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A collaborative study on serotyping of *Salmonella* amongst the National Reference Laboratories for *Salmonella*

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ABSTRACT

A collaborative study on serotyping in which the National Reference Laboratories (NRLs) for *Salmonella* of the EU Member States participated was organized by the Community Reference Laboratory (CRL) for *Salmonella*. The aim of this study was to be informed about the results of serotyping of *Salmonella enterica* by the NRLs.
The strains were identified with the serotyping method performed routinely in the laboratories. Seven of the 17 participating laboratories identified the 20 selected strains correctly.
The main reasons for incorrect results were the incorrect detection of antigens, the identification of strains based on an incomplete antigenic formula and an incorrect interpretation of the antigenic formula.

SUMMARY

A collaborative study on serotyping of *Salmonella* was organized by the Community Reference Laboratory (CRL) for *Salmonella*. In this study the National Reference Laboratories for *Salmonella* (NRLs) of the Member States of the European Union (EU) participated. The aim of this first serotyping study was to be informed about the results of serotyping of *Salmonella enterica* by the NRLs.

Seventeen laboratories each examined 20 individually numbered serovars of *Salmonella enterica*. The participating laboratories were asked to identify these strains with the serotyping method performed routinely in their laboratory. Furthermore, inquiries were made to be informed about the method applied, the origin of the sera and the number of strains typed per year.

Of the 17 participating laboratories 7 laboratories identified the 20 selected strains correctly. One laboratory reported six strains as not typable. Three laboratories identified one strain incorrectly, while 6 laboratories identified two or more strains incorrectly. The main reasons for incorrect results were the incorrect detection of O and/or H antigens, the identification of strains based on an incomplete antigenic formula by means of the Kauffmann-White scheme and an incorrect interpretation of the antigenic formula. It is better to identify strains by giving the antigenic formula as far as detected. Definite conclusions can be based only on agglutination with mono-specific antisera.

SAMENVATTING

Het Communautair Referentie Laboratorium voor *Salmonella* heeft een ringonderzoek voor de serotypering van *Salmonella* georganiseerd met deelname van alle Nationale Referentie Laboratoria voor *Salmonella* (NRL) van de lidstaten van de Europese Unie. Het doel van dit ringonderzoek was meer informatie te krijgen over de resultaten van de serotypering van *Salmonella enterica* in de NRLs.
Zeventien laboratoria onderzochten elk 20 individueel genummerde serotypes van *Salmonella enterica*. De deelnemende laboratoria moesten de stammen identificeren volgens de serotyperingsmethode die routinematig in hun laboratorium werd uitgevoerd. Daarnaast werd er o.a. geinformeerd naar de toegepaste methode, de oorsprong van de sera en het aantal getypeerde stammen per jaar.
Zeven van de 17 deelnemende laboratoria identificeerden alle 20 geselecteerde stammen correct. Eén laboratorium kon zes stammen niet typeren. Drie laboratoria identificeerden één stam incorrect, terwijl zes laboratoria meer dan één stam incorrect identificeerden. De belangrijkste redenen voor de incorrecte resultaten waren een incorrecte detectie van de O en H antigenen, identificatie van de stammen op grond van een onvolledige antigeenstructuur met behulp van het Kauffmann-White schema en een incorrecte interpretatie van de antigeenstructuur. Het is beter om van stammen de antigene structuurformule op te geven voor zover die bekend is. Definitieve conclusies kunnen alleen gegeven worden als er geagglutineerd is met voldoende, monospecifieke antisera.

1. INTRODUCTION

The Council Directive 92/117/EEC concerns measures for protection against specific zoonoses and specified zoonotic agents in animals and products of animal origin in order to prevent outbreaks of food borne infections and intoxications.

The Directive lays down rules for the collection of information on zoonoses and zoonotic agents and the relevant measures to be taken in the Member States.
As far as *Salmonella* is concerned the Commission has to be informed about:

- the occurrence of *Salmonella* in animals, products of animal origin and animal feeding stuffs,
- the occurrence of clinical cases of salmonellosis in humans and animals.

Furthermore the Directive predescribes intervention measures to reduce the contamination in poultry breeding flocks.

The Directive provides rules for sampling and examination of samples. It is a task of the Community Reference Laboratory (CRL) for *Salmonella* to organize collaborative studies, which have as their goal that the examination of samples in Member States is carried out uniformly and that comparable results will be obtained.

