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**Test results of *Salmonella* serotyping  
in the Member States of the European Union**

Collaborative study III amongst the National  
Reference Laboratories for *Salmonella*

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## Abstract

A third collaborative study on serotyping of *Salmonella* was organized by the Community Reference Laboratory (CRL) for *Salmonella*. All National Reference Laboratories (NRLs) for *Salmonella* of the European Union participated. The main goal of this study was to compare the test results of the NRLs.

In total 20 strains of subspecies *enterica* of the species *Salmonella enterica* were selected by the CRL and had to be tested by each NRL with the typing method routinely performed. The majority of the laboratories typed the strains, including the frequently occurring serotypes, correctly. For the first time some laboratories carried out phagotyping. It seems useful to carry out phagotyping in further studies again.

## Summary

A third collaborative study on serotyping of *Salmonella* was organized by the Community Reference Laboratory (CRL) for *Salmonella*. All National Reference Laboratories (NRLs) for *Salmonella* of the European Union participated. The main goal of this study was to compare the test results of the NRLs.

In total 20 strains of subspecies *enterica* of the species *Salmonella enterica* were selected by the CRL and had to be tested by each NRL with the typing method routinely performed. The NRLs were allowed to send strains for serotyping to another reference laboratory in their country. If laboratories had the possibility to do phagetyping of *Salmonella* Typhimurium and *Salmonella* Enteritidis, they were asked to type these strains with their phages.

The results of the third collaborative study were better than those of the previous studies. The use of serotypes occurring frequently reveals no problems in sero- and phagetyping for the majority of the participants. For the first time some laboratories carried out phagetyping. It seems useful to carry out phagetyping in further studies again.

## Samenvatting

Het Communautair Referentie Laboratorium (CRL) voor *Salmonella* heeft een derde ringonderzoek voor de serotypering van *Salmonella* georganiseerd. Alle Nationale Referentie Laboratoria (NRLs) voor *Salmonella* van de Europese Unie namen eraan deel. Het doel van dit onderzoek was het vergelijken van de testresultaten van de NRLs.

In totaal werden er 20 stammen van de subspecies *enterica* van de species *Salmonella enterica* door het CRL geselecteerd. Deze stammen moesten door elk NRL getest worden met de methode die zij routinematig toepassen. De NRLs mochten de stammen voor serotypering ook naar een ander gespecialiseerd laboratorium in hun land opsturen. Laboratoria die daarvoor de mogelijkheid hadden, werden gevraagd om faagtypering van *Salmonella* Enteritidis en *Salmonella* Typhimurium uit te voeren. De resultaten van dit derde ringonderzoek waren beter vergeleken met resultaten uit eerdere ringonderzoeken. Het gebruik van veel voorkomende serotypen leverden voor het merendeel van de deelnemers geen problemen op. Voor de eerste keer werd door een aantal laboratoria faagtypering uitgevoerd. Het lijkt zinvol om faagtypering ook in volgende ringonderzoeken uit te voeren.

# 1 Introduction

In this report the third collaborative study on serotyping of *Salmonella* strains is described. This study was organized by the Community Reference Laboratory (CRL) for *Salmonella* in accordance with the Council Directive 92/117/EEC. It is one of the tasks of the CRL to organize this type of study in which the National Reference Laboratories (NRLs) for *Salmonella* participate. The main goal is that the examination of samples in the Member States will be carried out uniformly and comparable results will be obtained.

In the first collaborative study one strain of *Salmonella enterica* subspecies *salamae* and one strain of subspecies *houtenae* were included among the 20 strains to be tested (1). In the second collaborative study only strains belonging to subspecies *enterica* were included. The 20 strains for the second study were selected among the more frequently found serotypes (2).

In the third study, described here, again 20 frequently occurring serotypes were selected. The main objective of the collaborative study was to compare the results of serotyping among the NRLs. In this third study also phagotyping of *Salmonella* Enteritidis and *Salmonella* Typhimurium strains was included, as far as the NRLs were able to do so.

