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Test results of *Salmonella* sero- and phage typing by the National Reference Laboratories and the EnterNet Laboratories in the Member States of the European Union

Collaborative study IV on sero- and phage typing

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Abstract

Test results of Salmonella serotyping and phage typing by the National Reference laboratories and the EnterNet laboratories in the Member States of the European Union

The fourth collaborative study on serotyping and phage typing for *Salmonella* was organised by the Community Reference Laboratory in collaboration with the Public Health Laboratory Services. All the National Reference Laboratories for *Salmonella* and 12 EnterNet laboratories participated in the study. In total, 16 strains of the species *Salmonella enterica* were selected for serotyping, while 10 strains of *Salmonella* Typhimurium and 10 strains of *Salmonella* Enteritidis were selected for phage typing. In general, problems with the typing of the O antigens did not occur. Most problems occurred with the typing of the H antigens. The majority of the EnterNet Laboratories and National Reference Laboratories did not encounter major problems with the phage typing of STM and SE strains.

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Samenvatting

Het Communautair Referentie Laboratorium (CRL) voor *Salmonella* heeft een vierde ringonderzoek voor de serotypering van *Salmonella* georganiseerd in samenwerking met het Central Public Health Laboratory (PHLS) in Londen. Voor de geïnteresseerde laboratoria bestond de mogelijkheid om ook faagtypering uit te voeren. Het doel van dit onderzoek was het vergelijken van de testresultaten tussen de Nationale Referentie Laboratoria (NRLs) onderling, tussen de EnterNet laboratoria (ENLs) onderling en tussen de NRLs en de ENLs.
Alle NRLs voor *Salmonella* van de lidstaten van de Europese Unie namen deel aan het ringonderzoek. Van deze 16 laboratoria voerden er 6 ook faagtypering uit. Tevens namen 12 ENLs deel waarvan er 10 ook faagtypering uitvoerden. Van de 16 NRLs zijn twee laboratoria tevens ENL. Beide laboratoria voerden faagtypering uit.
In totaal werden 16 stammen van de subspecies *enterica* van de species *Salmonella enterica* door het CRL-*Salmonella* geselecteerd. Deze stammen moesten door elk laboratorium getypeerd worden met de methode die zij routinematig toepassen. Ook mochten de laboratoria de stammen voor serotypering opsturen naar een ander gespecialiseerd laboratorium in hun land.
Voor de faagtypering werden 20 stammen geselecteerd door het PHLS. Tien stammen waren van het serotype *Salmonella* Enteritidis (SE) en 10 stammen waren van het serotype *Salmonella* Typhimurium (STM). De faagtypering moest uitgevoerd worden met de routinematig toegepaste methode van het laboratorium.

Summary

A fourth collaborative study on serotyping of *Salmonella* was organised by the Community Reference Laboratory (CRL) for *Salmonella* in collaboration with the Public Health Laboratory Service (PHLS) in London. Laboratories which were interested, had the possibility to perform phage typing too. The main goal of this study was to compare the results between the National Reference Laboratories (NRLs) as such, between the EnterNet laboratories (ENLs) as such and between the NRLs and the ENLs.

All NRLs for *Salmonella* of the Member States of the European Union participated in the collaborative study. Six of the 16 participating NRLs also performed phage typing. Twelve ENLs participated of which 10 laboratories performed phage typing. Two of the NRLs are also ENLs, and both of these laboratories performed phage typing.

In total 16 strains of the subspecies *enterica* of the species *Salmonella enterica* were selected by the CRL-*Salmonella*. The strains had to be typed by the NRLs with the method used routinely in their laboratory. The NRLs were allowed to send strains for serotyping to another specialised institute in their country.

The PHLS selected 20 strains for phage typing, 10 were of the serotype *Salmonella* Enteritidis (SE) and 10 of the serotype *Salmonella* Typhimurium (STM). Phage typing had to be performed by the routine method as used in the NRL and ENL laboratories and described in the phage typing protocol.

1. Introduction

In this report the fourth collaborative study on serotyping of *Salmonella* strains is described. This study was organised 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 organise this type of study in which the National Reference Laboratories (NRLs) for *Salmonella* can 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 and third collaborative study only strains belonging to subspecies *enterica* were included (2,3). The 20 strains for the second and third study were selected from among the more frequently found serotypes.
In the fourth study, described in this report, 16 serotypes were selected. Most strains were serotypes occurring frequently and some strains were serotypes occurring infrequently. The main objective of the study was to compare the results of serotyping among the NRLs.
In cooperation with the Central Public Health Laboratory (PHLS), London, phage typing was included in this study. Six of the 16 NRLs performed phage typing on 10 *Salmonella* Enteritidis and 10 *Salmonella* Typhimurium strains.
Fourteen EnterNet laboratories (ENLs) participated in this study (two of them are also NRLs). All of the ENLs performed serotyping and 12 of them performed phage typing.

2. Participants

		National Reference Laboratory for *Salmonella* (NRL) or EnterNet Laboratory (ENL)	
Austria	Bundesstaatliche bakteriologisch-serologische Untersuchungsanstalt Graz	NRL	and ENL
Belgium	Veterinary and Agrochemical Research Center (VAR) Bruxelles	NRL	
Belgium	Institute Scientifique de Santé Publique - Louis Pasteur Section Bacteriology Bruxelles		ENL
Denmark	Danish Veterinary Laboratory Copenhagen	NRL	
Finland	National Veterinary and Food Research Institute Department of Bacteriology Helsinki	NRL	
Finland	National Public Health Institute (KTL) Laboratory of Enteric Pathogens National Salmonella Centre Helsinki		ENL
France	Centre National d'Etudes Vétérinaires et Alimentaires Laboratoire central de recherches avicole et porcine Ploufragan	NRL	
Germany	Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin Berlin	NRL	
Germany	Robert-Koch Institut Wernigerode/Harz		ENL

