research for
man and environment

RIJKSINSTITUUT VOOR VOLKSGEZONDHEID EN MILIEU
NATIONAL INSTITUTE OF PUBLIC HEALTH AND THE ENVIRONMENT

RIVM report 284500 016

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

Collaborative study V on sero-, phage and antibiotic resistance pattern typing

M. Raes, L.R. Ward, H.M.E. Maas, W.J.B Wannet and A.M. Henken

January 2001

This investigation has been performed by order and for the account of the European Commission, Legislation Veterinaire et Zootechnique, within the framework of project 284500, by the Community Reference Laboratory for *Salmonella.*

RIVM, P.O. Box 1, 3720 BA Bilthoven, telephone: 31 - 30 - 274 91 11; telefax: 31 - 30 - 274 29 71
European Commission, Legislation Veterinaire et Zootechnique, Rue de la Loi 86, B-1049 Bruxelles, Belgique, telephone: 32-2-2959 928; telefax: 32-2-2953 144

Abstract

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

The fifth collaborative typing study for *Salmonella* was organised by the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*, Bilthoven) in collaboration with the Public Health Laboratory Services (PHLS, London). All 17 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) and 15 EnterNet laboratories (ENLs) participated in the study. Three of the NRLs for *Salmonella* are also ENL. The results of these three NRL-ENL laboratories will be evaluated with the NRLs for *Salmonella* as well as with the ENLs. In total, 20 strains of the species *Salmonella enterica* were selected for serotyping and antibiotic resistance pattern typing, while 10 strains of *Salmonella* Typhimurium (STM) and 10 strains of *Salmonella* Enteritidis (SE) were selected for phage typing. In general, no problems were encountered with the typing of the O antigens. However, some laboratories had problems typing of the H antigens. Antibiotic-resistance pattern typing revealed data showing that standardisation of this technique would be required to allow for comparison between laboratories. The majority of the EnterNet Laboratories and National Reference Laboratories for *Salmonella* did not encounter major problems with phage typing of STM and SE strains.

Contents

Samenvatting

Het Communautair Referentie Laboratorium voor *Salmonella* (CRL-*Salmonella*, Bilthoven) heeft een vijfde ringonderzoek voor de typering van *Salmonella* georganiseerd in samenwerking met het Central Public Health Laboratory (PHLS) in Londen. Voor de geïnteresseerde laboratoria bestond de mogelijkheid om ook faagtypering en antibioticum resistentie bepaling uit te voeren. Het doel van dit onderzoek was het vergelijken van de testresultaten tussen de Nationale Referentie Laboratoria voor *Salmonella* (NRLs-*Salmonella*) onderling, tussen de EnterNet laboratoria (ENLs) onderling en tussen de NRLs-*Salmonella* en de ENLs.

Alle NRLs-*Salmonella* van de lidstaten van de Europese Unie en NRL Noorwegen namen deel aan het ringonderzoek. Van deze 17 laboratoria voerden er 6 ook faagtypering uit. Tevens namen 15 ENLs deel waarvan er 12 faagtypering uitvoerden. Van de 17 NRLs-*Salmonella* zijn drie tevens ENL. Alle drie deze laboratoria voerden faagtypering uit. Het antibioticum resistentie patroon werd bepaald door 17 NRLs-*Salmonella* en 10 ENLs.

In totaal werden 20 stammen van de species *Salmonella enterica* door het CRL-*Salmonella* geselecteerd. Hiervan waren er 18 van de subspecies *enterica,* 1 van de subspecies *salamae* en 1 van de subspecies *houtenae.* 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. De meeste problemen werden gevonden bij het typeren van de H antigenen. Tevens waren er veel problemen met het typeren van *S*. Glostrup.

De resultaten van antibioticum resistentie patroon bepalingen laten zien dat het belangrijk is om een gestandaardiseerde methode te gebruiken om vergelijkingen te kunnen maken tussen laboratoria. Overeenkomsten tussen de resultaten zijn bij sommige stammen te vinden. Bij sommige stammen wordt door de meeste laboratoria resistentie aangetoond met 1 of enkele van de gebruikte antibiotica.

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 methode beschreven in het faagtypering protocol. Er traden geen grote problemen op bij het bepalen van het faagtype van de geselecteerde stammen.

Summary

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

All NRLs-*Salmonella* of the Member States of the European Union and NRL Norway participated in the collaborative study. Six of the 17 participating NRLs-*Salmonella* also performed phage typing. Fifteen ENLs participated of which 12 laboratories performed phage typing. Three of the NRLs-*Salmonella* are also ENLs, and all three of these laboratories performed phage typing. Antibiotic resistance pattern typing was performed by all of the NRLs-*Salmonella* and 10 of the ENLs.

In total 20 strains of the species *Salmonella enterica* were selected by the CRL-*Salmonella*. Eighteen of the strains were of the subspecies *enterica*, one was of the subspecies *salamae* and one was of the subspecies *houtenae*. The strains had to be typed by the NRLs-*Salmonella* with the method used routinely in their laboratory. The NRLs-*Salmonella* were allowed to send strains for serotyping to another specialised institute in their country. Most problems were found when typing the H antigens. In addition, problems were encountered by typing *S.* Glostrup.

Results of antibiotic resistance pattern typing show that standardisation of the method is necessary for comparison of the results between laboratories. Most laboratories found resistance in the same strains.

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 method described in the phage typing protocol. No major problems occurred by assigning the correct phage type to the strains.

1. Introduction

In this report the fifth collaborative typing study of *Salmonella* strains is described. This study was organised by the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*, Bilthoven) in accordance with the Council Directive 92/117/EEC. It is one of the tasks of the CRL-*Salmonella* to organise this type of study in which the National Reference Laboratories for *Salmonella* (NRLs-*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, third and fourth collaborative study only strains belonging to subspecies *enterica* were included (2,3,4). The 20 strains for the second and third study were selected from the more frequently found serotypes.
In the fifth study, described in this report, 20 serotypes were selected. Eighteen of the strains were of the subspecies *enterica*, one was of the subspecies *salamae* and one was of the subspecies *houtenae.* Most strains were serotypes that occurred frequently with some serotypes occurring infrequently.
Seventeen NRLs-*Salmonella* and eighteen EnterNet laboratories (ENLs) participated in this study (three of them are also NRLs-*Salmonella*). The main objective of the study was to compare the results of serotyping among the NRLs-*Salmonella*, among the ENLs and between the NRLs-*Salmonella* and ENLs.
All participants performed serotyping of the strains. In cooperation with the Central Public Health Laboratory (PHLS), London, phage typing was included in this study. Six of the NRLs-*Salmonella* and 12 ENLs performed phage typing on 10 *Salmonella* Enteritidis and 10 *Salmonella* Typhimurium strains.
This study also included the possibility to perform antibiotic resistance pattern typing on the strains for serotyping. All NRLs-*Salmonella* and 10 ENLs typed the antibiotic resistance patterns of the strains.

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
Canada	Canadian Science Centre for Human & Animal Health Bureau of Microbiology Winnipeg, Manitoba		ENL
Denmark	Danish Veterinary Laboratory Copenhagen	NRL	
Denmark	Statens Seruminstitut Department of gastrointestinal infections Copenhagen		ENL
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	

France	Unite des Enterobacteries Institute Pasteur Paris		ENL
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 (Phage typing)/ National School of Public Health (Serotyping) Department of Microbiology / Department of Public & Administrative Health 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	
Luxembourg	Laboratoire National de Santé Luxembourg		ENL

The Netherlands	Rijksinstituut voor Volksgezondheid en Milieu (RIVM) Microbiologisch laboratorium voor Gezondheidsbescherming Bilthoven	NRL	and ENL
Northern Ireland	Department of Agriculture for Northern Ireland Veterinary Sciences Division; Bacteriology Department Belfast	NRL	
Norway	National Institute of Public Health Oslo	NRL	and ENL
Portugal	Laboratorio Nacional de Veterinaria Lisboa	NRL	
Portugal	Instituto Nacional de Saude Lisboa		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 sero- and antibiotic resistance pattern typing

As stated in the protocol (Appendix 2), which was sent to the participants before mailing of the strains, 20 strains for serotyping were sent to the participants. 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. The antigenic formulae according to the Kauffmann-White scheme, 1997 (5) of the 20 serovars are shown in Table 1.

