubation temperature in °C (min-max)**	**Time (h:min)**	**Incubation temperature in °C (min-max)**
SOP & ISO 6579	**Overnight**	**36-38**	**45**	**36-38**	**16 – 20**	**36-38**
2	o/n	37.5	50	37.5	20	37-37.5
6	o/n	37	45	37	20:15	37
19	o/n	37.5-37.6	35	36-36.8	18:10	36.2-37.1
20	o/n	36.6-36.7	45	34.1-36.2	20:15	36.8
21	o/n	36.4-36.9	45	35.8-36.4	18:40	35.8-36.2
23	o/n	36.6-36.9	45	36.6	20:30	36.6-36.7
30	o/n	37	45	37	21:00	37

o/n : over night Grey cell : deviating times and temperatures - = no information

Table 8 Composition (in g/L) and pH of BPW medium.

Lab code	Enzymatic digest of casein (Peptone)	Sodium Chloride (NaCl)	Disodium hydrogen Phosphate dode-cahydrate* ($Na_2HPO_4.12H_2O$)	Potassium dihydrogen phosphate (KH_2PO_4)	pH
ISO 6579	**10.0**	**5.0**	**9.0**	**1.5**	**6.8 – 7.2**
4	10	5	3.5	1.5	7.4
8, 27	10	5	9	1.5	-
12, 26, 28	10	5	3.5	1.5	7.3
16	-	-	-	-	7.0

Grey cell: deviating from ISO 6579 - = no information

* = 3.5 grams Disodium hydrogen phosphate (anhydrous) is equivalent to 9 grams disodium hydrogen phosphate dodecahydrate

Table 9 Incubation temperatures of selective enrichment medium RVS, MKTTn and MSRV.

	RVS	MKTTn	MSRV
Lab code	**Incubation temperature in °C (min-max)**	**Incubation temperature in °C (min-max)**	**Incubation temperature in °C (min-max)**
ISO 6579 & Annex D	**40.5 – 42.5**	**36–38**	**40.5 – 42.5**
26	41.5	41.5	41.5

Grey cell: deviating times and temperatures.

Table 10 Composition (in g/L) and pH of RVS.

Lab code	Enzymatic digest of soya (Peptone)	Sodium Chloride (NaCl)	Potassium Dihydrogen Phosphate* (KH_2PO_4 K_2HPO_4)	Magnesium chloride anhydrous ($MgCl_2$)**	Malachite green oxalate	pH
ISO 6579	**4.5**	**7.2**	**1.44**	**13.4**	**0.036**	**5.0 - 5.4**
2	4.5	7.2	1.26 + 0.2*	13.6	0.036	-
7,9 (RV)	5	8	1.6	40**	0.04	5.2
8	4.5	7.2	1.44	13.4	0.036	-
13	4.5	7.2	1.44	28.6**	0.037	-
14, 27	4.5	7.2	1.26 + 0.2*	13.4	0.036	-
15 (RV)	5	8	1.4 + 0.2*	13.4	0.036	5.2
25	5	8	1.4 + 0.2*	400	0.4	5.2
26 (RV)	5	8	1.6	40**	0.04	-
28, 29	4.5	7.2	1.26 + 0.2*	13.6	0.036	5.3
30	4.5	7.2	1.44	28.6**	0.036	-
31	4.5	8	0.6 + 0.4*	29**	0.036	5.2

Grey cell: deviating from ISO 6579. - = no information.

*= 1.4 g/L Potassium dihydrogen phosphate (KH_2PO_4) + 0.2 g/L Di-potassium hydrogen phosphate (K_2HPO_4) gives a final concentration of 1.44 g/L KH_2PO_4 K_2HPO_4.

** = 13.4 grams $MgCl_2$ (anhydrous) is equivalent to 28.6 grams $MgCl_2$ hexahydrate.

Table 11 Composition (in g/L) and pH of MKTTn.

Lab code	Meat Extract	Enzymatic digest of casein (Peptone)	Sodium chloride (NaCl)	Calcium Carbonate ($CaCO_3$)	Sodium Thiosulfate Penta hydrate ($Na_2S_2O_3.5H_2O$)	Ox bile	Brilliant green	Iodine	Potas-sium iodide (KI)	Novo-Biocin	pH
ISO 6579	**4.3**	**8.6**	**2.6**	**38.7**	**47.8**	**4.8**	**0.0096 (9.6 mg)**	**4**	**5**	**0.04**	**8.0 – 8.4**
2	4.3	8.6	2.6	38.7	30.3*	4.8	0.0095	4	5	0.04	7.9
5	4.3	8.6	2.6	38.7	30.5*	4.8	0.0096	4	5	0.01	8
6	7	2.3	2.3	25	40.7	4.8	0.01	20g/100ml	25g/100ml	0	8.2
10	4.3	8.6	2.6	38.7	30.5*	4.8	0.0096	3.9	4.9	0.039	7.8
12	4.3	8.6	2.6	38.7	30.5*	4.8	0.0096	4	5	0.04	7.8
13, 27	4.2	8.5	2.5	38	30.3*	4.8	0.0095	4	5	0.05	-
14	4.3	8.6	2.6	38.7	47.8	4.8	0.0096	-	-	-	-
15	4.3	8.6	2.6	38.7	30.5*	4.8	0.0096	20	25	0.04	8
16	-	-	-	-	-	-	-	-	-	-	7.8
25	4.3	8.6	2.6	38.7	47.8	4.8	0.0096	20	25	0.04	8.2
26	4.3	8.6	2.6	38.7	30.5*	4.8	0.0096	3.9	4.9	0.039	-
28	7	2.3	2.3	25	40.7	4.8	0.0095	3.9	4.8	0.04	-
30	4.3	8.6	2.6	38.7	47.8	4.8	0.0096	20	25	0.01	7.8
31	4.3	8.6	2.6	38.7	30.5*	4.8	0.0096	-	-	0.04	8.2

Grey cell: deviating from ISO 6579. - = no information.

* 30.5 grams Sodium thiosulphate (anhydrous) is equivalent to 47.8 grams Sodium thiosulphate pentahydrate.

Table 12 Composition (in g/L) and pH of MSRV.

Lab code	Enzymatic digest of casein (Tryptose)	Casein hydro-lysate	Sodium chloride (NaCl)	Potassium Dihydrogen Phosphate (KH_2PO_4 K_2HPO_4)	Magnesium chloride anhydrous ($MgCl_2$)	Malachite green oxalate	Agar	Novo Biocin	pH
Annex D ISO 6579	**4.6**	**4.6**	**7.3**	**1.5**	**10.9**	**0.04**	**2.7**	**0.01 (10mg/L)**	**5.1- 5.4**
2, 27	4.6	4.6	7.3	1.5	10.9	0.04	2.7	10	-
6	4.6	4.6	7.3	1.5	10.9	0.04	2.7	20	5.2
7, 9	4.6	4.6	7.3	1.5	10.9	0.04	2.7	20	5.4
10	4.6	4.6	7.3	1.5	10.9	0.04	2.5	10	5.0
14	4.6	4.6	7.3	1.5	10.9	0.04	2.7	20	5.2
15	4.6	4.6	7.3	1.5	10.9	0.04	2.7	50	5.4
20	4.6	4.6	7.3	1.5	10.9	0.04	2.7	-	5.2
21	2.3	4.6	7.3	1.5	10.9	0.04	2.5	10	5.1
25	-	-	-	-	-	-	-	-	5.2
28	4.6	4.6	7.3	1.5	10.9	0.04	2.7	10	5.6
31	4.6	4.6	7.3	1.5	10.9	0.04	2.7	50	5.2

Grey cell: deviating from Annex D of ISO 6579 - = no information

Table 13 Composition (in g/L) and pH of XLD.

Lab code	Xylose	L-lysine	Lactose	Sucrose (Saccharose)	Sodium chloride (NaCl)	Yeast extrac	Phenol red	Agar	Sodium deoxy-cholate ($C_{24}H_{39}NaO_4$)	Sodium thio-sulphate ($Na_2S_2O_3$)	Iron (III) Ammonium Citrate ($C_6H_8O_7 \cdot nFe \cdot nH_3N$)	pH
ISO 6579	**3.75**	**5.0**	**7.5**	**7.5**	**5.0**	**3.0**	**0.08**	**9-18**	**1.0**	**6.8**	**0.8**	**7.2 – 7.6**
6	-	5	3.5	7.5	5	3	0.08	13.5	2.5	6.8	0.8	7.2
7	3.75	5	7.5	7.5	5	2+1*	0.07	15	1	4.3	0.8	7.3
13, 30	3.5	5	7.5	7.5	5	3	0.08	13.5	2.5	6.8	0.8	-
15	3.75	5	7.5	7.5	5	3	0.08	15.5	2.5	6.8	0.8	7.4
18	3.5	5	7.5	7.5	5	3	0.08	13.5	2.5	6.8	0.8	7.5
23	3.5	5	7.5	7.5	5	3	0.08	13.5	2.5	6.8	0.8	7.4
25	-	-	-	-	-	-	-	-	-	-	-	7.4
26	3.75	5.3	7.5	7.5	5	3	0.08	12.5	1	6.8	0.8	-
27	3.5	5	7.5	7.5	5	3	0.08	13.5	6.8	6.8	0.8	-

Grey cell: deviating from ISO 6579 - = no information

* 2 grams yeast extract + 1 grams peptone

Table 14 Used media/tests for biochemical confirmation of Salmonella*.*

Lab code	TSI	UA	LDC	Gal	VP	Indole	Biochemical kit or Other
1	+	+	+	-	-	+	Enterotest 24 Plova
2	+	+	-	-	-	-	Lysine Iron Agar
3, 5	+	+	+	+	+	+	
4	+	+	-	-	-	+	API20E
6	+	+	+	-	-	+	ID 32E
7	-	-	-	-	-	-	PCR
8, 10, 28	+	+	+	-	-	-	
9, 29	+	+	+	+	+	+	PCR
11	+	+	+	+	+	+	Home made (Fung et al.)
12	-	-	-	-	-	-	Kohns No1 (Mast Diagnostics)
13	+	+	+	-	-	-	ID32E
14	-	-	-	-	-	-	ID32E
15, 21	+	+	-	-	-	+	API20E, PCR
16	-	-	-	-	-	-	VITEK
17	+	+	+	+	-	+	
18	+	+	+	+	+	+	API20E
19	+	+	+	+	-	+	Agar tryptose
20	-	-	-	-	-	-	Enterotube II, PCR
22	-	-	-	-	-	-	Chromagar
23	+	+	+	+	+	+	BAX system Q7
25	+	+	+	+	-	+	Semi-solid glucose agar
26	+	-	-	-	-	-	H_2S, Oxidase
27	-	-	-	-	-	-	Microbact 12A
30	+	-	-	-	-	-	Oxoid
31	+	+	+	-	-	+	

- = Not done/ not mentioned

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

Table 15 Used antigens for serological confirmation of Salmonella*.*

Lab code	Serological			
	O antigens	**H antigens**	**Vi Antigens**	**Other**
23, 30	+	+	+	
9	+	-	+	
1, 3, 5, 6, 11, 12, 21	+	+	-	
16, 17, 18, 19, 22, 25, 31	+	-	-	
7, 8, 10, 13, 14, 26, 28	-	-	-	
2	-	-	-	Latex Agglutination Test Oxoid
4	-	-	-	Omnivalent serum SIFIN TR1101
20	-	-	-	Poli-A-S+Vi
27	-	-	-	*Salmonella* test kit

- = Not done/ not mentioned

4.4 Control samples

4.4.1 General

None of the laboratories isolated *Salmonella* from the procedure control (C6: no capsule/no meat) nor from the meat control (C7: no capsule/negative meat). All laboratories scored correct results for all the control capsules. Laboratory 2 did not perform selective enrichment in MKTTn. Table 16 summarises the highest number of positive isolations found with all combinations of selective enrichment media and isolation media per laboratory.

Blank capsule without addition of meat (n=1)
The blank capsule contained only sterile milk powder. For the analyses, no meat was added. All laboratories correctly analysed the blank capsules negative for all used media.

Salmonella *Typhimurium 5 capsules (STM5) without addition of meat (n=3)*
All laboratories isolated *Salmonella* from the three capsules containing STM5.