Greece	Veterinary Laboratory of Halkis Halkis	NRL	
Greece	Medical School, University of Athens Department of Microbiology Athens		ENL
Ireland	Department of Agriculture and Food Veterinary Research Laboratory Dublin	NRL	
Ireland	University College Hospital Galway		ENL
Italy	Istituto Zooprofilattico Sperimentale delle Venezie Legnaro	NRL	
Italy	Istituto Superiore di Sanita Laboratory of Medical Bacteriology & Mycology Rome		ENL
Luxembourg	Laboratoire de Médecine vétérinaire de l'Etat (animal zoonosis) Luxembourg	NRL	
The Netherlands	Rijksinstituut voor Volksgezondheid en Milieu (RIVM) Bilthoven	NRL	and ENL
Northern Ireland	Department of Agriculture for Northern Ireland Veterinary Sciences Division; Bacteriology Department Belfast	NRL	
Portugal	Laboratorio Nacional de Veterindria Lisboa	NRL	
Portugal	Instituto Nacional de Saude Lisbon		ENL
Scotland (United Kingdom)	Scottish Salmonella Reference Laboratory Department of Bacteriology Glasgow		ENL

Spain	Laboratorio de Sanidad Y Produccion Animal de Algete Madrid	NRL	
Spain	Instituto de Salud Carlos III Laboratorio de Enterobacterias, Centro Nacional de Microbiologia Madrid		ENL
Sweden	National Veterinary Institute Department of Bacteriology Uppsala	NRL	
Sweden	Swedish Institute of Infectious Disease Control Department of Bacteriology Solna		ENL
Switzerland	University of Berne, Institute of Veterinary Bacteriology National Reference Laboratory for Foodborne Diseases Berne		ENL
United Kingdom	Central Veterinary Laboratory Bacteriology Department Weybridge Surrey	NRL	
United Kingdom	Laboratory of Enteric Pathogens Central Public Health Laboratory Public Health Laboratory Service (PHLS) London		ENL

3. Materials and Methods

3.1 Selected *Salmonella* strains

3.1.1 Strains for serotyping

As stated in the protocol, which was sent to the participants before mailing of the strains, 20 strains for serotyping would be sent to the participants. However, due to some problems with the mailing of the strains, nr 6, 7, 10 and 17 were omitted at the last moment.

The *Salmonella* strains used for the collaborative study on serotyping originated from the collection of the National *Salmonella* Centre in The Netherlands. The strains were typed once again before mailing. In total 16 strains of the species *Salmonella enterica* were selected. All strains belonged to the subspecies *enterica.*

The antigenic formulae according to the Kauffmann-White scheme of the 16 serovars are shown in Table 1.

Table 1 Antigenic formulas of the 16 *Salmonella* strains used in the collaborative study according to the Kauffmann-White scheme

No.	serotype	O antigens	H antigens	Origin of strains
1	*S.* Albany	8,20	z4,z24:-	animal feed
2	*S.* Weltevreden	3,10[15]	r:z6	human faeces
3	*S.* Goettingen	9,12	l,v:e,n,z15	Monkey
4	*S.* Adelaide	35	f,g:-	Turtle
5	*S.* Lexington	3,10[15][15,34]	z10:1,5	animal feed
8	*S.* Typhimurium	1,4,[5],12	i:1,2	Pigeon
9	*S.* Tennesssee	6,7,14	z29:[1,2,7]	Fishmeal
11	*S.* Enteritidis	1,9,12	g,m:-	human faeces
12	*S.* Goldcoast	6,8	r:l,w	human faeces
13	*S.* Alachua	35	z4,z23:-	Pig
14	*S.* Bovismorbificans	6,8,20	r:[i]:1,5	human faeces
15	*S.* Schwarzengrund	1,4,12,27	d:1,7	animal feed
16	*S.* Stanley	1,4,[5],12,27	d:1,2	Paunch
18	*S.* Brandenburg	1,4,12,[5],27	l,v:e,n,z15	human faeces
19	*S.* Cubana	1,13,23	z29:-	animal feed
20	*S.* Heidelberg	1,4,[5],12	r:1,2	human faeces

3.1.2 Strains for phage typing

The *Salmonella* strains used for the collaborative study on phage typing originated from the collection of the Laboratory of Enteric Pathogens, Public Health Laboratory Service (PHLS). Ten strains of *Salmonella* Enteritidis and 10 strains of *Salmonella* Typhimurium were selected. The phage types and the phage reaction patterns of the 20 strains are shown in Table 2 and 3.

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 (appendix 2 and 4). The protocol and test report (appendix 3 and 5) were mailed four weeks before the start of the study to the participants.

3.2.1 Serotyping

All 15 Member States of the European Union participated and the United Kingdom participated with three laboratories. The laboratories were assigned a labcode from 1 to 16. From the ENLs 14 participants performed the study (Labcode M to Z). The NRLs which are also ENL were assigned to the NRL group. For evaluation of the results, their results were also evaluated among the ENLs.

The 16 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 possible for a laboratory to send strains for serotyping to another reference laboratory in their country.

3.2.2 Phage typing

Six of the NRLs (Labcode 1, 3, 6, 9, 11 and 15) and 10 of the ENLs (Labcode M, N, P, S, T, U, V, W, X and Y) were interested in performing phage typing. The 20 strains had to be tested according to the Salmonella phage typing protocol from PHLS (appendix 4).

Table 2 Phage reactions of the *Salmonella* Enteritidis strains used in the collaborative study

		Phages at Routine Test Dilution															
QA number	Phage type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
E1	6a	-	SCL	-	<OL	3	<OL	-	-	OL	-	-	±	-	-	-	-
E2	20	OL	-	CL	-	CL	-	<CL	OL	-	OL	±	CL	±	CL	<CL	OL
E3	34	-	-	-	-	-	-	-	OL	-	<OL	-	-	-	-	-	-
E4	1	OL	SCL	CL	SCL	CL	SCL	CL	OL	OL	OL	CL	CL	CL	CL	-	-
E5	4	-	SCL	OL	OL	OL	SCL	OL	<OL	OL	SCL	OL	OL	OL	-	-	-
E6	8	4	-	SCL	SCL	CL	SCL	<CL	OL	OL	OL	±± <<	CL	-	-	-	-
E7	6	-	SCL	-	SCL	-	SCL	-	OL	OL	<OL	-	-	-	-	-	-
E8	13a	-	-	-	SCL	-	SCL	-	SCL	OL	SCL	-	-	-	-	-	-
E9	4	-	SCL	CL	SCL	CL	SCL	CL	OL	OL	<OL	CL	CL	CL	3	-	-
E10	21	OL	SCL	-	SCL	-	SCL	-	OL	OL	OL	-	-	-	CL	-	-