## **2 Participants**

### **2.1 National Reference Laboratories**

<b>Austria</b>	Bundesstaatliche bakteriologisch-serologische Untersuchungsanstalt Graz
<b>Belgium</b>	Institut National de Recherches Veterinaires Bruxelles
<b>Denmark</b>	Danish Veterinary Laboratory Copenhagen
<b>Finland</b>	National Veterinary and Food Research Institute Department of Bacteriology Helsinki
<b>France</b>	Centre National d'Etudes Vétérinaires et Alimentaires Laboratoire central de recherches avicole et porcine Ploufragan
<b>Germany</b>	Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin Berlin
<b>Greece</b>	Veterinary Laboratory of Halkis Halkis
<b>Ireland</b>	Department of Agriculture Food and Forestry Veterinary Research Laboratory Dublin
<b>Italy</b>	Istituto Zooprofilattico Sperimentale delle Venezie Legnaro (Padova)
<b>Luxembourg</b>	Laboratoire de Médecine vétérinaire de l'Etat Luxembourg

<b>The Netherlands</b>	National Institute of Public Health and the Environment Bilthoven
<b>Northern Ireland</b> <sup>1</sup>	Veterinary Sciences Division Bacteriology Department Belfast
<b>Portugal</b>	Laboratório Nacional de Investigação Veterinária Lisboa
<b>Spain</b>	Laboratorio de Sanidad y Producción Animal de Algete Madrid
<b>Sweden</b>	National Veterinary Institute Department of Bacteriology Uppsala
<b>United Kingdom</b> <sup>1</sup>	Central Veterinary Laboratory Bacteriological Department Surrey

<sup>1</sup> For the United Kingdom two laboratories participated



## 3 Materials and methods

### 3.1 Selected *Salmonella* strains

The *Salmonella* strains used for the collaborative study originated from the collection of the National *Salmonella* Centre in The Netherlands. The strains were typed once again before mailing.

In total 20 strains of the species *Salmonella enterica* were selected. All strains belonged to the subspecies *enterica*. In total four strains belonging to three different phagetypes of *S. Enteritidis* and five strains representing four different phagetypes of *S. Typhimurium* were included. *S. Dublin* had to be identified twice (a subculture was included).

All these data were unknown to the NRLs.

The antigenic formulae according to the Kauffmann-White scheme of the 20 serovars used are shown in Table 1.

### 3.2 Collaborative study

Two weeks before the actual performance of the study the strains were mailed with special delivery conditions by cargo freight to the participants. After arrival at the laboratory the strains had to be subcultured and stored until the performance of the study. All details about mailing and storing were mentioned in a protocol (annex 1). The protocol and test report (annex 2) were mailed four weeks before the start of the study to the participants.

All 15 Member States participated. The United Kingdom participated with two laboratories. The 20 strains had to be tested with the typing method routinely performed in the laboratories. If laboratories did not use a complete set of mono-specific antisera, they had to identify the strains by giving the antigenic formula as far as detected. It was also a possibility for an NRL to send strains for serotyping to another reference laboratory in their country. Phagetyping is used in many countries to subdivide several serotypes. Most of these countries uses the phagetyping sets developed at the CPHL Colindale in London. If laboratories had the possibility to do phagetyping of *S. Typhimurium* and *S. Enteritidis*, they were asked to type these strains. This information had to be stated in the test report.

Table 1 *Antigenic formulas according to the Kauffmann-White scheme of the 20 Salmonella strains*

strain no.	O antigens	H antigens	serovar
1	3,10,[15]	l,v:1,6	<i>S. London</i>
2	<u>1</u> ,9,12,[Vi]	g,p:-	<i>S. Dublin</i>
3	<u>1</u> ,9,12	g,m:-	<i>S. Enteritidis</i>
4	<u>1</u> ,4,[5],12	f,g:[1,2]	<i>S. Derby</i>
5	6,7, <u>14</u>	d:l,w	<i>S. Livingstone</i>
6	1,3,19	g,[s],t:-	<i>S. Senftenberg</i>
7	<u>1</u> ,9,12	g,m:-	<i>S. Enteritidis</i>
8	<u>1</u> ,4,[5],12	i:1,2	<i>S. Typhimurium</i>
9	6,8	z <sub>10</sub> :e,n,x	<i>S. Hadar</i>
10	<u>1</u> ,4,[5],12, <u>27</u>	l,v:e,n,z <sub>15</sub>	<i>S. Brandenburg</i>
11	<u>1</u> ,4,[5],12	i:1,2	<i>S. Typhimurium</i>
12	<u>1</u> ,9,12,[Vi]	g,p:-	<i>S. Dublin</i>
13	6,8	d:1,2:[z <sub>67</sub> ]	<i>S. Muenchen</i>
14	6,8	d:1,5	<i>S. Manhattan</i>
15	<u>1</u> ,4,[5],12	i:1,2	<i>S. Typhimurium</i>
16	<u>1</u> ,9,12	g,m:-	<i>S. Enteritidis</i>
17	<u>1</u> ,9,12	g,m:-	<i>S. Enteritidis</i>
18	6,7, <u>14</u>	z <sub>10</sub> :e,n,z <sub>15</sub>	<i>S. Mbandaka</i>
19	<u>1</u> ,4,[5],12	i:1,2	<i>S. Typhimurium</i>
20	<u>1</u> ,4,[5],12	i:1,2	<i>S. Typhimurium</i>

