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

A collaborative study amongst the National Reference Laboratories for *Salmonella*

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Abstract

A second collaborative study on *Salmonella* serotyping was organized by the Community Reference Laboratory (CRL) for *Salmonella*, with participation of the National Reference Laboratories (NRLs) for *Salmonella* from 14 of the 15 Member States of the European Union. The objective of the study was to investigate the capacity of the NRLs for correctly identifying serovars of *Salmonella enterica* subsp. *enterica.*

Twenty serovars of *Salmonella enterica* subsp. *enterica*, selected by the CRL, had to be examined by all NRLs according to their routine serotyping method.

Thanks to the use of many frequently occurring serovars and the possibility of NRLs to send strains to another specialized laboratory, better results were obtained than for an earlier study. The incorrect detection of H antigens was the reason for most of the incorrect identifications.

Summary

A second collaborative study on serotyping of *Salmonella* was organized by the Community Reference Laboratory (CRL) for *Salmonella*. The National Reference Laboratories (NRLs) for *Salmonella* of 14 of the 15 Member States of the European Union participated. The objective of the study was to investigate the ability of the NRLs to correctly identify serovars of *Salmonella enterica* subsp. *enterica*.
In total 20 serovars of subspecies *enterica* of the species *Salmonella enterica* had to be examined by the national laboratories. The strains (many of them occurring frequently) were individually numbered and had to be identified according to the serotyping method routinely used in each laboratory. If a laboratory could not identify a strain, it was allowed to send it to another specialized laboratory in their country. Compared to an earlier collaborative study on serotyping better results were obtained. This was due to the use of many serovars occurring frequently in this study and the possibility to send strains to another laboratory. The strains mainly isolated from chickens, S. Enteritidis and S. Typhimurium, were identified correctly by all participants.
Incorrect identifications were caused mainly by an incorrect detection of the H antigens, in particular in the case of the strains S. Heidelberg and S. Virchow.

Samenvatting

Het Communautair Referentie Laboratorium (CRL) voor *Salmonella* organiseerde een tweede ringonderzoek voor de serotypering van *Salmonella.* De Nationale Referentie Laboratoria (NRLs) voor *Salmonella* van 14 van de 15 lidstaten van de Europese Unie namen deel. Het doel van dit ringonderzoek was om te onderzoeken of de NRLs in staat waren om serotypen van *Salmonella enterica* subsp. *enterica* correct te identificeren.
In totaal moesten er 20 serotypes van de subspecies *enterica* van de species *Salmonella enterica* door de nationale laboratoria onderzocht worden. De stammen (veel van hen komen vaak voor) waren individueel genummerd en moesten geidentificeerd worden volgens de serotyperingsmethode die routinematig op elk laboratorium werd toegepast. Als een laboratorium de stam zelf niet kon identificeren, was het toegestaan deze door te sturen naar een ander gespecialiseerd laboratorium in hun land.
Vergeleken met een eerder ringonderzoek werden er betere resultaten verkregen. Dit werd mogelijk veroorzaakt door het, in deze tweede studie, gebruiken van veel serotypen die vaak voorkomen en de mogelijkheid de stammen naar een ander laboratorium op te sturen. De stammen die veelal geisoleerd worden uit kippen, S. Enteritidis and S. Typhimurium, werden door alle deelnemers correct geidentificeerd. Incorrecte identificaties werden over het algemeen veroorzaakt door een incorrecte detectie van de H antigenen, in het bijzonder in het geval van de stammen S. Heidelberg en S. Virchow.

1 Introduction

In this report the second 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.

A first collaborative study on serotyping was organized in December 1995 (1). In that study the NRLs had to identify 20 strains of *Salmonella*. The results revealed that the most frequently isolated serotypes could be identified correctly by the majority of the participants. The main reasons for incorrect results of less frequently found serotypes 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 schema and an incorrect interpretation of the antigenic formula.

