National Institute
for Public Health
and the Environment

Report 330604011/2008

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EU Interlaboratory comparison study veterinary XI (2008)

Bacteriological detection of Salmonella in chicken faeces

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This investigation has been performed by order and for the account of the European Commission, Health and Consumer Protection Directorate-General and the Laboratory for Zoonoses and Environmental Microbiology (LZO) of the RIVM, within the framework of V/330604/08/CS by the Community Reference Laboratory for *Salmonella*

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Abstract

EU Interlaboratory comparison study veterinary XI (2008)
Bacteriological detection of *Salmonella* in chicken faeces

In 2008 all of the 32 National Reference Laboratories (NRLs) for *Salmonella* were able to detect high and low levels of *Salmonella* in chicken faeces. Of these, 28 NRLs achieved the level of 'good performance' at once. Two laboratories needed a follow up test. One NRL was visited by the CRL *Salmonella* staff during this follow up study and reached the desired level with an excellent performance. One laboratory also scored 'good performance' in a follow up study and their problems were most probably caused by inexperience with the prescribed method. The two other laboratories could give a plausible explanation for their deviating results and they scored well for all the other samples. Their final outcome was determined as 'moderate' and no follow up was required.

These results were found in the eleventh veterinary interlaboratory comparison study, organized by the Community Reference Laboratory (CRL) for *Salmonella*. The comparison study was conducted in March 2008, with the follow up in July and September of that year. All European Member States are obliged to participate in this study. The CRL for *Salmonella* is part of the National Institute for Public Health and the Environment (RIVM).

For this study, each laboratory received a package containing chicken faeces (free for *Salmonella*) and 35 gelatin capsules containing *Salmonella* spp. at different levels. The laboratories were instructed to spike the chicken faeces with the capsules and test the samples for the presence of *Salmonella* using the internationally prescribed method for the detection of *Salmonella* in veterinary samples.

Key words: *Salmonella*; CRL-*Salmonella*; NRL-*Salmonella*; interlaboratory comparison study; chicken faeces; *Salmonella* detection methods

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Rapport in het kort

EU Ringonderzoek veterinair-XI (2008)
Bacteriologische detectie van *Salmonella* in kippenmest

Alle 32 Nationale Referentie Laboratoria (NRL's) waren in 2008 in staat hoge en lage concentraties *Salmonella* in kippenmest aan te tonen. Hiervan behaalden 28 laboratoria direct het gewenste niveau. Twee laboratoria hadden een herkansing nodig. Bij één NRL was het CRL-*Salmonella* aanwezig tijdens de herkansing. Dit NRL behaalde het gewenste niveau met een zeer goede uitvoering. Het andere NRL behaalde tevens het gewenste niveau tijdens een herkansing, bij hen was de oorzaak van de problemen mogelijk een gebrek aan ervaring met de voorgeschreven methode. De twee overige laboratoria gaven een aanvaardbare verklaring voor het afwijkende resultaat, bovendien was de rest van de uitvoering goed. Het eindoordeel van deze twee NRLs werd vastgesteld als 'matig' en een herkansing was niet nodig.

Dit zijn de resultaten van het elfde veterinair ringonderzoek dat het Communautair Referentie Laboratorium (CRL) voor *Salmonella* heeft georganiseerd. Het onderzoek is in maart 2008 gehouden, de herkansing in juli en september van dat jaar. Europese lidstaten zijn verplicht om aan dit onderzoek deel te nemen. Het CRL-*Salmonella* is gevestigd bij het Rijksinstituut voor Volksgezondheid en Milieu (RIVM).

Voor dit ringonderzoek kreeg ieder laboratorium een pakket toegestuurd met kippenmest (vrij van *Salmonella*) en 35 gelatine capsules met melkpoeder met verschillende besmettingsniveaus van *Salmonella*. De laboratoria moesten volgens voorschrift kippenmest en capsules samenvoegen en onderzoeken op de aanwezigheid van *Salmonella*. Zij gebruikten hiervoor de internationaal voorgeschreven methode om *Salmonella* aan te tonen in dierlijk mest.

Trefwoorden: *Salmonella*; CRL-*Salmonella*; NRL-*Salmonella*; ringonderzoek; kippenmest; *Salmonella* detectie methode

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Contents

List of abbreviations

BGA (mod)	Brilliant Green Agar (modified)
BPLSA	Brilliant Green Phenol-Red Lactose Sucrose Agar
BPW	Buffered Peptone Water
BxLH	Brilliant Green, Xylose, Lysine, Sulphonamide
Cfp	colony forming particles
CRL	Community Reference Laboratory
dPCA	double concentrated Plate Count Agar
dVRBG	double concentrated Violet Red Bile Glucose agar
EU	European Union
Gal	Galactosidase
hcmp	highly contaminated milk powder
ISO	International Standardisation Organisation
LDC	Lysine Decarboxylase
MKTTn	Mueller Kauffmann Tetrathionate novobiocin broth
MLCB	Mannitol Lysine Crystal violet Brilliant green agar
MS	Member State
MSRV	Modified Semi-solid Rappaport Vassiliadis
NRL	National Reference Laboratory
PCA	Plate Count Agar
PCR	Polymerase Chain Reaction
RIVM	Rijksinstituut voor Volksgezondheid en het Milieu (National Institute for Public Health and the Environment)
RM	Reference Material
RVS	Rappaport Vassiliadis Soya broth
SE	*Salmonella* Enteritidis
SM (ID)2	*Salmonella* Detection and Identification-2
SOP	Standard Operating Procedure
SPan	*Salmonella* Panama
SS	*Salmonella Shigella* medium
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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Summary

In March 2008 the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*) organised the eleventh interlaboratory comparison study on bacteriological detection of *Salmonella* in a veterinary matrix (chicken faeces). Participants were 32 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) of the EU Member States, Norway, candidate country Former Yugoslav Republic of Macedonia (FYROM) and, on request of DG-Sanco, two third countries (non-EU) Israel and Tunesia.

The main objective of this eleventh study was to see whether the participating laboratories could detect *Salmonella* at different contamination levels in a veterinary matrix. For a better testing of the performance of the laboratories the contamination levels in this study were lower than in earlier veterinary studies. As a result of this, a new proposal for good performance was made and the performance of the laboratories was compared to this new proposal. The prescribed method was Annex D of ISO 6579, with the selective enrichment on Modified Semi-solid Rappaport Vassiliadis (MSRV). Optionally a laboratory could also use other, own media or procedures for the detection of *Salmonella*.

Thirty five individually numbered capsules had to be tested by the participants for the presence or absence of *Salmonella.* Twenty five of the capsules had to be examined in combination with each 10 gram of *Salmonella* negative chicken faeces. These 25 capsules were divided over the following groups: 5 capsules with approximately 5 colony forming particles (cfp) of *Salmonella* Typhimurium (STM5), 5 capsules containing approximately 50 cfp of *S*. Typhimurium (STM50), 5 capsules with approximately 10 cfp of *S*. Enteritidis (SE10), 5 capsules containing approximately 100 cfp of *S*. Enteritidis (SE100) and 5 blank capsules. The other 10 capsules, to which no faeces had to be added, were control samples, existing of 3 capsules STM5, 2 capsules SE10, 1 capsule SE100, 2 capsules containing approximately 5 cfp of *S.* Panama (SPan5) and 2 blank capsules.

On average the laboratories found *Salmonella* in 87% of the (contaminated) samples using the method for testing veterinary samples (MSRV).

All 32 laboratories scored the desired results with the prescribed method. Of these, 28 NRLs achieved the level of ‘good performance’ at once. Two laboratories needed a follow up test. One NRL was visited by the CRL *Salmonella* staff during this follow up study and reached the desired level with an excellent performance. One laboratory also scored ‘good performance’ in a follow up study and their problems were most probably caused by inexperience with the prescribed method. The two other laboratories could give a plausible explanation for their deviating results and they scored well for all the other samples. Their final outcome was determined as ‘moderate’ and no follow up was required.

1 Introduction

An important task of the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*), as laid down in Regulation EC No 882/2004, is the organisation of interlaboratory comparison studies. The history of the interlaboratory comparison studies as organised by CRL-*Salmonella* since 1995 is summarised in Annex 1. For a better testing of the performance of the laboratories, the contamination levels of the samples used in the studies organised since 2007 were lower than in earlier studies. The first and most important objective of the study, organized by the Community Reference Laboratory (CRL) for *Salmonella* in March 2008, was to see if the participating laboratories could detect *Salmonella* at different contamination levels in animal faeces. The second objective was to compare the different methods for the detection of *Salmonella* in animal faeces. Furthermore it is important that the examination of samples in the EU Member States is carried out uniformly and comparable results should be obtained by all National Reference Laboratories for *Salmonella* (NRL-*Salmonella*).
The prescribed method is Modified Semi-solid Rappaport Vassiliadis (MSRV) as selective enrichment medium for the detection of *Salmonella* spp. in animal faeces (Annex D of ISO 6579, Anonymous 2007).
The set-up of this study was comparable to earlier interlaboratory comparison studies on the detection of *Salmonella* spp. in veterinary and food samples. The contamination level of the low level capsules was at the detection limit of the method; the level of the high level samples was approximately
5-10 times above the detection limit. Ten control samples consisting of different reference materials, had to be tested without the addition of chicken faeces. These latter reference materials consisted of
3 capsules with approximately 5 cfp of *Salmonella* Typhimurium (STM5), 2 capsules with approximately 10 cfp of *Salmonella* Enteritidis (SE10), 1 capsule with approximately 100 cfp of *Salmonella* Enteritidis (SE100), 2 capsules with approximately 5 cfp of *Salmonella* Panama (SPan5) and 2 blank capsules. Twenty-five samples of *Salmonella* negative chicken faeces spiked with
5 different reference materials (including blank capsules) had to be examined. The different reference materials consisted of two levels of *Salmonella* Typhimurium (STM5 and STM50) and two levels of *Salmonella* Enteritidis (SE10 and SE100).

2 Participation

Country	City	Institute
Austria	Graz	Austrian Agency for Health and Food Safety (AGES) Institute for Medical Microbiology and Hygiene
Belgium	Brussels	Veterinary and Agrochemical Research Center (VAR/ CODA) Centrum voor onderzoek in diergeneeskunde en agrochemie
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	Copenhagen	National Food Institute The Technical University of Denmark Department of Microbiology and Risk Assessment
Estonia	Tartu	Estonia Veterinary and Food Laboratory, Bacteriology-Pathology Department
Finland	Kuopio	Veterinary Bacteriology Finnish Food Safety Authority Evira Research Department, Veterinary Bacteriology
France	Ploufragan	l'Agence Française de Sécurité Sanitaire des Aliments (AFSSA/ LERAPP)
Germany	Berlin	Federal Institute for Risk Assessment (BFR) National Veterinary Reference Laboratory for *Salmonella*
Greece	Halkis	Veterinary Laboratory of Halkis Hellenic Republic Ministry of rural development and food
Hungary	Budapest	Central Agricultural Office, Food and Feed Directorate Central Microbiological Laboratory
Ireland	Kildare	Central Veterinary Research Laboratory (CVRL / DAFF) Department of Agriculture, Fisheries and Food
Israel	Kiryat Malachi	Beer Tuvia Regional Poultry Diseases Laboratory
Italy	Legnaro	Istituto Zooprofilattico Sperimentale delle Venezie, OIE National Reference Laboratory for *Salmonella*
Latvia	Riga	Nationaly Diagnostic Centre (NDC) of Food and Veterinary Service
Lithuania	Vilnius	National food and veterinary risk assessment institute
Luxembourg	Luxembourg	Laboratoire de Médecine Vétérinaire de l'Etat, Animal Zoonosis
Macedonia Former Yugoslav Republic of Macedonia (FYROM)	Skopje	Food institute Faculty of veterinary medicine
Malta	Valletta	Public Health Laboratory (PHL) Evans Building
Netherlands the	Bilthoven	National Institute for Public Health and the Environment(RIVM)

Country	City	Institute
Norway	Oslo	National Veterinary Institute, Section of Bacteriology
Poland	Pulawy	National Veterinary Research Institute (NVRI) Department of Microbiology
Portugal	Lisbon	Laboratório Nacional de Investigação Veterinária (LNIV)
Romania	Bucharest	Institute for diagnosis and Animal Health, Dept .Bacteriology
Slovak Republic	Bratislava	State Veterinary and Food Institute Reference Laboratory for *Salmonella*
Slovenia	Ljubljana	National Veterinary Institute, Veterinary Faculty
Spain	Madrid Algete	Laboratorio de Sanidad Y Produccion Animal de Algete Central de Veterinaria
Sweden	Uppsala	National Veterinary Institute (SVA), Department of Bacteriology
Tunesia	Tunis	Veterinary Research Institute of Tunesia, Bacteriology
United Kingdom	Addlestone	Veterinary Laboratories Agency (VLA) Weybridge Department of Food and Environmental Safety
United Kingdom	Belfast	Agri-Food and Bioscience Institute (AFBI) Veterinary Sciences Division Bacteriology

3 Materials and methods

3.1 Reference materials

Six 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 in gelatin capsules resulting in the final reference materials (RMs).
The target levels of the six batches of RMs were:

- 5 colony forming particles (cfp) per capsule for *Salmonella* Panama (SPan5);
- 5 and 50 colony forming particles (cfp) per capsule for *Salmonella* Typhimurium (STM5 and STM50);
- 10, 20 and 100 colony forming particles (cfp) per capsule for *Salmonella* Enteritidis (SE10, SE20 and SE100).

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 ^{o}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 ^{o}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) ^{o}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) ^{o}C for (4 ± ½) h;
- after incubation, 10 ml of molten double concentrated Violet Red Bile Glucose agar (dVRBG) was added as an overlayer and after solidification the plates were incubated at (37 ± 1) ^{o}C for (20 ± 2)h.