<< : Merging plaques towards semi-confluent lysis

Table 3 Phage reactions of the *Salmonella* Typhimurium strains used in the collaborative study

		Phages at routine test dilution																												Additional phages				
QA Number	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	32	35	O*	1	2	3	10	18
M11	66	-	-	-	-	-	-	-	-	CL	<OL	-	3	-	-	<CL	-	-	-	<CL	-	<OL	<CL	1	±	++	-	-	CL	CL	±	CL					
M12	104H	-	-	-	-	-	-	-	1	-	-	<OL	OL	-	-	-	3	SCL	-	±N	-	-	-	-	-	-	±	1	-	-	±	CL	-	-	-	OL	2
M13	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CL	+++	SCL	+++	+	-
M14	12	-	-	-	-	-	-	-	-	-	-	+<<	<OL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CL	+++	SCL	+++	OL	-
M15	1	CL	<OL	CL	OL	CL	CL	CL	±	CL	CL	SCL	CL	OL	OL	CL	OL	CL	CL	<OL	OL	OL	CL	CL	<CL	CL	CL	±	CL	CL	OL	CL					
M16	208	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CL	-	+	-	<OL	OL
M17	4	-	-	-	SCL	SCL	SCL	-	6	CL	CL	+	++ <<	-	CL	CL	-	-	<CL	CL	-	CL	CL	-	SCL	±±	CL	±	CL	CL	OL	CL					
M18	104L	-	-	-	-	-	-	-	2	-	-	++ <<	SCL	-	-	-	-	+++	-	-	-	-	-	-	-	-	-	-	-	-	4	CL	-	-	-	OL	-
M19	10	-	-	-	-	-	-	-	-	CL	<OL	<CL	CL	-	-	<CL	-	-	-	<CL	2	OL	CL	-	±	±±	-	-	CL	CL	-	CL					
M20	160	-	-	-	-	-	-	-	-	-	OL	-	<CL	-	-	-	SCL	CL	OL	OL	-	OL	-	-	-	-	-	-	-	OL	-	CL					

O*: O pooled

<< : Merging plaques towards semi-confluent lysis

4. Results

4.1 General data of serotyping by the participants

The labcodes for the NRLs used in this study are the same labcodes which were used in the third study. The ENLs were assigned a letter from M to Z.
In Tables 4 and 5 the frequency of typing and the total number of strains typed at the NRLs and the ENLs is shown. For the NRLs, there are no differences in the frequency of typing between the third and fourth collaborative study. There are only small differences in the total number of strains typed between 1997 and 1998.
Table 6 shows the origin of the sera used by the different laboratories.

Table 4 Frequency of serotyping and total number of strains typed by the NRLs

Labcode	frequency of typing	total no. of strains typed in 1998	total no. of strains typed in 1997
1	daily	13,128	13,550
2	daily	2,023	1,905
3	daily	15,976	14,000 - 15,000
4	± 200 a month	± 1,000	1,500
5	± 20 strains every week	± 1,200	± 1,000
6	daily	5,660	7,000
7	on arrival	102	36
8	daily	709	1,470
9	daily	1,450	2,000
10	twice a month	20	7
11	once a week	11,351	± 8,000
12	on arrival	463	298
13	twice a month	500	300
14	daily	1,000 - 1,500	± 1,000
15	daily	10,000	10,000
16	daily	1,500	2,000

Table 5 Frequency of serotyping and total number of strains typed by the ENLs

labcode	frequency of typing	total no. of strains typed in 1998
M	daily	14,515
N	daily	± 2,500
P	daily	10,813
R	twice a week	1,000
S	daily	550
T	once a week	± 200
U	twice a week	354
V	daily	2,320
W	daily	7,200
X	± 3 times a week	5,173
Y	daily	3,481
Z	daily	6,404

Table 6 The origin of the sera used by the different laboratories

collaborative study	number of laboratories	commercial available sera	sera prepared by other institutes	own prepared sera
I	17	12	4	7
II	15	10	2	5
III	16	11	3	3
IV	14 (NRLs)	14	4	2
	12 (ENLs)	10	3	7
	2 (NRL+ENL)	2	1	2

4.2 Taxonomy and nomenclature of the typed strains

All NRLs wrote the identified serotype with a first capital letter as proposed by the *Salmonella* WHO reference centre (4). In the previous study 15 (of 16) laboratories wrote the name of the serovar with a capital letter. From the ENLs three laboratories (labcode M, T and X) wrote the whole name of the serovar in capital and one laboratory (labcode S) wrote the name of the serovar without any capital.

No laboratory used name(s) of serovars which are withdrawn from the most recent Kauffmann-White scheme (5) for identification of the strains.

4.3 Serotyping of the strains

Fourteen NRLs and all of the ENLs typed all strains in their own laboratory. Table 7 shows the two NRLs who each sent 3 strains to another laboratory for serotyping. Laboratory 2 sent the strains to the ENL in their country.

Table 7 Laboratory which did not type all strains

labcode	number of strains typed in	
	own laboratory	other laboratory
2	13	3 (nr. 1, 4 and 13 [1])
5	13	3 (nr. 1, 4 and 13 [1])

[1] identified in national reference laboratory for serotyping

4.3.1 Detection of the O and H antigens by the NRLs

The detection of O and H antigens are evaluated per strain and per laboratory.
Eight laboratories (labcode 1, 3, 6, 7, 11, 13, 14 and 15) typed all O and H antigens correctly. Table 8 shows the results of those laboratories which typed the O or H antigens incorrectly as stated in the test report. Two laboratories (labcode 2 and 5) typed 1 strain only partly correct or incomplete. Laboratory 5 identified strain number 4 as *S.* Adelaide where the only phase 1 H antigen typed was g. In addition to H antigen g, antigen f should be typed, to be sure it is *S.* Adelaide and not one of the other serotypes reacting with g.
Three laboratories (labcode 4, 10 and 16) typed 1 strain incorrectly. Two laboratories (labcode 9 and 12) typed two strains partly correct and incorrect respectively. One laboratory (labcode 8) typed 7 strains partly correct and incorrectly.
Seven strains were typed correctly by all laboratories; *S.* Lexington (nr. 5), *S.* Typhimurium (nr. 8), *S.* Enteritidis (nr. 11), *S.* Bovismorbificans (nr. 14), *S.* Stanley (nr. 16), *S.* Brandenburg (nr. 18) and *S.* Heidelberg (nr. 20). Table 9 shows the results of the strains which were typed incorrectly by at least one laboratory.