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

No.	Serotype	O antigens	H antigens	Origin of strains
1	*S.* Enteritidis	1, 9, 12	g, m : -	Human faeces
2	*S.* subsp. salamae (II)	3, 10	a : l, v	Reptile
3	*S.* Hadar	6, 8	z10 : e, n, x	Human faeces
4	*S.* Saintpaul	1, 4, [5], 12	e, h : 1, 2	Human faeces
5	*S.* Glostrup	6, 8	z10 : e, n, z15	Bovine
6	*S.* Kentucky	8, 20	i : z6	Laying poultry
7	*S.* Urbana	30	b : e, n, x	Chameleon
8	*S.* subsp. houtenae (IV)	16	z4, z32 : -	Reptile
9	*S.* Berta	1, 9, 12	[f], g, [t] : -	Broiler
10	*S.* Kaapstad	4, 12	e, h : 1, 7	Human faeces
11	*S.* Infantis	6, 7, 14	r, 1, 5	Human faeces
12	*S.* Altona	8, 20	r, [i] : z6	Animal feed
13	*S.* Corvallis	8, 20	z4, z23 : [z6]	Human faeces
14	*S.* Agona	1, 4, 12	f, g, s: [1, 2]	Human faeces
15	*S.* Enteritidis	1, 9, 12	g, m : -	Human faeces
16	*S.* Virchow	6, 7	r : 1, 2	Human faeces
17	*S.* Poona	1, 13, 22	z : 1, 6	Human faeces
18	*S.* Albany	8, 20	z4, z24 : -	Animal feed
19	*S.* Typhimurium	1, 4, [5], 12	i : 1, 2	Human faeces
20	*S.* Uganda	3, 10 [15]	l, z13 : 1, 5	Environmental sample

The antibiotic resistance patterns of these strains as they were typed at the CRL-*Salmonella* are shown in Table 2.

Table 2: Antibiotic resistance patterns of the 20 Salmonella *strains used in the collaborative study as typed at the CRL-Salmonella*

No.	Serotype	Antibiotic resistance pattern
1	*S.* Enteritidis	*
2	*S.* subsp. salamae	*
3	*S.* Hadar	*
4	*S.* Saintpaul	T C A Trs
5	*S.* Glostrup	*
6	*S.* Kentucky	*
7	*S.* Urbana	*
8	*S.* subsp. houtenae	*
9	*S.* Berta	*
10	*S.* Kaapstad	*
11	*S.* Infantis	*
12	*S.* Altona	*
13	*S.* Corvallis	*
14	*S.* Agona	T Trs
15	*S.* Enteritidis	*
16	*S.* Virchow	Fu
17	*S.* Poona	*
18	*S.* Albany	*
19	*S.* Typhimurium	T A
20	*S.* Uganda	*

* Strain is fully sensitive

Antibiotics used:

1) Tetracycline (T)
2) Chloramphenicol (C)
3) Neomycin (N)
4) Ampicillin (A)
5) Trimethoprim + sulfamethoxazole (Trs)
6) Furazolidone (Fu)
7) Flumequin (Fl)

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 (LEP), 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 3 and 4.

Table 3: 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	1b	OL	SCL	OL	SCL	CL	SCL	CL	OL	<OL	SCL	<CL	CL	CL	CL	CL	CL
E2	4	-	SCL	CL	SCL	CL	SCL	CL	SCL	OL	OL	CL	CL	CL	-	-	-
E3	6	-	SCL	-	SCL	-	SCL	-	SCL	OL	OL	-	-	-	-	-	-
E4	13a	-	-	-	SCL	-	SCL	-	SCL	SCL	OL	-	-	-	-	-	-
E5	8	-	-	SCL	SCL	CL	SCL	<CL	OL	OL	OL	SCL	CL	-	-	-	-
E6	6a	-	SCL	-	SCL	-	SCL	-	-	OL	-	-	-	-	-	-	-
E7	1	OL	SCL	CL	SCL	CL	SCL	CL	OL	OL	OL	CL	CL	CL	CL	-	-
E8	4b	-	SCL	OL	SCL	CL	SCL	CL	OL	OL	OL	CL	CL	CL	-	-	SCL
E9	44	OL	SCL	CL	-	CL	SCL	CL	OL	-	OL	CL	CL	CL	CL	-	-
E10	19	-	-	-	-	-	+++	-	SCL	-	OL	-	-	-	-	-	-

Table 4: 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	32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	-	-	-	-	-	-	-	-	-	CL	-	CL					
M12	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CL	+++	SCL	+++	-	-
M13	U302	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CL	-	-	-	OL	-
M14	208	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CL	-	±	-	SCL	SCL
M15	170	-	-	-	-	-	-	-	-	-	-	CL	CL	+++	-	-	±	-	-	-	±	-	-	-	-	-	++ <<	-	-	-	<OL	CL					
M16	120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CL	-	-	-	-	-	-	-	-	-	-	-	-	-	CL	++	+++	++	OL	-
M17	2	CL	CL	CL	CL	CL	CL	-	-	CL	CL	CL	CL	CL	CL	CL	CL	-	CL	CL	CL	CL	CL	CL	CL	CL	CL	- + -	CL	CL	CL	CL					
M18	9	-	-	-	-	-	-	-	CL	SCL	CL	CL	CL	-	-	SCL	-	-	-	CL	-	CL	CL	-	±	+	-	-	CL	CL	-	CL					
M19	12a	-	-	-	-	-	-	-	±	-	-	CL	CL	-	-	-	-	CL	-	-	++	-	-	-	-	-	OL	-	-	-	OL	CL					
M20	104L	-	-	-	-	-	-	-	-	-	-	++ <<	SCL	-	-	-	-	+++	-	-	-	-	-	-	-	-	-	-	-	-	±	CL	-	-	-	OL	-

O*: O pooled (<)CL: clear lysis (<)OL: opaque lysis SCL: semi confluent lysis

<< : Merging plaques towards semi-confluent lysis

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 performance of the study. All details about mailing and storing were mentioned in a protocol (Appendix 2). The protocol and test report (Appendix 3) were mailed four weeks before the start of the study to the participants.
All 15 Member States of the European Union participated and the United Kingdom participated with three laboratories. In addition, a laboratory from Norway participated. The NRLs-*Salmonella* were assigned a labcode from 1 to 17. From the ENLs, 15 participants performed the study (Labcode A to Z). The NRLs-*Salmonella* which are also ENL were assigned to the NRL group. For evaluation of the results of serotyping, their results were also evaluated among the ENLs.

3.2.1 Serotyping

All participants performed serotyping of the strains. 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 possible for a laboratory to send strains for serotyping to another reference laboratory in their country.

3.2.2 Antibiotic resistance pattern typing

All of the NRLs-*Salmonella* and 13 of the ENLs (labcode 1, 11, 12, B, C, D, E, H, J, P, R, T and Z) performed antibiotic resistance pattern typing according to the method routinely used in the laboratories. Except for laboratories 3, 15, C and Z, that used a quantitative method, all laboratories used a disc diffusion method.

3.2.3 Phage typing

Six of the NRLs-*Salmonella* (Labcode 1, 3, 6, 9, 11 and 16) and 12 of the ENLs (Labcode A, B, C, D, E, F, H, J, K, L, N and P) performed phage typing. The 20 strains had to be tested according to the *Salmonella* phage typing protocol from PHLS (Appendix 2).

3.3 Guidelines for evaluation

Evaluation of the results of serotyping is performed according to the guidelines mentioned in Table 5. The O and H antigens and the name of the serovar are evaluated separately.
Writing errors and the use of names that are withdrawn from the most recent Kauffmann-White scheme are approved. In case of mixed sera for O and / or H antigens, this is evaluated as incorrect. Also, if more than one name is given to the serovar this is considered incorrect

because no comparison can be made if more names are given to one strain. In this case the antigenic formula as far as found should be given. Such a result is evaluated as partly correct.

