TEST REPORT

INTERLABORATORY COMPARISON STUDY ON THE DETECTION OF *SALMONELLA* spp. IN CHICKEN FAECES organised by EU-RL-*Salmonella*

STUDY XIV- 2011

Laboratory code	
Laboratory name (NRL)	
Address	
Country	
Date of arrival of the parcels	Date: …… - ……. – 2011 time: ……... h ……..…… min
Start time of storage at - 20 °C (capsules) Start time of storage at +5 °C (faeces)	Date: …… - ……. – 2011 time: ……... h ……..…… min Date: …… - ……. – 2011 time: ……... h ……..…… min
Parcels damaged?	☐ Yes ☐ No
Starting date testing	…… - ……. – 2011

Is your laboratory accredited according to ISO 17025, or planning to become accredited, for the determination of *Salmonella*? For which *Salmonella* 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 in 2011* ☐ 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	
Name manufacturer and 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	…… - ……. – 2011
pH after preparation	…….., measured at …… °C
pH at the day of use	…….., measured at …… °C
Did you perform quality control of BPW?	□yes □no

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

Containers with BPW	
Did you use containers with pre filled BPW ?	□ yes □ no
What kind of containers did you use for the pre-enrichment in BPW ?	□ plastic bags □ jars □ bottles □ ……

Equilibration of the BPW	
At which temperature did you equilibrate the BPW ?	□ at 37 °C □ at room temperature □ ……. °C
For how long did you equilibrate the BPW ?	……. h

Mix the samples (BPW, lenticules, faeces)	
How did you mix the samples ?	□ shake □ knead □ vortex □ pulsifier □ stomacher □ ……
□ did not mix the samples	

Incubation time and temperature for pre-enrichment (18 ± 2) hrs after adding faeces and lenticules	
Start at	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
End at	Date: …… - ……. - 2011 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	
Name manufacturer and 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	…… - …….. - 2011
pH after preparation	…….., measured at …… °C
pH at the day of use	…….., measured at …… °C
Did you perform quality control of MSRV?	□yes □no

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

Incubation time and temperature for selective enrichment	
Start of the first period (first 24 h)	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
End of the first period (first 24 h)	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
Start of the second period (48 h)	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
End of the second period (48 h)	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C

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

If you use more selective media, please give relevant information in 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	
Name manufacturer and 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	…… - …….. - 2011
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 (optional) (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	
Start of the first period (first 24 h)	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
End of the first period (first 24 h)	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
Start of the second period (48 h)	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
End of the second period (48 h)	Date: …… - ……. - 2011 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	
Name manufacturer and 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)

Size of petri dishes	
Size of petri dishes used	□90 mm □100 mm □140 mm

Preparation of XLD	
Date of preparation	…… - ……. - 2011
pH after preparation	……., measured at …… °C
pH at the day of use	……., measured at …… °C
Did you perform quality control of XLD ?	□yes □no

Incubation time and temperature for isolation	
Start incubation of XLD, inoculated from 24 h MSRV	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
End incubation of XLD, inoculated from 24 h MSRV	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
Start incubation of XLD, inoculated from 48 h MSRV	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
End incubation of XLD, inoculated from 48 h MSRV	Date: …… - ……. - 2011 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	
Name manufacturer and 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)

Size of petri dishes			
Size of petri dishes used	□90 mm	□100 mm	□140 mm

Preparation of the second isolation medium	
Date of preparation	…… - ……. - 2011
pH after preparation	……., measured at …… °C
pH at the day of use	……., measured at …… °C
Did you perform quality control ?	□yes □no

Incubation time and temperature for isolation	
Start incubation of second medium, inoculated from 24 h MSRV	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
End incubation of second medium, inoculated from 24 h MSRV	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
Start incubation of second medium, inoculated from 48 h MSRV	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
End incubation of second medium, inoculated from 48 h MSRV	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C

FIRST AND SECOND ISOLATION – Own Isolation medium routinely used In your laboratory (optional) (I)

If you use more selective media, please give relevant information in 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	
Name manufacturer and 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 laboratory (optional) (II)