This report describes a collaborative study on serotyping of 20 *Salmonella* strains in which all National Reference Laboratories (NRLs) for *Salmonella* participated.

2. PARTICIPANTS

2.1. National Reference Laboratories

Austria	Bundesstaatliche bakteriologisch-serologische Untersuchungsanstalt Graz Graz
Belgium	Institut National de Recherches Veterinaires Bruxelles
Denmark	Danish Veterinary Laboratory Copenhagen
Finland	National Veterinary and Food Research Institute Department of Food Microbiology Helsinki
France	Centre National d'Etudes Vétérinaires et Alimentaires Laboratoire central de recherches avicole et porcine Ploufragan
Germany	Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinär-medizin Berlin
Greece	Centre of Athens Veterinary Institutions Microbiology Department Athens
Ireland	Department of Agriculture Food and Forestry Veterinary Research Laboratory Dublin
Italy	Istituto Zooprofilattico Sperimentale delle Venezie Vicenza

Luxembourg	Laboratoire de Médecine vétérinaire de l'Etat Luxembourg
The Netherlands	National Institute of Public Health and the Environment Bilthoven
Northern Ireland	Veterinary Sciences Division Bacteriology Department Belfast
Portugal	Laboratorio Nacional de Veterindria Lisboa
Spain	Laboratorio de Sanidad y Producción Animal de Algete Madrid
Sweden	National Veterinary Institute Laboratory of Bacteriology Uppsala
United Kingdom	Central Veterinary Laboratory Bacteriological Department Surrey

2.2. Other laboratories

Denmark	Danish Veterinary Laboratory Aarhus

3. MATERIALS AND METHODS

3.1. Taxonomy and nomenclature of the genus *Salmonella*

The genus *Salmonella* consists of two species (1):
1. *S.enterica*, which is divided into six subspecies;

S. enterica subsp. *enterica*	
S. enterica subsp. *salamae*	symbol: II
S. enterica subsp. *arizonae*	symbol: IIIa
S. enterica subsp. *diarizonae*	symbol: IIIb
S. enterica subsp. *houtenae*	symbol: IV
S. enterica subsp. *indica*	symbol: VI

2. *S. bongori* (formerly called *S.enterica* subsp. *bongori*) symbol: V

In the Kauffmann-White scheme the names of the serovars are maintained only for serovars belonging to *S.enterica* subspecies *enterica* which account for more than 99.5% of isolated *Salmonella* strains. These names must not be italicized. The first letter is a capital letter. Serovars of other subspecies of *S.enterica* and those of *S. bongori* are designated by a symbol (see above) followed by their antigenic formula.
Factors in square brackets, [ ], are O (= somatic) or H (= flagellar) factors that may be present or absent without relation to phage conversion. Factors in brackets, ( ), are often weakly expressed. The symbols for O-factors determined by phage conversion are underlined.

3.2. Selected *Salmonella* strains

The *Salmonella* strains used for the collaborative study originated from the collection of the Dutch National *Salmonella* Centre in Bilthoven (The Netherlands). The strains were identified once again before mailing.
In total twenty strains of the species *Salmonella enterica* were selected; eighteen strains belonged to the subspecies *enterica*. Strains were selected so that the most frequently isolated O groups (B, C, D, E and G) of *S. enterica* subspecies *enterica* were present. Among these, strains were included with common H antigens. One strain belonged to the subspecies *salamae* and one to the subspecies *houtenae*. Three strains (S. Llandoff, S. Give and S. Enteritidis) were included twice. These data were unknown to the participants. The antigenic formulas according to the Kauffmann-White scheme of the 20 serovars used in the study are shown in Table 1.

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

strain no.	O-antigens	H-antigens	serovar
1	1,3,19	z_{29}:[z_6]	S. Llandoff
2	3,10	[d],l,v:1,7	S. Give
3	6,7	r:1,2	S. Virchow
4	6,7,14	m,t:[z_{57}]	S. Oranienburg
5	47	d:z_{39}	S. II 47:d:z_{39}
6	1,9,12	g,m:-	S. Enteritidis
7	6,7,14	z_{38}:-	S. Lille
8	3,10	[d],l,v:1,7	S. Give
9	1,9,12	[f],g,t:-	S. Berta
10	1,4,[5],12	i:1,2	S. Typhimurium
11	1,13,22	z:1,6	S. Poona
12	1,3,19	z_{29}:[z_6]	S. Llandoff
13	16	z_4,z_{32}:-	S. IV 16:z_4,z_{32}:-
14	1,9,12,[Vi]	g,p:-	S. Dublin
15	6,7,14	r:1,5	S. Infantis
16	1,13,23	z:l,w	S. Worthington
17	6,14,18	b:1,5	S. Fluntern
18	8,20	i:z_6	S. Kentucky
19	1,9,12	g,m:-	S. Enteritidis
20	1,4,12	f,g,s:[1,2]	S. Agona