Table 5: Evaluation of the O- and H-antigens and the name of the serovar given

Result of laboratory	Evaluation
Autoagglutination Incomplete set of antisera (outside range of antisera)	Not typable (nt)
Partly typable due to incomplete set of antisera No name serovar Part of the formula (for the name of the serovar)	Partly correct (+/-)
Wrong serovar Mixed sera formula	Incorrect (-)

4. Results

4.1 General data of serotyping by the participants

The NRLs-*Salmonella* were assigned a labcode from 1 to 17 and the ENLs were assigned a labcode from A to Z. In Tables 6 and 7 the frequency of typing and the total number of strains typed at the NRLs-*Salmonella* and the ENLs is shown. For both NRLs-*Salmonella* and ENLs, there were no differences in the frequency of typing between this and the third and fourth collaborative study. There were only small differences in the total number of strains typed between 1998 and 1999 for both NRLs-*Salmonella* and ENLs. Table 8 shows the origin of the sera used by the different laboratories.

*Table 6: Frequency of serotyping and total number of strains typed by the NRLs-*Salmonella

Labcode	Frequency of typing	Total no. of strains typed in 1999	Total no. of strains typed in 1998
1	Daily	12,432	13,128
2	Once a week	1,800	2,023
3	Daily	13,432	15,976
4	± 120 a month	± 1,000	± 1,000
5	Once a week	± 1,000	± 1,200
6	Daily	5,000	5,660
7	Once a week	218	102
8	Daily	1,590	709
9	Daily	1,359	1,450
10	On arrival	58	20
11	Once a week	± 7,000	11,351
12	Daily	1,886	1,839
13	On arrival	367	463
14	Once a week	700	500
15	Daily	1,300	1,000 - 1,500
16	Daily	11,000	10,000
17	Daily	± 1,000	1,500

Table 7: Frequency of serotyping and total number of strains typed by the ENLs

Labcode	Frequency of typing	Total no. of strains typed in 1999	total no. of strains typed in 1998
A	Daily	15,774	14,515
B	Daily	2,700	± 2,500
C	Daily	8,360	10,813
D	Twice a week	1,050	1,000
E	Daily	346	550
F	Once a week	±120	± 200
H	Daily	3,184	2,320
J	Daily	8,000	7,200
K	3 times a week	3,219	5,173
L	Daily	3,249	3,481
N	Daily	1011	825
P	Daily	8,800	9,700
R	Daily	496	392
T	Daily	>3,377	>3,880
Z	Daily	6,578	6,404

Table 8: The origin of the sera used by the different laboratories

Collaborative study	Nr. of laboratories	Nr. of laboratories using commercial available sera	Nr. of laboratories using own prepared sera
I	17	12	7
II	15	10	5
III	16	11	3
IV	14 (NRLs-*Salmonella*)	14	2
	12 (ENLs)	10	7
	2 (NRL-*Salmonella* + ENL)	2	2
V	14 (NRLs-*Salmonella*)	14	2
	15 (ENLs)	12	5
	3 (NRL-*Salmonella* + ENL)	3	3

4.2 Taxonomy and nomenclature of the typed strains

All NRLs-*Salmonella* wrote the identified serotype with a first capital letter as proposed by the *Salmonella* WHO reference centre (4). From the ENLs two laboratories (labcode K and L) wrote the name of the serovar without any capital and one laboratory (labcode F) wrote the names of the serovars all in capital. In the previous study three ENLs wrote the name of the serovars all in capital and one laboratory wrote the names without capitals.
Two laboratories (labcode 10 and E) used the name *S*. Pikine, which is withdrawn from the most recent Kauffmann and White scheme to identify *S*. Altona. Three laboratories (labcode 10, E and R) used Chameleon for strain nr. 8 instead of IV.

4.3 Sero- and phage typing of the strains

Sixteen NRLs-*Salmonella* and all of the ENLs typed all strains in their own laboratory. One NRL (labcode 5) sent 4 strains (nr. 7, 8, 13 and 18) to another laboratory for serotyping.
All laboratories performed phage typing in their own laboratory except for NRL-*Salmonella* nr. 2, that sent the strains for phage typing to another laboratory in their country. Phage typing for this laboratory was performed by the ENL in that country and therefore, the results were not evaluated for NRL-*Salmonella* nr. 2 separately.

4.3.1 Rough strain

Problems arose with *Salmonella* Glostrup (strain nr. 5). Five laboratories (1 NRL-*Salmonella* (labcode 9), 2 ENLs (labcode H and T) and 2 NRL-*Salmonella* + ENL (labcode 1, 11)) typed strain 5 correctly. In total 14 NRLs-*Salmonella* and 13 ENLs could not type this strain correctly. Fourteen NRLs-*Salmonella* and 13 ENLs typed the O antigens incorrectly, as not typable or partly correct which resulted in incorrect identification of the strain. Three NRLs-*Salmonella* and 6 ENLs typed the H-antigens incorrectly, as not typable or partly correct. All laboratories were asked to send back strain nr. 5 to the CRL to be typed again. Table 9 shows the results obtained by the NRLs-*Salmonella,* ENLs and the result obtained by the CRL for strain nr. 5.
The results obtained by the different laboratories for strain nr. 5 can be divided into four groups, namely: 1) correct typing, *S.* Glostrup , 2) *S*. Chomedey, 3) not typable and 4) other serotype. An overview of the media used for subculturing of the strain has been made (Appendix 4). No correlation could be observed between the media used and the outcome of serotyping of strain nr. 5.

Table 9: Results of serotyping of strain nr. 5 by participants and CRL-Salmonella.

Labcode	Antigenic formula NRL	Serotype NRL-*Salmonella* / ENLs	Serotype CRL-*Salmonella*
1	6, 8 : z10 : enz15	*S.* Glostrup	*S.* Chomedey
2	Autoagglutinable	*S.* autoagglutinable	Not received
3	Autoagglutinable : z10 : enz15	Rough	*S.* Glostrup
4	4 : enx, z15, z10	*S.* Tokain	*S.* Glostrup
5	8: z10 : enz15	*S.* Chomedey	*S.* Glostrup
6	Rough : z10 : enz15	I Rough: z10 : enz15	*S.* Brandenburg / *S.* Chomedey *
7	8 : z10 : enz15	*S.* Istanbul	*S.* Glostrup
8	4 : z10 : enx	*S.* Albert	*S.* Glostrup
9	6, 8 : z10 : enz15	*S.* Glostrup	*S.* Chomedey
10	6,8 : z10 :enx	*S.* Hadar	Not received
11	6, 8 : z10 : enz15	*S.* Glostrup	*S.* Glostrup
12	8 : z10 : enz15	*S.* Chomedey	*S.* Glostrup
13	4,12 : z10 : enz15	*S.* Tokoin	*S.* Glostrup
14	8,20 : z10 : enz15	*S.* Chomedey	*S.* Glostrup
15	Spontaneous : z10 : enz15	Subsp. I - : z10 : enz15	Not received
16	8 : z10 : enz15	*S.* Chomedey	*S.* Glostrup
17	8 : z10 : enz15	*S.* Chomedey	*S.* Glostrup
A	Autoagglutinable	*S.* autoagglutinable	Not received
B	Rough : z10 : enz15	Ssp. I enterica	Not received
C	Rough : z10 : enz15	Ssp I enterica	*S.* Glostrup
D	8,20 : z10 : enz15	*S.* Chomedey	Not received
E	4 : z10 : enz15	*S.* Tokoin	*S.* Glostrup
F	4,12 : z13 : 1,6	*S.* Haduna	*S.* Glostrup
H	6, 8 : z10 : enz15	*S.* Glostrup	Not received
J	Autoagglutinable	-	*S.* Glostrup
K	Spontaneous	Subsp. I	Not received
L	Rough : z10 : enz15	I ? : z10 : enz15	*S.* Glostrup
N	9,12 : z10 : enz15	*S.* Ruanda	*S.* Chomedey
P	-	Not typable	*S.* Glostrup
R	6,8 : z10 : enx	*S.* Hadar	*S.* Chomedey
T	6, 8 : z10 : enz15	*S.* Glostrup	Not received
Z	Rough : z10 : enz15	*S.* Unnamed	*S.* Chomedey

* The strain from NRL-*Salmonella* nr. 6 was typed as *S*. Brandenburg. After typing the strain once more, the serotype revealed was *S*. Chomedey.