Salmonella *Typhimurium 50 capsules (STM50) without addition of meat (n=1)*
All participating laboratories tested the capsule containing STM50 positive for *Salmonella*.

The results of all control samples were compared with the definition of 'good performance' (see section 3.6) and all laboratories fulfilled the pre-set criteria.

Table 16 Total number of positive results of the control samples (capsule without meat) for all laboratories.

Lab code	The highest number of positive isolations found with all combinations of selective enrichment media and isolation medium		
	Blank n=1	STM5 n=3	STM50 n=1
Good Performance	**0**	**≥ 2**	**1**
All laboratories 1 - 31	0	3	1

4.4.2 Specificity, sensitivity and accuracy rates of the control samples

Table 17 shows the specificity, sensitivity and accuracy rates found with the control capsules without the addition of meat. The rates are calculated for the different selective enrichment media (RVS, MKTTn and MSRV) and plating-out medium XLD. The calculations were performed on the results of all participants and on the results of only the EU-MS (without the countries of the European Free Trade Association). As expected, the high level STM50 capsules showed a rate of 100% but also the low level materials (STM5) and the blank capsules showed a rate of 100%. There was no difference between rates of EU-MSs and the four non-EU-MSs.

Table 17 Specificity, sensitivity and accuracy rates of the control samples (capsules without the addition of meat).

Control capsules		RVS/XLD		MKTTn/XLD*		MSRV/XLD	
		All n=31	EU n=28	All n=30	EU n=28	All n=31	EU n=28
Blank	No. of samples	31	28	30	28	31	28
	No. of negative samples	31	28	30	28	31	28
	Specificity in%	**100**	**100**	**100**	**100**	**100**	**100**
STM5	No. of samples	93	84	90	84	93	84
	No. of positive samples	93	84	90	84	93	84
	Sensitivity in%	**100**	**100**	**100**	**100**	**100**	**100**
STM50	No. of samples	31	28	30	28	31	28
	No. of positive samples	31	28	30	28	31	28
	Sensitivity in%	**100**	**100**	**100**	**100**	**100**	**100**
All capsules with *Salmonella*	No. of samples	124	112	120	112	124	112
	No. of positive samples	124	112	120	112	124	112
	Sensitivity in%	**100**	**100**	**100**	**100**	**100**	**100**
All Capsules	No. of samples	155	140	150	140	155	140
	No. of correct samples	155	140	150	140	155	140
	Accuracy in%	**100**	**100**	**100**	**100**	**100**	**100**

*One laboratory (non-EU) did not use MKTTn
All = results of all laboratories
EU = results of only the laboratories of the EU Member States

4.5 Results of meat samples artificially contaminated with *Salmonella* spp.

4.5.1 Results per type of capsule and per laboratory

General
Twenty-one laboratories scored correct results for all the samples. Ten laboratories showed deviations which are shown in Table 18. Laboratory 2 did not perform selective enrichment in MKTTn. Laboratory 4 made an initial transcription error as they reported two blank samples and one STM5 sample positive for *Salmonella*, while they originally had tested these samples negative. In table 18 the results are given after correction. This is discussed in more detail in section 4.7. The highest number of positive isolations found with all combinations of selective enrichment media and isolation media per laboratory is summarised in Annex 6.

Table 18 Number of positive results of the artificially contaminated meat samples (with capsules) per selective enrichment medium and isolation medium for the participating laboratories which found one ore more deviating results. When only one number is mentioned, both isolation media used gave the same result.

Lab code	RVS XLD/2nd			MKTTn XLD/2nd			MSRV XLD/2nd		
	Blank n=8	STM5 n=8	STM50 n=8	Blank n=8	STM5 n=8	STM50 N=8	Blank n=8	STM5 n=8	STM50 n=8
Good Performance	≤ **1**	**≥ 4**	**≥ 6**	≤ **1**	**≥ 4**	**≥ 6**	≤ **1**	**≥ 4**	**≥ 6**
2*	0	**6**	8	**-**	**-**	**-**	0	**6**	8
3	0	8	8	0	8	**7**/8	0	8	8
4	0	**7**	8	0	**6/7**	8	0	**7**	8
7	0	**6**/8	**7**/8	0	8	8	0	8	8
9**	**1**	8	8	**1**	8	8	**1**	8	8
11	0	**7**	8	0	**7**	8/**7**	0	**7**	8
21	**1**	8/**7**	8	0	8	8/**7**	0	8	8
23	0	8	8	0	8/**5**	8	0	8	8
25	**2**	8	8	0	8	8	0	8	8
30	0	**7**	8	0	**7**	8	0	**7**	8

* Laboratory 2 did use MKTTn

**Laboratory 9 found two different blank samples positive: one with MKTTn and RVS and another one with MSRV.

Bold number: deviating result
Grey cell: result is below good performance

Blank capsules with negative minced meat (n=8)
Twenty-eight laboratories correctly did not isolate *Salmonella* from the blank capsules with the addition of negative minced meat. Two laboratories (9 and 25) tested two blank samples false positive. Laboratory 9 found two different blank samples positive, one with RVS and MKTTn and another positive sample only with MSRV. Laboratory 25 tested two blank samples positive for *Salmonella* with RVS in combination with both used isolation media, XLD and Rambach. Laboratory 21 found one blank sample positive for *Salmonella* after selective enrichment in RVS and isolation on XLD and BGA.
In theory all blank samples should be tested negative. However, as no 100% guarantee about the *Salmonella* negativity of the matrix can be given, 1 positive out of 8 blank samples (85% negative) is still considered acceptable. Finding more than one blank positive result is not very likely. Possible causes for finding a blank sample positive may be caused by cross-contamination, limited confirmation or by misinterpretation of the results.

S. *Typhimurium 5 capsules (STM5) with negative minced meat (n=8)*
Twenty-seven laboratories isolated *Salmonella* from all the eight capsules containing *Salmonella* Typhimurium at a level of approximately 5 cfp/ capsule in combination with minced meat. Laboratories 4, 11 and 30 missed one capsule and laboratory 2 found two capsules negative. These capsules contained STM at a low level. Due to variation between capsules, 1 out of 8 capsules containing STM5 may be negative. It is not very likely to find more than two negative results because of negative capsules.

S. *Typhimurium 50 capsules (STM50) with negative minced meat (n=8)*
All laboratories isolated *Salmonella* from all eight capsules containing *Salmonella* Typhimurium at a level of approximately 50 cfp/ capsule in combination with minced meat with all the selective enrichment media: RVS, MKTTn and MSRV.

The results of all artificially contaminated minced meat samples were compared with the definition of 'good performance' (see section 3.6). Two laboratories (lab code 4 and 25) scored below these criteria.

4.5.2 Results per selective enrichment medium, capsule and per laboratory

The Figures 1, 2, and 3 show the number of positive isolations per type of artificially contaminated minced meat sample per laboratory after pre-enrichment in BPW, selective enrichment in RVS, MKTTn and on MSRV, followed by isolation on a selective plating agar. To determine good performance per laboratory, all combinations of selective enrichment media and isolation media used by the laboratory were taken into account. The results of all artificially contaminated minced meat samples were compared with the definition of 'good performance' (see section 3.6). The black horizontal line in Figures 1-3 indicates the border of good performance.

Table 19 gives the differences in the number of positive isolations after 24 and 48 hours of incubation of the selective enrichment media. The choice of plating-out medium does not seem to have a large effect on the number of positive isolations. When MKTTn is used for selective enrichment, XLD gave 1% more positive results than other plating-out media. The majority of the laboratories used BGA as the second plating-out medium (see Table 6).
The difference in the number of positive isolations after 24 hours and after 48 hours of incubation of the selective enrichment media was the highest for MKTTn (Table 19): 2% more positive isolations were found after 48 hours of incubation. For RVS and MSRV the difference between the two incubation times was nihil.

Table 19 Mean percentages of positive results of all participating laboratories after selective enrichment in RVS, MKTTn and on MSRV, incubated for 24 hours and for 48 hours and followed by isolation on different plating out media, when analyzing the artificially contaminated minced meat samples.

Plating out medium	Selective enrichment medium		
	RVS	MKTTn	MSRV
	24 / 48 h	24 / 48 h	24 / 48 h
XLD	98 / 98%	97 / 99%	99 / 99%
Other (most often BGA)	99 / 99%	96 / 98%	99 / 99%

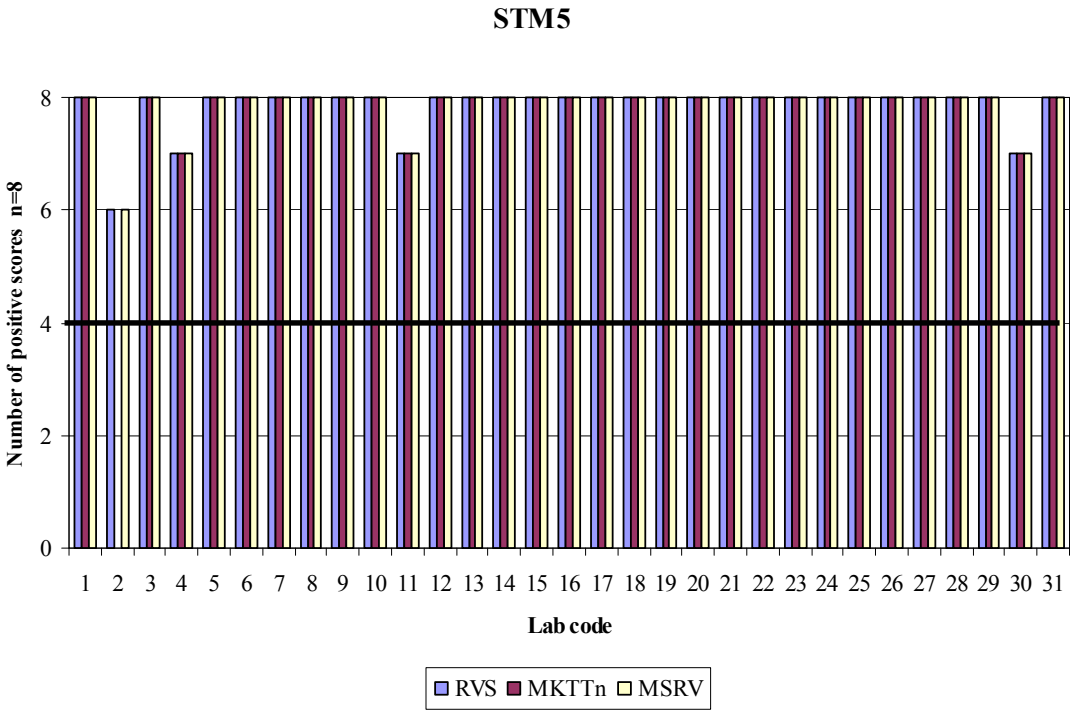

Figure 1 Results per laboratory found with the minced meat samples artificially contaminated wit STM5 capsules (n=8) after selective enrichment in RVS, MKTTn and on MSRV followed by isolation on selective plating agar.