3.2 Chicken faeces samples

3.2.1 General

Chicken faeces was sampled by the Animal Health Service (GD) Deventer at a *Salmonella* free farm (SPF-farm). A large batch of 10 kilogram from this farm arrived at the CRL-*Salmonella* on 21 January 2008. For the follow-up study 3 kilogram of faeces arrived on 19 May 2008. The faeces was stored at 5 ^{o}C and checked for the absence of *Salmonella* by testing 10 portions of 10 g chicken faeces randomly picked from the large batch. For the testing for *Salmonella* Annex D of ISO 6579 (Anonymous, 2007) was followed. For this purpose 10 portions of 10 g were each added to 90 ml Buffered Peptone Water (BPW). After pre-enrichment at (37 ± 1) ^{o}C for 16-18 h, selective enrichment was carried out on Modified Semi-solid Rappaport Vassiliadis (MSRV). Next, the suspect plates were plated-out on

Xylose Lysine Deoxycholate agar (XLD) and Brilliant Green Agar (BGA) and confirmed biochemically. The chicken faeces was stored at 5 ºC until further use.

3.2.2 Total bacterial count in chicken faeces

The total number of aerobic bacteria was investigated in the chicken faeces. The procedure of ISO 4833 (Anonymous, 2003) was followed for this purpose. Portions of 20 gram faeces were homogenized into 180 ml 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 one ml of each dilution was brought into 2 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) h and the total number of aerobic bacteria was counted after incubation.

3.2.3 Number of Enterobacteriaceae in chicken faeces

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. Portions of 20 gram faeces was homogenized into 180 ml 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 one ml of each dilution was brought into 2 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. These plates were incubated at (37 ± 1) ^{o}C for (24 ± 2) h 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 chicken faeces

On 18 February 2008 (two weeks before the study) the reference materials (35 individually numbered capsules) and 300 grams of *Salmonella* negative chicken faeces were packed with cooling devices as biological substance category B (UN 3373) and sent by door-to-door courier service to the participants. After arrival at the laboratory the capsules had to be stored at –20 ^{o}C and the faeces 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 5) and Standard Operation Procedure (Annex 6). The test report which was used during the study can be found at the CRL-*Salmonella* website: http://www.rivm.nl/crlsalmonella/prof_testing/detection_stud/ or can be obtained through the corresponding author of this report.

Ten control capsules had to be tested without faeces (numbered C1-C10). Twenty-five capsules (numbered 1 – 25) were each tested in combination with 10 grams of faeces (negative for *Salmonella*). The types and the number of capsules and faeces samples which had to be tested are shown in Table 1.

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

Capsules	Control capsules (n=10) No faeces added	Test samples (n=25) with 10 g *Salmonella* negative chicken faeces
S. Panama 5 (SPan5)	2	---
S. Enteritidis 10 (SE10)	2	5
S. Enteritidis 100 (SE100)	1	5
S. Typhimurium 5 (STM5)	3	5
S. Typhimurium 50 (STM50)	---	5
Blank	2	5

3.3.2 Sample packaging and temperature recording during shipment

The capsules and the chicken faeces were packed in 2 plastic containers firmly closed with screw caps (biopacks). Both biopacks were placed in one large shipping box, together with six frozen (-20 ^{o}C) cooling devices. Each shipping box was sent as biological substances category B (UN3373) by door-to-door courier services. 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 CRL-*Salmonella* to measure the temperature every hour. Each NRL had to return the temperature recorder, immediately after receipt of the parcel, to the CRL. At the CRL-*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 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 based methods.

In summary:

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

Selective enrichment on:
- Modified semi-solid Rappaport Vassiliadis medium (MSRV) (prescribed)
- 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, commercial 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 chicken faeces (negative for *Salmonella* spp.). The specificity, sensitivity and accuracy rates were calculated according to the following formulae:

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

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

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

3.6 Good performance

New proposal for definition of 'good performance'

During the tenth CRL-*Salmonella* workshop in April 2005 a proposal was made to define 'good performance' in interlaboratory comparison studies on detection of *Salmonella*. For a better testing of the performance of the laboratories the contamination level of STM and SE capsules in this study are lower than in earlier studies. The contamination level of the low level capsules is at the detection limit of the method; the high level samples approximately 5-10 times above the detection limit.
As a result of lowering the contamination levels, a new proposal for 'good performance' is needed.

Table 2 Proposed criteria for good performance in the Faeces-XI study (2008).

Control samples (capsules, no matrix)	Minimum result	
	Percentage positive	No. of positive samples / total No. of samples
SE100	100%	1/1
STM5	60%	2/3
Span5 and SE10	50%	1/2
Blank control capsules	0%	0/2

Samples (capsules with matrix)	Minimum result	
	Percentage positive	No. of positive samples / Total No. of samples
Blank[1]	20% at max[1]	1/5
STM50	80%	4/5
SE100	80%	4/5
STM5	50%	2-3/5
SE10	20%	1/5

1: All should be negative. However, as no 100% guarantees about the *Salmonella* negativity of the matrix can be given, 1 positive out of 5 blank samples (20% pos.) will still be considered as acceptable.

For determining good performance per laboratory the results found with MSRV together with all combinations of isolation media used by the laboratory were taken into account. For example if a laboratory found for the STM5 capsules with matrix 3/5 positive with MSRV/BGA but no positives with MSRV/XLD, this was still considered as good result. The opposite was performed for the blank capsules. Here also all combinations of isolation media used per laboratory were taken into account. If for example a laboratory found 2/5 blank capsules positive with MSRV/BGA but no positives with the other isolation media, this was still considered as a 'no-good' result.
When testing samples with a contamination level close to the detection limit it is expected that approximately 50% of the total number of tested samples will be found positive. For the *Salmonella* Enteritidis samples used in this study the detection limit was close to 10 cfp. As the mean contamination level of the low level SE reference materials was below 10 cfp/capsule (7 cfp), the demand for good performance of this type of RM added to a matrix was amended from 50% to 20% of the total number of samples to be positive.

4 Results

4.1 Reference materials

The level of contamination and the homogeneity of the final batches of capsules are presented in Tables 3A and 3B.The enumerated minimum and maximum levels within each batch of capsules are also given in the tables. 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 date of testing all batches fulfilled the pre-set-criteria as stated in section 3.1. However, at the second date of testing the mean contamination level of the SE10 capsules was decreased to the minimum level as demanded for this batch. Furthermore, the variation between capsules was increased. Because of these results, the demand for good performance with SE10 was amended (see section 3.6).
As it was expected that the contamination level of the low level SE capsules would decrease in the initial period after preparation, the contamination level of the SE20 test batch was kept at the high side. This resulted in a contamination level of the final batch close to the target level, however with a high variation between capsules. Still, the final batch of SE20 was useful for the cause of the study.

Table 3A Level of contamination and homogeneity of SE, SPan and STM capsules.

	SE10	SE100	SPan5	STM5	STM50
Final batch; Test 1					
Date testing capsules	28-11-07	19-11-07	09-01-08	13-11-07	07-11-07
Number of capsules tested	50	50	50	50	50
Mean cfp per capsule	9	90	5	6	47
Min-max cfp per capsule	3-19	63-120	1-15	1-11	29-63
T_2 / (I-1)	1.76	1.88	1.43	1.18	1.15
Final batch; Test 2					
Date testing capsules	12-03-08	12-03-08	18-03-08	13-03-08	13-03-08
Number of capsules tested	25	25	25	25	25
Mean cfp per capsule	7	91	6	5	44
Min-max cfp per capsule	0-17	65-113	2-14	2-11	30-67
T_2 / (I-1)	2.54	1.86	1.36	1.09	1.33

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

Table 3B Level of contamination and homogeneity of SE20 capsules only used in the follow up study.

	SE20
Test batch;	
Date testing capsules	23-05-08
Number of capsules tested	25
Mean cfp per capsule	28.2
Min-max cfp per capsule	17-39
T_2 / (I-1)	1.98
Final batch; Test 1	
Date testing capsules	13-06-08
Number of capsules tested	50
Mean cfp per capsule	18.92
Min-max cfp per capsule	8-49
T_2 / (I-1)	3.46

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 Chicken faeces samples

The faeces was tested negative for *Salmonella* and stored at 5 °C. On Monday 18 February 2008 the faeces was mailed to the NRLs. After receipt, the NRLs had to store the faeces at 5 °C. One NRL (labcode 20) stored the faeces at – 20 °C and the capsules at 5 °C for 19 hours.

The number of aerobic bacteria and the number of Enterobacteriaceae were tested twice; firstly at the day the faeces arrived at the CRL (22/01/2008) and secondly at the planned date (03/03/2008) of the interlaboratory comparison study. The results are shown in Table 4.

Table 4 Number of aerobic bacteria and the number of Enterobacteriaceae per gram of chicken faeces.

Date	Aerobic bacteria cfp/g	Enterobacteriaceae cfp/g
22 Januari 2008	$2.1*10^8$	$7.7*10^4$
3 March 2008	$3.3*10^8$	$3.3*10^4$

The majority of the laboratories (twenty-six) performed the study on the planned date (week 10 starting on 03/03/2008). However three laboratories (labcode 15, 19 and 26) performed the study one week earlier (25/02/2008), one laboratory (labcode 22) performed it two weeks earlier (18/02/2008) and one laboratory (labcode 1) performed the study one week later (10/03/2008). One laboratory (labcode 32) did not confirm their participation timely and their parcel was sent on 10 March to the laboratory. They performed the study 11 days later (13/03/2008).

4.3 Technical data interlaboratory comparison study

4.3.1 General

In this study 32 NRLs participated: 28 NRLs from 27 EU-Member States, 1 NRL from a European Economic Area country, 1 NRL from a EU-candidate country and, on request of DG-Sanco, 2 NRLs from third countries (non-EU).

4.3.2 Accreditation/certification

Twenty-two laboratories mentioned to be accredited for their quality system according to ISO/IEC 17025 (Anonymous, 2005) (labcodes 1, 2, 3, 4, 5, 6, 7, 10, 12, 13, 14, 15, 18, 19, 21, 22, 24, 25, 26, 29, 30 and 31). For two laboratories (labcodes 8 and 9) the accreditation is in process. Seven laboratories (labcodes 11, 16, 17, 20, 23, 27 and 28) are planning to become accredited or certified in the near future. One laboratory (labcode 32) has not (yet) planned to become accredited.

4.3.3 Transport of samples

An overview of the transport times and the temperatures during transport of the parcels is given in Table 5. The NRLs returned the temperature recorders immediately after receipt to the CRL-*Salmonella.* The majority of the laboratories received the materials within 1 day. However, the parcel of laboratory 27 was delayed at the border and finally arrived on Friday afternoon 22 February at the airport and it was stored there until Monday before it was delivered at the institute. The total transport time of this parcel was 172 hours. When this latter parcel and the two parcels from the Third countries (non-EU) are not taken into account, the average transport time was 39 hours. For the majority of the parcels the transport temperature did not exceed 5 ^{o}C. Although the parcel of laboratory 27 was delayed for 6 days, most of the time it was stored below 5 ^{o}C. For fourteen NRLs 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 at the institute the parcel arrived later at the laboratory of the NRL. The delay varied between 1 to 48 hours. In three laboratories (labcodes 8, 10 and 24) the storage temperature during the delay was at approximately 20 ^{o}C or higher. For the laboratories 8 and 24 this was only for one hour. However, in laboratory 10 the information from the temperature recorder showed a delay of 48 h at variable temperatures (11-23 ^{o}C). It was not clear whether this delay concerned the complete parcel or only the temperature recorder. For the other laboratories the storage temperature during delay was below 10 ^{o}C.

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

		Time (h) at					
Labcode	Transport time[1] total in hours	< 0 °C	0 °C - 5 °C	5 °C - 10 °C	10 °C - 15 °C	> 15 °C	Additional Storage[2]
1	45	40	5				42 hrs at – 20 °C
2	48	47				1	
3	26	26					
4	24	21	3				4 hrs < 5 °C
5	50	45	4	1			
6	71	18	53				
7	78	41	37				
8	48	26	22				1 hr between 4 °C – 24 °C
9	24	18	6				6 hrs at < 5 °C
10	27	25				2	48 hrs between11°C – 23 °C
11	48	46				2	
12	48	48					
13	25	20	5				
14	50	50					
15	71	31	40				2 hrs at 10 °C
16	24	24					
17	68	68					1 hr at 8.5 °C
18	24	21	3				
19	24	20	4				
20	45	31	14				3 hrs at < 5 °C
21	24	24					1 hr at 1 °C
22	1	1					
23	27	21	6				1 hr at 1 °C
24	25	24				1	1 hr at 24 °C
25	24	1					
26	24	18	6				
27	172	16	102	42	12		
28	51	13	37			1	
29	44	24	20				3 hrs at < 5 °C
30	24	17	7				
31	28	18	10				
32	71	33	38				5 hrs at < 5 °C
Average[3]	39						

1 = Transport time according to the courier

2 = Storage time of the samples at the institute before arriving at the laboratory of the NRL

3= Average without 2 NRLs of Third countries (non-EU) and lab 27

Table 6 Media combinations used per laboratory.