4.4 Typing by the NRLs-*Salmonella*

4.4.1 Evaluation per laboratory

The evaluation of the detection of O and H antigens and identification of the strains per laboratory are shown in Table 10. Three laboratories (labcode 1, 9 and 11) typed all O-antigens correctly. The H-antigens were typed correctly by 12 laboratories (labcode 1, 3, 4, 5, 6, 11, 12, 13, 14, 15, 16 and 17). The names given to the strains were assigned correctly for all 20 strains by 2 laboratories (labcode 1 and 11). Laboratory 5 concluded for strain 17, with the result for the O antigen 13, 22, 23, that the serotype should be *S.* Poona (13, 22 : z : 1, 6). With the results of the mixed serum this is not possible. The serotype could also be *S.* Farmsen (13, 23 : z : 1, 6).

Table 10: Evaluation of serotyping per laboratory

	O antigen detected				H antigen detected				Name serovar			
Labcode	+	nt	+/-	-	+	nt	+/-	-	+	nt	+/-	-
1	20				20				20			
2	17	1		2	14	1	1	4	13	1	4	2
3	19	1			20				19	1		
4	19			1	20				19			1
5	17		2	1	20				17			3
6	19	1			20				19	1		
7	19		1		19			1	17		1	2
8	17			3	15		4	1	14			6
9	20				19		1		19			1
10	18		1	1	16		2	2	15			5
11	20				20				20			
12	19		1		20				19			1
13	19			1	20				19			1
14	19		1		20				19			1
15	19	1			20				19	1		
16	19		1		20				19			1
17	19		1		20				19			1

+: correct nt: not typable

+/- : partly correct -: incorrect

4.4.2 Evaluation per strain

The evaluation of the detection of O and H antigens and identification of the strains per strain are shown in Table 11. The O-antigens of 14 strains were typed correctly by all laboratories. Most problems arose with strain nr. 5. The other strains (nr. 3, 6, 7, 8 and 17) were typed partly correct or incorrect by 1 or 2 laboratories. Eight strains were typed correctly (O, H and name) by all laboratories; *S.* Enteritidis (2x), *S.* Berta, *S.* Kaapstad, *S.* Infantis, *S.* Altona, *S.* Virchow, *S.* Typhimurium. An overview of partly correct or incorrect typing of O antigens, H antigens and assigning the name of the serovar, is shown in Table 12.

Table 11: Evaluation of serotyping per strain

Strain		O antigen detected				H antigen detected				Name serovar			
		+	nt	+/-	-	+	nt	+/-	-	+	nt	+/-	-
1	*S.* Enteritidis	17				17				17			
2	*S.* subsp. salamae	17				16		1		15		1	1
3	*S.* Hadar	15		1	1	16			1	15			2
4	*S.* Saintpaul	17				16		1		16			1
5	*S.* Glostrup	4	4	6	3	14	1	2		3	4		10
6	*S.* Kentucky	16		1		16			1	16			1
7	*S.* Urbana	15			2	15			2	15			2
8	*S.* subsp. houtenae	15			2	15		1	1	14			3
9	*S.* Berta	17				17				17			
10	*S.* Kaapstad	17				17				17			
11	*S.* Infantis	17				17				17			
12	*S.* Altona	17				17				17			
13	*S.* Corvallis	17				16			1	15		1	1
14	*S.* Agona	17				16			1	16		1	
15	*S.* Enteritidis	17				17				17			
16	*S.* Virchow	17				17				17			
17	*S.* Poona	16			1	16		1		16			1
18	*S.* Albany	17				16		1		16		1	
19	*S.* Typhimurium	17				17				17			
20	*S.* Uganda	17				15		1	1	13		1	3

+: correct nt: not typable

+/- : partly correct -: incorrect

Table 12: Identification per strain per laboratory that typed a strain partly correct or incorrect

Strain	Labcode						
	Correct identification	2	5	7	8	9	10
1	*S.* Enteritidis	S. Enteritidis	S. Enteritidis	S. Enteritidis	S. Enteritidis	S. Enteritidis	S. Enteritidis
2	*S.* subsp. salamae (II) 3,10 : a : l,v	3, 10 : v : ?	II 3, 10 : a : l, v	II 3, 10 : a : l, v	II 3, 10 : a : l, v	II 10 : a : l, v	? 3,10 : a : l,v
3	*S.* Hadar 6,8 : z10 : e,n,x	S. Hadar	S. Istanbul 8 : z10 : e, n, x	S. Hadar	S. Hadar	S. Hadar	S. Paratyphi A 1,2,12 : a : -
4	*S.* Saintpaul 1,4,[5],12 : e,h : 1,2	S. Saintpaul	S. Saintpaul	S. Saintpaul	S. Kaapstad 4 : e,h : 7	S. Saintpaul	S. Saintpaul
5	*S.* Glostrup	Table 9	Table 9	Table 9	Table 9	Table 9	Table 9
6	*S.* Kentucky 8, 20 : i : z6	S. Kentucky	S. Kentucky	S. Kentucky	S. Kentucky	S. Kentucky	S. Tulear 6,8 : z10 : e,n,x
7	*S.* Urbana 30 : b : e, n, x	O? ? : -	S. Urbana	S. Urbana	- - : e,h : -	S. Urbana	S. Urbana
8	*S.* subsp. houtenae (IV) 16 : z4,z32 : -	O? ? : -	IV 16 : z4,z32 : -	II 16 : z4,z24 : -	- - : z4 : -	IV 16 : z4,z32 : -	chameleon
9	*S.* Berta	S. Berta	S. Berta	S. Berta	S. Berta	S. Berta	S. Berta
10	*S.* Kaapstad	S. Kaapstad	S. Kaapstad	S. Kaapstad	S. Kaapstad	S. Kaapstad	S. Kaapstad
11	*S.* Infantis	S. Infantis	S. Infantis	S. Infantis	S. Infantis	S. Infantis	S. Infantis
12	*S.* Altona	S. Altona	S. Altona	S. Altona	S. Altona	S. Altona	S. Pikine
13	*S.* Corvallis 8,20 : z4,z23 : [z6]	8 : ? : ?	S. Dabou or S. Corvallis 8 : z4,z23 : ?	S. Corvallis	S. Corvallis	S. Corvallis	S. Corvallis
14	*S.* Agona 1,4,12 : f,g,s: [1,2]	S. Agona	S. Agona	- 4,12 : ? : ?	S. Agona	S. Agona	S. Agona
15	*S.* Enteritidis	S. Enteritidis	S. Enteritidis	S. Enteritidis	S. Enteritidis	S. Enteritidis	S. Enteritidis
16	*S.* Virchow	S. Virchow	S. Virchow	S. Virchow	S. Virchow	S. Virchow	S. Virchow
17	*S.* Poona 1,13,22 : z : 1,6	S. Poona	S. Poona 13,22,23 : z :1,6	S. Poona	S. Bristol 13,22 : z : 7	S. Poona	S. Poona
18	*S.* Albany 8,20 : z4,z24 : -	8 : ? : ?	S. Albany	S. Albany	S. Albany	S. Albany	S. Albany
19	*S.* Typhimurium	S. Typhimurium	S. Typhimurium	S. Typhimurium	S. Typhimurium	S. Typhimurium	S. Typhimurium
20	*S.* Uganda 3,10,[15] : l,z13 : 1,5	3,10 : ? : ?	S. Uganda	S. Uganda	S. Sinstorf 3,10 : l,v : 5	S. Sinstorf 10 : l,v : 1,5	S. Uganda or S. Kinshasa 3,10 or 3,15 : l,z13 : -

: Partly correct or incorrect identification of the strain

4.5 Typing by the ENLs

4.5.1 Evaluation per laboratory

The evaluation of the detection of O and H antigens and identification of the strains per laboratory are shown in Table 13. Five ENLs (labcode 1, 11, H, R and T) typed all O-antigens correctly. The H-antigens were typed correctly by 9 ENLs (labcode 1, 11, 12, B, C, E, L, N and Z). The names given to the strains were identified correctly for all 20 strains by 2 ENLs (labcode 1 and 11). Strain nr. 2 resulted in 5 partly correct identifications of the H antigen. For 3 laboratories (labcode A, H and R) this was caused by incorrect identification of the H antigen 'a'. These laboratories detected the H antigen z35.