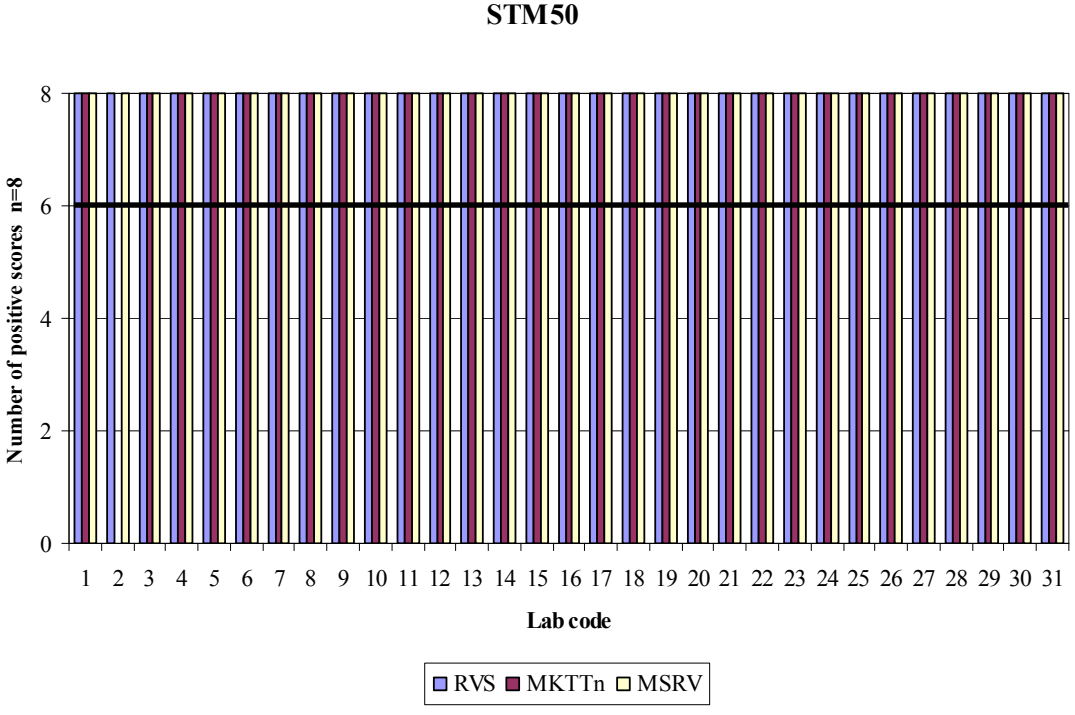

Figure 2 Results per laboratory found with the minced meat samples artificially contaminated with STM50 capsules (n=8) after selective enrichment in RVS, MKTTn and on MSRV followed by isolation on selective plating agar.

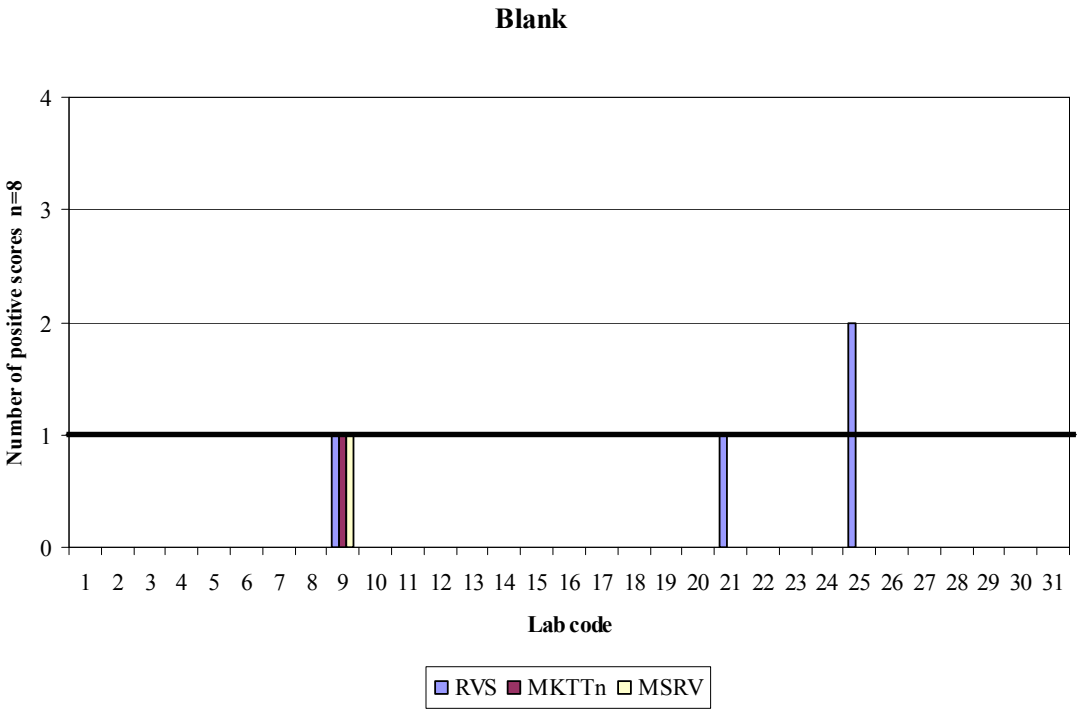

Figure 3 Results per laboratory found with the minced meat samples artificially contaminated with blank capsules (n=8) after selective enrichment in RVS, MKTTn and on MSRV followed by isolation on selective plating agar.

Tables 20 and 21 show the differences between selective enrichment media and isolation media per capsule as odds ratios (OR). In addition, the 95% confidence intervals and p-values are given.

In Table 20, 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* from the STM5 samples after selective enrichment in MKTTn is a factor 1.48 higher when XLD is used as isolation medium compared to an isolation medium other than XLD. In general, if MKTTn or MSRV are used as selective enrichment media, the ORs are greater than 1. If RVS is used, the ORs are in general less than 1. This means that, in combination with MKTTn and MSRV, it is easier to detect *Salmonella* if XLD is used compared to other isolation media. In combination with RVS, it is more difficult to detect *Salmonella* if XLD is used compared to other isolation media. However, none of the differences are significant.

The interpretation of Table 21 is similar to that of Table 20, except that selective enrichment media are mutually compared, given XLD as isolation medium.
For instance, the odds of finding *Salmonella* from all STM samples after selective enrichment in MKTTn is a factor 0.78 lower compared to MSRV. In general, if RVS is used as selective enrichment media the chance of finding *Salmonella* is greater than when MKTTn is used, but RVS compared with MSRV gives a smaller chance. However, none of the differences are significant.

Table 20 Number of positive isolations found with XLD compared to the number of positive isolations found with other isolation media, given a selective enrichment medium. Samples: minced meat, artificially contaminated with Salmonella *positive capsules*

Selective enrichment medium	Compared isolation media	Capsule	Odds Ratios	95% lower	95% upper	p-value*
RVS	XLD compared to Other than XLD	STM5	0.84	0.3	2.27	0.72
		STM50	0.59	0.07	4.33	0.61
		all STM	0.75	0.28	1.96	0.55
MKTTn	XLD compared to Other than XLD	STM5	1.48	0.51	4.59	0.48
		STM50	1.36	0.27	7.54	0.71
		all STM	1.54	0.59	4.30	0.39
MSRV	XLD compared to other than XLD	STM5	1.10	0.38	3.27	0.86
		STM50	1.00	0.10	10.22	1.00
		all STM	1.10	0.38	3.26	0.86
All enrichment media	XLD compared to other than XLD	STM5	1.11	0.58	2.13	0.76
		STM50	0.96	0.21	4.45	0.96
		all STM	1.09	0.59	2.02	0.78

*significant difference p < 0.05.

Table 21 Number of positive isolations found with a selective enrichment medium compared to the number of positive isolations found with another selective enrichment medium, given that the isolation is on XLD. Samples: minced meat, artificially contaminated with Salmonella *positive capsules*

Compared selective enrichment media	Isolation medium	Capsule	Odds Ratios	95% lower	95% upper	p-value*
RVS compared to MKTTn	XLD	STM5	0.88	0.41	1.90	0.75
		STM50	1.82	0.41	9.43	0.44
		all STM	1.03	0.50	2.10	0.94
RVS compared to MSRV	XLD	STM5	0.86	0.39	1.85	0.70
		STM50	0.60	0.07	4.39	0.62
		all STM	0.80	0.37	1.70	0.57
MKTTn compared to MSRV	XLD	STM5	0.97	0.44	2.16	0.94
		STM50	0.34	0.05	1.78	0.23
		all STM	0.78	0.36	1.65	0.52

*significant difference p < 0.05

Table 22 Specificity, sensitivity and accuracy rates for all participating laboratories of the artificially contaminated meat samples (each capsule added to 25 grams minced meat) for the selective enrichment in RVS, MKTTn and on MSRV and plating out medium XLD

Capsules with minced meat		**RVS/XLD**		**MKTTn/XLD***		**MSRV/XLD**	
		All n=31	EU n=28	All n=30	EU n=28	All n=31	EU n=28
Blank (n=8)	No. of samples	248	224	240	224	248	224
	No. of negative samples	244	220	239	223	247	223
	Specificity in%	**98**	**98**	**100**	**100**	**100**	**100**
STM5 (n=8)	No. of samples	248	224	240	224	248	224
	No. of positive samples	241	220	236	222	243	222
	Sensitivity in%	**97**	**98**	**98**	**99**	**98**	**99**
STM50 (n=8)	No. of samples	248	224	240	224	248	224
	No. of positive samples	247	223	239	223	248	224
	Sensitivity in%	**100**	**100**	**100**	**100**	**100**	**100**
All capsules with *Salmonella*	No. of samples	496	448	480	448	496	448
	No. of positive samples	488	443	475	445	491	446
	Sensitivity in%	**98**	**99**	**99**	**99**	**99**	**100**
All capsules	No. of samples	744	672	720	672	744	672
	No. of correct samples	732	663	714	668	738	669
	Accuracy in%	**98**	**99**	**99**	**99**	**99**	**100**

* One laboratory (non-EU) did not use MKTTn

All = results/of all laboratories

EU = results of only the laboratories of the EU Member States

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

Table 22 shows the specificity, sensitivity and accuracy rates for all types of capsules added to the minced meat. The results are given for the different medium combinations: pre-enrichment in BPW, followed by selective enrichment in RVS, MKTTn and on MSRV and isolation on selective plating agar XLD. The calculations were performed on the results of all participants and on the results of only the EU-MS (without the European Free Trade Association). It was expected that the blank capsules and the high-level materials would show rates close to 100%. For the low-level materials a minimum rate of 50% was expected. The rates showed the expected or even better results. The specificity rates (of the blank capsules) were for all three selective enrichment media

>98%. The sensitivity rates found for the high level samples with *S*. Typhimurium (STM50) were 100% for all tested media. The sensitivity rates for the low level contaminated samples (STM5) were 97% or higher. The highest rates were found with MSRV (99%). There was no difference between the rates found by the EU-MSs and the rates found by the three non-EU-MSs.

4.6 Own method

PCR

Six laboratories (lab codes 7, 9, 15, 20, 21 and 29) applied a PCR method as an additional detection technique. All laboratories use the PCR routinely except one (laboratory 15) and test the samples after pre-enrichment in BPW. PCR methods were validated (indicated with a reference in Table 23). Table 23 summarises further details on the volumes used in the PCR techniques.

Table 23 Details on Polymerase Chain Reaction procedures, used as own method during the interlaboratory comparison study by six laboratories

Lab code	Volume of BPW (µl)	Volume of DNA sample (µl)	Volume of DNA / PCR mix (µl)	PCR methode : Reference
7	1000	300	2/23	Real time: Malorny et al., 2004
9	10	200	50/tablet	BAX system: AFNOR, 2009
15	1000	200	5/50	Real time: Nordval, 2004
20	5	200	50/?	BAX system: Nordval, 2009
21	400	400	30/?	Real time: AFNOR, 2007
29	1000	75	5/12	non commercially, no further information

All laboratories found the same results with the PCR technique as with the bacteriological culture methods. Laboratories 7, 15, 20 and 29 scored all samples correctly. Laboratory 21 scored all samples correctly with the PCR which was comparable with their results after selective enrichment on MSRV. Laboratory 9 scored one blank sample positive with the PCR method, which was comparable with their results after selective enrichment in RVS. This false positive sample was the only sample of which they performed a PCR after selective enrichment in RVS for all the other, and correctly scored samples, they performed a PCR after pre-enrichment in BPW.

4.7 Performance of the NRLs

General

Twenty-eight NRLs fulfilled the criteria of good performance.

Laboratories 4, 9 and 25 scored below the criteria of good performance. The difficulties were found with the blank capsules. They found two blank capsules with meat positive.
All three laboratories were contacted by the EURL-*Salmonella* in November 2010 to ask for any explanations for the deviating results. To clarify the false positive results they were asked to perform additionally, when possible, serotyping and phagetyping.

