

**TEST REPORT OF THE SIXTH (2002)**

**BACTERIOLOGICAL COLLABORATIVE STUDY**

**ORGANISED BY CRL *SALMONELLA***

Detection of *Salmonella* in the presence of competitive micro-organisms  
The use of PCR technique for detection of *Salmonella* in faeces

Laboratory code	
Laboratory name	
Address	
Country	
Date of collecting the parcel	..... - ..... - 2002
Starting date testing	..... - ..... - 2002

Is your laboratory accredited or certified for the determination of <i>Salmonella</i> . If yes, according to which system ? If no, are you planning to be accredited or certified in the near future ?	
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**SHIPMENT**

<b>Cold chain monitor:</b>	
<b>Check at airport:</b>	date : ..... - .....2002 time : ..... h ..... min
Parcels damaged	<input type="checkbox"/> YES <input type="checkbox"/> NO
Colour of compartment 10°C Brief <input type="checkbox"/> completely coloured <input type="checkbox"/> partly coloured <input type="checkbox"/> white Moderate <input type="checkbox"/> completely coloured <input type="checkbox"/> partly coloured <input type="checkbox"/> white Prolonged <input type="checkbox"/> completely coloured <input type="checkbox"/> partly coloured <input type="checkbox"/> white	Colour of compartment 20°C Brief <input type="checkbox"/> completely coloured <input type="checkbox"/> partly coloured <input type="checkbox"/> white Moderate <input type="checkbox"/> completely coloured <input type="checkbox"/> partly coloured <input type="checkbox"/> white Prolonged <input type="checkbox"/> completely coloured <input type="checkbox"/> partly coloured <input type="checkbox"/> white
<b>Check at laboratory</b>	date : ..... - .....2002 time : ..... h ..... min
Parcels damaged	<input type="checkbox"/> YES <input type="checkbox"/> NO
Colour of compartment 10°C Brief <input type="checkbox"/> completely coloured <input type="checkbox"/> partly coloured <input type="checkbox"/> white Moderate <input type="checkbox"/> completely coloured <input type="checkbox"/> partly coloured <input type="checkbox"/> white Prolonged <input type="checkbox"/> completely coloured <input type="checkbox"/> partly coloured <input type="checkbox"/> white	Colour of compartment 20°C Brief <input type="checkbox"/> completely coloured <input type="checkbox"/> partly coloured <input type="checkbox"/> white Moderate <input type="checkbox"/> completely coloured <input type="checkbox"/> partly coloured <input type="checkbox"/> white Prolonged <input type="checkbox"/> completely coloured <input type="checkbox"/> partly coloured <input type="checkbox"/> white

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

**!!!! Record temperatures and times outside the range as indicated in the SOP on page 27 !!!!**

**Manufacturer of the BPW**

Name	
Code number	
Batch number	
Expire date	
pH of the BPW : .....	Measured at .....oC

**Incubation time and temperature for dissolving the capsules**

At the start	time: ..... h ..... min temperature incubator: ..... °C temperature of BPW at the start: <input type="checkbox"/> Room temperature <input type="checkbox"/> 37°C
At the end	time: ..... h ..... min temperature incubator: ..... °C

**Incubation time and temperature for pre-enrichment**

At the start	time: ..... h ..... min temperature incubator: ..... °C
At the end	time: ..... h ..... min temperature incubator: ..... °C

**SELECTIVE ENRICHMENT - Rappaport Vassiliadis Soya medium (RVS)****!!!! Record temperatures and times outside the range as indicated in the SOP on page 27 !!!!****Manufacturer of the RVS medium**

Name	
Code number	
Batch number	
Expire date	
pH of broth : .....	Measured at .....oC

**Specific data of composition of RVS medium. What is the concentration of the following compounds:**

Soya Peptone	
Sodium chloride	
Potassium dihydrogen phosphate	
Dipotassium hydrogen phosphate	
Magnesium chloride anhydrous	
Magnesium chloride.6H <sub>2</sub> O	
Malachite green	

**Incubation time and temperature for selective enrichment**

At the start of the first period	time: ..... h ..... min temperature incubator: ..... °C
At the end of the first period	time: ..... h ..... min temperature incubator: ..... °C
At the start of the second period	time: ..... h ..... min temperature incubator: ..... °C
At the end of the second period	time: ..... h ..... min temperature incubator: ..... °C

