TEST REPORT

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

FOOD STUDY III - 2009

Laboratory code	
Laboratory name	
Address	
Country	
Date of arrival of the parcels	…… - ……. – 2009
Start time of storage at - 20 °C (capsules) Start time of storage at + 5 °C (minced chicken meat)	Date:……. Time:…… Date:……. Time:……
Parcels damaged?	☐ Yes ☐ No
Starting date testing	…… - ……. – 2009

Is your laboratory accredited according to ISO 17025, or planning to become accredited, for the determination of *Salmonella*? For which method(s) and matrices are you, or planning to become, accredited? *According to EC Regulations No. 882/2004 each NRL should be accredited for their relevant work field before 31 December 2009 (EC Regulation No. 2076/2005)	Accredited : ☐ Yes ☐No Planning : ☐ Yes ☐ No Time schedule: ☐ Accredition before 2010* ☐ Other…… Method : ☐ ISO 6579 (RVS and MKTTn), matrices: ☐ Annex D of ISO 6579 (MSRV), matrices: ☐ Other……. matrices:

PRE-ENRICHMENT – Buffered Peptone Water (BPW) (I)

Medium information BPW
What did you use to prepare the BPW?
☐ Individual ingredients
☐ Dehydrated medium
☐ Ready-to-use medium

In case of dehydrated or ready-to-use medium , give information on the manufacturer of BPW	
Manufacturer & name medium	
Code number	
Batch number	
Expire date	

Specific data of composition of BPW medium. What is the concentration of the following compounds in 1000 ml water:	
Enzymatic digest of casein	
Sodium chloride	
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4.12H_2O$)	
Potassium dihydrogen phosphate (KH_2PO_4)	

Preparation of BPW	
Date of preparation	…… - ……. – 2009
pH after preparation	…….., measured at …… °C
pH at the day of use	…….., measured at …… °C
Did you perform quality control of BPW?	☐ Yes ☐ No

PRE-ENRICHMENT – Buffered Peptone Water (BPW) (II)

Prewarming time and temperature of the BPW	
At the start	Date: …… - ……. – 2009 time: ……... h …….…… min temperature incubator: ……..…… °C
At the end	Date: …… - ……. - 2009 time: …… h …… min temperature incubator: ……...…… °C

Incubation time and temperature for dissolving the capsules (45 min)	
At the start	Date: …… - ……. - 2009 time: ……... h …….…… min temperature incubator: ……..…… °C
At the end	time: …… h …… min temperature incubator: ……...…… °C

Incubation time and temperature for pre-enrichment (18 ± 2) hrs after adding the minced chicken meat	
At the start	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C

SELECTIVE ENRICHMENT - Rappaport Vassiliadis Soya medium (RVS) (I)

Medium information RVS
What did you use to prepare the RVS?
☐ Individual ingredients
☐ Dehydrated medium
☐ Ready-to-use medium

In case of dehydrated or ready-to-use medium , give information on the manufacturer of RVS	
Manufacturer & name medium	
Code number	
Batch number	
Expire date	

Specific data of composition of RVS medium. What is the concentration of the following compounds in 1000 ml water:	
Enzymatic digest of Soya	
Sodium chloride (NaCl)	
Potassium dihydrogen phosphate (KH_2PO_4)	
Dipotassium hydrogen phosphate (K_2HPO_4)	
Magnesium chloride anhydrous ($MgCl_2$)	
Magnesium chloride hexahydrate ($MgCl_26H_2O$)	
Malachite green oxalate	

Preparation of RVS	
Date of preparation	…… - ……. - 2009
pH after preparation	…….., measured at …… °C
pH at the day of use	…….., measured at …… °C
Did you perform quality control of RVS?	☐ Yes ☐ No

SELECTIVE ENRICHMENT - Rappaport Vassiliadis Soya medium (RVS) (II)

Incubation time and temperature for selective enrichment	
At the start of the first period (24 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the second period (48 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C

SELECTIVE ENRICHMENT - Muller Kauffmann Tetra Thionate + novobiocin (MKTTn) (I)

Medium information MKTTn
What did you use to prepare the MKTTn?
☐ Individual ingredients
☐ Dehydrated medium
☐ Ready-to-use medium

In case of dehydrated or ready-to-use medium , give information on the Manufacturer of MKTTn	
Manufacturer & name medium	
Code number	
Batch number	
Expire date	

Specific data of composition of MKTTn medium. What is the concentration of the following compounds in 1000 ml water:	
Meat extract	
Enzymatic digest of casein	
Sodium chloride (NaCl)	
Calcium carbonate ($CaCO_3$)	
Sodium thiosulfate pentahydrate ($Na_2S_2O_3.5H_2O$)	
Ox bile for bacteriological use	
Brilliant green	
Iodine	
Potassium iodide (KI)	
Novobiocin	

SELECTIVE ENRICHMENT - Muller Kauffmann Tetra Thionate + novobiocin (MKTTn) (II)

