

TEST REPORT

BACTERIOLOGICAL COLLABORATIVE STUDY VII (2003) ON THE DETECTION OF *SALMONELLA* spp. organised by CRL-*Salmonella*

Laboratory code	
Laboratory name	
Address	
Country	
Date of arrival of the parcels - - 2003
Start time of storage at - 20°C	Date:..... Time:.....
Parcels damaged?	<input type="checkbox"/> YES <input type="checkbox"/> NO
Starting date testing - - 2003

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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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

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

Potassium dihydrogen phosphate

Preparation of BPW

Date of preparation

..... - - 2003

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: - - 2003 time: h min temperature incubator: °C
At the end	Date: - - 2003 time: h min temperature incubator: °C

Incubation time and temperature for dissolving the capsules

At the start	Date: - - 2003 time: h min temperature incubator: °C
At the end	time: h min temperature incubator: °C

Incubation time and temperature for pre-enrichment (after adding the faeces)

At the start	Date: - - 2003 time: h min temperature incubator: °C
At the end	Date: - - 2003 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

Name

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:

Soya Peptone

Sodium chloride

Potassium dihydrogen phosphate

Dipotassium hydrogen phosphate

Magnesium chloride anhydrous

Magnesium chloride.6H₂O

Malachite green

Preparation of RVS

Date of preparation

..... - - 2003

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	Date: - - 2003 time: h min temperature incubator: °C
At the end of the first period	Date: - - 2003 time: h min temperature incubator: °C
At the start of the second period	Date: - - 2003 time: h min temperature incubator: °C
At the end of the second period	Date: - - 2003 time: h min temperature incubator: °C

SELECTIVE ENRICHMENT - Muller Kauffmann Tetra Thionate + novobiocin (MKTTn) (I)
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Medium information MKTTn

What did you use to prepare the MKTTn?
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- | |
|---|
| <input type="checkbox"/> Individual ingredients |
| <input type="checkbox"/> Dehydrated medium |
| <input type="checkbox"/> Ready-to-use medium |

In case of dehydrated or ready-to-use medium , give information on the manufacturer of MKTTn

Name	
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Code number	
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Batch number	
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Expire date	
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Specific data of composition of MKTTn medium. What is the concentration of the following compounds in 1000 ml water:

Enzymatic digest of meat extract	
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Enzymatic digest of casein	
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Sodium chloride (NaCl)	
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Calcium carbonate (CaCO ₃)	
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Sodium thiosulfate pentahydrate (Na ₂ S ₂ O ₃ ·5H ₂ O)	
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Ox bile for bacteriological use	
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Brilliant green	
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Preparation of MKTTn

Date of preparation - - 2003
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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 MKTTn?	<input type="checkbox"/> yes	<input type="checkbox"/> no
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SELECTIVE ENRICHMENT - Muller Kauffmann Tetra Thionate + novobiocin (MKTTn) (II)

Incubation time and temperature for selective enrichment	
At the start of the first period	Date: - - 2003 time: h min temperature incubator: °C
At the end of the first period	Date: - - 2003 time: h min temperature incubator: °C
At the start of the second period	Date: - - 2003 time: h min temperature incubator: °C
At the end of the second period	Date: - - 2003 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	
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:

Preparation of MSRV

Date of preparation - - 2003
pH after preparation, measured at °C
pH at the day of use, measured at °C
Did you perform quality control of MSRV?	<input type="checkbox"/> yes <input type="checkbox"/> no

SELECTIVE ENRICHMENT - Modified Semi solid Rappaport Vassiliadis medium (MSRV) (II)
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Incubation time and temperature for selective enrichment	
At the start of the first period	Date: - - 2003 time: h min temperature incubator: °C
At the end of the first period	Date: - - 2003 time: h min temperature incubator: °C
At the start of the second period	Date: - - 2003 time: h min temperature incubator: °C
At the end of the second period	Date: - - 2003 time: h min temperature incubator: °C

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

If you use more selective media, please write these 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

Name

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

..... - - 2003

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

SELECTIVE ENRICHMENT - Selective medium, routinely used in your laboratory (II)
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Further details concerning the medium	
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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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Incubation time and temperature for selective enrichment	
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At the start of the first period	Date: - - 2003 time: h min temperature incubator: °C
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At the end of the first period	Date: - - 2003 time: h min temperature incubator: °C
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At the start of the second period	Date: - - 2003 time: h min temperature incubator: °C
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At the end of the second period	Date: - - 2003 time: h min temperature incubator: °C
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FIRST AND SECOND ISOLATION - Phenol red/brilliant green agar (BGA) (I)

Medium information BGA

What did you use to prepare the BGA ?