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

**!!!! Record temperatures and times outside the range as indicated in the SOP on page 27 !!!!**

**Manufacturer of the MKTTn medium**

Name	
Code number	
Batch number	
Expire date	
pH of the broth : .....	Measured at .....oC

**Specific data of composition of MKTTn medium. What is the concentration of the following compounds:**

Enzymatic digest of meat extract	
Enzymatic digest of casein	
Sodium chloride (NaCl)	
Calcium carbonate (CaCO <sub>3</sub> )	
Sodium thiosulfate pentahydrate (Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .5H <sub>2</sub> O)	
Ox bile for bacteriological use	
Brilliant green	

**Incubation time and temperature for selective enrichment**

At the start of the first period	time: ..... h ..... min temperature incubator: ..... °C
At the end of the first period	time: ..... h ..... min temperature incubator: ..... °C
At the start of the second period	time: ..... h ..... min temperature incubator: ..... °C
At the end of the second period	time: ..... h ..... min temperature incubator: ..... °C

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

**!!!! Record temperatures and times outside the range as indicated in the SOP on page 27 !!!!**

**How much samples did you test in 2000 and 2001 using MSRV as selective enrichment**

2000:..... 2001:.....

**Manufacturer of the MSRV medium**

Name	
Code number	
Batch number	
Expire date	
pH of the broth :.....	Measured at .....oC

**Incubation time and temperature for selective enrichment**

At the start of the first period	time: ..... h ..... min temperature incubator: ..... °C
At the end of the first period	time: ..... h ..... min temperature incubator: ..... °C
At the start of the second period	time: ..... h ..... min temperature incubator: ..... °C
At the end of the second period	time: ..... h ..... min temperature incubator: ..... °C

**SELECTIVE ENRICHMENT - Selective medium, routinely used in your laboratory**

**!!!! Record temperatures and times outside the range as indicated in the SOP on page 27 !!!!**

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

Medium:

**Manufacturer of the medium**

Name	
Code number	
Batch number	
Expire date	
pH of the broth : .....	Measured at .....oC
Volume of the medium per jar/tube in ml	
Inoculation volume of BPW	
Prescribed incubation temperature in °C	

**Incubation time and temperature for selective enrichment**

At the start of the first period	time: ..... h ..... min temperature incubator: ..... °C
At the end of the first period	time: ..... h ..... min temperature incubator: ..... °C
At the start of the second period	time: ..... h ..... min temperature incubator: ..... °C
At the end of the second period	time: ..... h ..... min temperature incubator: ..... °C

**FIRST AND SECOND ISOLATION - Phenol red/brilliant green agar (BGA)**

**!!!! Record temperatures and times outside the range as indicated in the SOP on page 27 !!!!**

<b>Manufacturer of the phenol red/brilliant green agar</b>	
Name	
Code number	
Batch number	
Expire date	
pH of the broth:.....	Measured at .....°C
Volume of the medium per jar/tube in ml	
Inoculation volume of BPW	
Prescribed incubation temperature in °C	

<b>Size of petri dishes</b>			
Size of petri dishes used	<input type="checkbox"/> 90 mm	<input type="checkbox"/> 100 mm	<input type="checkbox"/> 140 mm

<b>Incubation time and temperature for first isolation</b>	
At the start	time: ..... h ..... min temperature incubator: ..... °C
At the end	time: ..... h ..... min temperature incubator: ..... °C

<b>Incubation time and temperature for second isolation</b>	
At the start	time: ..... h ..... min temperature incubator: ..... °C
At the end	time: ..... h ..... min temperature incubator: ..... °C



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

**!!!! Record temperatures and times outside the range as indicated in the SOP on page 27 !!!!**

**Manufacturer of the XLD medium**

Name	
Code number	
Batch number	
Expire date	
pH of the broth : .....	Measured at .....oC

**Size of petri dishes**

Size of petri dishes used	<input type="checkbox"/> 90 mm	<input type="checkbox"/> 100 mm	<input type="checkbox"/> 140 mm
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**Incubation time and temperature for first isolation**

At the start	time: ..... h ..... min temperature incubator: ..... °C
At the end	time: ..... h ..... min temperature incubator: ..... °C