As a result of the discussions during a workshop of the CRL *Salmonella* with the NRLs in May 1996 (2), the second collaborative study included many serotypes occurring frequently. The main objective of the study was to investigate the ability of the NRLs to correctly identify serovars of *Salmonella enterica* subsp. *enterica.*

2 Participants

2.1 National Reference Laboratories [1]

Austria	Bundesstaatliche bakteriologisch-serologische Untersuchungsanstalt 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 gesuntheitlichen Verbraucherschutz und Veterinärmedizin Berlin
Ireland	Department of Agriculture Food and Forestry Veterinary Research Laboratory Dublin
Italy	Istituto Zooprofilattico Sperimentale delle Venezie Legnaro (Padova)
Luxembourg	Laboratoire de Médecine vétérinaire de l'Etat Luxembourg
The Netherlands	National Institute of Public Health and the Environment Bilthoven

Northern Ireland [2]	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 [2]	Central Veterinary Laboratory Bacteriological Department Surrey

[1] Greece did not participate

[2] 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 identified once again before mailing.
In total 20 strains of the species *Salmonella enterica* were selected; all strains belonged to the subspecies *enterica.* In comparison with the first collaborative study on serotyping, many serovars occurring frequently, in general, and serotypes frequently isolated in poultry, e.g., S. Alachua, S. Paratyphi B var. Java, S. Virchow, S. Infantis and S. Saintpaul, were chosen. S. Tennessee and S. Paratyphi B var. Java had to be identified twice in this study (two subcultures were included). Furthermore various strains isolated from different sources of the serotypes S. Enteritidis (four strains) and S. Typhimurium (three strains) were included.
All these data were unknown to the NRLs.
The antigenic formulas 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 they arrived at the laboratory the strains had to be subcultured and stored until the performance of the study. All details about the mailing and storing were mentioned in the protocol (annex 1). The protocol and the test report (annex 2) were mailed five weeks before the start of the study to the participants.
In total 15 NRLs participated. Due to functional changes, the NRL of Greece was not able to carry out the study, while 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 a NRL to send strains for serotyping to another reference laboratory in their country. 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	6,7,14	g,m,[p],s:[1,2,7]	S. Montevideo
2	1,9,12 [Vi]	g,p:-	S. Dublin
3	1,9,12	g,m:-	S. Enteritidis
4	1,9,12	g,m:-	S. Enteritidis
5	1,4,[5],12	f,g:[1,2]	S. Derby
6	35	z_4,z_{23}:-	S. Alachua
7	1,4,[5],12	i:1,2	S. Typhimurium
8	1,9,12	g,m:-	S. Enteritidis
9	6,7,14	r:1,5	S. Infantis
10	1,4,[5],12	b:1,2	S. Paratyphi B [1]
11	1,4,[5],12	i:1,2	S. Typhimurium
12	1,9,12	g,m:-	S. Enteritidis
13	6,7,14	f,g:-	S. Rissen
14	1,4,[5],12	r:1,2	S. Heidelberg
15	6,7,14	z_{29}:[1,2,7]	S. Tennessee
16	1,4,[5],12	e,h:1,2	S. Saintpaul
17	1,4,[5],12	i:1,2	S. Typhimurium
18	6,7	r:1,2	S. Virchow
19	6,7,14	z_{29}:[1,2,7]	S. Tennessee
20	1,4,[5],12	b:1,2	S. Paratyphi B [1]

underlined = O factors determined by phage conversion

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

[1] = variety L (+) tartrate (= *d*-tartrate) positive is often called variety Java

4 Results

4.1 General data of serotyping by the participants

The labcodes used in this second study differed from those used in the first one.
As presented in Table 2 three NRLs are not the reference laboratory for serotyping in their country. A number of the other NRLs are the reference laboratory for serotyping of strains of animal origin only. The frequency of typing and number of strains typed in 1996 are also presented in Table 2. The corresponding data of the first study are shown in Table 11 (annex 3). There are no remarkable differences in the frequency and number of strains typed in the laboratories between the two collaborative studies. Labcode 6 identified 3,000 strains more compared to 1995. In 1996 labcode 12 typed 200 compared to nine strains in 1995.
The origin of the sera used by the different NRLs is shown in Table 3.

4.2 Taxonomy and nomenclature of the typed strains

The first letter of serovars belonging to *S. enterica* subspecies *enterica* is a capital letter. Ten of the 15 participants wrote the identified serotype with a capital letter. Of the remaining NRLs three (labcode 2, 7 and 15) reported the name with a small letter and two participants (labcode 4 and 8) wrote the whole name in capital letters.

One laboratory (labcode 7) used S. Ardwick as well as S. Rissen to characterize strain no. 13. S. Ardwick is the name of the serotype withdrawn from the Kauffmann-White scheme, 1992 (3). Other laboratories (labcode 5 and 16) used the withdrawn name S. St. Paul instead of S. Saintpaul. In this study these withdrawn names were interpreted as incomplete (±). The identification S. Tenesse instead of S. Tennessee was interpreted as incomplete (±) as well, because incorrect writing of a name could give misleading in surveillance results.