Labcode	Selective enrichment media	Plating-out Media
1	MSRV	XLD SMID2
2	MSRV MKTTn	XLD BGA
3	MSRV	XLD BGA^{MOD}
4	MSRV	XLD Rambach
5	MSRV RVS	XLD BGA^{MOD} MacConkey
6	MSRV	XLD Rambach
7	MSRV RVS	XLD BGA^{MOD}
8	MSRV	XLD BxLH
9	MSRV RVS	XLD BGA^{MOD}
10	MSRV RVS	XLD BGA^{MOD} Rambach
11	MSRV	XLD Rambach
12	MSRV MKTTn	XLD Rambach
13	MSRV RVS	XLD+novo BGA^{MOD}
14	MSRV	XLD BGA^{MOD}
15	MSRV RVS	XLD BGA^{MOD}
16	MSRV	XLD XLT4
17	MSRV	XLD BGA
18	MSRV	XLD BGA^{MOD}
19	MSRV MSRV*	XLD SM2
20	MSRV	XLD SMID2
21	MSRV RVS	XLD BGA
22	MSRV	XLD BGA^{MOD}
23	MSRV RVS	XLD MLCB BGA^{MOD}
24	MSRV	XLD BGA^{MOD}
25	MSRV	XLD Rambach
26	MSRV	XLD Rambach
27	MSRV	XLD Rambach
28	MSRV RVS	XLD BGA^{MOD}
29	MSRV	XLD Onoz
30	MSRV	XLD BGA^{MOD} Rapid *Salmonella* XLT4
31	MSRV	XLD BPLSA
32	MSRV MKTTn	XLD SS

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
* Laboratory 19 used MSRV with different concentrations of novobiocin
+ novo = with novobiocin

4.3.4 Media

Each laboratory was asked to test the samples with the prescribed (Annex D of ISO 6579) method. All laboratories used the selective enrichment medium MSRV with the plating out medium XLD and a second plating out medium of own choice. Thirteen laboratories used also another selective enrichment medium: RVS (nine laboratories), MKTTn (three laboratories) and one laboratory used also MSRV with a higher concentration of novobiocin. Four laboratories used more than two isolation media.
The media used per laboratory are shown in Table 6. Details on the media which are not described in ISO 6579 are given in Annex 3. In Tables 7-11 information is given on the composition of the media which were prescribed and on incubation temperatures and times. In these tables only the laboratories are indicated who reported deviations. Laboratories 16 and 30 did not mention the pH of the media and laboratory 17 and 25 did not mention de composition of the media used. Two laboratories incubated the selective enrichment on MSRV at deviating temperatures (labcodes 1 and 6) and two laboratories (labcode 1 and 7) used MSRV without novobiocin. Laboratory 13 used XLD with novobiocin 0.015 g/L.

A second plating-out medium for choice was obligatory. Twelve laboratories used BGA modified (ISO 6579, 1993) as a second plating-out medium and laboratory 31 used BPLSA (Merck) this is very closely related to BGA. Eight laboratories used Rambach, three laboratories used SM (ID) 2 agar and two laboratories used XLT4. The following media were used only by one laboratory: BGA, Onoz, MLCB, BxLH, Rapid *Salmonella* agar, SS medium and MacConkey.
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), MacConkey, Imuna zivny agar and Bromthymol blue lactose sucrose agar).

Table 7 Incubation time and temperature of BPW.

	Prewarming BPW		Dissolving capsules in BPW		Pre-enrichment in BPW	
Labcode	**Time (h:min)**	**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**
7	Overnight	37	45	37	21.10	37
13	Overnight	36.5	60	36.5-38.4	18.25	37.7-38.1
15	Overnight	37	45	37	24	37
32	Overnight	37	45	37	20:38	37

Grey cell : deviating times and temperatures - = no info

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

Labcode	Enzymatic digest of casein (Peptone)	Sodium Chloride (NaCl)	Disodium hydrogen Phosphate dodecahydrate* ($Na_2HPO_4.12H_2O$)	Potassium dihydrogen phosphate (KH_2PO_4)	pH
ISO 6579	**10.0**	**5.0**	**9.0**	**1.5**	**6.8 – 7.2**
9	10	5	3.5	1.5	7.3
16	10	5	3.5	1.5	-
17	-	-	-	-	7.3
18	10	5	3.5	1.5	7.3
30	10	5	3.5	1.5	-

Grey cell: deviating from ISO 6579 - = no info

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

Table 9 Composition (in g/L) and pH of MSRV.

Labcode	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**
1	8.25*		7.3	1.5	12.4	0.04	2.6	0	5.6
3	4.6	4.6	7.3	1.5	10.9	0.04	2.7	0.1	5.3
7	4.6	4.6	7.3	1.5	10.9	0.04	2.7	0	5.5
8	4.6	4.6	7.3	1.5	10.9	0.04	2.7	0.01	5.6
9	4.6	4.6	7.3	1.5	10.9	0.04	2.7	0.01	5.5
10	4.6	4.6	7.3	1.5	10.9	0.04	2.7	0.02	5.5
13	4.6	4.6	7.3	1.5	10.9	0.04	2.7	0.01	5.5
17	-	-	-	-	-	-	-	-	5.0
21	4.6	4.6	7.30	1.5	10.9	0.04	2.7	0.02	5.2
23, 29	4.6**	4.6	7.3	1.5	10.9	0.04	2.5	0.01	5.3
25	-	-	-	-	-	-	-	-	5.2
27	4.6	4.6	7.3	1.5	10.9	0.04	2.7	0.02	5.4
30	9.2***	-	7.3	1.5	10.9	0.04	2.7	0.02	-
31	4.6	4.6	7.3	1.5	10.9	0.04	2.7	0.01	5.6
32	4.6	4.6	7.3	1.5	10.9	0.04	2.7	0.02	5.2

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

* 8.25 g Peptone mixture with 0.92 g Yeast extract ** 2.3 g Tryptone with 2.3 gram Meat peptone

***9.2 g peptone

Table 10 Incubation times and temperatures of selective enrichment medium MSRV.

	MSRV	
Labcode	**Incubation time in h:min**	**Incubation temperature in ^{o}C (min-max)**
ISO 6579 Annex D	**2 x (24 ± 3) h**	**40.5 – 42.5**
1	50	42.4-42.8
6	44.5	36.8-37.5

Grey cell: deviating temperatures

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

Lab Code	Xylose	L-lysine	Lactose	Sucrose (Saccharose)	Sodium chloride (NaCl)	Yeast Extract	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**
4	3.75	5.0	7.5	7.5	5.0	3.0	0.08	15	1.0	6.8	0.8	7.0
6	3.5	5.0	7.5	7.5	5.0	3.0	0.08	13.5	2.5	6.8	0.8	7.4
10	3.75	5.0	7.5	7.5	5.0	3.0	0.08	13.5	2.5	6.8	0.8	7.6
15	3.75	5.0	7.5	7.5	5.0	3.0	0.08	13.5	2.5	6.8	0.8	7.4
16	3.75	5.0	7.5	7.5	5.0	3.0	0.08	15	1.0	6.8	0.8	-
17	-	-	-	-	-	-	-	-	-	-	-	7.5
25	-	-	-	-	-	-	-	-	-	-	-	7.4
30	3.75	5.0	7.5	7.5	5.0	3.0	0.08	13.5	1.0	6.8	0.8	-
32	3.5	5.0	7.5	7.5	5.0	3.0	0.08	13.5	2.5	6.8	0.8	7.3

Grey cell: deviating from ISO 6579 - = no info

Thirteen laboratories (labcode 5, 6, 7, 12, 14, 15, 16, 24, 25, 26, 27, 28, 29) used both biochemical and serological test for the confirmation of *Salmonella.* Five laboratories (labcode 1, 3, 4, 9 and 31) used only a serological test(s) for the confirmation of *Salmonella.* Laboratories 1 and 31 reported a rather limited confirmation with only one antigen test and no biochemical tests. Nine laboratories (labcodes 5, 6, 15, 16, 17, 19, 26, 29 and 32) used a biochemical identification kit (which is also allowed).
The used confirmation media or tests are summarised in Tables 12 and 13.

Table 12 Biochemical confirmation of *Salmonella.*

Labcode	UA	TSI	LDC	Gal	VP	Indole	Kit	Other
1, 3, 4, 31	-	-	-	-	-	-	-	
2, 21	+	+	+	+	+	+	-	
5	+	+	+	+	+	+	API 20^{E}	
6	+	+	+	-	-	-	API Rapid 20^{E} or API 20^{E}	and /or BBL BD Crystal E/NF
7, 11, 13, 18, 20, 22	+	+	+	-	-	-	-	
8	+	-	-	-	-	+	-	Kligler, indol, mannitol, nitrate ONPG, FDA, motility test
9	-	-	-	-	-	-	-	KOHNS No.1 MAST
10	+	+	+	+	+	+	-	Manitol, simmons citrate, SIM
12	-	+	+	+	+	-	-	UA-Indole broth
14, 25	+	+	+	+	-	+	-	
15	-	+	-	-	-	-	GN-ID A panel microgen bioproducts	
16, 29	-	-	-	-	-	-	API 20^{E}	
17	-	-	-	-	-	+	HY Enterotest	
19	-	-	-	-	-	-	Microbact 12A	
23	-	-	-	-	-	-	-	MacConkey / Chromagar *Salmonella* plus
24, 28	+	+	+	-	-	+	-	
26	+	+	+	-	-	-	API 20^{E}	
27	+	+	+	+	+	+	-	
30	-	+	+	-	-	-	-	Sorbitol mobility medium
32	-	+	+	+	+	+	API 20^{E}	Uree Indol

- = Not done/mentioned Explanations of the abbreviations are given in the 'List of abbreviations'

Table 13 Serological confirmation of *Salmonella.*

Labcode	Serological			Other
	O antigens	**Vi antigens**	**H antigens**	
1	-	-	-	Serological Enteroclon anti-*Salmonella* (Sifin)
2, 8, 10, 11,13, 17, 18, 19, 20, 21, 22, 30, 32	-	-	-	
3, 4, 6, 7, 12, 16, 23, 24, 26, 27, 28, 29	+	-	+	
5, 14, 15, 25, 31	+	-	-	
9	+	+	+	

- = Not done/mentioned

4.4 Control samples

4.4.1 General

None of the laboratories isolated *Salmonella* from the procedure control (C11: no capsule/no faeces) nor from the faeces control (C12: no capsule/negative chicken faeces). Nineteen laboratories scored correct results for all the control capsules containing *Salmonella*. The results of all control samples (capsules without faeces) are given in Table 14. In this table the highest number of positive isolations found with MSRV in combination with any isolation medium is given per laboratory. Fourteen laboratories used an additional selective enrichment medium (own method). The results found with these own methods are given in Annex 4 Table A.3.

Blank capsules (n=2) without addition of faeces
The blank capsules contained only sterile milk powder. For the analyses no faeces was added.
Thirty participating laboratories correctly analysed the blank capsules negative.
Laboratory 10 and 18 found one blank capsule positive on all media used by the laboratory. Possible causes for finding a blank sample positive may be cross-contamination, mixing up positive and negative samples or limited confirmation or misinterpretation of confirmation results. The relevant laboratories are advised to check their procedures.

Salmonella Enteritidis 10 capsules (SE10) without addition of faeces (n=2)
Twentyone laboratories isolated *Salmonella* Enteritidis at a mean level of approximately 10 cfp/ capsule from both capsules. Laboratory 9 could not detect *Salmonella* Enteritidis in both SE10 control capsules on both the isolation media inoculated from MSRV and RVS. These capsules contained SE at a low level (approx 10 cfp/capsule). Due to change, one out of two capsules containing SE10 may be negative. However, it is not very likely to find both SE10 capsules negative.
Ten laboratories could not detect *Salmonella* in one control capsule with any of the used media.

Salmonella Enteritidis 100 capsules (SE100) without addition of faeces (n=1)
All participating laboratories tested the capsule containing SE100 positive.

Salmonella Panama 5 capsules (Span5) without addition of faeces (n=2)
Thirty laboratories isolated *Salmonella* from both capsules. Two Laboratories (labcode 10 and 18) could not detect *Salmonella* Panama (SPan5) in one control capsule on any of the media used by the laboratory. These capsules contained *S*. Panama at a low level (approximately 5 cfp/ capsule). Due to change one out of two capsules containing Span5 may be negative.

Salmonella Typhimurium 5 capsules (STM5) without addition of faeces (n=3)
Thirty laboratories tested all three capsules containing STM5 positive. Laboratory 7 could not detect *Salmonella* (STM5) in one control capsule on both isolation media inoculated from MSRV and RVS. Laboratory 10 could not detect *Salmonella* Typhimurium in two of the three STM5 control capsules, on all the isolation media inoculated from MSRV and RVS. These capsules contained STM at a low level (approximately 5 cfp/ capsule). Due to change, one out of two capsules containing STM5 may occasionally be negative. However, it is less likely to find two STM5 capsules negative.

The results of all control samples were compared with the new definition of ‘good performance’ (see section 3.6). The score for the control samples was below these criteria for three laboratories (labcodes 9, 10 and 18).

Table 14 Total number of positive results of the control samples (capsule without faeces) per laboratory.

Labcode	The highest number of positive isolations found with MSRV in combination with any isolation medium				
	Blank n=2	SE10 n=2	SE100 n=1	SPan5 n=2	STM5 n=3
Good Performance	**0**	**≥ 1**	**1**	**≥ 1**	**≥ 2**
1	0	**1**	1	2	3
2	0	2	1	2	3
3	0	2	1	2	3
4	0	**1**	1	2	3
5	0	2	1	2	3
6	0	**1**	1	2	3
7	0	2	1	2	**2**
8	0	**1**	1	2	3
9	0	**0**	1	2	3
10	**1**	**1**	1	**1**	**1**
11	0	2	1	2	3
12	0	2	1	2	3
13	0	**1**	1	2	3
14	0	2	1	2	3
15	0	2	1	2	3
16	0	2	1	2	3
17	0	2	1	2	3
18	**1**	2	1	**1**	3
19	0	**1**	1	2	**2**
20	0	**1**	1	2	3
21	0	2	1	2	3
22	0	**1**	1	2	3
23	0	2	1	2	3
24	0	2	1	2	3
25	0	2	1	2	3
26	0	2	1	2	3
27	0	2	1	2	3
28	0	**1**	1	2	3
29	0	2	1	2	3
30	0	**1**	1	2	3
31	0	2	1	2	3
32	0	2	1	2	3

Bold numbers: deviating results
Grey cell: results are below criterion of good performance

4.4.2 Specificity, sensitivity and accuracy rates of the control samples

In Table 15 the specificity, sensitivity and accuracy rates for the control capsules without the addition of faeces are shown. The rates are calculated for the selective enrichment MSRV with plating-out medium XLD and non XLD media. The calculations were performed on the results of all participants and on the results of only the EU Member States (without the results of Norway, candidate and third countries). Only small differences (if any) were found between these groups.
The combination MSRV/XLD resulted in general 1% higher rates than the combination MSRV/non-XLD. As expected the high level control (SE100) showed rates of 100%. For the low level materials (Span5, STM5 and SE10) the rates were expected to lie between 50% and 100%. However, Span5 and STM5 were obviously easier to detect than SE10 as for the first two materials the sensitivity rates were even close to 100%. For SE10 the sensitivity rates were approximately 80%.