**Incubation time and temperature for second isolation**

At the start	time: ..... h ..... min temperature incubator: ..... °C
At the end	time: ..... h ..... min temperature incubator: ..... °C

**FIRST AND SECOND ISOLATION - Isolation medium routinely used in your lab.**

**!!!! Record temperatures and times outside the range as indicated in the SOP on page 27 !!!!**

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

Name of the medium	
Prescribed incubation temperature in °C	

**Manufacturer of this medium**

Name	
Code number	
Batch number	
Expire date	
pH of the broth : .....	Measured at .....°C

**Size of petri dishes**

Size of petri dishes used	<input type="checkbox"/> 90 mm	<input type="checkbox"/> 100 mm	<input type="checkbox"/> 140 mm
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**Incubation time and temperature for first isolation**

At the start	time: ..... h ..... min temperature incubator: ..... °C
At the end	time: ..... h ..... min temperature incubator: ..... °C

**Incubation time and temperature for second isolation**

At the start	time: ..... h ..... min temperature incubator: ..... °C
At the end	time: ..... h ..... min temperature incubator: ..... °C

**CONFIRMATION – Nutrient agar**

!!!! Record temperatures and times outside the range as indicated in the SOP on page 27 !!!!

**Manufacturer of the nutrient agar**

Name	
Code number	
Batch number	
Expire date	
pH of the broth:.....	Measured at.....°C

**Size of petri dishes**

Size of petri dishes used	<input type="checkbox"/> 90 mm	<input type="checkbox"/> 100 mm	<input type="checkbox"/> 140 mm
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**Incubation time and temperature for confirmation**

At the start	time: ..... h ..... min temperature incubator: ..... °C
At the end	time: ..... h ..... min temperature incubator: ..... °C

**BIOCHEMICAL CONFIRMATION**

**Manufacturer of the TSI agar**

Name	
Code number	
Batch number	
Expire date	
pH of the broth:.....	Measured at.....°C

**Manufacturer of the urea agar**

Name	
Code number	
Batch number	
Expire date	
pH of the broth:.....	Measured at.....°C

**Manufacturer of the l-Lysine decarboxylation medium**

Name	
Code number	
Batch number	
Expire date	
pH of the broth:.....	Measured at.....°C

**Manufacturer of other confirmation tests - .....**

Name	
Code number	
Batch number	
Expire date	
pH of the broth:.....	Measured at.....°C

**DETECTION BY PCR****General questions**

Is the PCR used commercially available	<input type="checkbox"/> Yes <input type="checkbox"/> No
If yes, name of PCR, manufacturer and batch used in the study:	
Is the PCR validated	<input type="checkbox"/> Yes <input type="checkbox"/> No
How much samples did you test for <i>Salmonella</i> using this PCR in 2001 ?	
At what moment did you start with the extraction/detection?	before or after incubation of BPW
Volume of pre-enrichment 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 cycles	
What kind of detection system is used	

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

no.	RV 24 hours						RV 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col <sup>a</sup>	Sal <sup>b</sup>	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
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15												
16												
17												
18												
19												
20												
21												
22												
23												
24												
25												

Col<sup>a</sup> = number of colonies used for confirmation  
 Sal<sup>b</sup> = number of colonies confirmed as *Salmonella*

Table 1 (continued): Results of isolation using RVS (dish numbers N1-N20)

no.	RV 24 hours						RV 48 hours					
	BGA		XLD		third medium		BGA		XLD		third medium	
	Col <sup>a</sup>	Sal <sup>b</sup>	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
N1												
N2												
N3												
N4												
N5												
N6												
N7												
N8												
N9												
N10												
N11												
N12												
N13												
N14												
N15												
N16												
N17												
N18												
N19												
N20												

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

Sal<sup>b</sup> = number of colonies confirmed as *Salmonella*

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

no.	RV 24 hours						RV 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col <sup>a</sup>	Sal <sup>b</sup>	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
C1												
C2												
C3												
C4												
C5												
C6												
C7												
C8												
C9												
C10												
C11												
C12												

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 MKTTn (dish numbers 1-25)

no.	RV 24 hours						RV 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col <sup>a</sup>	Sal <sup>b</sup>	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
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15												
16												
17												
18												
19												
20												
21												
22												
23												
24												
25												