Table 8 Number of laboratories which detected an O or H antigen partly correct or incorrect

labcode	O antigen detected			H antigen detected		
	correct	partly correct[1)]	incorrect	Correct	Partly correct[1)]	incorrect
2	16	-	-	15	1	-
4	16	-	-	15	-	1
5	16	-	-	15	1	-
8	14	2	-	10	1	5
9	16	-	-	14	2	-
10	15	-	1	16	-	-
12	15	-	1	15	-	1
16	15		1	16	-	-

[1)] partly correct or incomplete

Table 9 Strains where the O or H antigens were detected partly correct or incorrectly by one of the participating NRLs

strain no.	serotype	O antigen detected			H antigen detected		
		correct	partly correct[1)]	Incorrect	correct	partly correct[1)]	incorrect
1	*S.* Albany	14	-	2	13	1	2
2	*S.* Weltevreden	16	-	-	14	-	2
3	*S.* Goettingen	16	-	-	15	-	1
4	*S.* Adelaide	15	1	-	14	2	-
9	*S.* Tennessee	16	-	-	15	1	-
12	*S.* Goldcoast	16	-	-	15	-	1
13	*S.* Alachua	15	1	-	16	-	-
15	*S.* Schwarzengrund	16	-	-	15	-	1
19	*S.* Cubana	15	-	1	15	1	-

[1)] partly correct or incomplete

4.3.2 Identification of the strains by the NRLs

The results of six laboratories who identified at least one of the strains incorrectly are shown in Table 10. Strain nr. 1 (*S.* Albany) gave most problems. Four laboratories identified this serotype incorrectly. Strain nr. 2 (*S.* Weltevreden) and strain nr. 19 (*S.* Cubana) were identified incorrectly and/or incomplete by two (different) laboratories. Laboratory 8 seems to have difficulties with their A-S sera since they found no reaction with O antigens 35.

Table 10 Typing results of strains which were typed incomplete/incorrect by at least one laboratory

Strain	Labcode						
	Correct identification	4	8	9	10	12	16
1.	8,20:z4,z24:- Albany	8: d: 2 Virginia	8: i: z6 Kentucky	Albany	6, 8: z4, z24:- Duesseldorf	6, 8: z4, z24:- Duesseldorf	Albany
2.	3,10,15:r:z6 Weltevreden	Weltevreden	3, 10: d: l, w Birmingham	Weltevreden	Weltevreden	3, 10: i: z6 Yeerongpilly	Weltevreden
3.	9,12:l,v:e,n,z15 Goettingen	Goettingen	9: l, v: e, n, x Toronto or Zaiman	Goettingen	Goettingen	Goettingen	Goettingen
4.	35:f,g:- Adelaide	Adelaide	no reaction A-S: f:- -	Adelaide	Adelaide	Adelaide	Adelaide
9.	6,7,14:z29:1,2,7 Tennessee	Tennessee	Tennessee	6, 7, 14:-:- -	Tennessee	Tennessee	Tennessse
12.	6,8:r:l,w Goldcoast	Goldcoast	6, 7, 8: l, v: z15 Edmonton	Goldcoast	Goldcoast	Goldcoast	Goldcoast
13.	35:z4,z23:- Alachua	Alachua	no reaction A-S: z4: z24 -	Alachua	Alachua	Alachua	Alachua
15.	1,4,12,27:d:1,7 Schwarzengrund	Schwarzengrund	4: l, v: 7 Bredeney	Schwarzengrund	Schwarzengrund	Schwarzengrund	Schwarzengrund
19.	1,13,23:z29:- Cubana	Cubana	Cubana	13, 23:- -	Cubana	Cubana	13, 22: z29:- Agoueve

Incorrect or incomplete identification of the strain

4.3.3 Detection of the O and H antigens by ENLs

The detection of O and H antigens are evaluated per strain and per laboratory.

Table 11 shows the results of those laboratories which typed the O or H antigens incorrectly as stated in the test report. Ten laboratories (labcode 1, 11, M, N, P, V, W, X, Y and Z) typed all O and H antigens correctly. One laboratory (labcode V) typed 1 strain incomplete or partly correct. One laboratory (labcode T) typed 9 strains incorrectly and 4 strains incompletely. One laboratory (labcode S) typed 2 strains incorrect and 2 strains incompletely. One laboratory (labcode U) typed 2 strains incorrectly. One laboratory (labcode R) typed 1 strain incompletely and 2 strains incorrectly.

Table 12 shows the results of the strains which were typed incorrectly by at least one laboratory. Three strains were typed correctly by all laboratories: *S.* Adelaide (nr. 3), *S.* Lexington (nr. 5) and *S.* Typhimurium (nr. 8).

Table 11 Laboratories which detected an O or H antigen partly correct or incorrect

labcode	O antigen detected			H antigen detected		
	Correct	partly correct[1)]	incorrect	Correct	partly correct[1)]	Incorrect
R	15	-	1	13	1	2
S	14	1	1	13	2	1
T	10	4	2	7	2	7
U	15	1	-	14	-	2
V	15	1	-	16	-	-

[1)] partly correct or incomplete

Laboratory T was unable to type strain nr 11. As this was *S.* Enteritidis we asked them to send back the strain in order to be typed again by the CRL. The strain was retyped at the CRL and the serotype was confirmed as *S.* Enteritidis.

Laboratory V and R typed O antigens of strain nr 19 as rough. Both laboratories were asked to send back the strain to the CRL. Typing of the strain which was sent back to the CRL by laboratory V resulted in serotype *S.* Cubana. Laboratory R did not return the strain to the CRL.

