

TEST REPORT

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

STUDY XI- 2008

Laboratory code	
Laboratory name (NRL)	
Address	
Country	
Date of arrival of the parcels	Date: - – 2008 time: h min
Start time of storage at - 20°C (capsules)	Date: - – 2008 time: h min
Start time of storage at +5°C (faeces)	Date: - – 2008 time: h min
Parcels damaged?	<input type="checkbox"/> Yes <input type="checkbox"/> No
Starting date testing - – 2008

Is your laboratory accredited for the determination of <i>Salmonella</i> . If yes, according to which system (e.g. ISO 17025) ? If no, are you planning to become accredited in the near future?*	Accredited : <input type="checkbox"/> No <input type="checkbox"/> Yes System : Planning : <input type="checkbox"/> Yes <input type="checkbox"/> No
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* According to EC Regulations No. 882/2004 each NRL should be accredited for their relevant workfield before 31 December 2009 (EC Regulation No. 2076/2005)

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
($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$)

Potassium dihydrogen phosphate (KH_2PO_4)

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

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

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) (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 (KH₂PO₄)

Magnesium chloride anhydrous (MgCl₂)

Malachite green oxalate

Agar

Novobiocin

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

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: - - 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 - Selective medium, routinely used in your laboratory (optional) (I)

If you use more selective media, please write these on an annex.

Medium:	
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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 - - 2008
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 °C	

Incubation time and temperature for own 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

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
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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 MSR V	Date: - - 2008 time: h min temperature incubator: °C
End incubation of XLD, inoculated from 24 h MSR V	Date: - - 2008 time: h min temperature incubator: °C
Start incubation of XLD, inoculated from 48 h MSR V	Date: - - 2008 time: h min temperature incubator: °C
End incubation of XLD, inoculated from 48 h MSR V	Date: - - 2008 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 °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
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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 MSR V	Date: - - 2008 time: h min temperature incubator: °C
End incubation of second medium, inoculated from 24 h MSR V	Date: - - 2008 time: h min temperature incubator: °C
Start incubation of second medium, inoculated from 48 h MSR V	Date: - - 2008 time: h min temperature incubator: °C
End incubation of second medium, inoculated from 48 h MSR V	Date: - - 2008 time: h min temperature incubator: °C

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

If you use more selective media, please write these on an annex.

Name of the medium	
Prescribed incubation temperature in °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 lab. (II)

Size of petri dishes

Size of petri dishes used	90 mm	100 mm	140 mm
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Preparation of your own 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 own medium, inoculated from 24 h selective enrichment medium	Date: - - 2008 time: h min temperature incubator: °C
End incubation of own medium, inoculated from 24 h selective enrichment medium	Date: - - 2008 time: h min temperature incubator: °C
Start incubation of own medium, inoculated from 48 h selective enrichment medium	Date: - - 2008 time: h min temperature incubator: °C
End incubation of own medium, inoculated from 48 h selective enrichment medium	Date: - - 2008 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	

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, give name of PCR, manufacturer and batch used in the study:		
Is the PCR validated ?	Yes Matrices :.....	
If yes, for which matrix/matrices and by whom?	Validated by :.....	
	No	
How much samples did you test for <i>Salmonella</i> using this PCR in 2007 ?		
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	

Composition of PCR-mixture

Compound	Volume per sample	Manufacturer and batch of specific compound

Name of thermocycler	
Number of cycles	
Detection system	

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

sample no.	MSRV 24 hours						MSRV 48 hours					
	XLD		Second isolation medium		Own isolation medium		XLD		Second isolation medium		Own isolation medium	
	Col ^a	Sal ^b	Col ^a	Sal ^b	Col ^a	Sal ^b	Col ^a	Sal ^b	Col ^a	Sal ^b	Col ^a	Sal ^b
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
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 **MSRV** (dish numbers C1-C12)

sample no.	MSRV 24 hours						MSRV 48 hours					
	XLD		Second isolation medium		Own isolation medium		XLD		Second isolation medium		Own isolation medium	
	Col ^a	Sal ^b	Col ^a	Sal ^b	Col ^a	Sal ^b	Col ^a	Sal ^b	Col ^a	Sal ^b	Col ^a	Sal ^b
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 **OWN** selective enrichment (dish numbers 1-25)

sample no.	Own * 24 hours						Own * 48 hours					
	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												
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6												
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21												
22												
23												
24												
25												

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-C12)

sample no.	Own * 24 hours						Own * 48 hours					
	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												
C10												
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 1-25 & C1-C12)

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		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		

Please send the completed test report before 22 March, 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

P.O. Box 1

3720 BA Bilthoven

The Netherlands

E-mail : Angelina.Kuijpers@rivm.nl

<http://www.rivm.nl/crlsalmonella>

Tel. number: + 31 30 274 2093

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