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 MKTTn (dish numbers N1-N20)

no.	RV 24 hours						RV 48 hours					
	BGA		XLD		third medium		BGA		XLD		third medium	
	Col <sup>a</sup>	Sal <sup>b</sup>	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
N1												
N2												
N3												
N4												
N5												
N6												
N7												
N8												
N9												
N10												
N11												
N12												
N13												
N14												
N15												
N16												
N17												
N18												
N19												
N20												

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 MKTTn (dish numbers C1-C12)

no.	RV 24 hours						RV 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col <sup>a</sup>	Sal <sup>b</sup>	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
C1												
C2												
C3												
C4												
C5												
C6												
C7												
C8												
C9												
C10												
C11												
C12												

Col<sup>a</sup> = number of colonies used for confirmation  
 Sal<sup>b</sup> = number of colonies confirmed as *Salmonella*

Table 3: Results of isolation using MSR V (dish numbers 1-25)

no.	MSRV 24 hours						MSRV 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col <sup>a</sup>	Sal <sup>b</sup>	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
1												
2												
3												
4												
5												
6												
7												
8												
9												
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20												
21												
22												
23												
24												
25												

Col<sup>a</sup> = number of colonies used for confirmation  
 Sal<sup>b</sup> = number of colonies confirmed as *Salmonella*

Table 3 (continued): Results of isolation using MSR/V (dish numbers N1-N20)

no.	MSRV 24 hours						MSRV 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col <sup>a</sup>	Sal <sup>b</sup>	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
N1												
N2												
N3												
N4												
N5												
N6												
N7												
N8												
N9												
N10												
N11												
N12												
N13												
N14												
N15												
N16												
N17												
N18												
N19												
N20												

Col<sup>a</sup> = number of colonies used for confirmation  
 Sal<sup>b</sup> = number of colonies confirmed as *Salmonella*

Table 3 (continued): Results of isolation using MSR/V (dish numbers C1-C12)

no.	MSRV 24 hours						MSRV 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col <sup>a</sup>	Sal <sup>b</sup>	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
C1												
C2												
C3												
C4												
C5												
C6												
C7												
C8												
C9												
C10												
C11												
C12												

Col<sup>a</sup> = number of colonies used for confirmation  
 Sal<sup>b</sup> = number of colonies confirmed as *Salmonella*

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

no.	Own enrichment 24 hours						Own enrichment 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col <sup>a</sup>	Sal <sup>b</sup>	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
1												
2												
3												
4												
5												
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23												
24												
25												

Col<sup>a</sup> = number of colonies used for confirmation  
 Sal<sup>b</sup> = number of colonies confirmed as *Salmonella*

Table 4 (continued): Results of isolation using own enrichment (dish numbers N1-N20)

no.	Own enrichment 24 hours						Own enrichment 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col <sup>a</sup>	Sal <sup>b</sup>	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
N1												
N2												
N3												
N4												
N5												
N6												
N7												
N8												
N9												
N10												
N11												
N12												
N13												
N14												
N15												
N16												
N17												
N18												
N19												
N20												

Col<sup>a</sup> = number of colonies used for confirmation  
 Sal<sup>b</sup> = number of colonies confirmed as *Salmonella*



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

no.	Own enrichment 24 hours						Own enrichment 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col <sup>a</sup>	Sal <sup>b</sup>	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
C1												
C2												
C3												
C4												
C5												
C6												
C7												
C8												
C9												
C10												
C11												
C12												

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

Sal<sup>b</sup> = number of colonies confirmed as *Salmonella*

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

PCR + or -					
no.		no.		no.	
1		N1		C1	
2		N2		C2	
3		N3		C3	
4		N4		C4	
5		N5		C5	
6		N6		C6	
7		N7		C7	
8		N8		C8	
9		N9		C9	
10		N10		C10	
11		N11		C11	
12		N12		C12	
13		N13			
14		N14			
15		N15			
16		N16			
17		N17			
18		N18			
19		N19			
20		N20			
21					
22					
23					
24					
25					

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

Name of person carrying out the sixth bacteriological collaborative study	
Date and signature	

Name of person in charge	
Date and signature	