- Individual ingredients
- Dehydrated medium
- Ready-to-use medium

In case of dehydrated or ready-to-use medium , give information on the manufacturer of BGA

Name	
Code number	
Batch number	
Expire date	

Specific data of composition of BGA medium. What is the concentration of the following compounds in 1000 ml water:

Preparation of BGA

Date of preparation - - 2003
pH after preparation, measured at °C
pH at the day of use, measured at °C
Did you perform quality control of BGA ?	<input type="checkbox"/> yes <input type="checkbox"/> no

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

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 isolation

At the start of the first period	Date: - - 2003 time: h min temperature incubator: °C
At the end of the first period	Date: - - 2003 time: h min temperature incubator: °C
At the start of the second period	Date: - - 2003 time: h min temperature incubator: °C
At the end of the second period	Date: - - 2003 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	
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:

Preparation of XLD

Date of preparation - - 2003
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

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

At the start of the first period	Date: - - 2003 time: h min temperature incubator: °C
At the end of the first period	Date: - - 2003 time: h min temperature incubator: °C
At the start of the second period	Date: - - 2003 time: h min temperature incubator: °C
At the end of the second period	Date: - - 2003 time: h min temperature incubator: °C

FIRST AND SECOND ISOLATION - Isolation medium routinely used in your lab. (I)

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

Name of the medium	
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Prescribed incubation temperature in °C	
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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	
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Code number	
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Batch number	
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Expire date	
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Specific data of composition of your own medium. What is the concentration of the following compounds in 1000 ml water:

FIRST AND SECOND ISOLATION - Isolation medium routinely used in your lab. (II)

Preparation of your own medium	
Date of preparation - - 2003
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

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

Incubation time and temperature for isolation	
At the start of the first period	Date: - - 2003 time: h min temperature incubator: °C
At the end of the first period	Date: - - 2003 time: h min temperature incubator: °C
At the start of the second period	Date: - - 2003 time: h min temperature incubator: °C
At the end of the second period	Date: - - 2003 time: h min temperature incubator: °C

CONFIRMATION – Nutrient agar (I)

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	
Code number	
Batch number	
Expire date	

Specific data of composition of nutrient agar medium. What is the concentration of the following compounds in 1000 ml water:

CONFIRMATION – Nutrient agar (II)**Preparation of the nutrient agar**

Date of preparation - - 2003
pH after preparation, measured at °C
pH at the day of use, measured at °C
Did you perform quality control of nutrient agar ?	<input type="checkbox"/> yes <input type="checkbox"/> no

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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BIOCHEMICAL CONFIRMATION

Manufacturer of the TSI agar

Name	
Code number	
Batch number	
Expire date	
pH of the broth:.....	Measured at.....°C Date - 2003

Manufacturer of the urea agar

Name	
Code number	
Batch number	
Expire date	
pH of the broth:.....	Measured at.....°C Date - 2003

Manufacturer of the l-Lysine decarboxylation medium

Name	
Code number	
Batch number	
Expire date	
pH of the broth:.....	Measured at.....°C Date - 2003

Manufacturer of other confirmation tests -

Name	
Code number	
Batch number	
Expire date	
pH of the broth:.....	Measured at.....°C Date - 2003

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 2002 ?	
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)

Sample no.	RVS 24 hours						RVS 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col ^a	Sal ^b	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^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 N1-N20)

sample no.	RVS 24 hours						RVS 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col ^a	Sal ^b	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^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)

Sample no.	RVS 24 hours						RVS 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col ^a	Sal ^b	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
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)

sample no.	MKTTn 24 hours						MKTTn 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col ^a	Sal ^b	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
1												
2												
3												
4												
5												
6												
7												
8												
9												
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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 2 (continued): Results of isolation using MKTTn (dish numbers N1-N20)

sample no.	MKTTn 24 hours						MKTTn 48 hours					
	BGA		XLD		third medium		BGA		XLD		third medium	
	Col ^a	Sal ^b	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^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)

sample no.	MKTTn 24 hours						MKTTn 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col ^a	Sal ^b	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
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 MSR/V (dish numbers 1-25)

sample no.	MSRV 24 hours						MSRV 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col ^a	Sal ^b	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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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 3 (continued): Results of isolation using MSR/V (dish numbers N1-N20)

sample no.	MSRV 24 hours						MSRV 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col ^a	Sal ^b	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^a = number of colonies used for confirmation
 Sal^b = number of colonies confirmed as *Salmonella*

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

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

Col^a = number of colonies used for confirmationSal^b = number of colonies confirmed as *Salmonella*

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

sample no.	Own enrichment 24 hours						Own enrichment 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col ^a	Sal ^b	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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16												
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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 enrichment (dish numbers N1-N20)

sample no.	Own enrichment 24 hours						Own enrichment 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col ^a	Sal ^b	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^a = number of colonies used for confirmation

Sal^b = number of colonies confirmed as *Salmonella*

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

sample no.	Own enrichment 24 hours						Own enrichment 48 hours					
	BGA		XLD		Third medium		BGA		XLD		Third medium	
	Col ^a	Sal ^b	Col	Sal	Col	Sal	Col	Sal	Col	Sal	Col	Sal
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.		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:

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Name of person (s) carrying out the seventh bacteriological collaborative study (2003)	
Date and signature	

Name of person in charge	
Date and signature	