Table 15 Specificity, sensitivity and accuracy rates of the control samples (capsules without the addition of (faeces) for the selective enrichment on MSRV and plating out on XLD or non-XLD.

Control capsules		MRVS/ XLD		MSRV/ non-XLD*	
		All n= 32	EU MS n=28	All n= 32	EU MS n=28
Blank	No. of samples	64	56	74	66
	No. of negative samples	62	55	71	64
	Specificity in%	**96.9**	**98.2**	**95.9**	**97.0**
Span5	No. of samples	64	56	74	66
	No. of positive samples	62	55	71	64
	Sensitivity in%	**96.9**	**98.2**	**95.9**	**97.0**
STM5	No. of samples	96	84	111	99
	No. of positive samples	92	80	105	93
	Sensitivity in%	**95.8**	**95.2**	**94.6**	**93.9**
SE10	No. of samples	64	56	74	66
	No. of positive samples	51	43	58	50
	Sensitivity in%	**79.7**	**76.8**	**78.4**	**75.8**
SE100	No. of samples	32	28	37	33
	No. of positive samples	32	28	37	33
	Sensitivity in%	**100**	**100**	**100**	**100**
All capsules with *Salmonella*	No. of samples	256	224	296	264
	No. of positive samples	237	206	271	240
	Sensitivity in%	**92.6**	**92.0**	**91.6**	**90.9**
All capsules	No. of samples	320	280	370	330
	No. of correct samples	299	261	342	304
	Accuracy in%	**93.4**	**93.2**	**92.4**	**92.1**

*Four laboratories used more than one non XLD isolation medium

4.5 Results faeces samples artificially contaminated with *Salmonella* spp.

4.5.1 Results per type of capsule and per laboratory

General
The results of the *Salmonella* negative chicken faeces samples artificially contaminated with capsules are given in Table 16. The highest number of positive isolations found with MSRV in combination with any isolation medium is given per laboratory. Fourteen laboratories used an additional selective enrichment medium (own method). The results found with these own methods are given in Annex 4 Table A.4.
In general the number of positive results of the samples containing *S*. Enteritidis is lower than those samples containing *S.* Typhimurium.

Blank capsules with negative chicken faeces (n=5)
Thirty laboratories correctly did not isolate *Salmonella* from these blank capsules with the addition of negative chicken faeces. Only laboratory 5 and 7 found one positive blank with the addition of negative chicken faeces. This was after selective enrichment in RVS and after inoculation on only one isolation medium, respectively Mac Conkey and XLD. On the prescribed selective enrichment medium MSRV they correctly found all blank samples negative.
All blanks should be tested negative. However, as no 100% guaranty about the *Salmonella* negativity of the matrix can be given, 1 positive out of 5 blank samples (80% neg.) is still considered acceptable.

S. Enteritidis 10 capsules (SE10) with negative chicken faeces (n=5)
Only five laboratories (labcodes 5, 19, 23, 26, and 29) were able to isolate *Salmonella* from all the five capsules containing *Salmonella* Enteritidis at a level of approximately 10 cfp/ capsule in combination with chicken faeces. Thirty laboratories isolated *Salmonella* from at least one capsule containing SE10 and two laboratories (labcodes 10 and 32) found none of the SE10 capsules positive.

S. Enteritidis 100 capsules (SE100) with negative chicken faeces (n=5)
Thirty-one laboratories isolated *Salmonella* from all the five capsules containing *Salmonella* Enteritidis at a level of approximately 100 cfp/ capsule in combination with chicken faeces. Only one laboratory (labcode 32) was not able to find *Salmonella* in three SE100 capsules.

S. Typhimurium 5 capsules (STM5) with negative chicken faeces (n=5)
Twenty-six laboratories isolated *Salmonella* from all the five capsules containing *Salmonella* Typhimurium at a level of approximately 5 cfp/ capsule in combination with chicken faeces. Five laboratories 6, 19, 21, 22 and 32 found one capsules negative and one laboratory (labcode 10) found two capsules negative.

S. Typhimurium 50 capsules (STM50) with negative chicken faeces (n=5)
All laboratories isolated *Salmonella* from all five capsules containing *Salmonella* Typhimurium at a level of approximately 50 cfp/ capsule in combination with chicken faeces with the selective enrichment on MSRV.

The results of all artificially contaminated chicken faeces samples were compared with the new definition of ‘good performance’ (see section 3.6). The score for the artificially contaminated samples was below the set criteria for two laboratories (labcodes 10 and 32).

Table 16 Total number of positive results of the artificially contaminated chicken faeces samples per laboratory.

Labcode	The highest number of positive isolations found with MSRV in combination with any isolation medium				
	Blank n=5	SE10 n=5	SE100 n=5	STM5 n=5	STM50 n=5
Good performance	**≤ 1**	**≥ 1**	**≥ 4**	**≥ 2**	**≥ 4**
1	0	**1**	5	5	5
2	0	**3**	5	5	5
3	0	**2**	5	5	5
4	0	**1**	5	5	5
5	0	5	5	5	5
6	0	**2**	5	**4**	5
7	0	**2**	5	**4**	5
8	0	**3**	5	5	5
9	0	**3**	5	**4**	5
10	0	**0**	5	**3**	5
11	0	**2**	5	5	5
12	0	**4**	5	5	5
13	0	**3**	5	5	5
14	0	**4**	5	5	5
15	0	**2**	5	5	5
16	0	**3**	5	5	5
17	0	**4**	5	5	5
18	0	**3**	5	5	5
19	0	5	5	**4**	5
20	0	**4**	5	5	5
21	0	**3**	5	**4**	5
22	0	**2**	5	**4**	5
23	0	5	5	5	5
24	0	**3**	5	5	5
25	0	**2**	5	5	5
26	0	5	5	5	5
27	0	**3**	5	5	5
28	0	**1**	5	5	5
29	0	5	5	5	5
30	0	**1**	5	5	5
31	0	**3**	5	5	5
32	0	**0**	**2**	**4**	**4**

Bold numbers: deviating results
Grey cell: results are below good performance

4.5.2 Results per medium, capsule and per laboratory

In the Figures 1, 2, 3 and 4 the number of positive isolations per artificially contaminated chicken faeces sample is given per laboratory after pre-enrichment in BPW and selective enrichment on MSRV followed by isolation on selective plating agar XLD and another selective isolation agar (non-XLD).

The results of all artificially contaminated chicken faeces samples were compared with the proposed definition of 'good performance' (see section 3.6). In the figures 1-4 the border of good performance is indicated with a black horizontal line. According to this definition the score for the artificially contaminated samples with *Salmonella* was below the set criteria for laboratory 10 and 32.

Thirty laboratories found the highest number of positive isolations with selective enrichment on MSRV and twenty nine with the combination of MSRV and isolation on XLD medium. Three laboratories found differently when analysing the faeces samples artificially contaminated with *Salmonella*. Laboratory 7 found one sample (STM5) and laboratory 9 found two samples (SE10 and STM 5) more positive with the combination RVS/XLD. On the other hand, laboratory 9 found three more samples (SE100) positive with the combination MSRV/XLD than with the combination RVS/XLD. Laboratory 32 found one STM50 sample more positive with selective enrichment on MSRV and isolation on SS agar compared to XLD.

The difference in the number of positive isolations after 24 and 48 hours of incubation of the selective enrichment on MSRV was 5-6% and this is summarised in Table 17. The choice of plating out medium does not seem to have a large effect on the number of positive isolations, XLD gave 4% more positive results than other plating-out media. The majority of the laboratories used BGA as the second plating out medium (see Table 6).

Table 17 Mean percentages of positive results of all participating laboratories after selective enrichment on MSRV, incubated for 24 and 48 hours and followed by incubation on different plating out media, when analyzing the artificially contaminated chicken faeces samples.

Plating out medium	**Selective enrichment medium MSRV**
	24 / 48 h
XLD	81 / 87%
Other (most often BGA)	78 / 83%

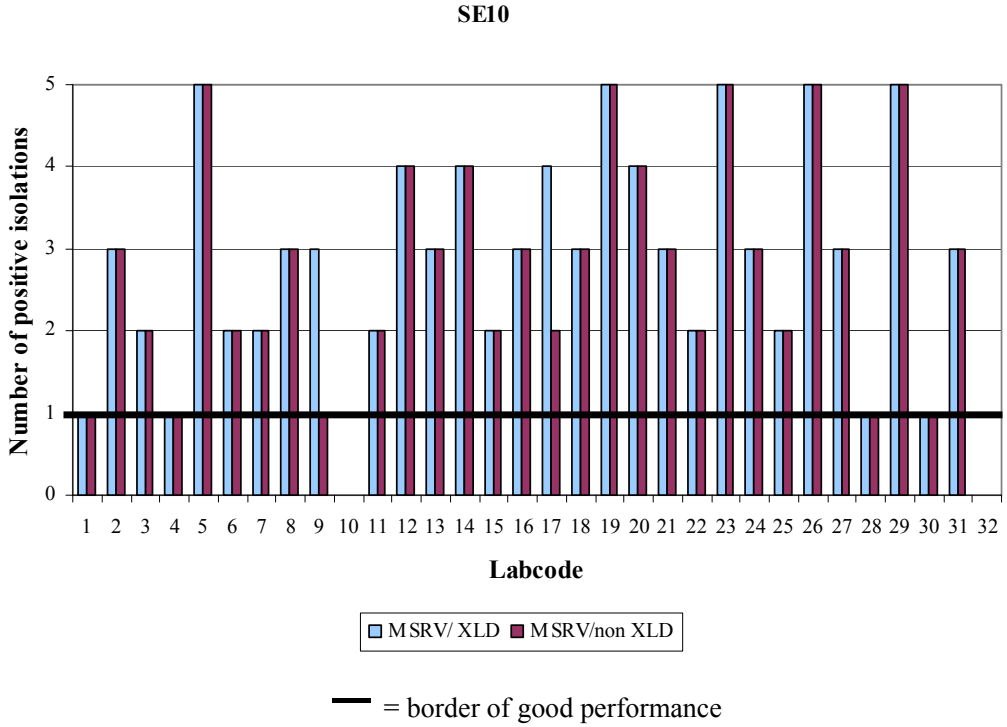

Figure 1 Results of chicken faeces artificially contaminated with SE10 capsules (n=5) after selective enrichment on MSRV followed by isolation on selective plating agars XLD and non XLD.

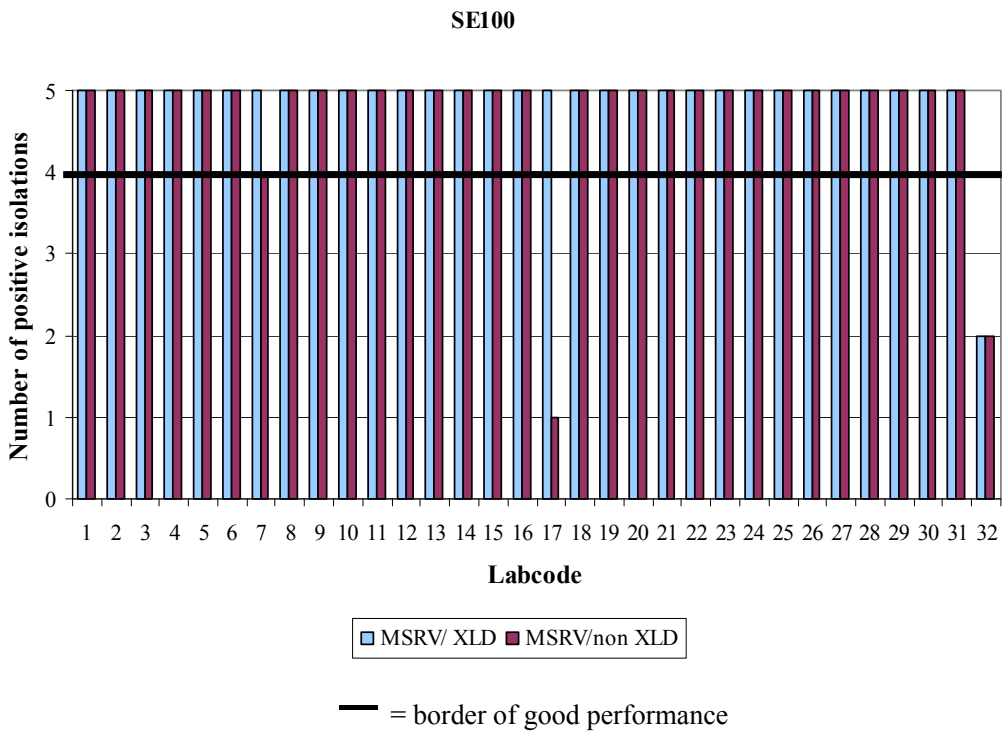

Figure 2 Results chicken faeces artificially contaminated with SE100 capsules (n=5) after selective enrichment on MSRV followed by isolation on selective plating agars XLD and non XLD.