Table 13: Evaluation of serotyping per laboratory

	O antigen detected				H antigen detected				Name serovar			
Labcode	+	nt	+/-	-	+	Nt	+/-	-	+	nt	+/-	-
1	20				20				20			
11	20				20				20			
12	19		1		20				19			1
A	19	1			18	1	1		18		1	1
B	19	1			20				19		1	
C	19	1			20				19		1	
D	19		1		19		1		18		1	1
E	19			1	20				18		1	1
F	17		1	2	16		1	3	16		1	3
H	20				19		1		19			1
J	18	1	1		18	1	1		18	1		1
K	19	1			19	1			19	1		
L	19	1			20				19		1	
N	19			1	20				19			1
P	19	1			19	1			19	1		
R	20				18		2		18			2
T	20				19			1	19			1
Z	19	1			20				20			

+: correct nt: not typable
+/- : partly correct -: incorrect

4.5.2 Evaluation per strain

The evaluation of the detection of O and H antigens and identification of the strains per strain are shown in Table 14. The O-antigens of 17 strains were typed correctly by all ENLs. Most problems arose with strain nr. 5. H-antigens of 6 strains (nr. 2, 5, 8, 14, 18 and 20) were not typed correctly by 1 to 6 laboratories. Most partly correct identifications of the H-antigens were revealed with strain nr. 2. Strain nr. 8, 14, 18 and 20 were each identified partly correct or incorrect by one laboratory. Identification of the strains was performed correctly by all laboratories for 14 strains. The six strains that were not identified correctly were the same strains as those of which the H-antigens were not typed correctly. An overview of partly correct or incorrect typing of O antigens, H antigens and assigning the name of the serovar, is shown in Table 15.

Table 14: Evaluation of serotyping per strain

Strain		O antigen detected				H antigen detected				Name serovar			
		+	nt	+/-	-	+	nt	+/-	-	+	nt	+/-	-
1	*S.* Enteritidis	18				18				18			
2	*S.* subsp. salamae	18				13		5		12		3	3
3	*S.* Hadar	17		1		18				18			
4	*S.* Saintpaul	18				18				18			
5	*S.* Glostrup	5	8	2	3	12	4	1	1	4	4	4	6
6	*S.* Kentucky	18				18				18			
7	*S.* Urbana	18				18				18			
8	*S.* subsp. houtenae	18				17			1	17			1
9	*S.* Berta	18				18				18			
10	*S.* Kaapstad	18				18				18			
11	*S.* Infantis	18				18				18			
12	*S.* Altona	17		1		18				18			
13	*S.* Corvallis	18				18				18			
14	*S.* Agona	18				17		1		17			1
15	*S.* Enteritidis	18				18				18			
16	*S.* Virchow	18				18				18			
17	*S.* Poona	18				18				18			
18	*S.* Albany	17			1	17			1	17			1
19	*S.* Typhimurium	18				18				18			
20	*S.* Uganda	18				17			1	17			1

+: correct nt: not typable +/-: partly correct -: incorrect

Table 15: Identification per strain per laboratory that typed a strain partly correct or incorrect

Strain	Labcode								
	Correct identification	A	D	E	F	H	J	R	T
1	*S.* Enteritidis	S. Enteritidis	S. Enteritidis	S. Enteritidis	S. Enteritidis	S. Enteritidis	S. Enteritidis	S. enteritidis	S. Enteritidis
2	*S.* subsp. salamae (II) 3, 10 : a : l, v	S. Sinchew 3, 10 : l, v : z35	II 3, 10 : l, v : -	- 3, 10 : a : l, v	II 3, 10, 15 : a : z39	S. Sinchew 3, 10 : l, v : z35	II 3, 10 : a : l, v	S. sinchew 3, 10 : l, v : z35	II
3	*S.* Hadar	S. Hadar	S. Hadar	S. Hadar	S. Hadar	S. Hadar	S. Hadar	S. hadar	S. Hadar
4	*S.* Saintpaul	S. Saintpaul	S. Saintpaul	S. Saintpaul	S. Saintpaul	S. Saintpaul	S. Saintpaul	S. saintpaul	S. Saintpaul
5	*S.* Glostrup	Table 9	Table 9	Table 9	Table 9	Table 9	Table 9	Table 9	Table 9
6	*S.* Kentucky	S. Kentucky	S. Kentucky	S. Kentucky	S. Kentucky	S. Kentucky	S. Kentucky	S. kentucky	S. Kentucky
7	*S.* Urbana	S. Urbana	S. Urbana	S. Urbana	S. Urbana	S. Urbana	S. Urbana	S. urbana	S. Urbana
8	*S.* subsp. houtenae (IV) 16 : z4, z32 : -	IV 16 : z4, z32 : -	IV 16 : z4, z32 : -	Chameleon 16 : z4, z32 : -	II 16 : z4, z23 : mal+	IV 16 : z4, z32 : -	IV 16 : z4, z32 : -	Chameleon 16 : z4, z32 : -	IV 16 : z4, z32 : -
9	*S.* Berta	S. Berta	S. Berta	S. Berta	S. Berta	S. Berta	S. Berta	S. berta	S. Berta
10	*S.* Kaapstad	S. Kaapstad	S. Kaapstad	S. Kaapstad	S. Kaapstad	S. Kaapstad	S. Kaapstad	S. kaapstad	S. Kaapstad
11	*S.* Infantis	S. Infantis	S. Infantis	S. Infantis	S. Infantis	S. Infantis	S. Infantis	S. infantis	S. Infantis
12	*S.* Altona 8,20:r,[i]:z6	S. Altona	S. Altona	S. Pikine	S. Altona	S. Altona	S. Altona 6, 8 : r : z6	S. altona	S. Altona
13	*S.* Corvallis	S. Corvallis	S. Corvallis	S. Corvallis	S. Corvallis	S. Corvallis	S. Corvallis	S. corvallis	S. Corvallis
14	*S.* Agona 1, 4, 12 : f, g, s: [1, 2]	S. Agona	S. Agona	S. Agona	S. Agona	S. Agona	S. Derby 4 : f : -	S. agona	S. Agona
15	*S.* Enteritidis	S. Enteritidis	S. Enteritidis	S. Enteritidis	S. Enteritidis	S. Enteritidis	S. Enteritidis	S. enteritidis	S. Enteritidis
16	*S.* Virchow	S. Virchow	S. Virchow	S. Virchow	S. Virchow	S. Virchow	S. Virchow	S. virchow	S. Virchow
17	*S.* Poona	S. Poona	S. Poona	S. Poona	S. Poona	S. Poona	S. Poona	S. poona	S. Poona
18	*S.* Albany 8, 20 : z4, z24	S. Albany	S. Albany	S. Albany	S. Stanleyville 4, 12 : z4, z23 : 1, 2	S. Albany	S. Albany	S. albany	S. Albany
19	*S.* Typhimurium	S. Typhimurium	S. Typhimurium	S. Typhimurium	S. Typhimurium	S. Typhimurium	S. Typhimurium	S. typhimurium	S. Typhimurium
20	*S.* Uganda 3,10[15]: l,z13 : 1, 5	S. Uganda	S. Uganda	S. Uganda	S. Uganda	S. Uganda	S. Uganda	S. uganda	S. Assinie (3),10:l,w:z6

: Partly correct or incorrect identification of the strain

4.6 Comparison of NRLs-*Salmonella* with ENLs

The identification of the strains is also evaluated between the NRLs-*Salmonella* and the ENLs. Table 16 shows the numbers of laboratories which identified a strain (in)correctly per strain. Eight strains (nr. 1, 9, 10, 11, 12, 15, 16 and 19) were identified correctly by all laboratories. The greatest difference between the NRLs-*Salmonella* and ENLs occurred for 2 strains (nr. 2 and 20).