Table 12 Strains of which the O or H antigens were detected partly correct or incorrectly by one of the participating ENLs

strain no.	Serotype	O antigen detected			H antigen detected		
		correct	partly correct[1)]	Incorrect	Correct	Partly correct[1)]	incorrect
1	*S.* Albany	11	2	1	12	-	2
2	*S.* Weltevreden	14	-	-	12	-	2
3	*S.* Goettingen	14	-	-	12	-	2
9	*S.* Tennessee	13	-	1	14	-	-
11	*S.* Enteritidis	13	1	-	13	1	-
12	*S.* Goldcoast	13	1	-	13	-	1
13	*S.* Alachua	13	1	-	12	1	1
14	*S.* Bovismorbificans	13	1	-	13	1	-
15	*S.* Schwarzengrund	14	-	-	12	1	1
16	*S.* Stanley	14	-	-	13	-	1
18	*S.* Brandenburg	14	-	-	13	1	-
19	*S.* Cubana	11	1	2	13	-	1
20	*S.* Heidelberg	14	-	-	13	-	1

[1)] partly correct or incomplete

4.3.4 Identification of the strains by the ENLs

Five of the 16 strains were identified correctly by all participants. The results of the laboratories who identified at least one of the strains incorrectly are shown in Table 13. Serotypes *S.* Alachua and *S.* Cubana gave most problems for identification. Both strains were identified differently by 4 laboratories. Two laboratories typed a wrong H antigen and 2 laboratories performed biotyping which resulted in the wrong identification. Typing of the strain at the CRL revealed no biotype III. Furthermore, *S.* Albany was identified incorrectly by 3 laboratories. Laboratory T mentioned that they have problems with their typing sera.

Table 13 Typing results of strains which were typed incomplete/incorrect by at least one laboratory

Strain	Labcode					
	correct identification	R	S	T	U	V
1.	8,20:z4,z24:- Albany	Albany	4,27:d:2 Cairo	8:d:1,2 Muenchen	8:a:e,n,z15 Leith	Albany
2.	3,10,15:r:z6 Weltevreden	Weltevreden	Weltevreden	3,10,15:r:1,7 Elisabethville	3,10:y:1,5 Orion	Weltevreden
3.	9,12:l,v:e,n,z15 Goettingen	9,12:l,v:1,7 Kapemba	Goettingen	9:l,v:1,5 Panama	Goettingen	Goettingen
9.	6,7,14:z29:1,2,7 Tennessee	Tennessee	Tennessee	62:z29 -	Tennessee	Tennessee
11.	1,9,12:g,m:- Enteritidis	Enteritidis	Enteritidis	12: -	Enteritidis	Enteritidis
12.	6,8:r:l,w Goldcoast	Goldcoast	Goldcoast	8:r:1,2 Bsilla	Goldcoast	Goldcoast
13.	35:z4,z23:- Alachua	IIIa 35:z4, z23:-*	35:z4,z23 no monovalent z23 or z24	35:z4,z24:- Westfalia	IIIa 35:z4, z23:-*	Alachua
14.	6,8,20:r:i:1,5 Bovismorbificans	Bovismorbificans	Bovismorbificans	poly II: r -	Bovismorbificans	Bovismorbificans
15.	1,4,12,27:d:1,7 Schwarzengrund	Schwarzengrund	4,27:r:7 Remo	4:y:1,2 Coeln	Schwarzengrund	Schwarzengrund
16.	1,4,5,12,27:d:1,2 Stanley	Stanley	Stanley	4:d:1,5 Eppendorf	Stanley	Stanley
18.	1,4,12,5,27:l,v: e,n, z15 Brandenburg	4,12:l,v:e,n,x Kimuenza	Brandenburg	Brandenburg	Brandenburg	Brandenburg
19.	1,13,23:z29:- Cubana	rough strain	13,22:z29 no monovalent o22 or o23	62:z29 -	Cubana	rough:z29:- Rough strain (-:z29:-)
20.	1,4,5,12:r:1,2 Heidelberg	Heidelberg	Heidelberg	4:r:1,5 Bradford	Heidelberg	Heidelberg

* Serotyping correct. Biotyping was necessary to differentiate between Alachua and SIIIa.

Incorrect or incomplete identification of the strain

4.3.5 Comparison of NRLs with ENLs

The identification of the strains is also evaluated between the NRLs and the ENLs. Table 14 shows the percentages of laboratories which identified a strain incorrectly. Two strains (nr. 5 and 8) were identified correctly by all laboratories. The greatest difference between the NRLs and ENLs occurred for two strains (nr. 13 and 19).

The percentage of ENLs which have identified a strain incorrectly is higher than the percentage of NRLs which have identified a strain incorrectly. If, however, the results of EnterNet laboratory T are excluded from the results, the differences observed in the comparison between NRLs and ENLs decreased. When those results are excluded there are three extra strains (nr. 11, 14 and 16) which were identified correctly by all laboratories.

Table 14 Strains which are identified incorrectly by NRLs or ENLs

strain no.	Serotype	Percentage of NRLs which identified the strain		Percentage of ENLs which identified the strain	
		correct	incorrect	correct	incorrect
1	*S.* Albany	75.0 (12/16)	25.0 (4/16)	78.6 (11/14)	21.4 (3/14)
2	*S.* Weltevreden	87.5 (14/16)	12.5 (2/16)	85.7 (12/14)	14.3 (2/14)
3	*S.* Goettingen	93.7 (15/16)	6.3 (1/16)	85.7 (12/14)	14.3 (2/14)
4	*S.* Adelaide	93.7 (15/16)	6.3 (1/16)	100 (14/14)	- (0/14)
9	*S.* Tennessee	93.7 (15/16)	6.3 (1/16)	92.9 (13/14)	7.1 (1/14)
11	*S.* Enteritidis	100 (16/16)	- (0/16)	92.9 (13/14)	7.1 (1/14)
12	*S.* Goldcoast	93.7 (15/16)	6.3 (1/16)	92.9 (13/14)	7.1 (1/14)
13	*S.* Alachua	93.7 (15/16)	6.3 (1/16)	71.4 (10/14)	28.6 (4/14)
14	*S.* Bovismorbificans	100 (16/16)	- (0/16)	92.9 (13/14)	7.1 (1/14)
15	*S.* Schwarzengrund	93.7 (15/16)	6.3 (1/16)	85.7 (12/14)	14.3 (2/14)
16	*S.* Stanley	100 (16/16)	- (0/16)	92.9 (13/14)	7.1 (1/14)
18	*S.* Brandenburg	100 (16/16)	- (0/16)	92.9 (13/14)	7.1 (1/14)
19	*S.* Cubana	87.5 (14/16)	12.5 (2/16)	71.4 (10/14)	28.6 (4/14)