Strains number 10 and 20 were S. Paratyphi B var. Java. This serotype is isolated mainly from animal sources, while the serotype S. Paratyphi B is a 'human type'. The difference between S. Paratyphi B var. Java and S. Paratyphi B depends on biotyping. If a strain is d-tartrate positive it is called variety Java. The strain is called S. Paratyphi B when the d-tartrate reaction is negative. Three laboratories (labcode 3, 5 and 10) reported strain 10 as S. Paratyphi B var. Java. Just one laboratory (labcode 10) identified strain 20 as S. Paratyphi B var. Java.
The withdrawn name from the Kauffmann-White scheme is S. Java. Three laboratories (labcode 13, 14 and 15) still used this name, probable to make clear that it was not a

S. Paratyphi B. Therefore it was interpreted as correct (+). All identifications of strain 10 and 20 reported by the participants and the interpretation in this study are shown in Table 4.

Table 2: *Frequency of serotyping and total number of strains typed in 1996 by the participants*

labcode	NRL = reference lab.	frequency of typing	number of strains typed in 1996
1	yes (all sources)	daily	14,314
2	no	daily	2,094
3	no	daily	± 12,000
4	yes (animal origin)	30-40 strains a week	1,500
5	no	± 20-50 strains a week	± 1,200
6	yes	daily	7,000
7	yes	daily	1,680
8	yes (not officially appointed)	daily	1,416
9	yes	not reported	8
10	yes	once a week	± 9,500
11	yes (for agriculture and fisheries)	as soon as cultures arrive at the laboratory	362
12	yes (for animals)	twice a month	200
13	yes (for animals)	daily	± 800
14	yes	daily	12,000
15	yes (for animals)	daily	2,000

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

number of laboratories	commercial sera	sera prepared by other institutes	own prepared sera
15	10	2	5

Table 4: *Nomenclature given to S. Paratyphi B var. Java by the laboratories*

name of strain	number of laboratories	identification[1]
S. Paratyphi B var. Java	3	+
S. Paratyphi B	7	+
S. Paratyphi B 05 neg	1	+
S. Java	3	+
Salm. groupe B	1	±
S. I 1,4,12:-:-	1	±

[1] see also Table 10

+ = correct

± = incomplete

4.3 Serotyping of the strains

In Table 5 the laboratories where the selected strains were typed are presented. Three NRLs (labcode 4, 12 and 15) which are the reference laboratory for animal sources, have sent some strains to the reference laboratory for human sources. In total five laboratories have sent one or more strains to another laboratory. Mostly strain number 6 (= S. Alachua), 10 and 20 (both S. Paratyphi B var. Java) were sent to a reference laboratory for further typing.

Table 5: *Laboratory in which selected strains are typed*

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

[1] = identified in National Public Health Institute

[2] = 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 6 presents the detection of the antigens per laboratory as stated in the test report and Table 7 the detection per strain.
The interpretation of the results was divided into correct (+), partly correct/incomplete (±) and incorrect (-).

Eleven participants detected the O antigens of all 20 selected strains correctly (Table 6). Two laboratories (labcode 5 and 9) detected the O antigens of one or two strains partly correctly and two other laboratories (labcode 2 and 7) could not type the O antigens of three and one strain(s), respectively.
Five laboratories detected all H antigens correctly (Table 6). Eight laboratories detected the H antigens of one to five strains partly correctly. Five laboratories detected one up to three H antigens as not typable.

Labcode 2 was not able to detect the O and H antigens of three strains, because the strains agglutinated spontaneously with saline. These were both S. Paratyphi B var. Java strains and S. Tennessee, which were included twice in this study. The other S. Tennessee strain was identified correctly.
Laboratory 5 detected H antigens of five strains partly correctly and detected two incorrectly. Five of these seven strains were sent to the reference laboratory for further identification. There was a difference in the identification of the two strains of S. Paratyphi B var. Java (strain no. 10 and 20). The O and H antigen of strain no. 20 were detected correctly, while the detection of the O antigens of strain no. 10 was incomplete. The H antigen of strain no. 10 agglutinated spontaneously.