underlined = O-factors determined by phage conversion

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

3.3. Collaborative study

The 20 strains were mailed with special delivery conditions by cargo freight two weeks before the actual performance of the study. After arrival at the laboratory the strains had to be subcultured and stored until analyzed. In the protocol (annex 1) all details about the mailing and storing were mentioned.
In total 17 laboratories participated and they each tested 20 strains of *Salmonella enterica* with the serotyping method performed routinely in the laboratory. To get more insight into the serotyping in the different Member States, information about the methods applied, the numbers of strains typed yearly and the origin of the sera applied were obtained. No data were collected about the set of sera used for serotyping and about the quality and the quality control of the antisera used by the different NRLs.
The protocol and test report (annex 2) were mailed to the participants four weeks before the actual performance of the study.

4. RESULTS

4.1. General data of serotyping by the participants

The frequency of serotyping and the total number of strains identified yearly by the laboratories are presented in Table 2. Laboratory 14 started its activities as NRL 6 months ago. Among the participants the number of strains typed yearly varied considerably. From less than 10 to 20,000. Besides the detection of O and H antigens 7 laboratories are able to detect the Vi antigen.

The different methods of agglutination are shown in Table 3. All laboratories used the object glass agglutination. Two participants (laboratory 12 and 16) used both tubes and microtitre trays as well. Laboratory 3 used microtitre trays besides the object glass agglutination. Laboratory 13 used object glass agglutination for the detection of O antigens and tube agglutination for H antigens.

Table 4 presents the origin of the sera used by the participants. Participants used antisera of various origins: commercial sera, sera obtained by other institutes and own prepared sera.
Most of the 17 participants used commercial sera from various manufacturers. Four participants obtained their sera from other institutes. In three cases that institute was in their own country.
Three laboratories only used sera prepared by themselves. Four laboratories used their own prepared sera in combination with commercial sera.

4.2. Taxonomy and nomenclature of the typed strains

Eight participants reported the identified strains with a capital letter, three reported a small letter, one reported both and the remaining five participants wrote the whole serovar in capital letters.
Some strains were indicated with the name of the serovar withdrawn from the Kauffmann-White scheme, 1992 (1). S. Chameleon was used instead of S. IV 16:z_4,z_{32}:- by laboratory 3 and 13. S. Quimbamba II instead of S. II 47:d:z_{39} by laboratory 3 and S. Thielallee instead of S. Oranienburg and S.Bornum instead of S. Lille were mentioned by laboratory 9, while laboratory 11 reported S. Typhimurium var. Copenhagen instead of S. Typhimurium. In this study these names were interpreted as correct.

Table 2: *Frequency of serotyping and total number of strains identified yearly by the laboratories*

labcode	frequency of typing	number of strains typed yearly
1	daily	12,000-15,000
2	daily	± 1,700
3	daily	10,000-20,000
4	daily	± 7,000
5	± 150 strains a month	± 2,000
6	twice a month	n.r.
7	daily	4,000
8	daily	± 2,000 (human origin)
9	daily	± 1,050
10	daily	1,200-1,600
11	n.r.	n.r.
12	once a week	8,000-9,000
13	daily	± 350
14 [1]	n.r.	9
15	60-80 strains a month	± 1,000
16	daily	10,000
17	daily	1,750

n.r. = not reported

[1] NRL for only 6 months

Table 3: *Different methods of agglutination used by the NRLs*

number of laboratories	object glass agglutination	agglutination in tubes	agglutination in microtitre trays
17	17	3	3

Table 4: *The origin of the sera used by the different NRLs*

number of laboratories	commercial sera	sera prepared by other institutes	own prepared sera
17	12	4	7

4.3. Serotyping of the strains

4.3.1. Detection of the O and H antigens

The results of the detection of the O and H antigens are shown per laboratory in Table 5, and per strain in Tables 6 and 7. In these Tables a distinction is made between correct, partly correct/incomplete and incorrect detection. The detection of mixed (polyvalent) O antisera (2) only was interpreted as partly correct at best.