Size of petri dishes	
Size of petri dishes used	□90 mm □100 mm □140 mm

Preparation of your own medium	
Date of preparation	…… - ……. - 2011
pH after preparation	……., measured at …… °C
pH at the day of use	……., measured at …… °C
Did you perform quality control ?	□yes □no

Incubation time and temperature for isolation	
Start incubation of own medium, inoculated from 24 h selective enrichment medium	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
End incubation of own medium, inoculated from 24 h selective enrichment medium	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
Start incubation of own medium, inoculated from 48 h selective enrichment medium	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C
End incubation of own medium, inoculated from 48 h selective enrichment medium	Date: …… - ……. - 2011 time: …….. h ……..…… min temperature incubator: ……..…… °C

CONFIRMATION – Nutrient agar

Did you streak the colonies on Nutrient agar before starting confirmation?
□yes □no If yes give further information on nutrient agar below

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	
Name manufacturer and medium	
Code number	
Batch number	
Expire date	

CONFIRMATION of *Salmonella* suspect 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 (I)

General questions	
Is the PCR used commercially available ?	□Yes □No
If yes, give 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 which organisation? If no, is the PCR published in the open literature ?	□Yes □No Matrices:…….. Validated by:…… Ref. number:…… Reference literature :
Do you use the PCR routinely ? How many samples did you test for *Salmonella* using this PCR in 2010 ?	□Yes □No …… number/year
At what moment did you start with the extraction/detection?	□before pre-enrichment in BPW □after pre-enrichment in BPW □after selective enrichment on MSRV □ other …… …… (please complete)
Volume of (pre-)enrichment broth used for extraction	……
Volume of DNA-sample obtained from extraction	…….
Volume of DNA-sample added to PCR-mixture	…….

DETECTION BY PCR (II)

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

Name of thermocycler	
Number of cycles	
What kind of detection system is used ?	

Table 1: Results of isolation using **MSRV** (dish numbers B1-B25)

	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]
B1												
B2												
B3												
B4												
B5												
B6												
B7												
B8												
B9												
B10												
B11												
B12												
B13												
B14												
B15												
B16												
B17												
B18												
B19												
B20												
B21												
B22												
B23												
B24												
B25												

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-C9)

	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												

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 medium
(dish numbers B1-B25) * = fill in the name of the medium used

	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]
B1												
B2												
B3												
B4												
B5												
B6												
B7												
B8												
B9												
B10												
B11												
B12												
B13												
B14												
B15												
B16												
B17												
B18												
B19												
B20												
B21												
B22												
B23												
B24												
B25												

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

Table 2 (continued): Results of isolation using **Own** selective enrichment medium (dish numbers C1-C9)

	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												
C9												

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 (sample numbers B1-B25 & C1-C9)

PCR + or -			
Sample no.		sample no.	
B1		C1	
B2		C2	
B3		C3	
B4		C4	
B5		C5	
B6		C6	
B7		C7	
B8		C8	
B9		C9	
B10			
B11			
B12			
B13			
B14			
B15			
B16			
B17			
B18			
B19			
B20			
B21			
B22			
B23			
B24			
B25			

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

Name of person (s) carrying out the fourteenth veterinary interlaboratory Comparison study (2011).	
Is the person(s) carrying out the fourteenth veterinary interlaboratory Comparison study (2011) working in the laboratory of the 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 of the NRL. When not NRL (see page 1) mention also the name of the laboratory.	
Date and signature	

Please send the completed test report before 18 March 2011, by email to EU-RL-*Salmonella*. If the test report is e-mailed to the EU-RL it is not necessary to sent the original test report as well, unless it is not legible (to be indicated by EU-RL-*Salmonella*).
Use the address below:

Angelina Kuijpers
EU-RL *Salmonella* (internal Pb 63)
RIVM / LZO
P.O. Box 1
3720 BA Bilthoven
The Netherlands

E-mail : Angelina.Kuijpers@rivm.nl

Tel. number: + 31 30 274 2093
Fax. number: + 31 30 274 4434
http://www.rivm.nl/crlsalmonella