underlined = O factors determined by phage conversion

[ ] = O or H factor that may be present or absent without relation to phage conversion

## **4 Results**

### **4.1 General data of serotyping by the participants**

The labcodes used in this third study differed partly from those used in the first and second one and, thus, can not be compared directly among reports.

Three participating NRLs (labcodes 2, 3 and 5) were not the reference laboratory for serotyping in their country.

In Table 2 the frequency of typing in the laboratories is shown. There is no difference in the frequency of typing between the second and third collaborative study. The total number of strains typed in 1996 and 1997 is also presented in Table 2. There are only small differences within a NRL between years.

In Table 3 the origin of the sera used by the different NRLs in all studies organized until now is shown.

### **4.2 Taxonomy and nomenclature of the typed strains**

As proposed by the *Salmonella* WHO reference centre (3), 15 of the 16 participants wrote the identified serotype with a capital letter. In the previous two studies eight (of 17) and ten (of 15) laboratories respectively, reported the name of the serovar with a capital letter.

In the first and second study several laboratories used name(s) of serovars, which were withdrawn from the Kauffmann-White scheme (3) at that time. In the third study this did not happen and names were in accordance with the most recent Kauffmann-White scheme (4) for identification of the strains.

Table 2 *Frequency of serotyping and total number of strains typed by the participants*

labcode	frequency of typing	total no. of strains typed in 1996	total no. of strains typed in 1997
1	daily	14,314	13,550
2	daily	2,094	1,905
3	daily	± 12,000	14,000-15,000
4	± 150 a month	1,500	±1,500
5	± 20 strains every week	± 1,200	± 1,000
6	daily	7,000	7,000
7	at the moment strains arrive at the laboratory	-	36
8	daily	1,680	1,470
9	daily	1,416	2,000
10	once a week	8	7
11	once a week	± 9,500	± 8,000
12	at the moment strains arrive at the laboratory	362	298
13	twice a month	200	300
14	daily	± 800	± 1,000
15	daily	12,000	10,000
16	daily	2,000	2,000

Table 3 *The origin of the sera used by the different NRLs*

collaborative study	number of laboratories	commercial sera	sera prepared by other institutes	own prepared sera
I	17	12	4	7
II	15	10	2	5
III	16	11	3	3

### 4.3 Serotyping of the strains

Table 4 shows the laboratories in which the selected strains were typed. Three laboratories have sent in total seven strains to another, reference, laboratory for further typing. In the second study five laboratories had sent strains to another laboratory for typing.

Table 4 *Laboratory in which selected strains are typed*

labcode	number of strains typed in	
	own laboratory	reference laboratory
1	1 - 20	-
2	1 - 13, 15 - 17 and 19 - 20	14 and 18 <sup>1</sup>
3	1 - 20	-
4	1 - 20	-
5	1 - 3, 5 - 12, 14 - 20	4 and 13 <sup>1</sup>
6	1 - 20	-
7	1 - 7, 9 - 18	8, 19 and 20 <sup>2</sup>
8	1 - 20	-
9	1 - 20	-
10	1 - 20	-
11	1 - 20	-
12	1 - 20	-
13	1 - 20	-
14	1 - 20	-
15	1 - 20	-
16	1 - 20	-

<sup>1</sup> = identified in national reference laboratory for serotyping

<sup>2</sup> = identified in reference laboratory for human sources

#### **4.3.1 Detection of the O and H antigens**

The results of the detection of the O and H antigens are shown in two Tables. Table 5 presents the detection of the antigens per laboratory as stated in the test report and Table 6 the detection per strain.