Table 7 shows that the detection of the antigens of S. Alachua (strain no. 6), S. Paratyphi B var. Java (no. 10 and 20), S. Heidelberg (no. 14) and S. Virchow (no. 18) caused the most problems. The O and H antigens of S. Alachua were detected partly correctly by two laboratories (labcode 5 and 9), which used a limited number of sera. Laboratory 5 sent the strain to the reference laboratory for further typing, while laboratory 9 identified it as not typable. Laboratory 7 was not able to detect the O and H antigens of S. Alachua with their range of antisera and therefore reported this strain as not typable.
Three laboratories (labcode 1, 2 and 9) could not detect the H antigens of both S. Paratyphi B var. Java strains. One laboratory (labcode 4) once typed the H antigen of S. Paratyphi B var. Java correctly and could not type the H antigen the other time.
The false detections of the antigens of S. Heidelberg and S. Virchow, respectively four and three times, were due to a partly correct detection of the H antigens. The results of the laboratories which detected the H antigens of these two strains partly correctly are presented in Table 8a and 8b. Mostly the H antigen phase I was detected correctly, while the H antigen phase II was detected incorrectly.

Table 6: *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	-	-	-	18	-	-	2
2	17	-	-	3	15	2	-	3
3	20	-	-	-	19	1	-	-
4	20	-	-	-	19	-	-	1
5	18	2	-	-	13	5	2	-
6	20	-	-	-	20	-	-	-
7	19	-	-	1	17	2	-	1
8	20	-	-	-	18	2	-	-
9	19	1	-	-	16	2	-	2
10	20	-	-	-	20	-	-	-
11	20	-	-	-	20	-	-	-
12	20	-	-	-	19	1	-	-
13	20	-	-	-	19	1	-	-
14	20	-	-	-	20	-	-	-
15	20	-	-	-	20	-	-	-

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

Table 7: *Detection of the O and H antigens of the 20 strains by the 15 participants*

strain no.	serotype	O antigen				H antigen			
		detected				detected			
		+	±	-	not typable	+	±	-	not typable
1	S. Montevideo	15	-	-	-	13	2	-	-
2	S. Dublin	15	-	-	-	13	1	1	-
3	S. Enteritidis	15	-	-	-	15	-	-	-
4	S. Enteritidis	15	-	-	-	15	-	-	-
5	S. Derby	15	-	-	-	14	1	-	-
6	S. Alachua	12	2	-	1	12	2	-	1
7	S. Typhimurium	15	-	-	-	15	-	-	-
8	S. Enteritidis	15	-	-	-	15	-	-	-
9	S. Infantis	15	-	-	-	15	-	-	-
10 [1]	S. Paratyphi B var. Java	13	1	-	1	11	-	1	3
11	S. Typhimurium	15	-	-	-	15	-	-	-
12	S. Enteritidis	15	-	-	-	15	-	-	-
13	S. Rissen	15	-	-	-	13	2	-	-
14	S. Heidelberg	15	-	-	-	11	4	-	-
15 [2]	S. Tennessee	15	-	-	-	15	-	-	-
16	S. Saintpaul	15	-	-	-	15	-	-	-
17	S. Typhimurium	15	-	-	-	15	-	-	-
18	S. Virchow	15	-	-	-	12	3	-	-
19 [2]	S. Tennessee	14	-	-	1	14	-	-	1
20 [1]	S. Paratyphi B var. Java	14	-	-	1	10	1	-	4

\+ = correct
± = partly correct/incomplete
\- = incorrect
[1, 2] = subcultures

Table 8a: *False detections of the antigens of S. Heidelberg (strain no. 14)*

	O antigens	H antigens Phase 1	H antigens Phase 2	serotype
Kauffmann-White scheme	1,4,[5],12	r	1,2	S. Heidelberg
labcode 2	4	r	1,5	S. Bradford
labcode 5	4,5	i	1,2	S. Heidelberg
labcode 7	4	r	5	S. Bradford
labcode 12	4,12	r	1,5	S. Bradford

Table 8b: *False detections of the antigens of S. Virchow (strain no. 18)*

	O antigens	H antigens Phase 1	H antigens Phase 2	serotype
Kauffmann-White scheme	6,7	r	1,2	S. Virchow
labcode 2	7	r	1,5	S. Infantis
labcode 7	6,7	r	5	S. Infantis
labcode 9	6,7	r	1,5	S. Infantis

4.3.2 Identification of the strains

Three participants identified all strains correctly (Table 9). Eight laboratories identified one up to three strains incompletely. The incomplete identifications were due to (a) an incorrect spelling of the name of the serovar, (b) the use of a withdrawn name and (c) the availability of a limited number of sera. In the case of (a) and (b) the detection of the O and H antigens was correct.