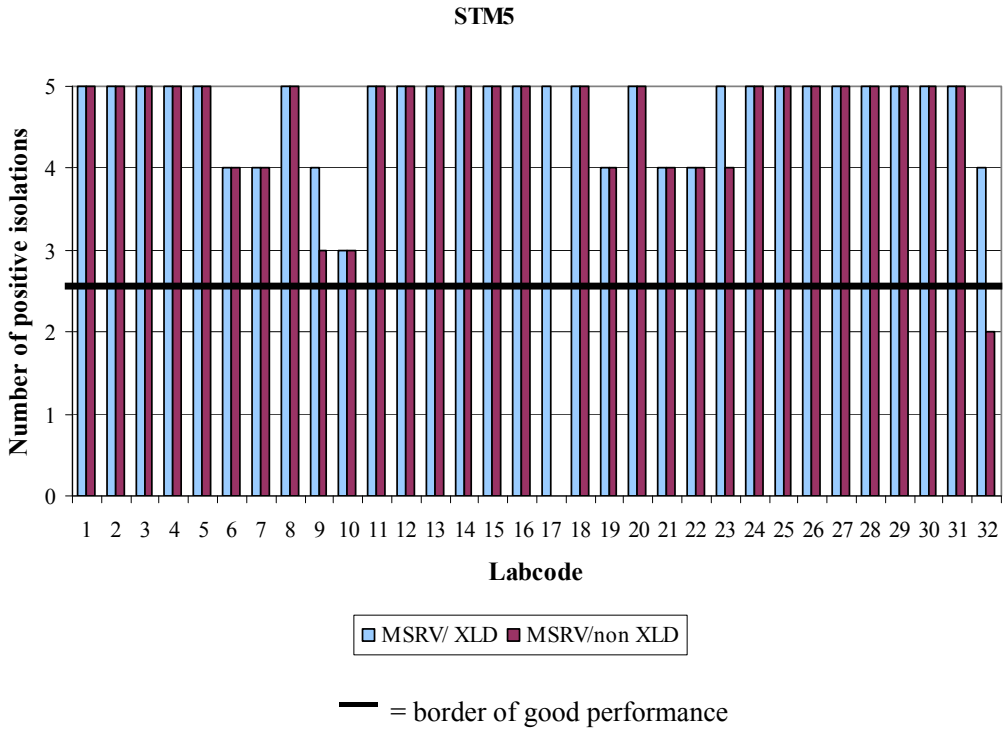

Figure 3 Results chicken faeces artificially contaminated with STM5 capsules (n=5) after selective enrichment on MSRV followed by isolation on selective plating agars XLD and non XLD.

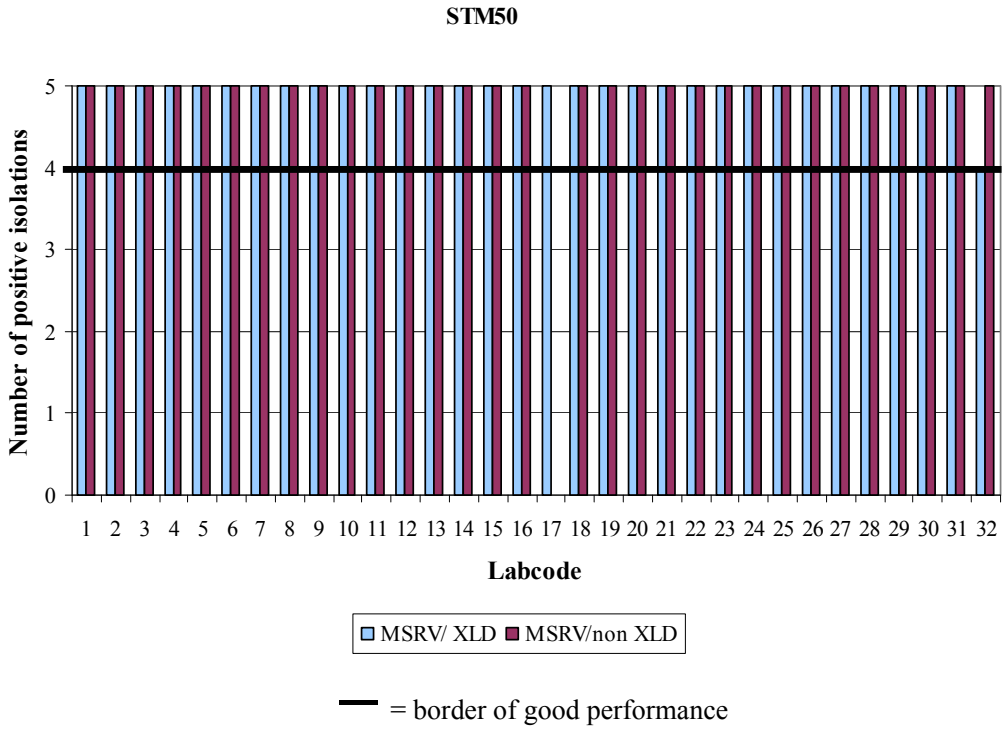

Figure 4 Results chicken faeces artificially contaminated with STM50 capsules (n=5) after selective enrichment on MSRV followed by isolation on selective plating agars XLD and non XLD.

Table 18 Specificity, sensitivity and accuracy rates of the artificially contaminated chicken faeces samples (each capsule added to 10 g chicken faeces) for the selective enrichment on MSRV and plating out on XLD or non-XLD.

Capsules with Chicken faeces		MRVS/ XLD		MSRV/ non-XLD*	
		All n=32	EU MS n=28	All n=32	EU MS n=28
Blank	No. of samples	160	140	185	165
(n=5)	No. of negative samples	160	140	185	165
	Specificity in%	**100**	**100**	**100**	**100**
STM5	No. of samples	160	140	185	165
(n=5)	No. of positive samples	151	132	163	151
	Sensitivity in%	**94.4**	**94.3**	**88.1**	**91.5**
STM50	No. of samples	160	140	185	165
(n=5)	No. of positive samples	159	140	177	162
	Sensitivity in%	**99.4**	**100**	**95.7**	**98.2**
SE10	No. of samples	160	140	185	165
(n=5)	No. of positive samples	89	79	97	89
	Sensitivity in%	**55.6**	**56.4**	**52.4**	**53.9**
SE100	No. of samples	160	140	185	165
(n=5)	No. of positive samples	157	140	176	163
	Sensitivity in%	**98.1**	**100**	**95.1**	**98.8**
All capsules with *Salmonella*	No. of samples	640	560	740	660
	No. of positive samples	556	491	613	565
	Sensitivity in%	**86.9**	**87.7**	**82.8**	**85.6**
All capsules	No. of samples	800	700	925	825
	No. of correct samples	716	631	798	730
	Accuracy in%	**89.5**	**90.1**	**86.3**	**88.5**

* Four laboratories used more than one non XLD isolation medium

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

The specificity, sensitivity and accuracy rates for all types of capsules added to the chicken faeces are shown in Table 18. The results are given for the different medium combinations: BPW followed by selective enrichment on MSRV and isolation on selective plating agar XLD and on other selective isolation agar medium (non-XLD). The calculations were performed on the results of all participants and on the results of only the EU Member States (without the results of Norway, candidate and third countries). Only small differences (if any) were found between these groups.
The specificity rates (of the blank capsules) were 100% for MSRV. For the capsules containing *Salmonella,* the rates for MSRV/XLD were 2-6% higher compared to MSRV/non-XLD.
The rates showed the expected results. As in this study the contamination level of the low level materials (STM5 and SE10) were close to the detection level, the sensitivity rates were expected to lie close to 50%. The samples with SE10 indeed showed these expected results. Obviously the low level materials of *S.* Typhimurium (STM5) were easier to detect than the low level materials of *S.* Enteritidis. The sensitivity rates of STM5 with MSRV/XLD were even > 94%.

4.6 PCR

Two laboratories (labcodes 5 and 29) applied a PCR method as additional detection technique. These laboratories tested the samples after incubation in BPW. In Table 19 the details are summarized.

Table 19 Details on the Polymerase Chain Reaction method, used as own Method during the interlaboratory comparison study by two laboratories.

Labcode	Volume of BPW (µl)	Volume of DNA sample (µl)	Volume of DNA / PCR mix (µl)
5	1000	150	5/50
29	1000	150	5/10

Laboratory 5 used a commercial available real time PCR (Biorad iQ-Check *Salmonella* kit). Laboratory 29 used an Inva PCR which is non-commercial and normally is used for confirmation of bacterial cultures and not from pre-enrichment broths.

The PCR results and the bacteriological culture results are shown in Table 20. For the control samples (without faeces) both laboratories found the same results with the PCR-technique and the bacteriological culture method. Laboratory 5 found one blank sample with chicken faeces (weak) positive with the PCR technique. Laboratory 29 found ten samples of the artificially contaminated chicken faeces negative (four SE10 /STM5 and two SE100) while with the culture method, from the same BPW, they found correct results.

Table 20 PCR results compared to bacteriological culture results of control capsules and of artificially contaminated chicken faeces samples of laboratories 5 and 29.

Capsules	Lab 5		Lab 29	
	MSRV	PCR	MSRV	PCR
Controls without faeces (n=10)				
Span 5 (n=2)	2	2	2	2
SE10 (n=2)	2	2	2	2
SE100 (n=1)	1	1	1	1
STM5 (n=3)	3	3	3	3
Blank (n=2)	0	0	0	0
BPW (n=1)	0	0	0	0
Faeces (n=1)	0	0	0	0
Test samples with faeces (n=25)				
SE10 (n=5)	5	5	5	**1**
SE100 (n=5)	5	5	5	**3**
STM5 (n=5)	5	5	5	**1**
STM50 (n=5)	5	5	5	5
Blank (n=5)	0	**1**	0	0

Grey cells: unexpected results
Bold numbers: different results found with MSRV or PCR
MSRV: the results found with the prescribed selective enrichment medium MSRV in combination with any isolation medium giving the highest number of positive isolations.

4.7 Performance of the NRLs

4.7.1 General

Twenty-eight NRLs fulfilled the (new) criteria of good performance. Four laboratories scored below these criteria (labcodes 9, 10, 18 and 30). The results from these laboratories are summarised in Tables 21 and 22.

Laboratory 9 could not detect *Salmonella* in both SE10 control samples (without faeces).
Laboratory 10 could not detect *Salmonella* in two of the three STM5 control capsules, they found a positive blank control capsule (without faeces) and they could not detect *Salmonella* in any of the five SE10 capsules with the addition of faeces. The same results were observed for both used selective enrichment media MSRV and RVS.
Laboratory 18 found one blank control sample positive while they missed one Span5 control capsule.
Laboratory 32 had some problems with the artificially contaminated samples with SE. They could not detect *Salmonella* in any of the five SE10 capsules and they could not detect *Salmonella* in three of the five SE100 capsules. Additional to MSRV, laboratory 32 used MKTTn as own selective enrichment medium. However, with this medium they did not find any of the artificially contaminated samples positive.

Table 21 Summary of laboratories finding some low scores for the control capsules. The number of positive isolations per laboratory and per capsule without the addition of faeces found on MSRV in combination with the isolation media giving the highest number of positive isolations are given.

Capsule	Blank	SE10	SE100	Span5	STM5
No. of capsules	n=2	n=2	n=1	n=2	n=3
Good performance	0	≥1	1	≥ 1	≥ 2
Labcode					
9	0	**0**	1	2	3
10	**1**	**1**	1	**1**	**1**
18	**1**	2	1	**1**	3
32	0	2	1	2	3

Bold numbers: Deviating results Grey cells: Results below the level of good performance

Table 22 Summary of laboratories finding some low scores for the artificially contaminated samples.The number of positive isolations per laboratory and per capsule (n=5) with the addition of 10 g *Salmonella* negative chicken faeces found on MSRV in combination with the isolation media giving the highest number of positive isolations are given.

Capsule	Blank	SE10	SE100	STM5	STM50
No. of capsules	n=5	n=5	n=5	n=5	n=5
Good performance	≤1	≥1	≥ 4	≥ 2	≥ 4
Labcode					
9	0	**3**	5	**4**	5
10	0	**0**	5	**3**	5
18	0	**3**	5	5	5
32	0	**0**	**2**	**4**	**4**

Bold numbers: Deviating results Grey cells: Results below the level of good performance

The four laboratories were contacted by the CRL-*Salmonella* in April 2008 to ask for any explanation for the deviating results.

Laboratory 9 reviewed all the information relevant for this trial but they were unfortunately not able to suggest a plausible reason for why their laboratory failed to isolate at least one of the SE10 control samples. They mentioned that they used the same batch of medium and followed the same procedures for the artificially contaminated faeces samples for which the results reached the level of good performance. Considering the low contamination level, it is possible (by chance) to find 1/2 SE10 capsules negative. Finding 2/2 SE10 capsules negative is less likely, but statistically there is still a small chance to find such a result in a large batch.

Laboratory 18 explained that they possibly mixed-up C1 (Blank) and C2 (Span5) in their laboratory at the very first stages when the capsules were added to the BPW. They will check their procedures for possible weak points.

Laboratory 10 explained that there was a low occupation of veterinary doctors during the time of the ringtrial and different people have been responsible for the ringtrails in the last years. For the positive blank they supposed they mixed up samples or there was a cross-contamination.
From the information of the temperature recorder it could have been the case that the parcel of laboratory 10 has been laying at 11-23 ^{o}C for 48 h. However from the information received of the laboratory it was not clear whether the parcel with its content has undergone this storage at elevated temperatures or whether it only concerned the temperature recorder (section 4.3.3). Long storage (> 1 day) at elevated temperatures (> 20 ^{o}C) may result in die off of *Salmonella* in the reference materials. If this has been the case it might (partly) explain the low number of positives. However, it doe not explain the positive blank result.

Laboratory 32 indicated that they routinely use MKTTn and RVS for selective enrichment and that they have problems with MKTTn at the moment of the ringtrail. They also mentioned that they have little to no experience with MSRV and XLD.