Laboratory 4 (non-EU) reported two blank capsules (with meat) positive after selective enrichment in RVS and isolation on Blasak medium. The same samples were correctly scored negative after selective enrichment in MKTTn and on

MSRV. The laboratory critically went through the raw data and the confirmation tests. They concluded that they made an initial transcription error. After showing the raw data it was agreed to introduce a correction to the data. The results used in this report were the results after correction of the transcription error. An initial transcription error is no good performance but a follow up study was not considered necessary. The results of laboratory 4 were indicated as 'moderate performance'.
Laboratory 9 found two blank capsules (with meat) positive. One false positive sample was found after selective enrichment in RV and MKTTn and another false positive sample was found after selective enrichment on MSRV. The laboratory investigated possible reasons and checked their procedures. Furthermore they performed additional phagetyping to the false positive isolated strains. As a check, they also performed phagetyping on two control samples with only capsules and two samples with meat and capsules. They found two different phagetypes. Phagetype U302 was found after selective enrichment in RV and MKTTn. Phagetype 192 was found from the false positive sample after selective enrichment on MSRV, from the control samples with only capsules and from the samples with meat and capsules. Phagetype U302 is currently frequently found in naturally contaminated samples. These findings indicate problems with cross-contamination, not only between positive and negative samples of the ringtrial but also between routine samples.
Laboratory 25 found two blank capsules (with meat) positive after selective enrichment in RVS. The laboratory investigated possible reasons and checked their procedures. The same samples were correctly found negative after selective enrichment in MKTTn and on MSRV; therefore it was concluded that the problems were not caused by contamination of the pre-enrichment broth. Possibly there was a cross-contamination at the inoculation of RVS or at the plating out step. The *Salmonella* strains isolated from RVS of the blank samples were biochemical typical to *Salmonella* and agglutinated with polyvalent antisera (Sifin). The laboratory eliminated all the samples after the ringtrial; therefore additional tests were not possible. The NRL mentioned that detection of *Salmonella* is not part of their routine activity but is a task of the regional laboratories. Therefore the circumstances in the laboratory of the NRL were not optimal. One of the problems they faced was that they did not posses the right pipettes to subsample from stomacher bags. These pipettes were ordered after the ringtrial.
To check whether the actions taken have been successful, laboratory 9 and 25 participated in a follow-up study organised by the EURL-*Salmonella* in January 2011.

One laboratory (lab code 2, a non EU-MS) used only one (RVS) of the two prescribed selective enrichment media, as described in ISO 6579 (2002). Laboratory 12, a non-EU-MS, did not use either one of the prescribed selective enrichment media (RVS and MKTTn). The laboratory indicated that it routinely uses only RV for selective enrichment of *Salmonella* from food samples. Additional, the laboratories 2 and 12 followed Annex D of ISO 6579 (MSRV) to analyse the meat samples. The results found with RV(S) and MSRV fulfilled the criteria of good performance and no further actions were deemed necessary.

Follow-up study
The set-up of the follow-up study was the same as the full interlaboratory comparison study in October 2010, but with a lower number of samples (see section 4.1 'Reference materials'). In this follow-up study, more blank capsules were tested, as these samples were causing most of the problems. Table 24 gives an overview.

Table 24 Overview of the types and the number of capsules tested by the laboratories 9 and 25 in the follow-up of the interlaboratory comparison study

Capsules	Control capsules (n=4) no meat added	Test samples (n=10) with 25g *Salmonella* negative minced meat
S. Typhimurium 50 (STM50)	2	4
Blank	2	6

On Monday 10 January 2011, one parcel with two plastic containers was sent to laboratory 4 and 25 containing: four control capsules (numbered C1 – C4), ten capsules (numbered 1-10), 400 grams minced meat and one temperature recorder.
The performance of this follow-up study started on 17 January 2011. The laboratory had to follow the same SOP and protocol as in the study of October 2010 (see Annexes 4 and 5). The test report was different from the October study (see Annex 7). For the media used, only the differences with the October study needed to be indicated.
For the media compositions, incubation times and temperatures, no differences were observed in comparison with the full study.
Both Laboratories correctly scored all blank samples negative and detected *Salmonella* Typhimurium in all the STM50 capsules.
Laboratory 9 and 25 fulfilled the criteria of good performance (see section 3.6) for the test samples in this follow-up study.

5 Discussion

Transport of the samples
Neither transport time nor transport temperature seems to have negatively affected the results for the majority of the laboratories. The laboratories with the longest transport times and/or the highest transport temperatures (lab codes 2, 4, 5, 6 and 30) still produced good results. However, laboratory 2 scored the lowest number of positive results in comparison with the other participants. There are different possible clarifications for this; they had one of the longest transport time in combination with the highest transport temperature (4 days > 5 ºC) but they also stored their reference material at 5 ºC instead of –20 ºC after arrival at the laboratory.

Performance of the laboratories
The prescribed method (ISO 6579: with selective enrichment in RVS and MKTTn) and the requested method (Annex D of ISO 6579: with selective enrichment on MSRV) were used by 30 laboratories. One laboratory (lab code 2), a non-EU-MS, did not use MKTTn. Four laboratories (lab codes 7, 9, 15 and 26) used RV instead of the prescribed RVS but this did not influence their results: they found all *Salmonella* containing samples correctly positive.
The EURL-*Salmonella* requested all participants to use MSRV in addition to the prescribed method for food and feed matrices. Although the scope of Annex D of ISO 6579 is detection of *Salmonella* spp. in samples of the primary production, selective enrichment medium MSRV showed, in this and in earlier comparison studies, equal or better results when compared to the results found with the prescribed selective enrichment media for food analyses (RVS and MKTTn of ISO 6579).

To determine 'good performance' per laboratory all combinations of selective enrichment media and isolation media used by each laboratory were taken into account. Twenty-eight out of 31 laboratories scored a 'good performance'. Three laboratories (4, 9 and 25) tested two blank samples in combination with *Salmonella* negative minced meat positive for *Salmonella*. Finding more than one blank meat sample positive is not very likely and may have been caused by cross-contamination or by misinterpretation of the results. The laboratories who found more than one blank sample positive during the study were advised to check their procedures. Laboratory 4 (non-EU-MS) concluded after tracing back the raw data that they made an initial transcription error and their results were indicated as a moderate performance. In earlier studies this laboratory had also shown some deviations (moderate performances or performance just within the lines of good performance). However, follow-up studies were never deemed necessary albeit that this laboratory is regularly performing on the edge of good performance.
The laboratories 9 and 25 were asked to perform some additional tests to find the possible source of a cross contamination.
Laboratory 25 mentioned that the circumstances in their laboratory are not optimal because the detection of *Salmonella* is not their routine activity. They required a new pipette for the inoculation from BPW which was not yet available at the date of the study. As they did not store the isolates obtained during the study, laboratory 25 was not able to perform additional tests.
Laboratory 9 performed additional tests by serotyping and phagetyping on the false positive samples and found two different phagetypes in the different

samples. It is most likely that cross-contamination did not only occur with the samples of the interlaboratory study, but also with the routine samples.
Both laboratories (lab code 9 and 25) participated in a follow-up study and scored all samples correctly. Herewith they reached the desired level of good performance.

Specificity, sensitivity and accuracy rates
The rates of the control samples showed the most optimal score: all rates were 100%.
The rates of the artificially contaminated samples were also high and were for all samples at least 97%. This also confirmed the, in general, good performance of the laboratories.

Media
Deviations in media compositions or incubation temperatures were reported but no effects were found on the results.
The increase in the number of positive results after 48 hours of incubation of the selective enrichment media was only small when compared to 24 hours of incubation: 2-3% for MSRV and RVS; 3-4% for MKTTn.

The choice of the plating-out medium does not seem to have a large effect on the number of positive isolations. When MKTTn is used for selective enrichment medium, XLD gave 3% more positive results than other plating-out media. For MSRV and RVS the difference between XLD and another plating-out medium was only 1%.

PCR
Six laboratories used a PCR technique additional to the prescribed and requested methods. The results found with the PCR methods were comparable to the results found with the bacteriological detection methods.

Evaluation of this study
The chosen matrix in this study, minced meat, contained comparable background flora as the matrices used in earlier food, feed and veterinary studies. In earlier studies combinations of different reference materials were used, containing different levels of *S*. Typhimurium and *S*. Enteritidis. From each study it was concluded that *S*. Typhimurium was easier to detect from the capsules than *S*. Enteritidis. The use of only STM capsules in the current food study may have positively influenced the outcome of this study.
During the workshop of 2010 it was discussed with the NRLs to adopt the EURL interlaboratory comparison studies more to the normal routine procedures (Mooijman, 2010). In the current study the first adaptation was made by increasing the amount of matrix per sample from 10 grams to 25 grams. The next amendment necessary is to adopt the handling of the ringtrial samples. With the current reference materials this latter is not possible, as the capsules first need to be fully reconstituted (45 min. 37 ºC) before the matrix can be added to the BPW. It is therefore considered to change to other reference materials in future studies.

6 Conclusions

- Thirty participants achieved the level of 'good performance' for the detection of *Salmonella* in minced meat. Two laboratories needed a follow-up study to reach the desired level. One laboratory scored a 'moderate performance'.

- The accuracy, specificity and sensitivity rates for the control samples (without matrix) found after selective enrichment in RVS, MKTTn and on MSRV were 100%.

- The specificity rates of the minced meat samples artificially 'contaminated' with blank capsules was 98% for RVS and 100% for MKTTn and MSRV.

- For all artificially contaminated minced meat samples with *Salmonella*, the rates found with MSRV were higher than the rates of MKTTn and RVS.

- The sensitivity rates for artificially contaminated minced meat samples with *Salmonella* were 97-100% after selective enrichment in RVS, MKTTn and on MSRV.

- The accuracy rates for the artificially contaminated minced meat samples were 99% for MKTTn and MSRV and 98% for RVS.

- The number of positive isolations is more influenced by the choice of the selective enrichment medium than by the choice of the plating-out medium.

- A longer incubation time than 24 hours gives only 2% more positive results after 48 hours for the selective enrichment MKTTn.

- RVS, MKTTn and MSRV are good selective enrichment media for the detection of *Salmonella* Typhimurium in the matrix used (minced pork/beef).

List of abbreviations

BGA (mod)	Brilliant Green Agar (modified)
BPLSA	Brilliant Green Phenol-Red Lactose Sucrose Agar
BPW	Buffered Peptone Water
BSA	Brilliance Salmonella Agar
cfp	colony forming particles
CRL	Community Reference Laboratory (new name EURL)
dPCA	double concentrated Plate Count Agar
dVRBG	double concentrated Violet Red Bile Glucose agar
EFTA	European Free Trade Association
EU	European Union
EURL	European Union Reference Laboratory
Gal	Galactosidase
hcmp	highly contaminated milk powder
ISO	International Standardisation Organisation
LDC	Lysine DeCarboxylase
MKTTn	Mueller Kauffmann TetraThionate novobiocin
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 Milieu (Dutch National Institute for Public Health and the Environment)
RM	Reference Material
RV(S)	Rappaport Vassiliadis (Soya) broth
SM2	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 History of EURL-*Salmonella* interlaboratory comparison studies on the detection of *Salmonella*

*Table A1.1 History of EURL-*Salmonella *interlaboratory comparison studies on detection of* Salmonella *in veterinary samples. In the studies the RM existed of gelatine capsules containing artificially contaminated milk powder.*

Study Year Reference [1]	Number of samples	RM	Actual number of cfp/RM	Matrix amount | type		Selective enrichment medium	Plating-out medium
I 1995 Voogt et al., 1996 RIVM report 284500003	26 4	STM5 Blank	6 0	No No		RV and SC	BGA and own
II 1996 Voogt et al., 1997 RIVM report 284500007	15 15 2 1 1	STM100 STM1000 SPan5 STM100 Blank	116 930 5 116 0	1 gram 1 gram No No No	Chicken faeces mixed with glycerol[2]	RV, SC and own	BGA and own
III 1998 Raes et al, 1998 RIVM report 284500011	14 14 7 14 4 2 5	STM10 STM100 STM100 SE100 STM10 SPan5 Blank	11 94 94 95 11 5 0	1 gram 1 gram 1 gram* 1 gram No No No	Chicken faeces mixed with glycerol[2]	RV and own	BGA and own
IV 1999 Raes et al, 2000 RIVM report 284500014	5 5 5 5 5 3 3 2 2	STM10 STM100 SE100 SE500 Blank STM10 SE100 SPan5 Blank	4 210 60 220 0 5 60 5 0	10 gram 10 gram 10 gram 10 gram 10 gram No No No No	Chicken faeces mixed with glycerol[2]	RV or RVS, MSRV and own	BGA and own
V 2000 Raes et al, 2001 RIVM report 284500018	5 5 5 5 5 3 3 2 2 20	STM10 STM100 SE100 SE500 Blank STM10 SE100 SPan5 Blank None	4 47 63 450 0 4 63 5 0 -	10 gram 10 gram 10 gram 10 gram 10 gram No No No No 25 gram**	Chicken faeces mixed with glycerol[2]	RV or RVS, MSRV and own	BGA and XLD