In Table 15 a comparison is made of the percentage of strains typed correctly in this study, the number of strains typed in 1998 by the laboratories and the average number of strains typed by the laboratories. From these data it can be concluded that the higher the number of strains as routinely typed yearly by a laboratory the better the results of serotyping in this study were. Typing on regular basis and experience with the procedure are necessary to get the best results.

Table 15 Comparison serotyping of total number of strains typed by the NRLs and ENLs in 1998 and number of strains assigned correctly in this collaborative study.

% of strains assigned correctly in this study (n=16)	Number of laboratories	Number of strains typed by the laboratories in 1998	Average number of strains typed per laboratory in 1998
25	1	200	200
50-75	2	550-709	630
80-93	4	354-1450	817
94	5	20-2023	1149
100	15	102-15976	7013

4.4 Phage typing of the strains

All laboratories which asked for strains for phage typing, performed phage typing in their own laboratory. One NRL (labcode 4) send their strains for phage typing to the ENL (labcode N) in their country. For that NRL the phage typing is not evaluated separately.

4.4.1 Phage typing results by the NRLs

The phage typing results are evaluated per strain and per laboratory. Table 16 and 17 show the results of phage typing as stated in the test report. Three laboratories (labcode 3, 6 and 15) assigned all strains the correct phage type. Four strains of SE (PT 1, 6, 20 and 21) and 6 strains of STM (PT 66, 104^{H}, 193, 12, 104^{L} and 160) were assigned correctly by all laboratories.

Table 16 Results of *Salmonella* Enteritidis phage typing by the NRLs

		Phagetypes of each laboratory					
Strain	PT	1	3	6	9	11	15
E1	6a	6a	6a	6a	6a	35	6a
E2	20	20	20	20	20	20	20
E3	34	34	34	34	19	34	34
E4	1	1	1	1	1	1	1
E5	4	4	4	4	4	37	4
E6	8	8	8	8	28	8	8
E7	6	6	6	6	6	6	6
E8	13a	28	13a	13a	13a	13a	13a
E9	4	4	4	4	4	4a	4
E10	21	21	21	21	21	21	21

Table 17 Results of *Salmonella* Typhimurium phage typing by the NRLs

		Phagetypes of each laboratory					
Strain	PT	1	3	6	9	11	15
M11	66	66	66	66	66	66	66
M12	104^{H}	104^{H}	104^{H}	104^{H}	104^{H}	104^{H}	104^{H}
M13	193	193	193	193	193	193	193
M14	12	12	12	12	12	12	12
M15	1	1	1	1	1	36	1
M16	208	RDNC[1)]	208	208	208	208	208
M17	4	4	4	4	4	52a	4
M18	104^{L}	104^{L}	104^{L}	104^{L}	104^{L}	104^{L}	104^{L}
M19	10	10	10	10	10	193	10
M20	160	160	160	160	160	160	160

1) RDNC: Reactions do not conform to a recognised pattern.
Laboratory 1 lacked the STM additional phage 18 and therefore was unable to assign a type to M16.

4.4.2 Phage typing results by the ENLs

The phage typing results are evaluated per strain and per laboratory. Table 18 and 19 show the results of phage typing as stated in the test report. Only one laboratory (labcode N) assigned all phage types correctly. Two strains of STM (PT 66 and 1) were assigned correctly by all laboratories but no strain of SE was assigned correctly by all laboratories. *Salmonella* Enteritidis PT 13a appears to give most problems. Laboratory T did not use additional phages on STM strains. Laboratory T had a problem with SE phage 1 obtaining false positive reactions for E3, E5, E7, E8 and E9. Without this reaction E3, E5, E7 and E9 were correct.

Table 18 Results of *Salmonella* Enteritidis phage typing by the ENLs

		Phage types of each laboratory									
Strain	PT	M	N	P	S	T	U	V	W	X	Y
E1	6a	5-like	6a	6a	6a	6a	6a	6a	6a	6a	6a
E2	20	20-like	20	20	20	20	20	20	20	20	RDNC
E3	34	3-like	34	34	34	3	34	34	34	34	34
E4	1	1	1	1	RDNC	1/4	1	1	1	1	1
E5	4	4-like	4	4	4	1/4	4	4	4	4	4
E6	8	8-like	8	8	8	2	14	8	28	8	8
E7	6	6-like	6	6	6	21	6	6	6	6	6
E8	13a	2-like	13a	13a	13	?	14b	28	13a	NST	RDNC
E9	4	4-like	4	4	4	1/4	4	4	4	4	4
E10	21	21-like	21	21	21C	21	21	21	21	21	21

RDNC: Reactions do not conform to a recognised pattern
?: No phage type given

Table 19 Results of *Salmonella* Typhimurium phage typing by the ENLs

		Phage types of each laboratory									
Strain	PT	M	N	P	S	T	U	V	W	X	Y
M11	66	66	66	66	66	66	66	66	66	66	n.d.
M12	104^{H}	12a or 104	104^{H}	104^{H}	104^{H}	104^{H}	104^{H}	104^{H}	104^{H}	104^{H}	n.d.
M13	193	*A.P. 1, 2, 3	193	193	193	Untyp	*A.P. 1, 2, 3	193	193	193	n.d.
M14	12	12	12	12	12	?	12	12	12	12	n.d.
M15	1	1	1	1	1	1	1	1	1	1	n.d.
M16	208	A.P. 18**	208	208	208	Untyp	A.P. 10	208	RDNC	208	n.d.
M17	4	4	4	52A	RDNC	135	4	4	4	4	n.d.
M18	104^{L}	151 or 104	104^{L}	104^{L}	104^{L}	?	12	104^{L}	104^{L}	104^{L}	n.d.
M19	10	67	10	10	10	10	9	10	10	10	n.d.
M20	160	160	160	160	RDNC	?	95	160	160	160	n.d.