Although laboratories 9 and 18 found some deviating results for the control samples, they found very good results for all the artificially contaminated samples. Their explanations for deviating results were plausible and a follow up study was not considered necessary. Their results were indicated as 'moderate performance'.
Laboratories 10 and 32 were advised to participate in a follow up study after they followed a training.

4.7.2 Follow-up study

The set-up of the follow-up study for laboratories 10 and 32 was the same as the full interlaboratory comparison study in March, with the same batch of capsules except for the low level SE. For this a new batch was made with approximately 20 cfu/capsule (SE20, see section 4.1 'Reference materials'). An overview on the type and number of samples is given in Table 23.

Table 23 Overview of the types and the number of capsules tested by laboratory 10 and 32 in the follow-up of the interlaboratory comparison study.

Capsules	Control capsules no faeces added		Test samples with 10 g *Salmonella* negative chicken faeces	
Labcode	**10**	**32**	**10**	**32**
S. Enteritidis 20 (SE20)	3	3	6	6
S. Enteritidis 100 (SE100)	1	1	5	6
S. Typhimurium 5 (STM5)	3	2	5	5
S. Typhimurium 50 (STM50)	-	-	2	2
Blank	1	1	5	5
Total number of capsules	n= 8	n=7	n=23	n=24

The laboratories had to follow the same Protocol and SOP as in the study of March 2008 (see Annexes 5 and 6). The test report was different from the March study (see Annex 7). For the media only the differences with the March study needed to be indicated.

Laboratory 10
Laboratory 10 showed repeatedly deviating results in ringtrails since 2006 (veterinary as well as food studies). Two staff members of the CRL-*Salmonella* visited this laboratory while they performed a follow up for this study (starting on 30 June 2008). During a two days visit (30 June and 1 July 2008) the procedures were checked for possible (technical) problems explaining the deviating results.

On Tuesday 16 June 2008 one parcel with one plastic container was send to laboratory 10 containing:
8 control capsules (C1 – C8), 23 capsules (1 – 23), 250 g chicken faeces and 1 temperature recorder.
On 20 May 2008, the number of aerobic bacteria ($6.6*10^8$ cfu/g) and Enterobacteriaceae ($2.1*10^5$ cfu/g) in the faeces were tested. These numbers were comparable to the numbers of the chicken faeces as used for the full study (see Table 4).
During the visit of the two staff members of the CRL-*Salmonella* of the veterinary, food and media (preparation) department of laboratory 10 some technical deviations were observed. These deviations were seen during the handling of the samples and during the several steps in the *Salmonella* detection procedure. At the end of the visit, a report with observations and advices for possible improvements was made by the staff members of the CRL-*Salmonella* and handed over to the staff members of laboratory 10.
During the follow up study laboratory 10 used some different or additional media or tests compared to the full study of March 2008.The deviations are summarised in the scheme below.

	March 2008	July 2008
Selective Enrichment	MSRV with 0.02 g/L novobiocin	MSRV with 0.01 g/L novobiocin (prescribed) and MSRV with 0.02 g/L novobiocin
	inoculation MSRV by one drop	inoculation MSRV by three drops spreaded on the plate
Isolation medium	XLD, BGA and Rambach	XLD and BGA
Conformation	Biochemical	Biochemical and Serotyping

The visit was considered successful: laboratory 10 scored all the eight control samples and twenty three test samples correct. With this result, laboratory 10 fulfilled the criteria of good performance (see section 3.6) for the test samples in this follow-up study.

Laboratory 32
Laboratory 32 was advised to participate in a follow up study after they followed a training to get more experience with MSRV and XLD and to solve the problems with MKTTn. After discussion with the laboratory, it was agreed to organise the follow-up study in September 2008.

On Monday 8 September 2008 one parcel with one plastic container was send to laboratory 32 containing:
7 control capsules (C1 – C7), 24 capsules (1 – 24), 300 g chicken faeces and 1 temperature recorder.
On 7 October 2008 the number of aerobic bacteria ($5.2*10^8$ cfu/g) and Enterobacteriaceae ($<1*10^2$ cfu/g) in the faeces were tested.
The performance of this follow up study was on 22 September 2008.
During the follow up study laboratory 32 used some different or additional media compared to the full study of March 2008.The deviations are summarised in the scheme below.

	March 2008	September 2008
Selective Enrichment	MSRV with 0.02 g/L novobiocin	MSRV with 0.01 g/L novobiocin (prescribed)
	MKTTn	RVS
Isolation medium	XLD and SS	XLD and BGA

Laboratory 32 scored all the seven control samples and twenty three out of 24 test samples correct with the prescribed method (MSRV). They could not detect *Salmonella* in one out of six SE100 samples with faeces using the prescribed method (MSRV) but they scored all the samples correct with their own method (RVS).
With this result laboratory 32 fulfilled the criteria of good performance (see section 3.6) for the tested samples in this follow-up study.

5 Discussion

Transport of the samples
In general the transport time or the transport temperature of the parcels does not seem to have negatively affected the results. The laboratory with the longest transport time and highest transport temperature (laboratory 27) still found good results. In some laboratories a delay was noticed between the delivery time of the courier and the receipt time by the laboratory. It is not always clear whether this delay concerns the parcel including the contents or only the temperature recorder.
For laboratory 10 a delay of 48 h at elevated temperatures (11-23 ^{o}C) was seen. If the content of the parcel was indeed exposed to this it could have been an explanation to the low number of positives found by laboratory 10. however, it can not explain the false positive result.

Performance of the laboratories
The prescribed method (Annex D of ISO 6579: MSRV) was used by all laboratories. Twelve laboratories used an 'own' selective enrichment medium (RVS or MKTTn) additional to MSRV. For the majority of these laboratories MSRV gave the highest scores compared to RVS or MKTTn. For determining 'good performance' per laboratory the best performing isolation medium after selective enrichment on MSRV was taken into account. Twenty-eight out of 32 laboratories scored 'good performance'. Two laboratories scored a 'moderate performance' and two laboratories scored under the level of 'good performance'.
The two 'moderate' performing laboratories gave a plausible explanation for their deviating result with the control sample. In addition they scored 92% and 96% of the artificially contaminated samples correct, while the other laboratories observed the main problems with these faeces samples. For the faeces, contaminated with SE10 capsules, the laboratories scored overall 55% positive while the two 'moderate' laboratories scored with 60 % and 80 % positive. It was therefore considered not necessary to send extra samples to the two 'moderate performing laboratories'.

Laboratory 10 scored for the third time in a ringtrial under the level of good performance and it was decided to visit this laboratory at the time they performed a follow up study. The visit was focused on possible technical deviations so that advises could be given on possible improvements. This worked well as the laboratory scored 100 % correct results with this follow up study.

Laboratory 32 participated for the first time and several aspects may have been of influence for their poor performance. They did not use the prescribed method (MSRV) as a routine method, the communication in English and the very late decision to participate were not the optimal conditions for their preparation to participate in this ringtrail. The laboratory scored under the level of good performance for the prescribed method (MSRV) and their own routine method (MKTTn). The laboratory was advised to follow a training to become more acquainted with MSRV and XLD and to solve their problems with MKTTn before organising a follow up study. The laboratory solved their problems and showed good results in a follow up study organised in September 2008.

One laboratory (labcode 7) mentioned that they made a transcription error with a blank capsule tested with chicken faeces. This was reported after the CRL send the results and interim summary report to all participating laboratories. By that time the results are not blind anymore and changes are not longer allowed.

Specificity, sensitivity and accuracy rates
The rates of the control samples were high. As expected the high level control sample (SE100) showed rates of 100%. For the low level materials (Span5, STM5 and SE10) the rates were expected to lie between 50% and 100%. However, Span5 and STM5 were obviously easier to detect than SE10 as for the first two materials the sensitivity rates were even close to 100%. For SE10 the sensitivity rates were approximately 80%.
For the artificially contaminated faeces samples the rates found with MSRV in combination with XLD were 1-6% higher compared to non-XLD isolation media. As in this study the contamination level of the low level materials (STM5 and SE10) were close to the detection limit, the sensitivity rates were expected to lie close to 50%. The samples with SE10 indeed showed these expected results. Obviously the low level materials of *S.* Typhimurium (STM5) were easier to detect than the low level materials of *S.* Enteritidis. The sensitivity rates of the STM5 samples with the medium combination MSRV/XLD were only a little lower than the high level contaminated samples STM50 and SE100.

Media
According to Annex D of ISO 6579 (Anonymous, 2007) the concentration of novobiocin in MSRV should be 10 mg/L and the pH between 5.1-5.4. Five laboratories reported the use of a higher concentration of novobiocin and four laboratories did not mention the use of novobiocin. Nine laboratories reported a higher or lower pH or did not mention the pH. Six laboratories (labcode 5, 19, 20, 23, 26 and 29) used MSRV with the prescribed novobiocin concentration and with the correct pH. These laboratories found the highest number of correct scores even for the artificially contaminated samples with SE10 capsules. A higher concentration of novobiocin in the MSRV can negatively influence the motility of *Salmonella* and may result in less positive results. A higher pH of MSRV may stimulate the growth of disturbing background flora which can negatively influence the growth of *Salmonella*. The poor performing laboratories 10 and 32 also reported deviations in MSRV which may have been an explanation for some of their negative results.

According to Annex D of ISO 6579 (Anonymous, 2007) the incubation temperature of MSRV should be between 40.5-42.5 ^{o}C. Two Laboratories (labcodes 1 and 6) mentioned deviating incubation temperatures. Laboratory 6 incubated MSRV at approximately 37 ^{o}C. This lower incubation temperature may stimulate the growth of disturbing background flora. Especially low numbers of *Salmonella* can than easily become overgrown. This might have been the problem with the faeces samples artificially contaminated with SE10 of laboratory 6.

Other deviations in media compositions or incubation temperatures were reported but no clear effects were found on the results.

In general XLD showed (slightly) more positive results than the other isolation media.

Confirmation of Salmonella
In an earlier interlaboratory comparison study on food (Kuijpers et al, 2008) a relatively high number of false positive blanks were observed. It was concluded that a complete confirmation of *Salmonella* suspect colonies is very important, especially when the number of background flora (Enterobacteriaceae) in a matrix is high and may negatively influence the reading of the isolation media. In this veterinary study special attention was asked for the confirmation of *Salmonella* suspect colonies. In the test report more details about the serological and biochemical confirmation was asked. Either the participating laboratories paid more attention to the confirmation, or the matrix contained less disturbing background flora, fact was that in this study no positive blank faeces samples were found with the prescribed method (MSRV).

PCR

Only two laboratories used a PCR technique additional to the prescribed method and found better results with the bacteriological detection methods inoculated from the same BPW. One laboratory found a positive blank with a commercially available PCR, while the other laboratory found more negatives with the PCR technique. A possible explanation for this could be that this latter laboratory routinely uses this technique not from pre-enrichment broths, as they did for this study, but for confirmation of *Salmonella*.

Evaluation of this study

For a better testing of the performance of the laboratories the contamination levels of the capsules used in this study were lower than in the former veterinary studies (see Annex 1 Table A.1). The contamination levels of the low level reference materials were close to the detection limits. The detection limit of *Salmonella* Typhimurium was obviously lower than the detection limit of *Salmonella* Enteritidis in chicken faeces tested with the same methods. From this study it can be concluded that the detection limit of STM in chicken faeces was close to 1 cfp/10 g. Technically it is not possible to prepare reference materials containing only 1 cfp/capsule and therefore the STM5 capsules can be considered as a right choice for the STM low level samples. The detection limit of *Salmonella* Enteritides in chicken faeces in this study was approximately 10 times higher than that of STM. The contamination level of the SE capsules used in this study was very close to this detection limit so that the positivity of the chicken faeces samples artificially contaminated with these low level SE capsules was sometimes hard to test. This was the reason to amend the criterium for good performance for these samples. Like concluded earlier for the Food-II ringtrail (Kuijpers et al, 2008), it may be better to adjust the contamination level of the low level SE capsules for the future studies, so that the level will be slightly above the detection limit (e.g. 15-20 cfp/capsule).

For this reason a batch of SE20 capsules was used in the follow up of this veterinary study and showed good results.

6 Conclusion

- All thirty-two NRLs for *Salmonella* were able to detect high and low levels of *Salmonella* in chicken faeces. Of these 28 laboratories achieved the level of 'good performance' at once. Two laboratories scored a 'moderate performance'. Two laboratories achieved the level of 'good performance' after a follow up study, one of these achieved this level after a 'training on the spot'.

- The accuracy, specificity and sensitivity rates for the control samples (without faeces) of MSRV were higher than 96% with the exception of the SE10 capsules which rates were approximately 80%.

- The specificity rate of the chicken faeces samples artificially 'contaminated' with blank capsules was 100% when tested with the prescribed method (MSRV).

- The sensitivity rates for artificially contaminated chicken faeces with STM5, STM50 and SE100 capsules were higher than 94% for MSRV in combination with isolation on XLD.

- The sensitivity rates for artificially contaminated chicken faeces with SE10 capsules were (as expected) approximately 55% for MSRV in combination with isolation on XLD.

- The low level materials of *S*. Typhimurium (STM5) were easier to detect than the low level materials of *S*. Enteritidis (SE10).

- The accuracy rates of the artificially contaminated chicken faeces samples were higher than 86% for MSRV.

- MSRV is a good selective enrichment medium for the matrix used (chicken faeces).

- The five laboratories without deviations for pH and novobiocin concentration in MSRV found the highest number of correct scores even for the SE10 capsules with matrix (chicken faeces).

- The contamination level of the chicken faeces samples artificially contaminated with the low level *S*. Enteritidis (SE10) capsules was very close to the detection limit of the method.

rivm

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Schulten SM, In ’t Veld PH, Ghameshlou Z, Schimmel H, Linsinger T, 2000. The certification of the number of colony forming particles of *Salmonella* Typhimurium and number fraction of negative capsules from artificially contaminated milk powder. Commission of European Communities, Community Bureau of Reference, Brussels, Luxembourg. CRM 507R, EUR 19622 EN.