Table A1.1 (continued)

Study Year Reference [1]	Number of samples	RM	Actual number of cfp/RM	Matrix amount	Matrix type	Selective enrichment medium	Plating-out medium
VI	5	STM10	11	10 gram	Chicken	RVS,	BGA,
2002	5	STM100	139	10 gram	faeces	MSRV,	XLD
	5	SE100	92	10 gram	mixed	MKTTn	and own
	5	SE500	389	10 gram	with	and own	
	5	Blank	0	10 gram	glycerol[2]		
Korver et al.,	3	STM10	11	No			
2003	3	SE100	92	No			
RIVM report	2	SPan5	5	No			
330300001	2	Blank	0	No			
	20	None	-	25 gram**			
VII	5	STM10	12	10 gram	Chicken	RVS,	BGA,
2003	5	STM100	96	10 gram	faeces	MSRV,	XLD
	5	SE100	127	10 gram	mixed	MKTTn	and own
	5	SE500	595	10 gram	with	and own	
	5	Blank	0	10 gram	glycerol[2]		
Korver et al.,	3	STM10	12	No			
2005	3	SE100	127	No			
RIVM report	2	SPan5	9	No			
330300004	2	Blank	0	No			
	20	None	-	10 gram**			
VIII	7	STM10	13	10 gram	Chicken	MSRV	XLD
2004	4	STM100	81	10 gram	faeces	and own	and own
	7	SE100	74	10 gram	mixed		
	4	SE500	434	10 gram	with		
	3	Blank	0	10 gram	glycerol[2]		
	3	STM10	13	No			
Korver et al.,	2	SE100	74	No			
2005	1	SE500	434	No			
RIVM report	2	SPan5	7	No			
330300008	2	Blank	0	No			
	20	None	-	10 gram**			
IX	5	STM10	9	10 gram	Chicken	MSRV	XLD
2005	5	STM100	86	10 gram	faeces[3]	and own	and own
	5	SE100	122	10 gram			
	5	SE500	441	10 gram			
	5	Blank	0	10 gram			
	3	STM10	9	No			
Berk et al.,	2	SE100	86	No			
2006	1	SE500	441	No			
RIVM report	2	SPan5	7	No			
330300011	2	Blank	0	No			
	10	None	-	10 gram***			

Table A1.1 (continued)

Study Year Reference [1]	Number of samples	RM	Actual number of cfp/RM	Matrix amount	 type	Selective enrichment medium	Plating-out medium
X	5	STM10	9	10 gram	Pig	MSRV	XLD
2006	5	STM100	98	10 gram	faeces[3]	and own	and own
	5	SE100	74	10 gram			
	5	SE500	519	10 gram			
	5	Blank	0	10 gram			
Kuijpers et al.,	3	STM10	9	No			
2007	2	SE100	98	No			
RIVM Report	1	SE500	519	No			
330604004	2	SPan5	5	No			
	2	Blank	0	No			
XI	5	STM5	6	10 gram	Chicken	MSRV	XLD
2008	5	STM50	47	10 gram	faeces[3]	and own	and own
	5	SE10	9	10 gram			
	5	SE100	90	10 gram			
	5	Blank	0	10 gram			
Kuijpers et al.,	3	STM5	6	No			
2008	2	SE10	9	No			
RIVM Report	1	SE100	90	No			
330604011	2	SPan5	5	No			
	2	Blank	0	No			
XII	5	STM5	6	10 gram	Chicken	MSRV	XLD
2009	5	STM50	53	10 gram	faeces[3]	and own	and own
	5	SE20	18	10 gram			
	5	SE100	84	10 gram			
	5	Blank	0	10 gram			
Kuijpers et al.,	3	STM5	6	No			
2009	2	SE20	18	No			
RIVM Report	1	SE100	84	No			
330604014	2	SPan5	7	No			
	2	Blank	0	No			
XIII	5	STM5	5	10 gram	Chicken	MSRV	XLD
2010	5	STM50	56	10 gram	faeces[3]	and own	and own
	5	SE20	13	10 gram			
	5	SE100	78	10 gram			
	5	Blank	0	10 gram			
Kuijpers et al.,	4	SE20	22	10 gram*			
2010	2	STM5	8	No			
RIVM Report	2	SE20	13	No			
330604018	1	SE100	78	No			
	1	Blank	0	No			

[1]The report of each study can be obtained through the corresponding author of this report or can be found at the EURL-*Salmonella* website: http://www.rivm.nl/crlsalmonella/publication/

[2] Faeces mixed (1:1) with a solution of peptone/glycerol. Final concentration glycerol in the faeces mixture was 15%(v/v)

[3] Faeces not mixed with any preservation medium

* = with antibiotics

** = Naturally contaminated chicken faeces with *Salmonella*

*** = Naturally contaminated dust with *Salmonella*

*Table A1.2 EURL-*Salmonella *interlaboratory comparison study on the detection of* Salmonella *in food samples. In the studies the RM existed of gelatine capsules containing artificially contaminated milk powder.*

Study Year Reference [1]	Number of samples	RM	Actual number of cfp/RM	Matrix amount	type	Selective enrichment medium	Plating-out medium
I	5	STM10	9	10 gram	Minced beef	RVS,	XLD
2006	5	STM100	98	10 gram		MKTTn,	and own
	5	SE100	74	10 gram		MSRV	
	5	SE500	519	10 gram		and own	
	5	Blank	0	10 gram			
Kuijpers et al.,	3	STM10	9	No			
2007	2	SE100	98	No			
RIVM Report	1	SE500	519	No			
330604003	2	SPan5	5	No			
	2	Blank	0	No			
II	5	STM5	4	10 gram	Minced beef	RVS,	XLD
2007	5	STM50	40	10 gram		MKTTn,	and own
	5	SE10	7	10 gram		MSRV	
	5	SE100	71	10 gram		and own	
	5	Blank	0	10 gram			
Kuijpers et al.,	3	STM5	4	No			
2008	2	SE10	7	No			
RIVM Report	1	SE100	71	No			
330604010	2	SPan5	7	No			
	2	Blank	0	No			
III	5	STM5	6	10 gram	Minced	RVS,	XLD
2009	5	STM50	54	10 gram	chicken meat	MKTTn,	and own
	5	SE20	12	10 gram		MSRV	
	5	SE100	50	10 gram		and own	
	5	Blank	0	10 gram			
Kuijpers et al.,	3	STM5	6	No			
2010	2	SE20	12	No			
RIVM Report	1	SE100	50	No			
330604017	2	SPan5	6	No			
	2	Blank	0	No			
IV	8	STM5	6	25 gram	Minced	RVS,	XLD
2010	8	STM50	55	25 gram	pork/beef	MKTTn,	and own
	8	Blank	0	25 gram	meat	MSRV	
	3	STM5	6	No		and own	
This report	1	STM50	55	No			
	1	Blank	0	No			

[1]The report of each study can be obtained through the corresponding author of this report or can be found at the EURL-*Salmonella* website: http://www.rivm.nl/crlsalmonella/publication/

*Table A1.3 EURL-*Salmonella *interlaboratory comparison study on the detection of* Salmonella *in animal feed samples. In the studies the RM existed of gelatine capsules containing artificially contaminated milk powder.*

Study Year Reference [1]	Number of samples	RM	Actual number of cfp/capsule	Matrix amount	type	Selective enrich-ment medium	Plating-out medium
I	5	STM5	5	25 gram	Chicken feed	RVS,	XLD
2008	5	STM50	43	25 gram	(mixed grains)	MKTTn,	and own
	5	SE20	15	25 gram		MSRV	
	5	SE100	48	25 gram		and own	
	5	Blank	0	25 gram			
Kuijpers et al.,	3	STM5	5	No			
2009	2	SE20	15	No			
RIVM Report	1	SE100	48	No			
330604012	2	SPan5	5	No			
	2	Blank	0	No			

[1]The report of each study can be obtained through the corresponding author of this report or can be found at the EURL-*Salmonella* website: http://www.rivm.nl/crlsalmonella/publication/

Annex 2 Calculation of T_2

The variation between capsules of one batch of reference materials is calculated by means of the so-called T_2 statistic (Heisterkamp et al., 1993).*

$$T_2 = \sum_i [(z_i - z_+ / I)^2 / (z_+ / I)]$$

where z_i = count of one capsule (*i*)
z_+ = sum of counts of all capsules
I = total number of capsules analysed

In case of a Poisson distribution, T_2 follows a χ^2 -distribution with (I-1) degrees of freedom. In this case, the expected T_2-value is the same as the number of degrees of freedom and thus $T_2/(I-1)$ is expected to be equal to one. For the variation between capsules of one batch, the Poisson distribution is the theoretical smallest possible variation which could be achieved. However, over-dispersion is expected and $T_2/(I-1)$ will mostly be larger than 1 (Heisterkamp et al., 1993). An acceptable variation for a batch of capsules will be $T_2/(I-1) \leq 2$.

*Heisterkamp SH, Hoekstra JA, van Strijp-Lockefeer NGWM, Havelaar A, Mooijman KA, In 't Veld PH, Notermans SHW, 1993. Statistical analysis of certification trials for microbiological reference materials. Commission of European Communities, Community Bureau of Reference, Brussels, Luxembourg. EUR Report; EUR 15008 EN.

Annex 3 Information on the media used

RV (Oxoid CM 0699, Hampshire, United Kingdom) (Biolife 4019812, Milan, Italy)
Vassiliadis P., Pateraki E., Papaiconomou N., Papadakis J.A. and Trichopoulos D. 1976 Annales de Microbiologie (Institut Pasteur) 127B. 195-200.
Composition of RV medium: the concentration of the compounds in g/L water: Soya Peptone 5, Sodium Chloride 8, Potassium dihydrogen phosphate 1.6, Magnesium Chloride 40, Malachiet green 0.04 pH 5.2- 5.4

BGA modified (Oxoid CM 0329; Hampshire, United Kingdom) (BPLS, Merck 1.10747, Darmstadt, Germany) (Biomark B439) (Lab M, lab 34 Bury, United Kingdom) (HImedia Laboratories M971, Mumbai, India) (Scharlau 01-309, Barcelona, Spain) (SIFIN TN 1110, Berlin, Germany) (AES CHEMUNEX, AEB 521500, Cranbury, USA)
Watson and Walker 1978 A modification of brilliant green agar for improved isolation of *Salmonella*. J. Appl.Bact. 45 195-204
Composition of BGA modified: Edel and Kampelmacher; according ISO 6579, 1993

BGA (Conda laboratories 136600, Madrid, Spain)
Composition of BGA medium: the concentration of the compounds in g/L water: Yeast extract 3, Tryptone 5, Peptic digest of animal tissue 5, Lactose 10, Saccharose 10, Sodium chloride 5, Phenol red 0.08, Sulfadiazine 0.08, Agar 20, pH 7.4

BGA (Oxoid CM 0263, Hampshire, United Kingdom)
Composition of BGA medium: the concentration of the compounds in g/L water: Proteose peptone 10, Yeast extract 3, Lactose 10, Sucrose 10, Sodium chloride 5, Phenol red 0.08, Brilliant green 0.0125, Agar 12, pH 6.8-7.0

Blaskal Bromthymol blue agar
Composition of Blaskal medium: the concentration of the compounds in g/L water: Beef Heart Infusion 10, Tryptose 10, Sodium Chloride 5, Agar 15, Laktose-Saccarose solution 60 ml (60 ml contains : Lactose 10, Saccarose 10, Sodium thisulfate.5H2O 4, Bromthymolblue 0.2% (40 ml), Crystal violet 0.1% (5 ml)) pH 7.5

BPLSA (Merck 107237.0500, Darmstadt, Germany)
Adam D., Zusatz von Natriumdesoxycholat zum Brilliantgrün-Phenolrot-Agar nach Kristensen-Kauffmann zur Hemmung des Schwärmvermögens von Proteuskeimen, 1966 Ärztl. Lab. 12, 245.
Composition of BPLSA medium: the concentration of the compounds in g/L water: Peptone from meat 5, Peptone from casein 5, Meat extract 5, Sodium chloride 3, di-sodium hydrogen phosphate 2, Lactose 10, Sucrose 10, Phenol red 0.08, brilliant green 0.0125, Agar agar 12, pH 7

Brilliance *Salmonella* Agar BSA (previous OSCM) (Oxoid CM 1092; PO 5098A, Hampshire, United Kingdom)
Schönenbrücher V, Mallinson ET, Bülte M. A comparison of standard cultural methods for the detection of foodborne *Salmonella* species including three new chromogenic plating media. Int J Food Microbiol. 2008 Mar 31;123(1-2):61-6.
Composition of BSA agar : the concentration of the compounds in g/L water: *Salmonella* Growth mix 14, Chromogen mix 25, Agar 15, Cefsulodin 0.012, novobiocin 0.05, pH 7.2

Compass *Salmonella*: (Biokar Diagnostics BM 06608, Beauvais, France)
Perez JM et al., Comparison of four chromogenic media and Hektoen agar for detection and presumptive identification of *Salmonella* strains in human stools, J Clin. Microbiol., 2003 Mar., 41(3), 1130 – 4.
Composition of Compass agar: the concentration of the compounds in g/L water: Pepton 10, Sodium chloride 5, Phosphate Buffer 7, Inhibitory agents 9, Chromogenic mixture 1.4, Bacteriological agar 15 pH 7.

