

**TEST REPORT**  
**INTERLABORATORY COMPARISON STUDY ON THE**  
**DETECTION OF SALMONELLA spp. IN PIG FAECES**  
 organised by EURL-Salmonella  
**STUDY XV- 2012**

Laboratory code	
Laboratory name (NRL)	
Address	
Country	
Date of arrival of the parcels	Date: ..... - ..... - 2012 time: ..... h ..... min
Start time of storage at - 20 °C (lenticule discs)	Date: ..... - ..... - 2012 time: ..... h ..... min
Start time of storage at +5 °C (faeces)	Date: ..... - ..... - 2012 time: ..... h ..... min
Parcels damaged?	<input type="checkbox"/> Yes <input type="checkbox"/> No
Starting date testing	..... - ..... - 2012
Is your laboratory accredited according to ISO 17025, or planning to become accredited, for the determination of <i>Salmonella</i> ? For which <i>Salmonella</i> 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 : <input type="checkbox"/> Yes <input type="checkbox"/> No Planning : <input type="checkbox"/> Yes <input type="checkbox"/> No Time schedule: <input type="checkbox"/> Accreditation in 2012* <input type="checkbox"/> Other..... Method : <input type="checkbox"/> ISO 6579 (RVS and MKTTn), matrices: <input type="checkbox"/> Annex D of ISO 6579 (MSRV), matrices: <input type="checkbox"/> 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 ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ )Potassium dihydrogen phosphate  
( $\text{KH}_2\text{PO}_4$ )**Preparation of BPW**

Date of preparation ..... - ..... – 2012

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 incubation

Date: ..... - ..... - 2012  
time: ..... h ..... min  
temperature incubator: ..... °C

End incubation

Date: ..... - ..... - 2012  
time: ..... h ..... min  
temperature incubator: ..... °C

<b>SELECTIVE ENRICHMENT - Modified Semi solid Rappaport Vassiliadis medium (MSRV) (I)</b>
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<b>Medium information MSR/V</b>
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What did you use to prepare the MSR/V?
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- |   |
|---|
| <input type="checkbox"/> Individual ingredients |
| <input type="checkbox"/> Dehydrated medium      |
| <input type="checkbox"/> Ready-to-use medium    |

<b>In case of dehydrated or ready-to-use medium , give information on the manufacturer of MSR/V</b>
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Name manufacturer and medium	
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Code number	
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Batch number	
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Expire date	
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<b>Specific data of composition of MSR/V medium. What is the concentration of the following compounds in 1000 ml water:</b>
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Enzymatic digest of casein	
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Acid hydrolysate of casein	
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Sodium chloride (NaCl)	
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Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )	
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Magnesium chloride anhydrous (MgCl <sub>2</sub> )	
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Malachite green oxalate	
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Agar	
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Novobiocin	
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<b>Preparation of MSR/V</b>
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Date of preparation	..... - ..... - 2012
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pH after preparation	....., measured at ..... °C
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pH at the day of use	....., measured at ..... °C
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Did you perform quality control of MSR/V?	<input type="checkbox"/> Yes <input type="checkbox"/> No
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**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: ..... - ..... - 2012 time: ..... h ..... min temperature incubator: ..... °C
End of the first period (first 24 h)	Date: ..... - ..... - 2012 time: ..... h ..... min temperature incubator: ..... °C
Start of the second period (48 h)	Date: ..... - ..... - 2012 time: ..... h ..... min temperature incubator: ..... °C
End of the second period (48 h)	Date: ..... - ..... - 2012 time: ..... h ..... min temperature incubator: ..... °C

<b>OWN SELECTIVE ENRICHMENT - Selective medium, routinely used in your laboratory (optional) (I)</b>
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<b>If you use more selective media, please give relevant information in an annex.</b>
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Medium:	
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<b>Medium information</b>
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What did you use to prepare the medium?
---

<input type="checkbox"/> Individual ingredients
---

<input type="checkbox"/> Dehydrated medium
--

<input type="checkbox"/> Ready-to-use medium
--

<b>In case of dehydrated or ready-to-use medium , give information on the manufacturer of the medium</b>
--

Name manufacturer and medium	
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Code number	
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Batch number	
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Expire date	
-------------	--

