TEST REPORT

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

FEED STUDY I - 2008

Laboratory code	
Laboratory name	
Address	
Country	
Date of arrival of the parcels	…… - ……. – 2008
Start time of storage at - 20 ^{o}C (capsules) Start time of storage at + 5 ^{o}C (chicken feed)	Date:……. Time:…… Date:……. Time:……
Parcels damaged?	☐ Yes ☐ No
Starting date testing	…… - ……. – 2008

Is your laboratory accredited or certified for the determination of *Salmonella*. If yes, according to which procedure (e.g. ISO 17025)? If no, are you planning to become accredited or certified in the near future?*	Accredited : ☐ No ☐Yes Procedure : Planning : ☐ Yes ☐ No

* 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	
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	…… - ……. – 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	
At the start	Date: …… - ……. – 2008 time: ……... h …….…… min temperature incubator: ……..…… °C
At the end	Date: …… - ……. - 2008 time: …… h …… min temperature incubator: ……...…… °C

Incubation time and temperature for dissolving the capsules (45 min)	
At the start	Date: …… - ……. - 2008 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 chicken feed	
At the start	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end	Date: …… - ……. - 2008 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	…… - ……. - 2008
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: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the second period (48 h)	Date: …… - ……. - 2008 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	……. - ……. - 2008
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: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the second period (48 h)	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	
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	…… - ……. - 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	
At the start of the first period (24 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the 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 (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	…… - ……. - 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 ^{o}C	

Incubation time and temperature for own selective enrichment	
At the start of the first period (24 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the 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	
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	…… - ……. - 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	
At the start of the first period (24 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the second period (48 h)	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 ^{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	…… - ……. - 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	
At the start of the first period (24 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the second period (48 h)	Date: …… - ……. - 2008 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	…… - ……. - 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	
At the start of the first period (24 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the first period (24 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the start of the second period (48 h)	Date: …… - ……. - 2008 time: …….. h ……..…… min temperature incubator: ……..…… °C
At the end of the second period (48 h)	Date: …… - ……. - 2008 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	…… - ……. - 2008
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:	
Is the PCR validated? If yes, for which matrix/matrices and by whom?	☐ Yes Matrices :…… Validated by :…… ☐ No
How many samples did you test for *Salmonella* 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
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												
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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												
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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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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 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			
15			
16			
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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 first feed interlaboratory Comparison study (2008).	
Is the person(s) carrying out the first feed interlaboratory Comparison study (2008) 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 or certified 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 24 October preferable by email to CRL-*Salmonella*. If the test report is e-mailed or faxed 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:

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