Table 16: Number of NRLs-*Salmonella or ENLs which identified the strain (in)correctly*

strain no.	Serotype	Number of NRLs-*Salmonella* which identified the strain		Number of ENLs which identified the strain	
		Correct	nt , +/- , -	Correct	nt , +/- , -
1	*S.* Enteritidis	17	-	18	-
2	*S.* subsp. salamae	15	2	12	6
3	*S.* Hadar	15	2	18	-
4	*S.* Saintpaul	16	1	18	-
5	*S.* Glostrup	3	14	5	13
6	*S.* Kentucky	16	1	18	-
7	*S.* Urbana	15	2	18	-
8	*S.* subsp. houtenae	14	3	17	1
9	*S.* Berta	17	-	18	-
10	*S.* Kaapstad	17	-	18	-
11	*S.* Infantis	17	-	18	-
12	*S.* Altona	17	-	18	-
13	*S.* Corvallis	15	2	18	-
14	*S.* Agona	16	1	17	1
15	*S.* Enteritidis	17	-	18	-
16	*S.* Virchow	17	-	18	-
17	*S.* Poona	16	1	18	-
18	*S.* Albany	16	1	17	1
19	*S.* Typhimurium	17	-	18	-
20	*S.* Uganda	13	4	17	1

In Table 17 a comparison is made of the percentage of strains typed correctly in this study, the number of strains typed in 1999 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 routinely typed annually by a laboratory the better the results of serotyping in this study were.

Table 17: Comparison serotyping of total number of strains typed by the NRLs-*Salmonella and ENLs in 1999 and number of strains assigned correctly in this collaborative study.*

% of strains assigned correctly in this study (n=20)	Number of laboratories	Number of strains typed by the laboratories in 1999	Average number of strains typed per laboratory in 1999
65 - 75	3	58 - 1,800	1,150
80 - 90	7	120 - 15,774	3,606
95	19	367 - 13,432	3,789
100	3	6,578 - 12,432	8,670

4.7 Antibiotic resistance pattern typing by NRLs-*Salmonella*

The number of antibiotics used per laboratory varied from 7 to 18 (Appendix 5). The names and the abbreviations used by the NRLs-*Salmonella* for the antibiotics are mentioned in Table 18. Most laboratories found resistance to one or more antibiotics for strain nr. 3, 4, 14, 16 and 19. Laboratory 5 found all strains resistant to erythromycin and laboratory 8 found all strains resistant to tetracycline.
Strain nr. 4 was found resistant to ampicillin, tetracycline, chloramphenicol, streptomycin and trimethoprim + sulfamethoxazole by most laboratories. Strain nr. 14 was found resistant to tetracycline, trimethoprim (+sulfamethoxazole), streptomycin and sulphonamides by most laboratories using these antibiotics. An overview of the results of antibiotic resistance pattern typing of the laboratories is given in Table 19.

*Table 18: Names and abbreviations of antibiotics used by NRLs-*Salmonella

Name of the antibiotic	Abbreviation
acide oxolinique	Oa
Amikacin	Ak
Amoxicillin + clavulanic acid	Amc
Amoxicilline	Amx
Ampicillin	A
Apramycin	Apr
Cefalotine	Cf
Cefixime	Cfm
Cefoperazone	Cfp
Cefotaxime	Ctx
Cefoxitine	Fox
Cefropepazone	Cf
Ceftazidime	Ce
Ceftiofur	Cef
Cefuroxim	Cxm
Cephalotin	Ce
Cephazoline	Cf
Chloramphenicol	C
Ciprofloxacin	Cp
Colistin sulphate	Ct
Colistine	Col

Name of the antibiotic	Abbreviation
Doxycyclin	D
Enrofloxacin	Enr
Erythromycin	E
Florfenicol	Ffn
Flumequin	Fl
Framycetin	Fy
Furazolidone	Fu
Gentamicin	G
Kanamycin	K
Minocycline	Mn
Nalidixic acid	Na
Neomycin	N
Nitrofurantoin	Ni
Polymyxin B	Pb
Spectinomycin	Sp
Streptomycin	S
Sulphamethoxazole	Smx
Sulphonamides (Compound)	Su
Tetracycline	T
Trimethoprim	Tr
Trimethoprim + Sulfamethoxazole	Trs

Table 19: Results of antibiotic resistance pattern typing by NRLs-Salmonella

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	*	*	*	*	E	*	*	T Fu	*	*	*	C:I	R: E, I: K T S Na D	*	*	*	Fu
2	*	*	*	*	E	*	*	T Su	*	*	*	*	R: S Fl D E, I: K T Na	*	*	*	*
3	Na	Trs Na	Na	Na	Na E	Na	Na	T Cp I: Enr	Na	Fl St	*	I: Cp C R: Na	R: S Na Fl E, I: K T N D	*	Enr (ds)	Na	Na Sp
4	A C S Su T Tr	T C A Trs Mn	Ac C S Smx T Tr Trs	Ap C S Sxt T	S E C T Trs Tr Ax	A C S Su Trs T W	A S Su T C Trs	T A Fu Trs C S Su Cfp	Trs T A S Su C	A T Trs Su St C	T C A Trs	A T C Trs	R: A Trs T C S D E, I: K Na Fl N	Ac A C Ni S T Trs	A T C S Su Trs	T A Trs Cxm S Su	A C S Su T Tr Fu Sp
5	*	I: Mn	*	*	E	*	*	T	*	*	*	I: C	R: K S Fl E I: A G C Na N D	*	*	*	*
6	*	I: Mn	*	*	E	*	*	T Su	*	*	*	I: C	R: Trs K S Na Fl N E I: G T D	*	*	*	*
7	*	*	*	*	E	*	*	T	*	*	*	I: T C	R: Trs Na D E I: K T S Fl N	*	*	*	*
8	*	*	*	*	E	*	*	T Su	Trs Su	I: Su	*	I: C	R: Na Fl E I: K T C S N D	*	*	*	*
9	*	I: Mn	*	*	E	*	*	T	*	*	*	I: T C	R: Trs T Na Fl D E I: G K S N	*	*	*	*
10	*	*	*	*	E	*	*	T	*	I: Su	*	I: T C	R: Trs T S Na Fl D E I: K C N	S	*	*	*
11	*	*	*	*	E	*	S	T	*	*	*	I: T C	R: Trs S N D E I: K T Fl	*	*	*	*
12	*	*	*	*	E	*	*	T	*	*	*	I: T C	R: Trs D E, I: T S Na	*	*	*	*
13	*	*	*	*	E	*	*	T	*	I: Su	*	I: T C	R: Trs K T Na Fl D E I: G S N	*	*	*	*
14	S Su T Tr	T Mn Trs Sp	Sp S Smx T Tr Trs	Sxt T	S E T Trs Tr	Su Trs T W	S Su T Trs	T Trs S Su	Trs G T S Su	T Trs Su St	T Trs	R: T Trs I: C	R: Trs T S Na Fl D E I: K	S T Trs	T Su Trs	T Trs Su	S Su T Tr Sp
15	*	*	*	*	E	*	*	T Fr	*	I: Su	*	I: T C	R: T Na D E I: K S Fl N	*	*	*	*
16	Na	I: Na / Mn	Na	Na	Na Oa E	Fr Na	Na	T N Fu Cp I: Enr	Na	I: Su Fl St	Fu Fl	I: Cp C Trs R: Na	R: T S Na Fl D E I: K	Ni	Enr (ds)	Na Fu	Fu Na
17	*	I: Mn	*	Na	E	*	*	T	*	R: Su I: Enr	*	I: T	R: Trs T S Na Fl N D E I: G K C	*	*	*	Su T
18	*	I: Mn	*	*	E	*	S	T	*	R: Cf I: Su St	*	I: T C	R: Trs S Na Fl D E I: A G K T N	S	*	*	*
19	A S Su T	Mn T A	Ac S Smx T	A S T	S E T Ax	A S Su T	A S Su T Ac	T A S Su Cfp	T A S Su	A T Su St	T A	R: A T D S Smx, I: C	R: A Trs T S Na D E I: G K Fl	Ac A S T	A T S Su Trs	T A S Su	A S Su T
20	*	*	*	*	E	*	*	T Su	*	I: Su St	*	I: T C	R: Trs S Na Fl D E I: G K T C N	*	*	*	*

*: Strain is sensitive for all antibiotics tested R: strain is resistant I: Intermediate reaction against antibiotic
(abbreviations in Table 18)

4.8 Antibiotic resistance pattern typing by ENLs

The number of antibiotics used per laboratory varied from 11 to 23 (Appendix 6). The names and the abbreviations used for the antibiotics are mentioned in Table 20.
Strain nr. 4 was found resistant for Ampicillin, Chloramphenicol, Streptomycin, Tetracycline and Trimethoprim (+sulfamethoxazole) by most laboratories using these antibiotics. Furthermore, strain nr. 14 was found resistant to streptomycin, sulphonamides, tetracycline and trimethoprim by most laboratories using these antibiotics. An overview of the results of antibiotic resistance pattern typing of the ENLs is given in Table 21.