Annex 1 History of CRL-*Salmonella* interlaboratory comparison studies on the detection of *Salmonella*

Table A.1 History of CRL-*Salmonella* interlaboratory comparison studies on detection of *Salmonella* in veterinary samples.

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

Table A.1 (continued)

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

Table A.1 (continued)

Study	Year	Number of samples	Capsules	Actual number of cfp/capsule	*Salmonella* negative faeces added[2]	Selective enrichment medium	Plating-out medium	Reference [3] (RIVM report)
X	2006	5	STM10	9	10 gram	MSRV and own	XLD and own	Kuijpers et al., 2007 (Report 330604004)
		5	STM100	98	10 gram			
		5	SE100	74	10 gram			
		5	SE500	519	10 gram			
		5	Blank	0	10 gram			
		3	STM10	9	No			
		2	SE100	98	No			
		1	SE500	519	No			
		2	SPan5	5	No			
		2	Blank	0	No			
XI	2008	5	STM5	6	10 gram	MSRV and own	XLD and own	This report
		5	STM50	47	10 gram			
		5	SE10	9	10 gram			
		5	SE100	90	10 gram			
		5	Blank	0	10 gram			
		3	STM5	6	No			
		2	SE10	9	No			
		1	SE100	90	No			
		2	SPan5	5	No			
		2	Blank	0	No			

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

[2] Faeces not mixed with any preservation medium

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

* = with antibiotics

** = Naturally contaminated chicken faeces with *Salmonella*

*** = Naturally contaminated dust with *Salmonella*

Table A.2 CRL-*Salmonella* interlaboratory comparison study on the detection of *Salmonella* in food samples.

Study	Year	Number of samples	Capsules	Actual number of cfp/capsule	*Salmonella* negative meat	Selective enrichment medium	Plating-out medium	Reference [1] (RIVM report)
I	2006	5	STM10	9	10 gram	RVS, MKTTn, MSRV and own	XLD and own	Kuijpers et al.,. 2007 (Report 330604003)
		5	STM100	98	10 gram			
		5	SE100	74	10 gram			
		5	SE500	519	10 gram			
		5	Blank	0	10 gram			
		3	STM10	9	No			
		2	SE100	98	No			
		1	SE500	519	No			
		2	SPan5	5	No			
		2	Blank	0	No			
II	2007	5	STM5	4	10 gram	RVS, MKTTn, MSRV and own	XLD and own	Kuijpers et al., 2008 (Report 330604010)
		5	STM50	40	10 gram			
		5	SE10	7	10 gram			
		5	SE100	71	10 gram			
		5	Blank	0	10 gram			
		3	STM5	4	No			
		2	SE10	7	No			
		1	SE100	71	No			
		2	SPan5	7	No			
		2	Blank	0	No			

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

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.

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Annex 3 Information on the media used

MKTTn
(Oxoid CM 1048 Hampshire, United Kingdom) (Biokar BK 169 HA, Beauvais, France)
Composition of MKTTn: according ISO 6579, 2002

RVS (Oxoid CM 0866, Hampshire, United Kingdom) (AES Laboratoire AEB140862, France) (Lab M. Ltd. LAB 86, Bury, United Kingdom) (Himedia M149I)
Composition of RVS: according ISO 6579, 2002

BGA modified (Oxoid CM 0329/PO5033A, Hampshire, United Kingdom) (BPLS Merck 1.10747 Darmstadt, Germany) (AEB 151492, AES Laboratoire, France) (Biomark B439) (Lab M, lab 34 Bury, United Kingdom) (HImedai M971)
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: according ISO 6579, 1993

BGA (Conda laboratories, 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 20.0, pH 6.9

BGA (Oxoid CM 0263, Hampshire, United Kingdom)
Composition of BGA medium: the concentration of the compounds in g/L water: Proteose peptone 10.0, Yeast extract 3.0, Lactose 10.0, Sucrose 10.0, Sodium chloride 5.0, Phenol red 0.08, Brilliant green 0.0125, Agar 12.0, pH 6.9

BPLSA (Merck 107237, Darmstadt, Germany)
Composition of BPLSA medium: the concentration of the compounds in g/L water: Peptone from meat 5.0, Peptone from casein 5.0, Meat extract 5.0, Sodium chloride 3.0, di-sodium hydrogen phosphate 2.0, Lactose 10, Sucrose 10, Phenol red 0.08, brilliant green 0.0125, Agar agar 12.0, pH 7

BxLH
Home made 12 ingredients, the medium is patented, pH 7.1

MacConkey (Oxoid CM0115, Hampshire, United Kingdom)
Composition of MacConkey Agar No. 3 : the concentration of the compounds in g/L water: Peptone 20, Lactose 10, Bile Salts No3. 1.5, Sodium Chloride 5, Neutral red 0.03, Crystal violet 0.0001, Agar 15 pH 7.1

MLCB (Lab M. Ltd. LAB 116, Bury, United Kingdom)
Inoue T, Takagi S, Ohnishi A, et al. Foodborne disease *salmonella* isolation medium (MLCB). Japanese Journal of Veterinary Science 1968;30 (suppl):26.
Composition of MLCB medium: the concentration of the compounds in g/L water: Yeast Extract 5.0, Tryptone 5.0, Meat Peptones 7.0, Sodium Chloride 4.0, Mannitol 3.0, L-Lysine HCL 5.0, Sodium Thiosulphate 4.0, Ferric Ammonium Citrate Green 1.0, Brilliant Green 0.012, Crystal Violet 0.01, Agar No.2 15.0, pH 6.7

Onöz (Merck 115034, Darmstadt, Germany)
Onoz E, Hoffmann K. 1978 [Experience with a new culture medium for *salmonella* diagnosis (author's transl)] Zentralbl Bakteriol [Orig A]. 1978 Jan;240(1):16-21. German.
Composition of Onöz medium: the concentration of the compounds in g/L water: Yeast 3.0, Meat extract 6.0, Pepton from meat 6.8, Lactose 11.5, Sucrose 13.0, Bile salt mixture 3.825, tri-Sodium nitrate 5,5-Hydrate 9.3, Sodium Thiosulfate 5-Hydrate 4.25, L-Phenylalanine 5.0, Iron(III) Citrate 0.5,Magnesiumsulfate 0.4, Brilliant Green 0.00166, Neutral Red 0.022, Aniline Blue 0.25, Metachrome Yellow 0.47, di-Sodium Hydrogen Phosphate2-Hydrate 1.0, Agar-Agar 15, pH 7.1-7.2

Rambach (Merck 107500.0001/2/3, 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 8.0, NaCl 5.0, sodium deoxycholate 1.0, Cromogenic mix 1.5, propylene glycol 10.5, agar-agar 15, Rambach agar supplement 10 ml, pH 7.1-7.3

Rapid Salmonella agar (Biorad 3563961, Marnes-La-Coquette, France)
Casein Peptone 5, Meat extract 5, Selective agents 14, Chromogenic mixture 310 mg, Agar 12, pH 7.2

Salmonella Shigella SS medium (Biokar BK022 HA, Beauvais, France)
Composition of SS agar : the concentration of the compounds in g/L water: Peptone pancreatique de viande 5, Extrait de viande 5, Lactose 10, Sels biliaires 8.5, Citrate de Sodium 10, Thiosulfate de sodium 8.5, Citrate ferrique amoniacal 1, Rouge neuter 0.025 g, Vert brilliant 0.00033 g, Agar 15 g pH 6.8

SM(ID)2 (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) 6.25, Tris 0.16, Lactose 6.0, Ox bile (bovine and swine) 1.5, Cromogenic mix 9.63, Sodium chloride 5.0, Selective mix 0.03, Agar 14
pH 7.1

XLT4 (Oxoid CM1061/ PO5116A, Hampshire, United Kingdom) (Biorad 356 3654, Marnes-La-Coquette, France)
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 4.6 ml, Phenol Red 0.08, Agar 18 pH 7.4

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Annex 4 Results samples analysed with an 'own method'

Table A.3 Results control samples analysed with an 'own method'

	The highest number of positive isolations found with the given selective enrichment medium in combination with any isolation medium									
Labcode	**Other than MSRV 'own method'**					**MSRV**				
	Blank n=2	**SE10 n=2**	**SE100 n=1**	**SPan5 n=2**	**STM5 n=3**	**Blank n=2**	**SE10 n=2**	**SE100 n=1**	**SPan5 n=2**	**STM5 n=3**
Good Performance	**0**	**≥ 1**	**1**	**≥ 1**	**≥ 2**	**0**	**≥ 1**	**1**	**≥ 1**	**≥ 2**
	MKTTn					**MSRV**				
2	0	2	1	2	3	0	2	1	2	3
12	0	2	1	2	3	0	2	1	2	3
32	0	2	1	2	3	0	2	1	2	3
	RVS					**MSRV**				
5	0	2	1	2	3	0	2	1	2	3
7	0	2	1	2	**2**	0	2	1	2	**2**
9	0	**0**	1	2	3	0	**0**	1	2	3
10	**1**	**1**	1	**1**	**1**	**1**	**1**	1	**1**	**1**
13	0	**1**	1	2	3	0	**1**	1	2	3
15	0	2	1	2	3	0	2	1	2	3
21	0	2	1	2	3	0	2	1	2	3
23	0	2	1	2	3	0	2	1	2	3
28	0	**1**	1	2	3	0	1	1	2	3
	MSRV with 0.02 novobiocin					**MSRV with 0.01 novobiocin**				
19	0	2	1	2	3	0	**1**	1	2	**2**

Bold numbers: Deviating results

Grey cells: Results below the level of good performance

Table A.4 Results faeces samples artificially contaminated with *Salmonella* analysed with an 'own method'

	The highest number of positive isolations found with the given selective enrichment medium in combination with any isolation medium									
Labcode	**Other than MSRV 'own method'**					**MSRV**				
	Blank n=5	**SE10 n=5**	**SE100 n=5**	**STM5 n=5**	**STM50 n=5**	**Blank n=5**	**SE10 n=5**	**SE100 n=5**	**STM5 n=5**	**STM50 n=5**
Good Performance	**≤ 1**	**≥ 1**	**≥ 4**	**≥ 2**	**≥ 4**	**≤ 1**	**≥ 1**	**≥ 4**	**≥ 2**	**≥ 4**
	MKTTn					**MSRV**				
2	0	**1**	5	**4**	**4**	0	**3**	5	5	5
12	0	**1**	5	5	5	0	**4**	5	5	5
32	0	**0**	**0**	**0**	**0**	0	**0**	**2**	**4**	**4**
	RVS					**MSRV**				
5	0	5	5	5	5	0	5	5	5	5
7	**1**	**2**	5	5	5	0	**2**	5	**4**	5
9	0	**4**	**2**	5	5	0	**3**	5	**4**	5
10	0	**0**	5	**3**	5	0	**0**	5	**3**	5
13	0	**1**	5	5	5	0	**3**	5	5	5
15	0	**2**	5	5	5	0	**2**	5	5	5
21	0	**3**	5	**4**	5	0	**3**	5	**4**	5
23	0	5	5	5	5	0	5	5	5	5
28	0	**1**	5	5	**4**	0	**1**	5	5	5
	MSRV with 0.02 novobiocin					**MSRV with 0.01 novobiocin**				
19	0	5	5	**4**	5	0	5	5	**4**	5

Bold numbers: Deviating results

Grey cells: Results below the level of good performance

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Annex 5 Protocol

INTERLABORATORY COMPARISON STUDY ON THE DETECTION OF *SALMONELLA* spp. IN CHICKEN FAECES
organised by CRL-*Salmonella*
STUDY XI – 2008

Introduction

This is the 11th interlaboratory comparison study on the detection of *Salmonella* spp. in animal faeces amongst the National Reference Laboratories (NRLs for *Salmonella*) in the EU. This study will have a comparable set-up as the earlier studies on the detection of *Salmonella* spp. in veterinary samples. The prescribed method is the procedure as described in 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). 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 chicken faeces samples (*Salmonella* negative) artificially contaminated with reference materials. The reference materials (RMs) consist of gelatine capsules containing sublethally injured *Salmonella* Typhimurium (STM), *Salmonella* Enteritidis (SE) or *Salmonella* Panama (SPan) at different contamination levels. Each laboratory will examine 25 faeces samples (10 g each) in combination with a capsule containing STM or SE and 10 control samples (capsules only).

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 container will contain the chicken faeces. 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.

Each box will be sent as biological substance category B (UN3373) by door-to-door courier service. Please contact CRL-*Salmonella* when the parcel has not arrived at your laboratory at 21th of February 2008 (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 poultry faeces.

Outline of the study

Each participant will receive (in week 8 of 2008) one box containing 2 plastic containers, packed with cooling elements. The containers contain:

Container 1:

- 25 numbered vials; each containing one *Salmonella* Typhimurium, one *Salmonella* Enteritidis or a blank capsule (numbered 1-25);
- 10 control vials; each containing one capsule with or without *Salmonella* (numbered C1-C10).

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:

- 300 g of chicken faeces (free from *Salmonella*).

Store container 2 at (5 ± 3) ºC immediately after receipt.