Preparation of MKTTn	
Date of preparation	……. - ……. - 2009
pH after preparation	……., measured at …… °C
pH at the day of use	……., measured at …… °C
Did you perform quality control of MKTTn?	☐ Yes ☐ No

Incubation time and temperature for selective enrichment	
At the start of the first period (24 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the second period (48 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C

SELECTIVE ENRICHMENT - Modified Semi solid Rappaport Vassiliadis medium (MSRV) (I)

Medium information MSRV
What did you use to prepare the MSRV?
☐ Individual ingredients
☐ Dehydrated medium
☐ Ready-to-use medium

In case of dehydrated or ready-to-use medium , give information on the manufacturer of MSRV	
Manufacturer & name medium	
Code number	
Batch number	
Expire date	

Specific data of composition of MSRV medium. What is the concentration of the following compounds in 1000 ml water:	
Enzymatic digest of casein	
Acid hydrolysate of casein	
Sodium chloride (NaCl)	
Potassium dihydrogen phosphate (KH_2PO_4)	
Magnesium chloride anhydrous ($MgCl_2$)	
Malachite green oxalate	
Agar	
Novobiocin	

Preparation of MSRV	
Date of preparation	…… - ……. - 2009
pH after preparation	…….., measured at …… °C
pH at the day of use	…….., measured at …… °C
Did you perform quality control of MSRV?	☐ Yes ☐ No

SELECTIVE ENRICHMENT - Modified Semi solid Rappaport Vassiliadis medium (MSRV) (II)

Incubation time and temperature for selective enrichment	
At the start of the first period (24 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the second period (48 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C

OWN SELECTIVE ENRICHMENT - Selective medium, routinely used in your laboratory (I)

If you use more selective media, please give information on an annex.	
Medium:	

Medium information
What did you use to prepare the medium?
☐ Individual ingredients
☐ Dehydrated medium
☐ Ready-to-use medium

In case of dehydrated or ready-to-use medium , give information on the manufacturer of the medium	
Manufacturer & name medium	
Code number	
Batch number	
Expire date	

Specific data of composition of the medium. What is the concentration of the compounds in 1000 ml water:	

Preparation of the medium	
Date of preparation	…… - ……. - 2009
pH after preparation	…….., measured at …… °C
pH at the day of use	…….., measured at …… °C
Did you perform quality control of the medium?	☐ Yes ☐ No

OWN SELECTIVE ENRICHMENT - Selective medium, routinely used in your laboratory (II)

Further details concerning the medium	
Volume of the medium per jar/tube in ml	
Inoculation volume of BPW	
Prescribed incubation temperature in ^{o}C	

Incubation time and temperature for own selective enrichment	
At the start of the first period (24 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the second period (48 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C

FIRST AND SECOND ISOLATION - Xylose Lysine Desoxycholate medium (XLD) (I)

Medium information XLD
What did you use to prepare the XLD ?
☐ Individual ingredients
☐ Dehydrated medium
☐ Ready-to-use medium

In case of dehydrated or ready-to-use medium , give information on the manufacturer of XLD	
Manufacturer & name medium	
Code number	
Batch number	
Expire date	

Specific data of composition of XLD medium. What is the concentration of the following compounds in 1000 ml water:	
Xylose	
L-lysine hydrochloride	
Lactose	
Sucrose	
Sodium chloride (NaCl)	
Yeast extract powder	
Phenol red	
Agar	
Sodium desoxycholate	
Sodium thiosulfate	
Iron(III) ammonium citrate	

FIRST AND SECOND ISOLATION - Xylose Lysine Desoxycholate medium (XLD) (II)

Preparation of XLD	
Date of preparation	…… - ……. - 2009
pH after preparation	……., measured at …… °C
pH at the day of use	……., measured at …… °C
Did you perform quality control of XLD ?	☐ Yes ☐ No

Incubation time and temperature for isolation	
At the start of the first period (24 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the second period (48 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C

FIRST AND SECOND ISOLATION – Second Isolation medium. (I)

Give information on the second isolation medium.	
Name of the medium	
Prescribed incubation temperature in ^{o}C	

Medium information of the second isolation medium
What did you use to prepare the second isolation medium?
☐ Individual ingredients
☐ Dehydrated medium
☐ Ready-to-use medium

In case of dehydrated or ready-to-use medium , give information on the manufacturer of the second isolation medium	
Manufacturer & name medium	
Code number	
Batch number	
Expire date	

Specific data of composition of the second isolation medium. What is the concentration of the compounds in 1000 ml water:	

FIRST AND SECOND ISOLATION – Second Isolation medium. (II)

Preparation of the second isolation medium	
Date of preparation	…… - ……. - 2009
pH after preparation	……., measured at …… °C
pH at the day of use	……., measured at …… °C
Did you perform quality control ?	☐ Yes ☐ No

Incubation time and temperature for isolation	
At the start of the first period (24 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the second period (48 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C

FIRST AND SECOND ISOLATION – Own Isolation medium routinely used in your lab. (I)