Table 20: Names and abbreviations of antibiotics used by ENLs

Name of the antibiotic	Abbreviation
Amikacin	Ak
Amocixillin+clavulanic acid	Amc
Amoxicilline	Amx
Ampicillin	A
Apramycin	Apr
Aztreonam	Atm
Cefalotine	Cf
Cefamandole	Ma
Cefotaxime	Ctx
Cefotiam	Ctm
Cefoxitim	Cox
Cefoxitin	Fox
Ceftazidim	Ce
Ceftiofur	Cef
Ceftriaxone	Cro
Cephalotin	Ce
Chloramphenicol	C
Ciprofloxacin	Cp
Colistine	Col
Colomycin	Co
Doxycycline	D
Flumequine	Fl
Furanes	Ft
Furazolidone	Fu

Name of the antibiotic	Abbreviation
Gentamicin	G
Imipenem	Imp
Kanamycin	K
Mecillinam	Me
Mezlocillin	Mzl
Mezlocillin+sulfalactam	Msu
Minocycline	Mn
Nalidixic acid	Na
Neomycin	N
Netilmicin	Ne
Nitrofurantoin	Ni
Nonrseothricin	Not
Ofloxacine	Ofx
Oxytetracyclin	Ote
Phosphomycin	Ph
Spectinomycin	Sp
Streptomycin	S
Sulfamerazin	Smz
Sulphamethoxazole	Smx
Sulphonamides	Su
Tetracycline	T
Tobramycine	Tb
Trimethoprim	Tr
Trimethoprim +sulfamethoxazole	Trs

Table 21: Results of antibiotic resistance pattern typing by ENLs

Strain	B	C	D	E	H	J	P	R	T	Z
1	*	I: Amp Cox Ote Smz	*	I: Ni	*	*	*	*	Ni	*
2	*	I: Cox S Smz	*	*	*	*	*	*	*	*
3	Nal	R: Na I: Cox Amp Gen Kan S Smz	*	*	Na CP	Na	Na	Na Cp	R: Na I: S	Na Cp
4	A C S Su T Tr	R: A C Mez Ote S Smz Trs I: Cox	A Trs Tmp T C	A C S Su T Tr Ni Mn	A C Sp S Sx T Tr	A C Su S T Trs	Amx S T C Su Tr	A C S Su T Trs	S T A Su C Ni Tr	A C S Su T Tr
5	*	I: Amp Cox Ote S Smz	*	*	*	I: T S	T S (I)	*	I: S	*
6	*	I: Amp Cox Ote S Smz	*	*	*	I: S	*	*	I: S	*
7	*	I: Amp Cox Ote Smz	*	*	*	*	*	*	*	*
8	*	I: Amk Amp Cox Ote S Smz	*	*	*	*	*	I: Su	I: Su	*
9	*	I: Amp Cox Smz	*	*	*	*	*	*	*	*
10	*	I: Amk Amp Cox Ote S Smz	*	*	*	I: T	*	*	*	*
11	*	I: Amp Cox Gen S Smz	*	*	*	*	*	*	*	*
12	*	I: Amp Cox Mez Smz	*	*	*	*	*	*	*	*
13	*	I: Cox Smz	*	*	*	*	*	*	I: Ni	*
14	S Su T Tr	R: Ote Smz S Trs I: Amp Cox	Trs Tmp C	R: S Su T Tr Mn Sp I: Ni	Sp S Sx T Tr	Su S T Trs	S T Su Tr	S Su T Trs	R: T Su Tr I: Sp S	S Su T Tr
15	*	I: Cox Ote Smz	*	Ni	*	*	*	*	I: Ni	*
16	Nal	R: Na I: Amp Cox Ote S Smz	*	Na Ni	Na CP	Na	Na Ft	Na Cp	Na Ni	Fu Na Cp
17	*	I: Amk Cox Ote Smz	*	*	*	*	*	*	*	*
18	*	I: Cox Smz	*	*	*	*	*	*	*	*
19	A S Su T	R: A Ote Smz S I: Cox Msu	A Amc C	A S Su T Mn	A S Sx T	A Su S T	Amx S T Su	A S Su T	S T A Su	A S Su T
20	*	I: Amk Cox Kan S Smz	*	*	*	*	*	*	*	*

*: Strain is sensitive for all antibiotics tested R: strain is resistant I: Intermediate reaction against antibiotic

4.9 Results phage typing by the NRLs-*Salmonella*

All laboratories that received strains for phage typing, performed phage typing in their own laboratory. The phage typing results were evaluated per strain and per laboratory. Table 22 and 23 show the result of phage typing as stated in the rest report. One laboratory (labcode 6) assigned all the *S.* Enteritidis (SE) strains the correct phage type and one laboratory (labcode 1) assigned all the *S.* Typhimurium (STM) strains correctly. Five laboratories (labcode 1, 3, 6, 9 and 16) achieved at least 90% correct identification for all the phage typable strains. Five strains of SE (PT 1b, 4, 6a, 44 and 19) and seven strains of STM (PT 32, 193, U302, 120, 2, 9 and 104) were assigned correctly by all laboratories.

Table 22: Results of Salmonella *Enteritidis phage typing by the NRLs*-Salmonella

		Phagetypes of each laboratory					
Strain	PT	1	3	6	9	11	16
E1	1b	1b	1b	1b	1b	1b	1b
E2	4	4	4	4	4	4	4
E3	6	6	6	6	21b	6	6
E4	13a	28	13a	13a	13a	13a	13a
E5	8	8	8	8	8	28	8
E6	6a	6a	6a	6a	6a	6a	6a
E7	1	1b	1	1	1	1	1
E8	4b	4b	4	4b	4b	4	4
E9	44	44	44	44	44	44	44
E10	19	19	19	19	19	19	19

Table 23: Results of Salmonella *Typhimurium phage typing by the NRLs*-Salmonella

		Phagetypes of each laboratory					
Strain	PT	1	3	6	9	11	16
M11	32	32	32	32	32	nd	32
M12	193	193	193	193	193	nd	193
M13	U302	U302	U302	U302	U302	nd	U302
M14	208	208	208	208	208	nd	208
M15	170	170	170	104A	108	nd	108
M16	120	120	120	120	120	nd	120
M17	2	2	2	2	2	nd	2
M18	9	9	9	9	9	nd	9
M19	12a	12a	104	12a	12a	nd	12a
M20	104	104	104	104	104	nd	104

nd: Not done

4.10 Results phage typing by the ENLs

The phage typing results were evaluated per strain and by laboratory. Tables 24 and 25 show the result of phage typing as stated in the test report. Seven laboratories (labcode B, C, F, H, J, K and P) assigned all the *S*. Enteritidis strains the correct phage type and three laboratories (labcode C, J and K) also assigned all the *S*. Typhimurium strains correctly. Nine laboratories (labcode B, C, E, F, H, J, K, L and N) achieved at least 90% correct identification for all the phage typable strains. Six strains of SE (PT 4, 6, 8, 6a, 1 and 44) and three strains of STM (PT 32, 2 and 9) were assigned correctly by all laboratories.