Rambach (Merck 107500.0002, Darmstadt, Germany)
Rambach, A.: New Plate Medium far Facilitated Differentiation of *Salmonella* spp. from Proteus sac. and Other Enteric Bacteria». - Appl. Environm. Microbiol., 56; 301-303 (1990).
Composition of Rambach medium: the concentration of the compounds in g/L water: Peptone 8, NaCl 5, Sodium deoxycholate 1.0, Chromogenic mix 1.5, Propylene glycol 10.5, Agar-agar 15, Rambach agar supplement 10 ml, pH 7.1-7.3

Rapid *Salmonella* (RS) agar (Biorad 356-4705, Marnes-La-Coquette, France)
Lauer W & Martinez F. 2009. RAPID'*Salmonella*TM Chromogenic Medium. Journal of AOAC Int. Vol. 92, No 6: 1871-1875
Composition of Rapid Salmonella agar: the concentration of the compounds in g/L water: Casein Peptone 5, Meat extract 5, Selective agents 14, Chromogenic mixture 0.31, Agar 12.7, pH 7.2

SM(ID)2 = Chrom ID (bioMérieux SM2 43621, Marcy l' Etoile, France)
Pignato, S., G. Giammanco, and G. Giammanco. 1995 Rambach agar and SM-ID medium sensitivity for presumptive identification of *Salmonella* subspecies I to VI. J. Med. Microbiol., Vol 43, Issue 1, 68-71
Composition of SM ID2 medium: the concentration of the compounds in g/L water: Peptones (swine and bovine) 6.3, Tris 0.2, Lactose 6, Ox bile (bovine and swine) 1.5, Chromogenic mix 9.6, Sodium chloride 5, Selective mix 0.03, Agar 14 pH 6.7- 7.3

XLT4 (Oxoid CM 1061, Hampshire, United Kingdom
Miller, R.G., C.R. Tate. 1990. XLT4: A highly selective plating medium for the isolation of *Salmonella*. The Maryland Poultryman, April: 2-7 (1990).
Composition of XLT4 medium: the concentration of the compounds in g/L water: Peptone 1.6, Yeast extract 3, L-Lysine 5, Lactose 7.5, Saccharose 7.5, Xylose 3.75, Sodium Chloride 5, Sodium Thiosulphate 6.8, Ferric Ammonium Citrate 0.8, 7-ethyl-2 methyl-4-undecanol hydrogen (Tergitol 4) 4.6 ml, Phenol Red 0.08, Agar 18 pH 7.4

Annex 4 Protocol

INTERLABORATORY COMPARISON STUDY ON THE DETECTION OF *SALMONELLA* spp. IN FOOD organised by CRL-*Salmonella*

FOOD STUDY IV - 2010

Introduction

This protocol describes the procedures for the fourth interlaboratory comparison study on the detection of *Salmonella* spp. in a food matrix amongst the National Reference Laboratories (NRLs for *Salmonella*) in the EU. This study will have a comparable set-up as the earlier studies (food, veterinary and feed) on the detection of *Salmonella* spp. The prescribed method is the procedure as described in ISO 6579 (Microbiology of food and feeding stuffs – Horizontal method for the detection of *Salmonella* spp. Fourth edition, 2002.) Beside ISO 6579 it is requested also to use Annex D of ISO 6579 (EN-ISO 6579:2002/Amd1: 2007: Amendment 1: Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage). The method in this annex is especially intended for the detection of *Salmonella* spp. in animal faeces and environmental samples from the primary production stage, but is also applicable for the analyses of food samples. Furthermore laboratories who are interested can also perform PCR on the samples and/or use additional methods (routinely) used in their laboratories.

The samples will consist of minced meat (*Salmonella* negative) artificially contaminated with reference materials. The reference materials (RMs) consist of gelatine capsules containing sublethally injured *Salmonella* strains at different contamination levels. Each laboratory will examine 24 meat samples of 25 g each. This is different from earlier studies in which samples of 10 g meat have been analysed.

The samples will be packed in 2 plastic containers in one large box together with cooling elements. One container will contain the capsules the other will contain the minced meat. The container with the capsules will also contain a temperature recorder to measure the temperature during transport of the samples. The recorder will be packed in a plastic bag, which will also contain your lab code. You are urgently requested to return this complete plastic bag with recorder and lab code to the CRL-*Salmonella*, immediately after receipt of the parcel. For this purpose a return envelope with a preprinted address label of the CRL-*Salmonella* has been included. Do not forget to note your lab code before returning it to the CRL.
Each box with samples will be sent as biological substance category B (UN3373) by door-to-door courier service. **Please contact the CRL-*Salmonella* when the parcel has not arrived at your laboratory on 23th of September 2010** (this is 4 working days after the day of mailing).

Objective

The main objective of the interlaboratory comparison study is to evaluate the performance of the NRLs for *Salmonella* for their ability to detect *Salmonella* spp. at different contamination levels in a food matrix using different methods.

Outline of the study

Each participant will receive (in week 38 of 2010) one box containing 2 biopacks, packed with cooling elements.

The containers contain:

Container 1:
one plastic bag with 35 numbered vials each containing one capsule with or without *Salmonella*
-24 vials numbered 1-24;
-5 vials numbered C1-C5;
This container will also contain the small electronic temperature recorder in a plastic bag with your lab code. **This recorder (in the plastic bag) should be returned to the CRL-*Salmonella* as soon as possible.**
Store container 1 at (-20 ± 5) ºC immediately after receipt.

Container 2:
One plastic bag with approximately 750 g of minced meat (free from *Salmonella*).
Store container 2 at (5 ± 3) ºC immediately after receipt.

The performance of the study will be in week 39 (starting on 27 September 2010).

The documents necessary for performing the study are:

- Protocol Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in food IV (2010) (this document);
- SOP Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in food IV (2010);
- Test report Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in food IV (2010);
- ISO 6579 (2002). Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.;
- Amendment ISO 6579:2002/Amd 1: 2007 Amendment 1 Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage.

The media used for the collaborative study will not be supplied by the CRL.

All data have to be reported in the test report and sent to the CRL-*Salmonella* **before 15 October 2010.** The CRL will prepare a summary report soon after the study to inform all NRLs on their own results and on the overall results. **Results which will be received after the deadline can not be used in the analyses for the interim summary report.**

If you have questions or remarks about the interlaboratory comparison study please contact:

Angelina Kuijpers (Tel. number: + 31 30 274 2093) or
Kirsten Mooijman (Tel. number: + 31 30 274 3537)
RIVM / LZO (internal Pb 63)
P.O. Box 1
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Kirsten.Mooijman@rivm.nl

Time table of interlaboratory comparison study FOOD IV (2010)

Week	Date	Topic
36	6 -10 September	Mailing of the protocol, Standard Operating Procedure and test report to the NRLs-*Salmonella*
38	20 -24 September	Mailing of the parcels to the NRLs as Biological Substance Category B (UN3373) by door-to-door courier service. Immediately after arrival of the parcels at the laboratory: - Check for any serious damages (**do not accept damaged packages)**; - Check for completeness; - Remove the **electronic temperature** recorder from the parcel (leave it in the plastic bag with lab code) and return it to **CRL-*Salmonella*** using the return envelope; - **Store the capsules at -20 ± 5 °C** - **Store the meat at +5 ± 3 °C** **If you did not receive the parcel at 23 September, do contact the CRL immediately.** Preparation of: 1. Non selective pre-enrichment medium (see SOP 6.1) 2. Selective enrichment media (see SOP 6.2) 3. Solid selective plating media (see SOP 6.3) 4. Confirmation media (see SOP 6.4)
39	27 September – 1 October	Performance of the study, following the instructions as given in the protocol and the SOP of study Food IV (2010).
41	Before 15 October	Completion of the test report. Send the test report by e-mail to the CRL *Salmonella* Angelina.kuijpers@rivm.nl *.
44	17 - 22 October	Checking the results by the National Reference Laboratories.
	December 2010	Sending of the final results to the NRLs together with an interim summary report. A follow-up will be discussed with NRLs who showed no good performance, according to pre-defined criteria.

* If the test report is e-mailed to the CRL it is not necessary to sent the original test report as well, unless it is not legible (to be indicated by CRL-*Salmonella*)

Annex 5 Standard Operating Procedure (SOP)

INTERLABORATORY COMPARISON STUDY ON THE DETECTION OF *SALMONELLA* spp. IN FOOD
organised by CRL-*Salmonella*
FOOD STUDY IV- 2010

1 Scope and field of application

This standard operating procedure (SOP) describes the procedure for the detection of *Salmonella* in the presence of competitive micro-organisms in a food matrix. For this purpose Reference Materials (RMs), containing sublethally injured *Salmonella* spp., as prepared by the Community Reference Laboratory (CRL) for *Salmonella,* are used. As matrix, minced meat (negative for *Salmonella*) is used. The application of this SOP is limited to the interlaboratory comparison study for *Salmonella* described in this SOP.

2 References

International Standard – ISO 6579: 2002(E)
Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

Amendment ISO 6579:2002/Amd 1 2007. Amendment 1 Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage.

3 Definitions

For the purpose of this SOP, the following definitions apply:

- *Salmonella*: micro-organisms which form typical colonies on isolation media for *Salmonella* and which display the serological and/or biochemical reactions described when tests are carried out in accordance with this SOP.
- *Reference Material*: a gelatine capsule containing a quantified amount of a test organism in spray dried milk.

4 Principle

The detection of *Salmonella* involves the following stages:
a) Pre-enrichment
b) Selective enrichment
c) Isolation
d) Confirmation of typical colonies as *Salmonella*.

5 List of abbreviations

BPW	Buffered Peptone Water
MKTTn	Muller Kaufmann Tetrathionate novobiocin broth
MSRV	Modified semi-solid Rappaport Vassiliadis medium
RM	Reference Material
RVS	Rappaport Vassiliadis medium with Soya
SOP	Standard Operating Procedure
XLD	Xylose Lysine Deoxycholate agar

6 Methods and culture media

For this study the prescribed method is ISO 6579, with an extra incubation step of 24 h of the selective enrichment media. Additional to ISO 6579 it is requested also to apply Annex D of ISO 6579.

Non selective pre-enrichment medium	BPW (6.1)
Selective enrichment medium	MKTTn & RVS (prescribed)(6.2)
	MSRV (requested) (6.2)
Selective plating medium for first and second isolation	XLD and a second medium for choice (obligatory!)(6.3)
Confirmation media	see 6.4

Composition and preparation of the media and reagents are described in Annex B, and in Annex D of the ISO 6579: 2002(E). In the list of media given in 6.1 up to 6.4, reference is made to the relevant part of ISO 6579. Complete ready-to-use media or dehydrated media are also allowed to be used, as long as the composition is in accordance with the information given below. Control the quality of the media before use.