Laboratory 2 identified S.Tennessee, which was included twice in the study once correctly and once incorrectly. Laboratory 3 did the same with S. Paratyphi B var. Java. Laboratory 7 and 9 did not send the not typable strains, respectively one and three strains, to another laboratory for further identification.

In Table 10 the identification of the 20 strains is shown. The strains mainly isolated from chickens, S. Enteritidis and S. Typhimurium, were identified correctly by all participants.
The serovars S. Heidelberg (strain no. 14) and S. Virchow (strain no. 18) were identified incorrectly by three laboratories. All these incorrect identifications were due to a partly correct detection of the H antigens (see also Table 8a and 8b).
Most laboratories did not carry out a biochemical test to make a distinction between the S. Paratyphi B and the S. Paratyphi B var. Java. Most identifications of this serotype (strains no. 10 and 20) were interpreted correct, because only serotyping results according to the Kauffmann-White scheme were taken into account.

Table 9: *Identification of the 20 selected strains per laboratory as stated in the test report*

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

\+ = correct
± = incomplete
\- = incorrect

Table 10: *Identification of the 20 strains by the 15 participants*

strain no.	serotype	Identification			
					not typable
		+	±	-	
1	S. Montevideo	15	-	-	-
2	S. Dublin	13	-	2	-
3	S. Enteritidis	15	-	-	-
4	S. Enteritidis	15	-	-	-
5	S. Derby	15	-	-	-
6	S. Alachua	13	-	-	2
7	S. Typhimurium	15	-	-	-
8	S. Enteritidis	15	-	-	-
9	S. Infantis	15	-	-	-
10	S. Paratyphi B var. Java	13	1	-	1
11	S. Typhimurium	15	-	-	-
12	S. Enteritidis	15	-	-	-
13	S. Rissen	13	1	1	-
14	S. Heidelberg	12	-	3	-
15	S. Tennessee	14	1	-	-
16	S. Saintpaul	13	2	-	-
17	S. Typhimurium	15	-	-	-
18	S. Virchow	12	-	3	-
19	S. Tennessee	13	1	1	-
20	S. Paratyphi B var. Java	11	2	1	1

\+ = correct
± = incomplete
\- = incorrect

5 Discussion

In this second collaborative study on serotyping the main objective was to investigate the ability of the NRLs to correctly identify serovars of *Salmonella enterica* subsp. *enterica.* In the first collaborative study (1) a reason for incorrect typing was the limited availability of a number of antisera for identification in several laboratories. During the workshop (2) it was recommended for those cases to send strains to another laboratory in their country with more experience in serotyping. Owing to this, better results were obtained in this second study. Five laboratories have sent in total 19 strains to another laboratory for identification or confirmation. All these strains were serotyped correctly, but sometimes the biotyping was not carried out to make a distinction between S. Paratyphi B var. Java and S. Paratyphi B.

Compared to the first study (results are shown in Table 12 and 13 of annex 3), the detection of the O antigens has improved, while the number of incomplete detected H antigens was nearly the same. The number of incorrect identifications was reduced in this second study.
There may be various reasons for the better results in this second study:
- many serotypes occurring frequently were included in the study. To identify these strains a laboratory did not need the whole set of mono-specific antisera. In the first study a limited number of antisera was one of the reasons of incomplete typing;
- laboratories could send strains to another laboratory for typing. This resulted in more correct identifications.

The strains had to be identified according to the Kauffmann-White scheme of 1992 (3). Some laboratories still used names which were withdrawn from the scheme. If laboratories used such a name, this was interpreted as an incomplete identification. After five years it should be possible to use the right names of the serotypes. Furthermore, the use of withdrawn names could lead to misinterpretation in surveillance results.

The serotype S. Paratyphi B var. Java was included twice in the study. In recent years the number of isolations in poultry has increased. Therefore a distinction between the S. Paratyphi B var. Java and the S. Paratyphi B is important. In this study a number of laboratories carried out the tartrate test to make this distinction. Although this test, like any other biochemical test, was beyond the protocol.