Six laboratories detected the O antigens of a number of strains partly correctly/incompletely (Table 5). This means one of the O antigens of the strain was not recorded. Four laboratories were not able to detect the O:20 antigen of S. Kentucky (strain no. 18) and three times the O:47 antigen *S. enterica* subspecies *salamae* (strain no. 5) was not detected. Five strains were detected partly correctly/incompletely by just one laboratory (see Tables 6 and 7).
Two laboratories (labcode 2 and 9) could not detect the O antigens of three different strains; it concerns the strains S. Fluntern, *S. enterica* subspecies *salamae* and subspecies *houtenae*.

Nine laboratories only detected a part of the H antigens of 12 different strains (Table 5). Laboratory 14 (6 times), laboratory 6 (5 times) and laboratory 17 (4 times) reported the most partly correctly/incompletely detected H antigens. In the case of laboratory 14 these strains included S. Enteritidis (strain no. 6 and 19). A H:m,t antigen instead of H:g,m was detected. Laboratory 3 detected the H antigen of the two S. Llandoff strains (strain no. 1 and 12) incorrectly.
Three times the H:t antigen and once the H:m antigen of S. Oranienburg (strain no. 4) was detected partly correctly/incompletely (Table 6). A H:r antigen instead of $H:z_{38}$ of S.Lille (strain no. 7) was detected by the laboratories 6 and 15.
Nine laboratories could not detect the $H:z_{39}$ antigen of *S. enterica* subspecies *salamae* and five of these laboratories also could not detect the $H:z_{32}$ antigen of *S. enterica* subspecies *houtenae* either (Table 7).

Table 5: *Detection of O and H antigens of all 20 selected strains per laboratory*

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

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

Table 6: *Detection of the O and H antigens of the 18 strains belonging to the subspecies enterica by the 17 participants*

strain no.	serotype	O antigen				H antigen			
		detected			not typable	detected			not typable
		+	±	-		+	±	-	
1	S. Llandoff	17	-	-	-	15	-	1	1
2	S. Give	17	-	-	-	16	1	-	-
3	S. Virchow	17	-	-	-	17	-	-	-
4	S. Oranienburg	16	-	1	-	13	4	-	-
6	S. Enteritidis	17	-	-	-	16	1	-	-
7	S. Lille	17	-	-	-	11	1	2	3
8	S. Give	16	1	-	-	15	1	1	-
9	S. Berta	17	-	-	-	15	2	-	-
10	S. Typhimurium	17	-	-	-	17	-	-	-
11	S. Poona	16	1	-	-	17	-	-	-
12	S. Llandoff	16	1	-	-	14	-	2	1
14	S. Dublin	17	-	-	-	17	-	-	-
15	S. Infantis	17	-	-	-	17	-	-	-
16	S. Worthington	16	1	-	-	16	1	-	-
17	S. Fluntern	13	-	1	3	15	-	-	2
18	S. Kentucky	13	4	-	-	16	1	-	-
19	S. Enteritidis	17	-	-	-	16	1	-	-
20	S. Agona	17	-	-	-	15	2	-	-

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

Table 7: *Detection of the O and H antigens of the 2 strains belonging to the subspecies salamae (S. II 47:d:z39) and houtenae (S. IV 16:z4,z32) by the 17 participants*

strain no.		O antigen				H antigen			
		detected			not typable	detected			not typable
		+	±	-		+	±	-	
5	S. II 47:d:-z39	10	3	1	3	8	6	-	3
13	S. IV 16:z4,z32	14	1	-	2	11	4	-	2

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

4.3.2. Identification of the strains

Seven participants identified correctly all strains (Table 8). The number of incorrectly identified strains varied between 1 and 5 per participant. Three of these laboratories (laboratory 9, 11 and 13) identified two strains as not typable. Laboratory 2 identified six strains as not typable.
Seven laboratories identified one strain partly correctly/incompletely. In the case of 6 laboratories this strain belonged to the subspecies *salamae* or *houtenae*.

Seven strains of the subspecies *enterica* were identified correctly by all participants (Table 9). The serovars S. Oranienburg (strain no. 4), S. Lille (no. 7) and S. Fluntern (no. 17) were identified incorrectly by three laboratories. Besides S. Lille was not typable by three laboratories. S. Fluntern and S. Dublin were not typable once.
S. Llandoff was included twice in this study (strain no. 1 and 12). The laboratories 11 and 15 reported two different identifications. The serovars S. Give (strain no. 2 and 8) and S. Enteritidis (no. 6 and 19) were also included twice. Within the laboratories no difference in identification of these strains were reported.