Beside the prescribed method (ISO 6579) and requested method (Annex D of ISO 6579) it is allowed to use other methods, e.g. the one(s) routinely used in your laboratory [‘Own’ method(s)]. Prepare media for the ‘own’ method(s) according to the relevant instructions. Record all relevant information in the test report.

6.1 Non selective pre-enrichment medium

- Buffered Peptone water (BPW) (ISO6579 Annex B.1)
  Distribute the BPW in portions of **225 ml** into suitable flasks before sterilisation.

6.2 Selective enrichment medium

- Rappaport Vassiliadis medium with soya (RVS broth) (ISO6579 Annex B.2);
- Muller Kauffmann tetrathionate-novobiocin broth (MKTTn) (ISO6579 Annex B.3);
- Modified Semi solid Rappaport Vassiliadis (MSRV) (requested) (ISO6579 Annex D);
- Own selective enrichment medium used in your laboratory (optionally).

6.3 Solid selective media for first and second isolation

- Xylose-Lysine-Desoxycholate (140 mm and 90 mm plates) (ISO6579 Annex B.4);
- Second isolation medium for choice (obligatory);
- Own medium used in your laboratory (optionally).

6.4 Confirmation media

- Nutrient agar (optionally) (ISO6579Annex B.5);
- Biochemical confirmation as described in ISO 6579 Annex B.6-B.11 or by reliable, commercial available identification kits.

7 Apparatus and glassware

The usual used microbiological laboratory equipment. If requested, note specifications of the apparatus and glassware on the test report.

7.1 Apparatus

- Oven (for dry sterilisation) or autoclave (for wet sterilisation);
- Water bath or incubator, capable of operating at 37 °C ± 1 °C ;
- Water bath or incubator, capable of operating at 41.5 °C ± 1 °C;
- Sterile loops of 1 µl and of 10 µl;
- pH-meter; having an accuracy of calibration of ± 0.1 pH unit at 25 °C.

7.2 Glassware

- Culture bottles or jars with nominal capacity of 300 ml;
- Culture tubes with approximate sizes: 8 mm in diameter and 160 mm in length;
- Micro-pipettes; nominal capacity 0.1 ml and 1 ml;
- Petri dishes; standard size (diameter 90 mm to 100 mm) and/or large size (diameter 140 mm).

8 Procedure

Below the prescribed and the requested method of the interlaboratory comparison study in a food matrix of CRL-*Salmonella* is described. The different steps in the procedure are also summarized in Annex A of this SOP. Beside these methods it is also allowed to use one or more own methods. Please record all relevant data in the test report. Details of the prescribed method can be found in ISO 6579. Details of the requested method can be found in Annex D of ISO 6579 (2007).

8.1 Prewarming BPW (day 0)

Label 31 jars, each containing 225 ml of BPW as follow:

- 24 jars from 1 to 24;
- 7 jars from C1 to C7 (control capsules).

Place all jars (at least) **overnight** at 37 °C (± 1 °C). Also place some extra non-labelled jars containing 225 ml of BPW at 37 °C in case some jars might have been contaminated. Record in the test report (page 2 & 3) the requested data on BPW.

8.2 Pre-enrichment (day 1)

Take the numbered vials with the *Salmonella* capsules and the control capsules out of the freezer one hour before they are added to the BPW, to allow them to equilibrate to room temperature.
Shortly before adding the capsules, take the jars with BPW from the 37 °C incubator and inspect them for visual growth. Discard infected jars.
Add to 29 labelled jars a gelatine capsule from the vial with the corresponding label number. Do not open the gelatine capsule and do not shake the BPW to dissolve the capsule more rapidly. Place the jars with the capsules in the 37 °C incubator for **45 minutes** for dissolving of the capsules. Record the temperature and time at the start and at the end of this period in the test report (page 3).
After 45 minutes add the faeces to the jars according to the following scheme:

- **Add 25 g of meat to each jar labelled 1-24 and C7;**
- **Add no meat to jars labelled C1 – C6.**

Do not shake the jars after adding the meat.

One jar is a procedure control (= C6) to which no capsule or meat is added and one jar is a negative meat control to which only 25 g meat is added (= C7). These control jars should be handled in the same way as the other jars.

Place all jars in the 37 °C (± 1 °C) incubator for 18 h ± 2 h. Record the temperature and time at the start and at the end of the incubation period and other requested data on page 3 of the test report.
If PCR is performed, fill in all requested data on page 20 & 29 of the test report.

8.3 Selective enrichment (day 2)

Allow the selective enrichment broths RVS and MKTTn (prescribes method) to equilibrate to room temperature, if they were stored at a lower temperature. Dry the surface of the MSRV plates (requested method) in a Laminair Air Flow cabinet if necessary. Record (page 4-11) the requested data of the selective enrichment broths (RVS and MKTTn), MSRV plates and own selective enrichment media (if used) in the test report.

Label 31 RVS tubes, MKTTn tubes and MSRV plates as follow:
- 24 tubes/plates from 1 to 24;
- 7 tubes/plates from C1 to C7 (control).

If other selective enrichment media are used, label them in the same way as described above.

After equilibration:
Prescribed methods:
- Transfer 0.1 ml of each BPW culture to each tube with a corresponding label containing 10 ml RVS medium. Incubate at 41.5 °C ± 1 °C for 24 h ± 3 h and later on for another 24 h ± 3 h;
- Transfer 1 ml of each BPW culture to each tube with a corresponding label containing 10 ml MKTTn medium. Incubate at 37 °C ± 1 °C for 24 h ± 3 h and later on for another 24h ± 3 h;

Requested method:
- Inoculate each MSRV plate with three drops of each BPW culture with a corresponding label. Inoculate a MSRV plate with a total volume of 0.1 ml. Incubate **(not upside down)** at 41.5 °C ± 1 °C for 24 h ± 3 h and if negative for another 24 h ± 3 h;

Optional method:
- Inoculate the routinely used selective medium/media (other than those mentioned above), with the corresponding BPW culture (note the inoculation volume of BPW used and the volume of the selective medium/media on the test report). Incubate at the temperature routinely used.

Place the jars/tubes/plates in the appropriate incubator(s)/water bath(s) and record the temperature and time for the different enrichment media at the start and at the end of the incubation period and other requested data in the test report (page 4-11).

8.4 Isolation media (first and second isolation) (day 3 and 4)

Record in the test report (page 12-17) the requested data of the isolation media used. Label two times 24 large size Petri dishes and 24 standard size Petri dishes of the isolation media from 1 to 24 and label two times 7 large size Petri dishes and 7 standard size Petri dishes from C1 to C7.

Note:
In case you do not have large dishes (140 mm) at your disposal use two standard size (90 -100 mm) dishes, one after the other, using the same loop.

First isolation after 24 h
Inoculation:

Inoculate, by means of a 10 µl loop, from MKTTn and RVS cultures the surface of isolation media in large size Petri dishes (or two standard size Petri dishes) with the corresponding label numbers. Use a 1 µl loop to inoculate from suspect MSRV plates, the surface of isolation media in one standard size Petri dish with the corresponding label number. Inoculate the isolation media in such a way that isolated colonies will be obtained.
The following isolation media will be used:

1) Xylose Lysine Desoxycholate agar (XLD)
   Place the inoculated plates with the bottom up in the incubator set at 37 °C (record temperature and time and other requested data in the test report, page 12-13).
2) Second isolation medium. Follow the instructions of the manufacturer (record temperature and time and other requested data in the test report, page 14-15).
3) Optionally: selective isolation medium/media routinely used in your laboratory. Incubate the medium/media at the temperature routinely used (record temperature and time and other requested data in the test report, page 16-17).

After incubation for 24 h ± 3 h, examine the Petri dishes for the presence of typical colonies of *Salmonella*.

Second isolation after 48 h
After a total incubation time of 48 h ± 3 h of the selective enrichment media, repeat the procedure described above (**First isolation after 24 h**). Repeat the full procedure only when the First isolation after 24 h on selective enrichment media is negative.

8.5 Confirmation of colonies from first and second isolation (day 4 and day 5)

For confirmation take from each Petri dish of each selective medium at least 1 colony considered to be typical or suspect (use only well isolated colonies). Store the plates at 5 °C ± 3 °C.
Before confirmation (see below), optionally, streak the typical colonies onto the surface of nutrient agar plates with the corresponding label numbers, in a manner which allows to develop well isolated colonies. Record on the test report (page 18) the requested data of the nutrient agar. Incubate the inoculated plates at 37 °C ± 1 °C for 24 h ± 3 h.
If the selected colony is not confirmed as *Salmonella*, test at maximum another 5 typical colonies from the original isolation medium (stored at 5 °C). Report the number of colonies tested and the number of colonies confirmed as *Salmonella* for each dish in Table 1 (isolation using RVS), Table 2 (isolation using MKTTn), Table 3 (isolation using MSRV) and Table 4 (isolation using own enrichment) on the test report pages 21-28. For the results of detection of *Salmonella* using PCR fill in Table 5 on the test report page 29.

Confirmation of identity
The identity from the colony selected above (either directly from the isolation medium, or from nutrient agar) is confirmed by means of appropriate biochemical and serological tests. Follow the instructions of ISO 6579. Note in the test report (page 19) which media/tests have been used for confirmation. The interpretation of the biochemical tests is given in Table 1 of ISO 6579:2002 on page 9. Optionally inoculate other media which are routinely used for confirmation. Record in the test report (page 19) the requested data.

Conserve one positive isolate (*Salmonella* strain) from each sample.

After the interlaboratory comparison study it may be necessary to perform some additional testing (in case of deviating results). Therefore it is requested to

conserve one *Salmonella* confirmed colony from one of the used isolation media of each of the used selective enrichment medium from the samples 1-25 and C1-C7.

Test report

The test report will contain all information that might influence the results and is not mentioned in this SOP. Some incidents or deviations from the specified procedures will also be recorded. The test report should include the name of the person in charge for the NRL, and the names of the persons who are carrying out the work. If the study was carried out by another laboratory than the NRL, please also give the details of this laboratory in the test report.

Scheme of Bacteriological Interlaboratory Comparison Study FOOD IV (2010) On detection of *Salmonella* spp. in minced meat (see Annex A)		
Day	Topic	Description
0	**Prewarming BPW**	Place at least at the end of the day sufficient jars, each containing 225 ml BPW, at 37 °C ± 1 °C.
1	**Pre-enrichment**	Add 1 capsule to 225 ml (prewarmed) BPW Do not shake Incubate 45 min. at 37 °C ± 1 °C Add 25 g minced meat to BPW Incubate 18 h ± 2 h at 37 °C ± 1 °C
2	**Selective enrichment**	0.1 ml BPW culture in 10 ml RVS, incubate at (41.5 ± 1) °C for (24 ± 3) h 1 ml BPW culture in 10 ml MKTTn, incubate at (37 ± 1) °C for (24 ± 3) h 0.1 ml BPW culture on MSRV plate, incubate at (41.5 ± 1) °C for (24 ± 3) h Own selective enrichment medi(um)(a)
3	**First isolation after 24 h**	Inoculate from RVS, MKTTn, suspect MSRV plates (24h) and own medi(um)(a): • Xylose Lysine Desoxycholate agar, incubate at (37 ± 1) °C for (24 ± 3) h • Second isolation medium* • Own selective medi(um)(a)* * incubate for specified time at the specified temperature
3	**Continue selective Enrichment**	Incubate RVS, MKTTn, MSRV and own medium another 24 (± 3) hours at the relevant temperatures
4	**Second isolation after 48 h**	If the first isolation was negative, inoculate from RVS, MKTTn, suspect MSRV plates (48 h) and Own medi(um)(a): • Xylose Lysine Desoxycholate agar, incubate at (37 ± 1) °C for (24 ± 3) h • Second isolation medium* • Own selective medi(um)(a)* * incubate for specified time at the specified temperature
4	**Confirmation of identity**	Confirm the identity of the *Salmonella* suspect colonies from the first isolation media (day 3).
5	**Confirmation of identity**	Confirm the identity of the *Salmonella* suspect colonies from the first isolation media (day 4).