The interpretation of the results was divided into correct (+), partly correct/incomplete ( $\pm$ ), incorrect (-) and not typable.

Thirteen of the 16 participants detected the O antigens of all 20 selected strains correctly (Table 5). Laboratory 2 could not detect the right O and H antigens of one strain.

Laboratory 10 detected one strain only partly correctly and another strain could not be typed, because it was polyagglutinable. Laboratory 12 detected the O antigens of three strains only partly correctly.

Nine NRLs detected the H antigens of all selected strains correctly. Six laboratories detected one to three strains partly correctly.

In total two strains were detected incorrectly (Table 6), which resulted in a wrong serotype. Two laboratories detected the O antigens of in total four of the strains incompletely, whereas six laboratories detected the H antigens of six different strains as incomplete.

All strains sent to another, reference, laboratory by NRLs (strain 4, 8, 13, 14, 18, 19 and 20) were typed correctly.

Table 5 *Detection of O and H antigens of all 20 selected strains per laboratory as stated in the test report*

labcode	O antigen				H antigen			
	detected			not typable	detected			not typable
	+	±	-		+	±	-	
1	20	-	-	-	20	-	-	-
2	19	-	1	-	19	-	1	-
3	20	-	-	-	19	1	-	-
4	20	-	-	-	20	-	-	-
5	20	-	-	-	19	1	-	-
6	20	-	-	-	19	1	-	-
7	20	-	-	-	20	-	-	-
8	20	-	-	-	17	2	1	-
9	20	-	-	-	20	-	-	-
10	18	1	-	1	16	3	-	1
11	20	-	-	-	20	-	-	-
12	17	3	-	-	20	-	-	-
13	20	-	-	-	20	-	-	-
14	20	-	-	-	20	-	-	-
15	20	-	-	-	20	-	-	-
16	20	-	-	-	18	2	-	-

+ = correct  
 ± = partly correct/incomplete  
 - = incorrect

Table 6 *Detection of the O and H antigens of the 20 strains by the 16 participants*

strain no.	serotype	O antigen				H antigen			
		detected			not typable	detected			not typable
		+	±	-		+	±	-	
1	<i>S. London</i>	15	-	1	-	15	-	1	-
2	<i>S. Dublin</i>	16	-	-	-	16	-	-	-
3	<i>S. Enteritidis</i>	16	-	-	-	16	-	-	-
4	<i>S. Derby</i>	16	-	-	-	16	-	-	-
5	<i>S. Livingstone</i>	15	1	-	-	15	-	1	-
6	<i>S. Senftenberg</i>	16	-	-	-	15	1	-	-
7	<i>S. Enteritidis</i>	16	-	-	-	16	-	-	-
8	<i>S. Typhimurium</i>	16	-	-	-	16	-	-	-
9	<i>S. Hadar</i>	15	1	-	-	15	1	-	-
10	<i>S. Brandenburg</i>	16	-	-	-	14	2	-	-
11	<i>S. Typhimurium</i>	16	-	-	-	16	-	-	-
12	<i>S. Dublin</i>	16	-	-	-	16	-	-	-
13	<i>S. Muenchen</i>	14	1	-	1 <sup>1</sup>	13	2	-	1 <sup>1</sup>
14	<i>S. Manhattan</i>	15	1	-	-	14	2	-	-
15	<i>S. Typhimurium</i>	16	-	-	-	16	-	-	-
16	<i>S. Enteritidis</i>	16	-	-	-	16	-	-	-
17	<i>S. Enteritidis</i>	16	-	-	-	16	-	-	-
18	<i>S. Mbandaka</i>	16	-	-	-	14	2	-	-
19	<i>S. Typhimurium</i>	16	-	-	-	16	-	-	-
20	<i>S. Typhimurium</i>	16	-	-	-	16	-	-	-

+ = correct  
 ± = partly correct/incomplete  
 - = incorrect  
 1 = polyagglutinable



### 4.3.2 Identification of the strains

Thirteen of the 20 strains were identified correctly by all participants (Table 7). These included the four *S. Enteritidis* and five *S. Typhimurium* strains.