A.P.: Additional phages; *: correct reactions for phage type 193; **correct reaction for phage type 208
n.d.: Not done
?: No phage type given

5. Discussion

Serotyping

The frequency of typing by the NRLs in 1998 was the same as the frequency of typing in 1997. There were only small differences in the total no. of strains typed in 1997 and 1998. Only two NRLs have each sent three strains to another laboratory for typing. In an earlier study, three laboratories sent strains to another laboratory. All of the ENLs typed the strains in their own laboratory. None of the laboratories used names of serovars which are withdrawn from the most recent Kauffmann-White scheme for identification of the strains. Four of the ENLs wrote the names of the serovars incorrectly.

For the NRLs this was the fourth collaborative study on serotyping. On request of the NRLs, not only strains occurring frequently were included in this study but also strains occurring infrequently. Most problems occurred with the typing of H antigens. Some laboratories mentioned that they do not have all the relevant monovalent antisera.
One strain was found as ‘rough’ by two laboratories, and therefore not typable. It is possible that subculturing on the laboratory’s medium could have caused this problem, because retyping of the strains after the culture was sent back to the CRL gave no typing problems.

Small differences in detecting the right antigens can lead to totally different *Salmonella* types, which will have consequences for international comparison of *Salmonella* surveillance or detection of international foodborne outbreaks.

Phage typing

The strains of STM and SE included in the collaborative study for phage typing were selected from recent isolates studied by the LEP and included phage types known to be occurring in the European Union.

The results obtained by the participating laboratories were encouraging considering that this was the first study undertaken by the ENLs and the first separate phage typing study for the NRLs.

Analysis of the results obtained show that certain laboratories were unable to identify phage types 193 and 208. This situation probably arose because the laboratories were either lacking the full complement of STM typing phages, particularly the additional phages necessary for this identification, or the most recent typing chart which identifies the reactions of the 193 and 208 phages. In addition, one of the ENLs experienced problems with SE phage 1 giving false positive reactions. This resulted in a number of identifications being confused.

6. Conclusions

Serotyping
In general there were no problems with typing of the O antigens. Most problems occurred with the typing of the H antigens. One of the reasons can be the missing of qualified monovalent antisera which are essential for the exact identification of *Salmonella* strains.
Laboratories that type a higher number of strains on a regular basis obtained the best results.

Phage typing
In general, the majority of the ENLs and the NRLs did not encounter major problems with the phage typing of strains of STM and SE.

Where phage typing is carried out it is important to ensure that all laboratories are supplied with a full complement of typing phages and complete and up to date information.

Standardisation of the methods used by the participating laboratories requires careful monitoring to ensure overall consistency of the results obtained.

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4. 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.

5. Antigenic formulas of the *Salmonella* serovars, 1997
   WHO Collaborating Centre for Reference and Research on *Salmonella*;
   Michel Y. Popoff and Léon Le Minor, Institut Pasteur, Paris.

Appendix 1 Mailing list

01	European Commission	A. Checchi Lang
02	European Commission	B. Hogben
03	European Commission	V. Niemi
04	Veterinary Public Health Inspector	drs. H. Verburg
05-32	Participants of the study (National Reference Laboratories and EnterNet laboratories)	
33	Board of Directors RIVM	dr. G. Elzinga
34	Director Sector Public Health Research	prof. dr. ir. D. Kromhout
35	Head of Microbiological Laboratory for Health Protection and Director CRL *Salmonella*	dr. ir. A.M. Henken
36-38	Project Workers	
39-43	Authors	
44	Dutch National Library for Publications and Bibliography	
45	SBD/Information and Public Relations	
46	Registration agency for Scientific Reports	
47-48	Library RIVM	
49-63	Sales department ot RIVM Reports	
64-75	Spare copies	

Appendix 2 Protocol Serotyping

COLLABORATIVE STUDY
ON SEROTYPING OF *SALMONELLA* STRAINS (4)
ORGANISED BY CRL SALMONELLA

PROTOCOL:

Introduction:
The Community Reference Laboratory (CRL) Salmonella organises a fourth 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.

Objective:
The main objective of the fourth 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 10 (starting on March 8th 1999) 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 (4)

The identification of the *Salmonella* cultures must take place in week 10 (starting on March 8th) or one week earlier or later.

1-5 February	Mailing the protocol and test report to the participating laboratories.
22-26 February	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 the parcel did not arrive at the airport before or on 26 February 1999, do contact the CRL immediately.**
1-5 March	Checking the presence of all necessary reagents and materials for the performance of the study.
8-12 March	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.
22-26 March	Completion of the test report and faxing it to the CRL. The original test report will be sent to the CRL.
29 March - 2 April	Checking the results by the NRLs.

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

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Appendix 3 Test Report Serotyping

COLLABORATIVE STUDY
ON SEROTYPING OF *SALMONELLA* STRAINS (4)
ORGANISED BY CRL SALMONELLA

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

Laboratory code	:
Laboratory name	:
Date of collecting the parcel	: ..... - ..... - 1999
Starting date for serotyping	: ..... - ..... - 1999

GENERAL QUESTIONS

1. What was the frequency of serotyping at your laboratory in **1998**?

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

2. How many strains did you serotype in **1998**?

.....

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: .....

PROTOCOL

Shipment:

Parcel damaged ❑YES
❑NO

date of receipt at the laboratory : ..... - 1999
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 : ..... - 1999

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!

TEST RESULTS OF THE COLLABORATIVE STUDY ON SEROTYPING

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

labcode:

starting date of serotyping: ..... - ..... - 1999

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			

Remarks and comments:

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

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

.....

signature:.....

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

Name of person in charge:

.....

signature:.....