<b>Specific data of composition of the medium. What is the concentration of the compounds in 1000 ml water:</b>
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<b>Preparation of the medium</b>
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Date of preparation	..... - ..... - 2012
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pH after preparation	....., measured at ..... °C
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pH at the day of use	....., measured at ..... °C
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Did you perform quality control of the medium?	<input type="checkbox"/> Yes <input type="checkbox"/> No
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<b>OWN SELECTIVE ENRICHMENT - Selective medium, routinely used in your laboratory (optional) (II)</b>
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<b>Further details concerning the medium</b>
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Volume of the medium per jar/tube in ml	
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Inoculation volume of BPW	
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Prescribed incubation temperature in °C	
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<b>Incubation time and temperature for own selective enrichment</b>
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Start of the first period (first 24 h)	Date: ..... - ..... - 2012 time: ..... h ..... min temperature incubator: ..... °C
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End of the first period (first 24 h)	Date: ..... - ..... - 2012 time: ..... h ..... min temperature incubator: ..... °C
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Start of the second period (48 h)	Date: ..... - ..... - 2012 time: ..... h ..... min temperature incubator: ..... °C
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End of the second period (48 h)	Date: ..... - ..... - 2012 time: ..... h ..... min temperature incubator: ..... °C
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**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)****Preparation of XLD**

Date of preparation	..... - ..... - 2012
pH after preparation	....., measured at ..... °C
pH at the day of use	....., measured at ..... °C
Did you perform quality control of XLD ?	<input type="checkbox"/> Yes <input type="checkbox"/> No

**Incubation time and temperature for isolation**

Start incubation of XLD, inoculated from 24 h MSR V	Date: ..... - ..... - 2012 time: ..... h ..... min temperature incubator: ..... °C
End incubation of XLD, inoculated from 24 h MSR V	Date: ..... - ..... - 2012 time: ..... h ..... min temperature incubator: ..... °C
Start incubation of XLD, inoculated from 48 h MSR V	Date: ..... - ..... - 2012 time: ..... h ..... min temperature incubator: ..... °C
End incubation of XLD, inoculated from 48 h MSR V	Date: ..... - ..... - 2012 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)****Preparation of the second isolation medium**

Date of preparation	..... - ..... - 2012
pH after preparation	....., measured at ..... °C
pH at the day of use	....., measured at ..... °C
Did you perform quality control ?	<input type="checkbox"/> Yes <input type="checkbox"/> No

**Incubation time and temperature for isolation**

Start incubation of second medium, inoculated from 24 h MSR V	Date: ..... - ..... - 2012 time: ..... h ..... min temperature incubator: ..... °C
End incubation of second medium, inoculated from 24 h MSR V	Date: ..... - ..... - 2012 time: ..... h ..... min temperature incubator: ..... °C
Start incubation of second medium, inoculated from 48 h MSR V	Date: ..... - ..... - 2012 time: ..... h ..... min temperature incubator: ..... °C
End incubation of second medium, inoculated from 48 h MSR V	Date: ..... - ..... - 2012 time: ..... h ..... min temperature incubator: ..... °C

<b>FIRST AND SECOND ISOLATION – Own Isolation medium routinely used In your laboratory (optional) (I)</b>
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<b>If you use more selective media, please give relevant information in an annex.</b>
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Name of the medium	
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Prescribed incubation temperature in °C	
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<b>Medium information of your own medium</b>
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What did you use to prepare your own medium ?
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<input type="checkbox"/> Individual ingredients
---

<input type="checkbox"/> Dehydrated medium
--

<input type="checkbox"/> Ready-to-use medium
--

<b>In case of dehydrated or ready-to-use medium , give information on the manufacturer of your own medium</b>
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Name manufacturer and medium	
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Code number	
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Batch number	
--------------	--

Expire date	
-------------	--

<b>Specific data of composition of your own medium. What is the concentration of the compounds in 1000 ml water:</b>
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<b>FIRST AND SECOND ISOLATION – Own Isolation medium routinely used in your laboratory (optional) (II)</b>
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<b>Preparation of your own medium</b>	
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Date of preparation	..... - ..... - 2012
pH after preparation	....., measured at ..... °C
pH at the day of use	....., measured at ..... °C
Did you perform quality control ?	<input type="checkbox"/> Yes <input type="checkbox"/> No