Annex 6 Number of positive results of the artificially contaminated meat samples

Results of meat samples with capsules per laboratory and per selective enrichment medium in combination with the isolation medium that gives the highest number of positive isolations.

Lab code	RVS			MKTTn			MSRV		
	Blank n=8	STM5 n=8	STM50 n=8	Blank n=8	STM5 n=8	STM50 n=8	Blank n=8	STM5 n=8	STM50 n=8
Good Performance	**≤ 1**	**≥ 4**	**≥ 6**	**≤ 1**	**≥ 4**	**≥ 6**	**≤ 1**	**≥ 4**	**≥ 6**
1	0	8	8	0	8	8	0	8	8
2	0	**6**	8	**-**	-	-	0	**6**	8
3	0	8	8	0	8	8	0	8	8
4	0	**7**	8	0	**7**	8	0	**7**	8
5	0	8	8	0	8	8	0	8	8
6	0	8	8	0	8	8	0	8	8
7	0	8	8	0	8	8	0	8	8
8	0	8	8	0	8	8	0	8	8
9	**1**	8	8	**1**	8	8	**1**	8	8
10	0	8	8	0	8	8	0	8	8
11	0	**7**	8	0	**7**	8	0	**7**	8
12	0	8	8	0	8	8	0	8	8
13	0	8	8	0	8	8	0	8	8
14	0	8	8	0	8	8	0	8	8
15	0	8	8	0	8	8	0	8	8
16	0	8	8	0	8	8	0	8	8
17	0	8	8	0	8	8	0	8	8
18	0	8	8	0	8	8	0	8	8
19	0	8	8	0	8	8	0	8	8
20	0	8	8	0	8	8	0	8	8
21	**1**	8	8	0	8	8	0	8	8
22	0	8	8	0	8	8	0	8	8
23	0	8	8	0	8	8	0	8	8
24	0	8	8	0	8	8	0	8	8
25	**2**	8	8	0	8	8	0	8	8
26	0	8	8	0	8	8	0	8	8
27	0	8	8	0	8	8	0	8	8
28	0	8	8	0	8	8	0	8	8
29	0	8	8	0	8	8	0	8	8
30	0	**7**	8	0	**7**	8	0	**7**	8
31	0	8	8	0	8	8	0	8	8

Bold number: deviating result
Grey cell: result is below good performance

Annex 7 Follow up Test report

INTERLABORATORY COMPARISON STUDY ON THE DETECTION OF *SALMONELLA* spp. IN FOOD organised by CRL-*Salmonella*

FOOD STUDY IV
FOLLOW UP January 2011

Laboratory code This is the same code as in FOOD IV 2010	
Laboratory name	
Address	
Country	
Date of arrival of the parcels	…… - …….. – 2011
Start time of storage at - 20 °C (capsules) Start time of storage at + 5 °C (meat)	Date:……. Time:…… Date:……. Time:……
Parcels damaged?	☐ Yes ☐ No
Starting date testing	…… - …….. – 2011

PRE-ENRICHMENT – Buffered Peptone Water (BPW)	
Medium information BPW	
Was the composition of BPW the same as used in BRO FOOD IV 2010?	
☐ Yes	
☐ No please give more details in an annex:	
Preparation of BPW	
Date of preparation	…… - …….. – 2011
pH after preparation	……., measured at …… °C
pH at the day of use	……., measured at …… °C
Did you perform quality control of BPW?	☐ Yes ☐ No
Prewarming time and temperature of the BPW (at least overnight)	
At the start	Date: …… - …….. – 2011 time: ……... h …….. min temperature incubator: ……..…… °C
At the end	Date: …… - …….. – 2011 time: …… h …….. min temperature incubator: ……..…… °C
Incubation time and temperature for dissolving the capsules (45 min)	
At the start	Date: …… - …….. – 2011 time: ……... h …….. min temperature incubator: ……..…… °C
At the end	time: …… h …….. min temperature incubator: ……..…… °C
Incubation time and temperature for pre-enrichment (18 ± 2) hrs after adding the meat	
At the start	Date: …… - …….. – 2011 time: ……... h ……..…… min temperature incubator: ……..…… °C
At the end	Date: …… - …….. – 2011 time: ……... h ……..…… min temperature incubator: ……..…… °C

SELECTIVE ENRICHMENT - Rappaport Vassiliadis Soya medium (RVS)	
Medium information RVS	
Was the composition of RVS the same as used in BRO FOOD IV 2010?	
☐ Yes	
☐ No please give more details in an annex:	
Preparation of RVS	
Date of preparation	…… - …….. - 2011
pH after preparation	……., measured at …… °C
pH at the day of use	……., measured at …… °C
Did you perform quality control of RVS?	☐ Yes ☐ No
Incubation time and temperature for selective enrichment	
At the start of the first period (24 h)	Date: …… - ……. – 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. – 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. – 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the second period (48 h)	Date: …… - ……. – 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C

SELECTIVE ENRICHMENT - Muller Kauffmann Tetra Thionate + novobiocin (MKTTn)	
Medium information MKTTn	
Was the composition of MKTTn the same as used in BRO FOOD IV 2010?	
☐ Yes	
☐ No please give more details in an annex:	
Preparation of MKTTn	
Date of preparation	…… - …….. – 2011
pH after preparation	……., measured at …… °C
pH at the day of use	……., measured at …… °C
Did you perform quality control of MKTTn?	☐ Yes ☐ No
Incubation time and temperature for selective enrichment	
At the start of the first period (24 h)	Date: …… - ……. – 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. – 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. – 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the second period (48 h)	Date: …… - ……. – 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C

SELECTIVE ENRICHMENT - Modified Semi solid Rappaport Vassiliadis medium (MSRV)	
Medium information MSRV	
Was the composition of MSRV the same as used in BRO FOOD IV 2010?	
☐ Yes	
☐ No please give more details in an annex:	
Specific data of composition of MSRV medium. What is the concentration of novobiocin in 1000 ml water:	
Novobiocin	☐ 0.01 g/L ☐ 0.02 g/L ☐ Other: …g/L
Preparation of MSRV	
Date of preparation	…… - ……. - 2011
pH after preparation	……., measured at …… °C
pH at the day of use	……., measured at …… °C
Did you perform quality control of MSRV?	☐ Yes ☐ No
Incubation time and temperature for selective enrichment	
At the start of the first period (24 h)	Date: …… - ……. – 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. – 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. – 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the second period (48 h)	Date: …… - ……. – 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C

OWN SELECTIVE ENRICHMENT - Own Selective enrichment medium, routinely used in your laboratory (optional)
Name of medium :
Was the composition of the Own selective the same as used in BRO FOOD IV 2010?
☐ Yes ☐ No Please give more details in an annex:

FIRST AND SECOND ISOLATION - Xylose Lysine Desoxycholate medium (XLD)	
Medium information XLD	
Was the composition of XLD the same as used in BRO FOOD IV 2010?	
☐ Yes	
☐ No please give more details in an annex:	
Preparation of XLD	
Date of preparation	…… - ……. – 2011
pH after preparation	……..., measured at …… °C
pH at the day of use	……..., measured at …… °C
Did you perform quality control of XLD?	☐ Yes ☐ No
Incubation time and temperature for isolation	
At the start of the first period (24 h)	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the second period (48 h)	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C

FIRST AND SECOND ISOLATION – Second Isolation medium.	
Medium information second isolation medium:	
Name of second isolation medium:	
Was the composition of the second medium the same as used in BRO FOOD IV 2010?	
☐ Yes	
☐ No please give more details in an annex:	
Preparation of the second isolation medium	
Date of preparation	…… - ……. – 2011
pH after preparation	……..., measured at …… °C
pH at the day of use	……..., measured at …… °C
Did you perform quality control?	☐ Yes ☐ No
Incubation time and temperature for isolation	
At the start of the first period (24 h)	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. – 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the second period (48 h)	Date: …… - ……. – 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C

FIRST AND SECOND ISOLATION – Own Isolation medium routinely used in your lab. (optional)
Name of medium:
Was the composition of the Own isolation medium the same as used in BRO FOOD IV 2010?
☐ Yes ☐ No
Please give more details in an annex:

CONFIRMATION – Nutrient agar	
Did you streak the colonies on Nutrient agar before starting confirmation?	
☐ Yes If yes give further information on nutrient agar below	☐ No
Medium Nutrient agar	
Name of Nutrient agar:	
Was the composition of Nutrient agar the same as used in BRO FOOD IV 2010?	
☐ Yes	
☐ No please give more details in an annex:	
Preparation of the nutrient agar	
Date of preparation	…… - ……. – 2011
pH after preparation	……., measured at …… °C
pH at the day of use	……., measured at …… °C
Did you perform quality control of agar?	☐ Yes ☐ No

CONFIRMATION of *Salmonella* suspected colonies
What media/tests did you use for confirmation?
☐ Biochemical: ☐TSI ☐UA ☐ LDC ☐ galactosidase ☐ Voges-Proskauer (VP) ☐ Indole ☐ Identification kit name of the kit : …….. ☐ Other: …….
☐ Serotyping: ☐ O antigen ☐ H antigen ☐ Vi antigen ☐ Other: ……..
☐ Other confirmation test: …….
DETECTION BY PCR
General questions
Did you use PCR? ☐ Yes ☐ No
If yes and when different from PCR-technique used during FOOD IV BRO 2010, please give more information in an annex.

*Table 1: Results of isolation using **RVS** (dish numbers 1-10, C1-C4, C6 and C7)*

sample no.	RVS 24 hours						RVS 48 hours					
	XLD		Second isolation medium		Own isolation medium		XLD		Second isolation medium		Own isolation medium	
	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
C1												
C2												
C3												
C4												
C6												
C7												

Col [a] = **number** of colonies used for confirmation
Sal [b] = **number** of colonies confirmed as *Salmonella*

*Table 2: Results of isolation using **MKTTn** (dish numbers 1-10, C1-C4, C6 andC7*

sample no.	MKTTn 24 hours						MKTTn 48 hours					
	XLD		Second isolation medium		Own isolation medium		XLD		Second isolation medium		Own isolation medium	
	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
C1												
C2												
C3												
C4												
C6												
C7												

Col [a] = **number** of colonies used for confirmation
Sal [b] = **number** of colonies confirmed as *Salmonella*

*Table 3: Results of isolation using **MSRV** (dish numbers 1-10, C1-C4, C6 andC7)*

	MSRV 24 hours						MSRV 48 hours					
sample no.	XLD		Second isolation medium		Own isolation medium		XLD		Second isolation medium		Own isolation medium	
	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
C1												
C2												
C3												
C4												
C6												
C7												

Col [a] = **number** of colonies used for confirmation
Sal [b] = **number** of colonies confirmed as *Salmonella*

*Table 4: Results of isolation using **OWN selective enrichment** medium (dish numbers 1-10, C1-C4, C6 and C7)*

sample no.	OWN 24 hours						OWN 48 hours					
	XLD		Second isolation medium		Own isolation medium		XLD		Second isolation medium		Own isolation medium	
	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
C1												
C2												
C3												
C4												
C6												
C7												

Col [a] = **number** of colonies used for confirmation
Sal [b] = **number** of colonies confirmed as *Salmonella*

Table 5: Results of detection using PCR (dish numbers 1-10, C1-C4, C6 and C7)

sample no.	PCR + or -		
		no.	
1		C1	
2		C2	
3		C3	
4		C4	
5		C6	
6		C7	
7			
8			
9			
10			

Comment(s) on operational details that might have influenced the test results:

Name of person (s) carrying out the follow up FOOD IV interlaboratory Comparison study.	
Is the person(s) carrying out the follow up FOOD IV interlaboratory Comparison study working in the laboratory of NRL mentioned on page 1?	☐ Yes ☐ No give more information of the laboratory carrying out the study : Laboratory name ……. Address …… Is this laboratory accredited or certified for the determination of *Salmonella*. ☐ Yes ☐ No
Date and signature	

Name of person in charge of the NRL	
Date and signature	

Please send the completed test report before 31 January 2011 preferable by email to CRL-*Salmonella*. If the test report is e-mailed to the CRL it is not necessary to send the original test report as well, unless it is not legible (to be indicated by CRL-*Salmonella*).
Use the address below:

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RIVM / LZO internal Pb 63
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