The results of the serotyping of the remaining seven strains are shown in Table 8. The deviating identifications were shown in italics/bold. Strain no. 13, *S. Muenchen*, gave apparently the most problems. Four laboratories could not identify this strain correctly. Three laboratories typed strain no. 14 (*S. Manhattan*) incorrectly, while strain no. 9 and 18 respectively *S. Hadar* and *S. Mbandaka*, were identified incorrectly by two laboratories. The three other strains, no. 1, 5 and 10, were typed incorrectly only by one laboratory.

Table 7 *Thirteen strains typed correctly by the 16 participants*

strain no.	serotype
2	<i>S. Dublin</i>
3	<i>S. Enteritidis</i>
4	<i>S. Derby</i>
6	<i>S. Senftenberg</i>
7	<i>S. Enteritidis</i>
8	<i>S. Typhimurium</i>
11	<i>S. Typhimurium</i>
12	<i>S. Dublin</i>
15	<i>S. Typhimurium</i>
16	<i>S. Enteritidis</i>
17	<i>S. Enteritidis</i>
19	<i>S. Typhimurium</i>
20	<i>S. Typhimurium</i>

Table 8 Strains typed differently by the participants

lab	strain no.						
	1	5	9	10	13	14	18
1	S. London	S. Livingstone	S. Hadar	S. Brandenburg	S. Muenchen	S. Manhattan	S. Mbandaka
2	<i>S. Hadar (-)</i>	S. Livingstone	S. Hadar	S. Brandenburg	S. Muenchen	S. Manhattan	S. Mbandaka
3	S. London	S. Livingstone	S. Hadar	S. Brandenburg	<i>S. Newport (-)</i>	S. Manhattan	S. Mbandaka
4	S. London	S. Livingstone	S. Hadar	S. Brandenburg	S. Muenchen	S. Manhattan	S. Mbandaka
5	S. London	S. Livingstone	S. Hadar	S. Brandenburg	S. Muenchen	S. Manhattan	S. Mbandaka
6	S. London	S. Livingstone	S. Hadar	S. Brandenburg	<i>S. I 6,8:-:1,2 monoph. var</i>	S. Manhattan	S. Mbandaka
7	S. London	S. Livingstone	S. Hadar	S. Brandenburg	S. Muenchen	S. Manhattan	S. Mbandaka
8	S. London	<i>S. Gombe (-)</i>	S. Hadar	S. Brandenburg	S. Muenchen	<i>S. Gombe (-)</i>	<i>S. Gombe (-)</i>
9	S. London	S. Livingstone	S. Hadar	S. Brandenburg	S. Muenchen	S. Manhattan	S. Mbandaka
10	S. London	S. Livingstone	<i>S. Narashino (-)</i>	<i>S. Kimuenza (-)</i>	<i>S. spp</i>	S. Manhattan	<i>mixture</i>
11	S. London	S. Livingstone	S. Hadar	S. Brandenburg	S. Muenchen	S. Manhattan	S. Mbandaka
12	S. London	S. Livingstone	<i>S. C<sub>2</sub>-C<sub>3</sub> group (±)</i>	S. Brandenburg	<i>S. C<sub>2</sub>-C<sub>3</sub> group (±)</i>	<i>S. C<sub>2</sub>-C<sub>3</sub> group (±)</i>	S. Mbandaka
13	S. London	S. Livingstone	S. Hadar	S. Brandenburg	S. Muenchen	S. Manhattan	S. Mbandaka
14	S. London	S. Livingstone	S. Hadar	S. Brandenburg	S. Muenchen	S. Manhattan	S. Mbandaka
15	S. London	S. Livingstone	S. Hadar	S. Brandenburg	S. Muenchen	S. Manhattan	S. Mbandaka
16	S. London	S. Livingstone	S. Hadar	S. Brandenburg	S. Muenchen	<i>S 6,8:d (±)</i>	S. Mbandaka

± = partly correct/incomplete

- = incorrect

#### 4.4 Phagotyping of the strains

Six of the 16 laboratories did phagotyping of the strains of *S. Enteritidis* and *S. Typhimurium*. All laboratories used the Colindale typing system.

The results of the phagotyping of *S. Enteritidis* are shown in Table 9a. All six laboratories typed strain 3 and 7 as phagetype 4 and strain 17 as type 21. The phagetype of strain 16 differed between the laboratories. Four laboratories (labcode 1, 3, 4 and 11) typed the strain as 6a, one laboratory (labcode 6) as 5a and laboratory 15 could not confirm the reactions.