If you use more selective media, please give information on an annex.	
Name of the medium	
Prescribed incubation temperature in ^{o}C	

Medium information of your own medium
What did you use to prepare your own medium ?
☐ Individual ingredients
☐ Dehydrated medium
☐ Ready-to-use medium

In case of dehydrated or ready-to-use medium , give information on the manufacturer of your own medium	
Manufacturer & name medium	
Code number	
Batch number	
Expire date	

Specific data of composition of your own medium. What is the concentration of the compounds in 1000 ml water:	

FIRST AND SECOND ISOLATION – Own Isolation medium routinely used in your lab. (II)

Preparation of your own medium	
Date of preparation	…… - ……. - 2009
pH after preparation	……., measured at …… °C
pH at the day of use	……., measured at …… °C
Did you perform quality control ?	☐ Yes ☐ No

Incubation time and temperature for isolation	
At the start of the first period (24 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the second period (48 h)	Date: …… - ……. - 2009 time: …….. h ……..…… min temperature incubator: ……..…… °C

CONFIRMATION – Nutrient agar (I)

Did you streak the colonies on Nutrient agar before starting confirmation?	
☐ Yes If yes give further information on nutrient agar below	☐ No

Medium information Nutrient agar
What did you use to prepare the nutrient agar ?
☐ Individual ingredients
☐ Dehydrated medium
☐ Ready-to-use medium

In case of dehydrated or ready-to-use medium , give information on the manufacturer of the nutrient agar	
Manufacturer & name medium	
Code number	
Batch number	
Expire date	

Specific data of composition of nutrient agar medium. What is the concentration of the compounds in 1000 ml water:	

Preparation of the nutrient agar	
Date of preparation	…… - ……. - 2009
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	
Is the PCR used commercially available?	☐ Yes ☐ No
If yes, name of PCR, manufacturer and batch used in the study:	☐ Real time PCR ☐ Other PCR ……. Manufacturer : ……. Batch : …….
Is the PCR validated? If yes, for which matrix/matrices and by whom?	☐ Yes ☐ No Matrices : …… Validated by : …….ref. number:
How many samples did you test for *Salmonella* using this PCR in 2008 ?	
At what moment did you start with the extraction/detection?	☐ before pre-enrichment in BPW ☐ after pre-enrichment in BPW ☐ after selective enrichment in/on: RVS /MKTTn/MSRV ☐ Other…….
Volume of (pre-) enrichment broth used for extraction	……
Volume of DNA-sample obtained from extraction	…….
Volume of DNA-sample added to PCR-mixture	…….

Composition of PCR-mixture		
Compound	Volume per sample	Manufacturer and batch of specific compound

Name of thermocycler	
Write down the number of cycles	
What kind of detection system is used	

Table 1: Results of isolation using **RVS** (dish numbers 1-25)

	RVS 24 hours						RVS 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												
24												
25												

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

Table 1 (continued): Results of isolation using **RVS** (dish numbers C1-C12)

	RVS 24 hours						RVS 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												
C9												
C10												
C11												
C12												

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

Table 2: Results of isolation using **MKTTn** (dish numbers 1-25)

	MKTTn 24 hours						MKTTn 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												
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7												
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20												
21												
22												
23												
24												
25												

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

Table 2 (continued): Results of isolation using **MKTTn** (dish numbers C1-C12)

	MKTTn 24 hours						MKTTn 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												
C9												
C10												
C11												
C12												

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

Table 3: Results of isolation using **MSRV** (dish numbers 1-25)

	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												
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18												
19												
20												
21												
22												
23												
24												
25												

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

Table 3 (continued): Results of isolation using **MSRV** (dish numbers C1-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												
C9												
C10												
C11												
C12												

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

Table 4: Results of isolation using own selective enrichment (dish numbers 1-25)

	Own selective enrichment 24 hours						Own selective enrichment 48 hours					
sample no.												
	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												
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19												
20												
21												
22												
23												
24												
25												

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

Table 4 (continued): Results of isolation using own selective enrichment (dish numbers C1-C12)

	Own selective enrichment 24 hours						Own selective enrichment 48 hours					
sample no.												
	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												
C9												
C10												
C11												
C12												

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

Table 5: Results of detection using PCR (dish numbers 1-25)

sample no.	PCR + or -		
		no.	
1		C1	
2		C2	
3		C3	
4		C4	
5		C5	
6		C6	
7		C7	
8		C8	
9		C9	
10		C10	
11		C11	
12		C12	
13			
14			
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20			
21			
22			
23			
24			
25			

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

Name of person (s) carrying out the interlaboratory Comparison study (2009).	
Is the person(s) carrying out the interlaboratory Comparison study (2009) 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 page 1) mention also the name of the laboratory.	
Date and signature	

Please send the completed test report before 23 October 2009 by email to CRL-*Salmonella*. If the test report is e-mailed or faxed to the CRL it is not necessary to sent the original test report as well, unless it is not legible (to be indicated by CRL-*Salmonella*).
Use the address below:

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