The performance of the study will be in week 10 (starting on 3 March 2008).
The documents necessary for performing the study are:
-Protocol Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in animal faeces XI (2008);
-SOP Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in animal faeces XI (2008);

-Test report Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in animal faeces XI (2008);
-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 **22 March 2008.** The CRL will prepare a summary report soon after the study to inform all NRLs on the overall results.
Results which will be received after the deadline can not be used in the analyses for the summary report.
If you have questions or remarks about the interlaboratory comparison study please contact:

Angelina Kuijpers (Tel. number: + 31 30 274 2093)
Kirsten Mooijman (Tel. number: + 31 30 274 3537)
RIVM / LZO (Pb 63)
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Fax. number: + 31 30 274 4434
E-mail : Angelina.Kuijpers@rivm.nl or Kirsten.Mooijman@rivm.nl

Time table of interlaboratory comparison study ANIMAL FAECES XI (2008)

Week	Date	Topic
5	28 January – 1 February	Mailing of the protocol, standard operating procedure and test report to the NRLs-*Salmonella*
8	18-22 February	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 container (leave it in the plastic bag with lab code) and return it to CRL-*Salmonella* using the return envelope; **-Store the capsules at -20°C ± 5 °C** **-Store the faeces at +5°C ± 3 °C** **If you did not receive the parcel at 21 February, do contact the CRL immediately.**
9	25-29 February	**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)
10	3-7 March	Performance of the study, following the instructions as given in the protocol and the SOP of study Animal faeces XI (2008).
12	Before 22 March	Completion of the test report. Send the test report, preferably by e-mail to the CRL *Salmonella* (Angelina.Kuijpers@rivm.nl)* .
13	24-28 March	Data input at CRL-*Salmonella* and sending these data to NRLs. Checking these results by the National Reference Laboratories.
	April-May 2008	Sending of the final results to the NRLs together with a short report. As a follow-up, actions will be undertaken for those NRLs which scored below the average results of all NRLs.

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

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Annex 6 Standard Operating Procedure

(SOP)

INTERLABORATORY COMPARISON STUDY ON THE DETECTION OF *SALMONELLA* spp. IN CHICKEN FAECES
organised by CRL-*Salmonella*
STUDY XI - 2008

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 chicken faeces. For this purpose Reference Materials (RMs) containing sublethally injured *Salmonella* Typhimurium (STM), *Salmonella* Enteritidis (SE) or *Salmonella* Panama (SPan) as prepared by the Community Reference Laboratory (CRL) for *Salmonella* are used. As matrix, chicken faeces 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 faces 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.
- *Detection of Salmonella*: detection of *Salmonella* from reference materials in the presence of competitive organisms, when the test is 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
MSRV	Modified semi-solid Rappaport Vassiliadis medium
RM	Reference Material
SOP	Standard Operating Procedure
XLD	Xylose Lysine Deoxycholate agar

6 Culture media

For this study the prescribed method is the procedure as described in Annex D of ISO 6579, for which the following media are needed.

Non selective pre-enrichment medium	BPW
Selective enrichment medium	MSRV
Selective plating medium for first and second isolation	XLD and a second medium for choice (obligatory!)

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 (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. Note all relevant information in the test report.

6.1 Non selective pre-enrichment medium

- Buffered Peptone water (BPW) (ISO6579 Annex B.1)

Mind to distribute the BPW in portions of **90 ml** into suitable flasks before sterilisation.

6.2 Selective enrichment medium

- Modified Semi solid Rappaport Vassiliadis (MSRV) (ISO6579 Annex D)
- Own selective enrichment medium routinely used in your laboratory (optionally)

6.3 Solid selective media for first and second isolation

- Xylose-Lysine-Desoxycholat (90 mm plates) (ISO6579 Annex B.4)
- Second isolation medium for choice (obligatory)
- Own medium (optionally)

6.4 Confirmation media

Biochemical confirmation as described in ISO 6579 Annex B.6-B.11 or by reliable, commercial available identification kits.

- Nutrient agar (optionally) (ISO6579 Annex B.5)

7 Apparatus and glassware

The usual 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;
- 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 200 ml;
- Culture tubes with approximate sizes: 8 mm in diameter and 160 mm in length;
- Micro-pipettes; nominal capacity 0,1 ml;
- Petri dishes; standard size (diameter 90 mm to 100 mm)

8 Procedure

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

8.1 Prewarming BPW (day 0)

Label 25 jars, each containing 90 ml of BPW, from 1 to 25. Also label 12 jars of BPW from C1 to C12 (control capsules). One jar is a procedure control (= C11) to which no capsule or faeces is added and one jar is a negative faeces control to which only 10 g faeces is added (= C12). These control jars should further be handled in the same way as the other jars. Place all jars (at least) **overnight** at 37 °C (± 1 °C). Also place some extra non-labelled jars containing 90 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 of 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 35 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 10 g of faeces to jars labelled 1-25 and C12,**
- **Add no faeces to jars labelled C1 - C11,**

Do not shake the jars after adding the faeces.

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 16 & 21 of the test report.

8.3 Selective enrichment (day 2)

Allow the MSRV plates to equilibrate to room temperature, if they were stored at a lower temperature. Dry the surface of the MSRV plates in a Laminair Air Flow cabinet if necessary. Record (page 4-7) the requested data of the MSRV and own selective enrichment media (if used) in the test report. Label 25 MSRV plates from 1 to 25. Also label 12 MSRV plates from C1 to C12. Incubate the MSRV plates for 24 h and later on for another 24 h. If own selective enrichment media are used, label them in the same way as described for MSRV.

After equilibration:
Prescribed method:

- Inoculate the MSRV plates with three drops of BPW culture, 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 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 4-7).

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

Record in the test report (page 8-13) the requested data of the isolation media used. Label 25 (standard size) Petri dishes of each isolation medium from 1 to 25 and label 12 (standard size) Petri dishes from C1 to C12.

First isolation after 24 h
Inoculation:
Inoculate from suspect MSRV plates, the surface of an isolation medium in one standard size Petri dish with the corresponding label number in such a way that well isolated colonies will be obtained. The following isolation media will be used:

1)Xylose Lysine Desoxycholate agar (XLD)
Place the Petri dishes with the bottom up in the incubator set at 37 °C (record temperature and time and other requested data in test report, page 8 & 9).
2)Second isolation medium. Follow the instructions of the manufacturer (record temperature and time and other requested data in test report, page 10 & 11).
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 test report, page 12 &13).

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 isolation medium at least 1 colony considered to be typical or suspect (use only well isolated colonies). Store the plates at 5 ^{o}C ± 3 ^{o}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 the requested data of the nutrient agar on the test report (page 14). Incubate the inoculated plates at 37 ± 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 MSRV) and Table 2 (isolation using own enrichment) on the test report (pages 17-20).
If a PCR method has been used, report the results in Table 3 of the test report (page 21).

Confirmation of identity
The identity from the colony selected as described 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. The interpretation of the biochemical tests is given in Table 1 of ISO 6579:2002 on page 9.

9 Test report

The test report should contain all information that might influence the results and is not mentioned in this SOP. Some incidents or deviations from the specified procedures have to also be recorded. The test report shall include the name of the person in charge for the NRL, and the names of the persons who are carrying out the work and has to be signed by these persons. 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 ANIMAL FAECES XI (2008)
on the detection of *Salmonella* spp. in chicken faeces

Day	Topic	Description
0	**Prewarming BPW**	Place at least at the end of the day sufficient jars, each containing 90 ml BPW, at 37 °C ± 1 °C.
1	**Pre-enrichment**	Add 1 capsule to 90 ml (prewarmed) BPW Do not shake Incubate 45 min. at 37 °C ± 1 °C Add 10 g faeces to BPW Incubate 18 h ± 2 h at 37 °C ± 1 °C
2	**Selective enrichment**	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 suspect MSRV (24h) plates and Own medi(um)(a) ➢ XLD 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 MSRV medium another 24 (± 3) hours at the relevant temperatures
4	**Second isolation after 48 h**	If the first isolation was negative, inoculate from suspect MSRV (48h) plates and Own medi(um)(a) ➢ XLD agar ➢ Second isolation medium ➢ Own selective medi(um)(a)
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 second isolation media (day 4).

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Annex 7 Test report follow up study

INTERLABORATORY COMPARISON STUDY ON THE DETECTION OF *SALMONELLA* spp. IN CHICKEN FAECES
organised by CRL-*Salmonella*
STUDY XI- FOLLOW UP
June 2008

Laboratory code This is the same code as in FAECES XI February 2008	
Laboratory name (NRL)	
Address	
Country	
Date of arrival of the parcels	Date: …… - ……. – 2008 time: ……... h ……..…… min
Start time of storage at - 20 °C (capsules) Start time of storage at +5 °C (faeces)	Date: …… - ……. – 2008 time: ……... h ……..…… min Date: …… - ……. – 2008 time: ……... h ……..…… min
Parcels damaged?	☐ Yes ☐ No
Starting date testing	…… - ……. – 2008

PRE-ENRICHMENT – Buffered Peptone Water (BPW)	
Medium information BPW	
Was the composition of BPW the same as used in Faeces XI February 2008 ?	
☐ Yes	
☐ No, please give more details in an annex :	
Preparation of BPW	
Date of preparation	…… - ……. – 2008
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 (overnight)	
Start at	Date: …… - ……. – 2008 time: ……... h ……..…… min temperature incubator: ……..…… °C
End at	Date: …… - ……. - 2008 time: …… h …… min temperature incubator: ……...…… °C
Incubation time and temperature for dissolving the capsules (45 min)	
Start at	Date: …… - ……. - 2008 time: ……... h ……..…… min temperature incubator: ……..…… °C
End at	time: …… h …… min temperature incubator: ……...…… °C
Incubation time and temperature for pre-enrichment (18 ± 2) hrs after adding the faeces	
Start at	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
End at	Date: …… - ……. - 2008 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 Faeces XI February 2008 ?	
□ 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	…… - ……. - 2008
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	
Start of the first period (first 24 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
End of the first period (first 24 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
Start of the second period (48 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
End of the second period (48 h)	Date: …… - ……. - 2008 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 FAECES XI February 2008 ?
☐ 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 FAECES XI February 2008 ?		
☐ Yes		
☐ No please give more details in an annex :		
Size of petri dishes		
Size of petri dishes used	□90 mm □100 mm	□140 mm
Preparation of XLD		
Date of preparation	…… - ……. - 2008	
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		
Start incubation of XLD, inoculated from 24 h MSRV	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C	
End incubation of XLD, inoculated from 24 h MSRV	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C	
Start incubation of XLD,	Date: …… - ……. - 2008	

inoculated from 48 h MSRV	time: …….. h ……..…… min temperature incubator: ……..…… °C
End incubation of XLD, inoculated from 48 h MSRV	Date: …… - ……. - 2008 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 FAECES XI February 2008 ?		
☐ Yes		
☐ No please give more details in an annex :		
Size of petri dishes		
Size of petri dishes used	□90 mm □100 mm	□140 mm
Preparation of the second isolation medium		
Date of preparation	…… - ……. - 2008	
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		
Start incubation of second medium, inoculated from 24 h MSRV	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C	
End incubation of second medium, inoculated from 24 h MSRV	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C	
Start incubation of second medium, inoculated from 48 h MSRV	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C	
End incubation of second medium, inoculated from 48 h MSRV	Date: …… - ……. - 2008 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 FAECES XI February 2008? ☐ 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 FAECES XI February 2008 ?		
☐ Yes		
☐ No please give more details in an annex :		
Preparation of the nutrient agar		
Date of preparation	…… - ……. – 2008	
pH after preparation	……., measured at …… °C	
pH at the day of use	……., measured at …… °C	
Did you perform quality control of agar ?	☐ Yes ☐ No	

CONFORMATION of *Salmonella* suspected colonies
What media/test did you use for confirmation ?
☐ Biochemical: ☐ Triple sugar/iron agar (TSI) ☐ Urea Agar (UA) ☐ L-Lysine decarboxylation medium (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 faeces BRO February 2008, please give more information in an annex .	

Table 1: Results of isolation using **MSRV** (dish numbers 1-23)

	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												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												

22												
23												

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

Table 1 (continued): Results of isolation using **MSRV** (dish numbers C1-C8, C11 and C12)

	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]
C1												
C2												
C3												
C4												
C5												
C6												
C7												
C8												
C11												
C12												

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

Table 2: Results of isolation using **OWN** selective enrichment (dish numbers 1-23)

	Own * 24 hours						Own * 48 hours					
sample no.	XLD		*		*		XL:D		*		*	
	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												
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14												
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16												

17												
18												
19												
20												
21												
22												
23												

Col [a] = **number** of colonies used for confirmation
Sal [b] = **number** of colonies confirmed as *Salmonella*
* = fill in the name of the medium used

Table 2 (continued): Results of isolation using **Own** selective enrichment
(dish numbers C1-C8, C11 and C12)

	Own * 24 hours						Own * 48 hours					
sample no.	XLD		*		*		XLD		*		*	
	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]	Col [a]	Sal [b]
C1												
C2												
C3												
C4												
C5												
C6												
C7												
C8												
C11												
C12												

Col [a] = **number** of colonies used for confirmation
Sal [b] = **number** of colonies confirmed as *Salmonella*
* = fill in the name of the medium used

Table 3: Results of detection using PCR (dish numbers 1-23 & C1-C8, C11 and C12)

Sample no.	PCR + or -		
		no.	
1		C1	
2		C2	
3		C3	
4		C4	
5		C5	
6		C6	
7		C7	
8		C8	
9		C11	
10		C12	
11			
12			

13	
14	
15	
16	
17	
18	
19	
20	
21	
22	
23	

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

Name of person (s) carrying out the follow up faeces XI interlaboratory Comparison study (2008).	
Is the person(s) carrying out the follow up faeces XI interlaboratory Comparison study (2008) 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 for the determination of *Salmonella*. □YES □NO
Date and signature	

Name of person in charge When not NRL (see Pg 1) mention also the name of the laboratory.	
Date and signature	

Please send the completed test report **before 11 July**, preferably by email, to CRL-*Salmonella*. If the test report is e-mailed to the CRL, it is not longer necessary to sent the original test report as well, unless it is not legible (to be indicated by CRL-*Salmonella*).
Use the address below:

Angelina Kuijpers
CRL *Salmonella* (Pb 63)
RIVM / LZO
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