The results of phagotyping of *S. Typhimurium* are shown in Table 9b. All laboratories found the strains 8 and 15 as phagetype 104 and strain 20 as phagetype 17. Laboratory 4 typed strain 11 as 182var, while all other laboratories found phagetype 124. Most differences were found in the results of strain 19. Three laboratories were not able to phagetype this strain, while the remaining laboratories found different phagetypes, respectively 193, 195 and 20a.

Table 9a Results phagotyping *Salmonella* Enteritidis

strain no.	labcode					
	1	3	4	6	11	15
3	4	4	4	4	4	4
7	4	4	4	4	4	4
16	6a	6a	6a	5a	6a	RDNC <sup>1</sup>
17	21	21	21	21	21	21

Table 9b Results phagotyping *Salmonella* Typhimurium

strain no.	labcode					
	1	3	4	6	11	15
8	104	104	104	104	104	104
11	124	124	182 var.	124	124	124
15	104	104	104	104	104	104
19	RDNC <sup>1</sup>	NT <sup>2</sup>	RDNC <sup>1</sup>	193	195	20a
20	17	17	17	17	17	17

<sup>1</sup>RDNC = reactions do not confirm (i.e. the phages react, but the pattern of lysis does not confirm to a recognised phage type)

<sup>2</sup>NT = not typable

## 5 Discussion

The total number of strains typed by the different NRLs in 1997 was nearly the same as in 1996. In 1997, a number of laboratories typed many strains (> 5,000), while some other laboratories typed less than 2,000 strains. In the earlier, second, study five laboratories had sent their strains to another, reference, laboratory for further typing, while in the present, third, study only three laboratories have sent some strains to another laboratory. Also the number of strains sent to another, reference, laboratory decreased (from 1 to 8 in 1996 to 2 or 3 in 1997). None of the laboratories used names which were withdrawn from the most recent Kauffmann-White scheme.

In this third collaborative study the number of incorrect and partly correct typed O and H antigens decreased in comparison with the first and second study. The identification of the strains was also better compared with previous studies. Thirteen of the 20 strains were identified correctly by all participants. A reason for this could be that only serotypes occurring frequently were used in this study, including four and five strains of *S. Enteritidis* and *S. Typhimurium*, respectively. In future collaborative studies strains which are not so common can be included to challenge the participating laboratories to detect also less frequently occurring strains.

For the first time some laboratories carried out phagetyping. All the laboratories used the Colindale system. It seems useful to include phagetyping in future studies.

## 6 Conclusions

The conclusions of this study are:

- The majority of the NRLs had no problems with the identification of serotypes occurring frequently.
- All participants used up-to-date names (Kauffmann-White scheme, 1997) for the serotypes detected.
- Phagetyping is done only by a minority of the NRLs. However, inclusion of phagetyping in future collaborative studies needs to be considered.

## Literature

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## **Annex 1**

### **COLLABORATIVE STUDY ON SEROTYPING OF *SALMONELLA* STRAINS (3) ORGANIZED BY CRL SALMONELLA**

#### **PROTOCOL:**

##### **Introduction:**

The Community Reference Laboratory (CRL) for Salmonella organizes a third collaborative study on serotyping of *Salmonella* strains amongst the National Reference Laboratories (NRLs).

In this study again a total number of 20 *Salmonella* strains, supplied by the CRL, must be identified. The results will be evaluated by the CRL.

The typing method routinely performed in the laboratory will be used in the study. Definite conclusions can be based only on agglutination with mono-specific antisera. Otherwise it is better to identify the strains by giving the antigenic formula as far as detected. A NRL is allowed to send strains for serotyping to another reference laboratory in their country.

Those laboratories who have also the possibility to do phage-typing of *S. Typhimurium* and *S. Enteritidis* strains could type these strains with their phages and send the results back to the CRL.

##### **Objective:**

The main objective of the third study on serotyping is to confirm the test results of the NRLs in cooperation with the CRL Salmonella.

##### **Outline of the study:**

Each NRL will receive a parcel containing 20 *Salmonella* cultures (numbered 1 to 20). On arrival the cultures must be subcultured on agar plates.

The performance of the study will be in week 51 (starting on 15 December 1997) or one week earlier or later. All data will be reported on the test report to the CRL Salmonella and will be used for analysis.

**Time table of the collaborative study on serotyping of *Salmonella* strains (3)**

The identification of the *Salmonella* cultures must take place in week 51 (starting on 15 December) or one week earlier or later.