6 Conclusions

The conclusions of this study are:

- The use of many serotypes occurring frequently and the possibility to confirm the serotype of a strain in a reference laboratory yields better results of the serotyping of *Salmonella* strains.
-The detection of the H antigens, particularly in the case of the detection of S. Virchow and S. Heidelberg, gave the most incorrect identifications.
- A number of laboratories still uses names that are withdrawn from the Kauffmann-White scheme of 1992.
- The strains mainly isolated from chickens, S. Enteritidis and S. Typhimurium, were identified correctly by all participants.
- Not all laboratories made the distinction between S. Paratyphi B var. Java and S. Paratyphi B by means of d-tartrate test.

Literature

1. A collaborative study on serotyping of *Salmonella* amongst the National Reference Laboratories for *Salmonella* (report 284500 004)
N. Voogt, H.M.E. Maas, W.J. van Leeuwen and A.M. Henken, July 1996.

2. Report on the second workshop organized by CRL *Salmonella*;
Bilthoven, the Netherlands, 29-30 May 1996
N. Voogt, A.M. Henken (editors), October 1996.

3. Antigenic formulas of the *Salmonella* serovars, 1992
WHO Collaborating Centre for Reference and Research on *Salmonella*;
Michel Y. Popoff and Léon Le Minor, Institut Pasteur, Paris.

Annex 1

COLLABORATIVE STUDY (1996/1997) ON SEROTYPING OF *SALMONELLA* STRAINS ORGANIZED BY CRL SALMONELLA

PROTOCOL:

Introduction:

The Community Reference Laboratory (CRL) Salmonella organizes a second collaborative study on serotyping of *Salmonella* strains amongst the National Reference Laboratories (NRLs). In the first study on serotyping 7 of the 17 participating laboratories identified the 20 selected strains correctly. The main reasons for incorrect results by the remaining participants 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.

In this second 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.

Objective:

The main objective of the second 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 04 (starting on 20 January 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

The identification of the *Salmonella* cultures must take place in week 04 (starting on 20 January) or one week earlier or later.

16-20 December	Mailing the protocol and test report to the NRLs.
06-10 January	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't 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 10 January 1997, do contact the CRL immediately.**
13 - 17 January	Checking the presence of all necessary reagents and materials for the performance of the study.
20 - 24 January	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.

03 February	Completion of the test report and faxing it to the CRL. The original test report will be sent by express to CRL.
24 February	Checking the results by the NRLs.

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

Nelly Voogt
(research assistant CRL)
P.O. Box 1
3720 BA Bilthoven
tel. number : ..-31-30-2743927
fax. number : ..-31-30-2744434

Annex 2

COLLABORATIVE STUDY (1996/1997) page 1 of 5
ON SEROTYPING OF *SALMONELLA* STRAINS
ORGANIZED BY CRL SALMONELLA

TEST REPORT
OF THE SECOND 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 **1996**?

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

2. How many strains did you identify in **1996**?

.....

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 identified by

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

PLEASE WRITE YOUR REMARKS AND COMMENTS ON PAGE 5 OF THE TEST REPORT!

PROTOCOL

Shipment:

Parcel damaged YES
NO

date of receipt at the laboratory : ..... - 1997
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 : ..... - 1997

Medium used for subculturing the strains:

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

Did you store the strains after subculturing?

YES temperature: ..... °C
NO

PLEASE WRITE YOUR REMARKS AND COMMENTS ON PAGE 5 OF THE TEST REPORT!

page 4 of 5

TEST RESULTS OF THE COLLABORATIVE STUDY ON SEROTYPING

Please fill in your results in the table 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			

page 5 of 5

Remarks and comments:

Date: ..... - ..... - 1997

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

.....

signature:.....

Name of person in charge:

.....

signature:.....

Annex 3

RESULTS OF THE FIRST COLLABORATIVE STUDY ON SEROTYPING (organized in 1995)

Table 11: *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	± 150 strains a month	± 2,000
5	twice a month	n.r.
6	daily	4,000
7	daily	± 1,050
8	daily	1,200-1,600
9	n.r.	n.r.
10	once a week	8,000-9,000
11	daily	± 350
12 [1]	n.r.	9
13	60-80 strains a month	± 1,000
14	daily	10,000
15	daily	1,750

n.r. = not reported

[1] NRL for only 6 months

Table 12: *Results of detection of O and H antigens of 20 selected strains per laboratory in 1995*

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 13: *Results of the identification of 20 selected strains per laboratory in 1995*

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

\+ = correct

± = partly correct/incomplete

\- = incorrect