The strains belonging to the subspecies *salamae* and *houtenae* were identified correctly 8 and 12 times respectively (Table 10). These strains were identified partly correctly/incompletely 4 and 2 times respectively, while these strains were not typable 2 and 3 times.

Table 8: *Identification of all 20 selected strains per laboratory*

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

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

Table 9: *Identification of the 18 strains belonging to the subspecies enterica by the 17 participants*

strain no	serotype	Identification		
		+	-	not typable
1	S. Llandoff	15	1	1
2	S. Give	16	1	-
3	S. Virchow	17	-	-
4	S. Oranienburg	14	3	-
6	S. Enteritidis	16	1	-
7	S. Lille	11	3	3
8	S. Give	16	1	-
9	S. Berta	17	-	-
10	S. Typhimurium	17	-	-
11	S. Poona	17	-	-
12	S. Llandoff	13	2	2
14	S. Dublin	16	1	-
15	S. Infantis	17	-	-
16	S. Worthington	17	-	-
17	S. Fluntern	13	3	1
18	S. Kentucky	17	-	-
19	S. Enteritidis	16	1	-
20	S. Agona	16	1	-

\+ = correct
\- = incorrect

Table 10: *Identification of the 2 strains belonging to the subspecies salamae (S. II 47:d:z39) and houtenae (S. IV 16:z4,z32) by the 17 participants*

strain no		Identification			
					not typable
		+	±	-	
5	S. II 47:d:z39	8	4	3	2
13	S. IV 16:z4,z32	12	2	-	3

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

5. DISCUSSION

Serotyping is an internationally accepted method for surveillance of *Salmonella*. In the different countries *Salmonella* strains were identified according to the Kauffmann-White scheme (1). This scheme is brought up to date yearly by the WHO Collaborating Centre for Reference and Research on *Salmonella* in Paris (Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France). The preparation of antisera and the purpose of standardization is described in the Guideline of the WHO for the preparation of *Salmonella* antisera (2).

This collaborative study was organized to be informed about the results of serotyping of *Salmonella enterica* by the NRLs of the EU Member States. It reveals that only 7 laboratories identified all strains completely correct. Most of these 7 laboratories have wide experience in serotyping based on the numbers of strains typed per year. Six of these laboratories produced at least partly their own sera.
The remaining 10 laboratories identified one or more strains incorrectly. The main reasons for incorrect typing were (i) the availability of a limited number of antisera for identification, (ii) the identification on basis of an incomplete antigenic formula by means of the Kauffmann-White scheme and (iii) an incorrect interpretation of a correct antigenic formula.

On account of the results, it seems some laboratories identified the strains with a limited number of antisera. Sometimes they obtained too little information about the antigens to be able to give a reliable identification. In most cases these laboratories also typed a limited number of strains per year. For different reasons it is possible that not all laboratories use a complete set of mono-specific antisera and definite conclusions can be based only on agglutination with mono-specific antisera. Sometimes laboratories use antisera dependent on the prevalence of the O groups in their country. Therefore it is not possible to interpret well the results in the case of partly correct/incomplete identifications.
A limited number of sera is also a possible reason for the large number of partly correct/incomplete identifications of the strains belonging to the subspecies *houtenae* and *salamae*. Other participants identified one or more strains based on an incomplete antigenic formula. This increased the chance of giving incorrect names of serovars. In these cases it is better to identify strains by giving only the antigenic formula as far as detected.

The laboratories should be careful of identifying strains based on an incomplete antigenic formula. It could lead to misinterpretation in the surveillance results among different countries. Although the most frequently isolated serovars were identified correctly by all participants, one laboratory did not identify S. Enteritidis.

The results of this study make clear that it is meaningful to repeat such a collaborative study on serotyping. It is a recommendation for laboratories with a limited number of antisera to check their results by sending strains to a national *Salmonella* centre, which uses the whole set of mono-specific antisera.

6. CONCLUSION

The conclusions of this study are:

- Seven of the 17 participants identified the 20 selected strains correctly; three laboratories typed one strain incorrectly and six laboratories typed 2-5 strains incorrectly. One laboratory reported six strains as not typable.

- Seven of the 18 strains of subspecies *enterica* were identified correctly by all participants; the strain belonging to the subspecies *salamae* was identified correctly by eight participants and the strain belonging to the subspecies *houtenae* by 12 participants.