17-20 November      Mailing the protocol and test report to the NRLs.

01-05 December      Mailing the strains to the NRLs.  
CRL will mail the parcel by cargo freight from the Dutch airport (Schiphol) to the airport of destination.  
The participants have to collect the parcel at the airport. For this you need the airway bill number. This number and other necessary information will be indicated in a fax in the week before mailing.

**The transport costs from the airport of destination to the laboratory can not be paid by the CRL, so this will be at the expense of the NRL.**

After arrival at the laboratory the strains need to be subcultured and stored until the performance of the serotyping.

**If you did not receive the parcel before or on 05 December 1997, do contact the CRL immediately.**

08-12 December      Checking the presence of all necessary reagents and materials for the performance of the study.

15-19 December      Starting with the identification of the strains.

**Note:** Each laboratory is free to identify the strains when they want as long as it will be done in the scheduled weeks.



- 05 January            Completion of the test report and faxing it to the CRL. The original test report will be sent to the CRL.
- 19 January            Checking the results by the NRLs.

If you have questions or remarks about the collaborative study please contact:

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(research assistant CRL)  
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## Annex 2

**COLLABORATIVE STUDY  
ON SEROTYPING OF *SALMONELLA* STRAINS (3)  
ORGANIZED BY CRL SALMONELLA**

page 1 of 7

**TEST REPORT**  
**OF THE THIRD COLLABORATIVE STUDY**  
**ON SEROTYPING OF *SALMONELLA* STRAINS**

Laboratory code :

Laboratory name :

Date of collecting the parcel : ..... - ..... - 1997

Starting date for serotyping : ..... - ..... - 1997

**GENERAL QUESTIONS**

1. What was the frequency of serotyping at your laboratory in **1997**?

- once a week
- twice a month
- once a month
- more frequent, namely .....
- less frequent, namely .....

2. How many strains did you serotype in **1997**?

.....

3. Which kind of sera do you use?

- commercial available sera
- manufacturer :.....  
.....  
.....
- prepared in own laboratory

4. Is your laboratory the reference laboratory for serotyping *Salmonella* in your country?

- YES
- NO, the name and address of the reference laboratory is:  
.....  
.....  
.....

5. The strains in this collaborative study were serotyped by

- own laboratory, strain no: .....
- other laboratory, namely:  
.....  
.....  
strain no: .....

**Questions 6 and 7 only when your laboratory does phage-typing:**

6. Do your laboratory phage-typing of

- Salmonella* Typhimurium
- Salmonella* Enteritidis

7. Which typing system is used for

- Salmonella* Typhimurium

.....

.....

- Salmonella* Enteritidis

.....

.....

PLEASE WRITE YOUR REMARKS AND COMMENTS ON PAGE 7 OF THE TEST REPORT!



**TEST RESULTS OF THE COLLABORATIVE STUDY ON SEROTYPING**

Please fill in your results in the table(s) below.

**labcode:**

starting date of serotyping: ..... - ..... - 1997

strain no.	O-antigens detected	H-antigens detected	serotype
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			

**labcode:**

starting date of phage-typing: ..... - ..... - 1997

strain no.	serotype	phagetype
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		

Remarks and comments:

Date: ..... - ..... - .....

Name of technician/technologist carrying out the collaborative study on serotyping:

.....

signature:.....

Date: ..... - ..... - .....

Name of technician/technologist carrying out the phage-typing:

.....

signature:.....

Name of person in charge:

.....

signature:.....



## Annex 3

### Mailing list

01	European Commission	A. Checchi Lang
02	European Commission	B. Hogben
03	European Commission	R. Holma
04	Veterinary Public Health Inspector	drs. H. Verburg
05-20	Participants of the study (National Reference Laboratories for <i>Salmonella</i> )	
21	Board of Directors RIVM	dr. G. Elzinga
22	Director Sector Public Health Research	prof. dr. ir. D. Kromhout
23	Head of Microbiological Laboratory for Health Protection and Director CRL <i>Salmonella</i>	dr. ir. A.M. Henken
24	Head of Diagnostic Laboratory for Infectious Diseases and Perinatal Screening	dr. J.G. Loeber
25-27	Project Workers	
28-30	Authors	
31	Dutch National Library for Publications and Bibliography	
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