<b>Incubation time and temperature for isolation</b>	
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Start incubation of own medium, inoculated from 24 h selective enrichment medium	Date: ..... - ..... - 2012 time: ..... h ..... min temperature incubator: ..... °C
End incubation of own medium, inoculated from 24 h selective enrichment medium	Date: ..... - ..... - 2012 time: ..... h ..... min temperature incubator: ..... °C
Start incubation of own medium, inoculated from 48 h selective enrichment medium	Date: ..... - ..... - 2012 time: ..... h ..... min temperature incubator: ..... °C
End incubation of own medium, inoculated from 48 h selective enrichment medium	Date: ..... - ..... - 2012 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)**

<b>General questions</b>	
Is the PCR used commercially available?	<input type="checkbox"/> Yes <input type="checkbox"/> No
If yes, give name of PCR, manufacturer and batch used in the study:	<input type="checkbox"/> Real time PCR <input type="checkbox"/> 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 ?	<input type="checkbox"/> Yes <input type="checkbox"/> No  Matrices:.....  Validated by:.....  Ref. number:.....  Reference literature :
Do you use the PCR routinely ? How many samples did you test for <i>Salmonella</i> using this PCR in 2011 ?	<input type="checkbox"/> Yes <input type="checkbox"/> No ..... number/year
At what moment did you start with the extraction/detection?	<input type="checkbox"/> before pre-enrichment in BPW <input type="checkbox"/> after pre-enrichment in BPW <input type="checkbox"/> after selective enrichment on MSR/V <input type="checkbox"/> 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)**

<b>Composition of PCR-mixture</b>		
Compound	Volume per sample	Manufacturer and batch of specific compound
Total volume of PCR mix per sample		
Name of thermocycler		
Number of cycles		
What kind of detection system is used ?		

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

sample no.	MSRV 24 hours						MSRV 48 hours					
	XLD		Second isolation medium		Own isolation medium		XLD		Second isolation medium		Own isolation medium	
	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>
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<sup>a</sup> = **number** of colonies used for confirmationSal<sup>b</sup> = **number** of colonies confirmed as *Salmonella*

Table 1 (continued): Results of isolation using **MSRV** (dish numbers C1-C9)

sample no.	MSRV 24 hours						MSRV 48 hours					
	XLD		Second isolation medium		Own isolation medium		XLD		Second isolation medium		Own isolation medium	
	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>
C1												
C2												
C3												
C4												
C5												
C6												
C7												
C8												
C9												

Col<sup>a</sup> = **number** of colonies used for confirmation

Sal<sup>b</sup> = **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

sample no.	Own * 24 hours						Own * 48 hours					
	XLD		*		*		XLD		*		*	
	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>
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<sup>a</sup> = number of colonies used for confirmation  
 Sal<sup>b</sup> = number of colonies confirmed as *Salmonella*

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

sample no.	Own * 24 hours						Own * 48 hours					
	XLD		*		*		XLD		*		*	
	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>	Col <sup>a</sup>	Sal <sup>b</sup>
C1												
C2												
C3												
C4												
C5												
C6												
C7												
C8												
C9												

Col<sup>a</sup> = **number** of colonies used for confirmation  
 Sal<sup>b</sup> = **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 &amp; 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 fifteenth veterinary interlaboratory Comparison study (2012).	
Is the person(s) carrying out the fifteenth veterinary interlaboratory Comparison study (2012) working in the laboratory of the NRL mentioned on page 1?	<input type="checkbox"/> Yes <input type="checkbox"/> No give more information of the laboratory carrying out the study :  Laboratory name .....  Address .....  Is this laboratory accredited for the determination of <i>Salmonella</i> . <input type="checkbox"/> Yes <span style="margin-left: 100px;"><input type="checkbox"/> No</span>
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 9 March 2012, by email to EURL-*Salmonella*. If the test report is e-mailed to the EURL it is not necessary to send the original test report as well, unless it is not legible (to be indicated by EURL-*Salmonella*).

Use the address below:

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