- The 3 main reasons of incorrect identification were:
1. The O and/or H antigens were incorrectly detected;
2. The strains were identified on the grounds of an incomplete antigenic formula by means of the Kauffmann-White scheme;
3. The antigenic formula was interpreted incorrectly.

- It is better to identify strains by giving the antigenic formula as far as detected. Definite conclusions can be based only on agglutination with mono-specific antisera.

LITERATURE

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ANNEX 1

COMPARATIVE STUDY ON SEROTYPING OF *SALMONELLA* STRAINS ORGANIZED BY CRL SALMONELLA

PROTOCOL

Introduction:

The Community Reference Laboratory (CRL) for *Salmonella* is organizing a comparative study on serotyping of *Salmonella* strains amongst the National Reference Laboratories (NRLs). The typing method routinely performed in the laboratory will be used in this comparative study.
The CRL will supply *Salmonella* strains which will be used for serotyping. A total number of 20 *Salmonella* strains must be identified. The results will be evaluated by the CRL.

Objective:

The main objective of this serological study is to compare the test results of the NRLs in cooperation with the CRL *Salmonella*.

Outline of the study:

Each NRL will receive a parcel containing:

20 numbered cultures (numbered 1 to 20); each culture is a *Salmonella* strain from which the serotype needs to be identified.

On arrival the cultures must be subcultured on nutrient agar plates.
The performance of the study will be in **week 49** (starting at 04 December 1995) 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 comparative study on serotyping of *Salmonella* strains

The identification of the *Salmonella* cultures must take place in week 49 (starting at 04 December) or one week earlier or later.

06-10 November	Mailing the protocol and test report to the participating laboratories.
20-24 November	Mailing the strains to the participating laboratories. 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 costs for transport from the airport to the laboratory can't be paid by the CRL, so this will be at the expense of the NRL. For a small number of laboratories the CRL will mail the parcel by courier service. After arrival at the laboratory the strain needs to be subcultured and stored until the performance of the serotyping. **If you did not receive the parcel before or on 24 November, please contact the CRL immediately.**
27 November - 01 December	Checking the presence of all necessary reagents and materials for the performance of the study.
04-08 December	Starting with the identification of the strains. **Note:** Each laboratory is free to type the strains when they like as long as it will be done in the scheduled weeks.
18 December	Completion of the test report and faxing it to the CRL. The original test report will be sent by express to CRL.
08 January	Checking the results by the participating laboratories.

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

Nelly Voogt
(research assistant CRL)
P.O. Box 1
3720 BA Bilthoven
tel. number : ..-31-30-2742093
fax. number : ..-31-30-2744434

ANNEX 2

COMPARATIVE STUDY
ON SEROTYPING OF *SALMONELLA* STRAINS
ORGANIZED BY CRL SALMONELLA

TEST REPORT
OF THE FIRST COMPARATIVE STUDY
ON SEROTYPING OF *SALMONELLA* STRAINS

Laboratory code	:
Laboratory name	: .....
Date of collecting the parcel	: ..... - ..... - 1995
Starting date for serotyping	: ..... - ..... - 1995

GENERAL QUESTIONS

1. What is the frequency of serotyping at your laboratory?
   - ☐ once a week
   - ☐ twice a month
   - ☐ once a month
   - ☐ more frequent, namely .....
   - ☐ less frequent, namely .....

2. How many strains do you identify yearly?

   .....

3. Which kind of sera do you use?
   - ☐ commercial available sera

     manufacturer : .....
   - ☐ prepared in own laboratory

4. Which antigens do you identify?:
   - ☐ only O antigens
   - ☐ only H antigens
   - ☐ both O and H antigens
   - ☐ Vi antigen

5. Which method of agglutination do you use?
   - ☐ object glass agglutination
   - ☐ other, namely .....

PROTOCOL

Shipment:

Parcel damaged ☐ YES
☐ NO

date of receipt at the laboratory : ..... - 1995
time of receipt at the laboratory : ..... h ..... min

Did you store the strains before subculturing?

☐ YES temperature: ..... °C
☐ NO

Subculturing:

date the strains are subcultured : ..... - 1995

Medium used for subculturing the strains:

- name : .....
- manufacturer : .....
- catalogue number : .....

Did you store the strains after subculturing?

☐ YES temperature: ..... °C
☐ NO

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TEST RESULTS OF THE SEROLOGICAL COMPARATIVE STUDY

Please fill in your results in the table below.

labcode:

starting date of serotyping: ..... - ..... - 1995

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			