Appendix 4 Protocol phage typing

COLLABORATIVE STUDY
ON SEROTYPING OF *SALMONELLA* STRAINS (4)
ORGANISED BY CRL SALMONELLA

PROTOCOL:

Introduction:
The Community Reference Laboratory (CRL) Salmonella organises a fourth 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 receive the strains to do phage-typing of *S.* Typhimurium and *S.* Enteritidis strains type these strains with there phages and send the results back to PHLS London and to the CRL.

Objective:
The main objective of the fourth 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 two parcels containing 40 *Salmonella* cultures (numbered 1 to 20, E1 to E10 and M11 to M20). On arrival the cultures must be subcultured on agar plates. The performance of the study will be in week 10 (starting on March 8th 1999) 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. The results of phage typing will be sent to Linda Ward (PHLS London).

Time table of the collaborative study on serotyping of *Salmonella* strains (4)

The identification of the *Salmonella* cultures must take place in week 10 (starting on March 8th) or one week earlier or later.

1-5 February	Mailing the protocol and test report to the participating laboratories.
22-26 February	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 the parcel did not arrive at the airport before or on 26 February 1999, do contact the CRL immediately.**
1-5 March	Checking the presence of all necessary reagents and materials for the performance of the study.
8-12 March	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.
22-26 March	Completion of the test report and faxing it to the CRL and PHLS. The original test report will be sent to the CRL.
29 March - 2 April	Checking the results on serotyping by the NRLs.

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

Maurice Raes
(research assistant CRL)
P.O. Box 1
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tel. number : ..-31-30-2744263
fax. number : ..-31-30-2744434
e-mail : Maurice.Raes@rivm.nl

If you have questions or remarks on the phage typing you can also contact:

L.R. Ward
Public Health Laboratory Service
Laboratory of Enteric Pathogens
61 Colindale Avenue, London NW9 5HT-
tel. Number: ..-441-181-200 4400
fax number: ..-441-181-905 9929

As an example the Salmonella Phage typing protocol from PHLS (London) is included.

1. Media

1.1 Double strength nutrient broth

Bacto dehydrated nutrient broth (Difco laboratories)	20 grms
NaCl	8.5 grms
Distilled water	to 1000 ml

to sterilise: Autoclave for 10 minutes at 115°C and 15 lbs pressure

1.2 Nutrient agar

Bacto dehydrated nutrient broth (Difco laboratories)	20 grms
NaCl	8.5 grms
Bacto agar dyhydrated (Difco laboratories)	13 grms
Distilled water	to 1000 ml

to sterilise: Autoclave for 10 minutes at 115°C and 15 lbs pressure

The prepared agar is distributed in 30 ml volumes into 9 cm single vent Petri dishes. The nutrient agar plates are incubated overnight at 37°C and then examined for contamination. Contaminated plates are discarded. The plates are further dried open at 37°C for 1.5 hours.

2. Procedure

2.1 By means of a sterile inoculating loop or plastic pastette, inoculate the test strain from the culture slope asceptically into a test tube containing 4 mls of double strength Difco nutrient broth. Heavy inoculum to give visible turbidity for *S.* Enteritidis and a very light inoculum for *S.* Typhimurium to give a barely visible turbidity.

2.2 Incubate the inoculated broth tubes on a horizontal shaker at 37°C for 1-1.5 hours for S. Enteritidis. For S. Typhimurium incubate at 37°C without agitation for 1.25 hours to obtain a very light growth in early log. phase.

2.3 Flood the broth culture over the surface of a dried Difco nutrient agar plate using a flooding pipette or a plastic pastette. Remove the excess culture from the surface.

2.4 When the surface of the nutrient agar plate is dry, apply the appropriate typing phages at routine test dilution (RTD) to the dried surface. Suggested methods:

a) Multipoint inoculator
b) Sterile loops delivering approximately 0.01 ml phage lysate
c) Dropping pipettes delivering approximately 0.01 ml phage lysate

2.5 When the phage spots are dry, the Difco nutrient agar plates are incubated inverted at 37°C for 5-18 hours.

2.6 The phage typing plates are removed from the incubator and the phage reactions are read using a x10 aplanat hand lens (or alternative methods of magnification) through the bottom of the plates using both direct and oblique illumination.

Appendix 5 Test Report phage typing

COLLABORATIVE STUDY
ON SEROTYPING OF *SALMONELLA* STRAINS (4)
ORGANISED BY CRL SALMONELLA

TEST REPORT
OF THE FOURTH COLLABORATIVE STUDY
ON SERO- AND PHAGE TYPING OF *SALMONELLA* STRAINS

Laboratory code	:
Laboratory name	:
Date of collecting the parcel	: ..... - ..... - 1999
Starting date for serotyping	: ..... - ..... - 1999

GENERAL QUESTIONS

1. What was the frequency of serotyping at your laboratory in **1998**?

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

2. How many strains did you serotype in **1998**?

.....

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: .....

PLEASE WRITE YOUR REMARKS AND COMMENTS ON PAGE 9 OF THE TEST REPORT!

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 9 OF THE TEST REPORT!

PROTOCOL

Shipment:

Parcel damaged ❑YES
❑NO

date of receipt at the laboratory : ..... - 1999
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 : ..... - 1999

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 9 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: ..... - ..... - 1999

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			

TEST RESULTS OF THE COLLABORATIVE STUDY ON PHAGE TYPING

Salmonella Enteritidis phage typing QA Strains March 1999

Testing Lab: _____

_____

_____

Date of receipt: _____

Date of completion: _____

		Phages at Routine Test Dilution									
QA Number	Phage type	1	2	3	4	5	6	7	8	9	10
E1											
E2											
E3											
E4											
E5											
E6											
E7											
E8											
E9											
E10											

Salmonella Typhimurium phage typing QA Strains March 1999

Testing Lab: _____

_____

_____

Date of receipt: _____

Date of completion: _____

		Typing phages in routine test dilution																																**Additional phages**				
QA Number	Phage type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	pooled O	1	2	3	10	18
M11																																									
M12																																									
M13																																									
M14																																									
M15																																									
M16																																									
M17																																									
M18																																									
M19																																									
M20																																									

Remarks and comments:

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

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

.....

signature:.....

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

Name of person in charge:

.....

signature:.....