Table 24: Results of Salmonella *Enteritidis phage typing by the ENLs*

		Phage types of each laboratory											
Strain	PT	A	B	C	D	E	F	H	J	K	L	N	P
E1	1b	1c	1b	1b	1	1b	1b	1b	1b	1b	1b	1b	1b
E2	4	4	4	4	4	4	4	4	4	4	4	4	4
E3	6	6	6	6	6	6	6	6	6	6	6	6	6
E4	13a	13a	13a	13a	8	28	13a	13a	13a	13a	28	28	13a
E5	8	8	8	8	8	8	8	8	8	8	8	8	8
E6	6a	6a	6a	6a	6a	6a	6a	6a	6a	6a	6a	6a	6a
E7	1	1	1	1	1	1	1	1	1	1	1	1	1
E8	4b	4b	4b	4b	4	4b	4b	4b	4b	4b	4b	4b	4b
E9	44	44	44	44	44	44	44	44	44	44	44	44	44
E10	19	19 or 19a	19	19	19	19	19	19	19	19	19	19	19

Table 25: Results of Salmonella *Typhimurium phage typing by the ENLs*

		Phage types of each laboratory											
Strain	PT	A	B	C	D	E	F	H	J	K	L	N	P
M11	32	32	32	32	32	32	32	32	32	32	nd	32	32
M12	193		193	193	193	193	193	193	193	193	nd	193	193
M13	U302	NT	U302	U302	U302	U302	U302	U302	U302	U302	nd	U302	U302
M14	208		208	208	?	208	208	208	208	208	nd	208	12
M15	170	108	12	170	170	RDNC	104a	170	170	170	nd	108	104
M16	120	120	120	120	104b	120	120	104b	120	120	nd	120	120
M17	2	2	2	2	2	2	2	2	2	2	nd	2	2
M18	9	9	9	9	9	9	9	9	9	9	nd	9	9
M19	12a	149	12a	12a	12a	12a	12a	12a	12a	12a	nd	12a	104
M20	104	151 or 104	104	104	104	104	104	104	104	104	nd	104	12

n.d.: Not done

5. Discussion

Serotyping

Two laboratories wrote the name of the serovar in low case letters and 1 laboratory wrote the names all in capital letters. Two laboratories used a name which is withdrawn from the most recent Kauffman-White scheme, to identify a serotype. Three laboratories identified strain nr. 8 as S. enterica subsp. Chameleon. This is the original description for this serotype but it is no longer used in international *Salmonella* reports.

Several causes can be thought of for the incorrect typing of strain nr. 5. The components of the medium used for subculturing of the strain, like sugar or bacterial inhibitors can influence a strain to become rough. No clear correlation(s) could be observed between the media used and the outcome of the serotyping of strain nr. 5. Furthermore, the use of a batch of serum which is not good, can result in missing the O 6 antigen which results for strain nr. 5 in the serotype *S.* Chomedey. This problem also arose at the CRL-*Salmonella*, with another batch of O 6,7 serum, *S.* Glostrup could not be typed anymore, and the serovar found was *S*. Chomedey.

Salmonella subsp. salamae (strain nr. 2) resulted in 5 partly correct identifications of the H antigen. For 3 laboratories this was caused by incorrect identification of the H antigen ‘a’. These laboratories detected the H antigen z35. Probably, during the production of the antiserum, absorption with z35 was not performed well.

Antibiotic resistance pattern typing

In the test report, no data were asked on the exact method used by the participants. Most laboratories used a disc diffusion method (different manufacturers of the discs) and four laboratories used a quantitative method. The number of antibiotics varied from 7 to 23 per laboratory. Different methods and different antibiotics used can not be compared easily. Even though equivalence is observed between the methods, with strains that obtain resistance for several antibiotics, standardisation of antibiotic resistance pattern typing is required.

Phage typing

The strains represented phage types known to be occurring in the European Union, including phage type 44, a new type which was first defined in 1998 and is linked with travel to the Canary Isles. All the ENL and NRL laboratories identified this new type correctly. Phage type 4b was typed as PT4 by four laboratories. Missing phage 16 can be the cause of that.

The results obtained by the participating laboratories for phage typing was much improved compared to those obtained in the first study in 1999. SE PT 13a as in the 1999 study was the SE type giving most problems with incorrect identification by one NRL-*Salmonella* and four ENLs. For STM, PT170 was the most difficult strain with incorrect results from four NRLs-*Salmonella* and six ENLs due to the reading with phage 14 being missed. To be seen clearly this reading requires a hand lens or some form of magnification.

6. Conclusion

Serotyping
In general, problems with the typing of the O antigens did not occur. Most problems occurred with the typing of the H antigens. Typing on a regular basis and experience with the procedure apparently are essential to get the best results.

Antibiotic resistance pattern typing
Antibiotic resistance pattern typing revealed data which show that standardisation of this technique is required for comparison between laboratories. The type of antibiotic as well as the number of antibiotics used should be standardised.

Phage typing
Many lessons have been learned since the first phage typing study in 1999 and this is reflected in the improved results obtained by the NRLs-*Salmonella* and ENLs. The majority of laboratories obtained at least 90% correct identifications. Standardisation of the methods used in each laboratory and the use of identical phage typing preparations is essential for consistent results.

References

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

2. Test results of *Salmonella* serotyping in the Member States of the European Union. A collaborative study amongst the National Reference Laboratories for *Salmonella* (RIVM report 284500 008)
N. Voogt, H.M.E. Maas, W.J. van Leeuwen and A.M. Henken, September 1997, RIVM, Bilthoven

3. Test results of *Salmonella* serotyping in the Member States of the European Union. Collaborative study III amongst the National Reference Laboratories for *Salmonella* (RIVM report 284500 010)
N. Voogt, H.M.E. Maas, W.J. van Leeuwen and A.M. Henken, September 1999, RIVM, Bilthoven

4. 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 (RIVM report 284500 013)
M. Raes, L.R. Ward, H.M.E. Maas, W.J. van Leeuwen and A.M. Henken, February 2000, RIVM, Bilthoven

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, Director of Directorate D	P. Testori-Coggi
02	European Commission, head of Unit D.2	E. Poudelet
03	European Commission	J-C. Cavitte
04	European Commission	P. Mäkelä
05	President of the Council of Health, the Netherlands	prof. dr. J. J. Sixma
06	Veterinary Public Health Inspector	drs. H. Verburg
07-39	Participants of the study	
40	Board of Directors RIVM	dr. G. Elzinga
41	Director Sector Public Health Research	prof. dr. ir. D. Kromhout
42	Head of Microbiological Laboratory for Health Protection and Director CRL-*Salmonella*	dr. ir. A.M. Henken
43-46	Project Workers	
47-51	Authors	
52	Dutch National Library for Publications and Bibliography	
53	SBD/Information and Public Relations	
54	Registration agency for Scientific Reports	
55-56	Library RIVM	
57-71	Sales department of RIVM Reports	
72-81	Spare copies	

Appendix 2 Protocol Typing study

COLLABORATIVE STUDY
ON TYPING OF *SALMONELLA* STRAINS (5)
ORGANISED BY CRL *SALMONELLA*

PROTOCOL:

Introduction:
The Community Reference Laboratory (CRL) *Salmonella* organises a fifth collaborative typing study of *Salmonella* strains amongst the National Reference Laboratories (NRLs-*Salmonella*) and EnterNet laboratories (ENLs).
In this study again a total number of 20 *Salmonella* strains, supplied by the CRL, have to be identified. The results will be evaluated by the CRL. Laboratories, which are interested, can also perform resistance pattern typing with the method routinely used by the laboratory. Results of resistance pattern typing should be given as follows: R (resistant), S (sensitive) or I (intermediate).
The typing method routinely performed in the laboratory will be used in the study. Definite conclusions can only be based 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.
Also 20 *Salmonella* strains (10x *S*. Enteritidis and 10x *S*. Typhimurium), supplied by PHLS, London, can be send to the laboratories to perform phage typing. **As an example** the *Salmonella* phage typing protocol from PHLS (London) is included (page 4 and 5).

Objective:
The main objective of the fifth typing study is to compare the test results of sero- and resistance pattern typing of the participants with the results obtained at the CRL-*Salmonella*. Evaluation of the phage typing will be done by Linda Ward, PHLS, London.

Outline of the study:
Each laboratory will receive a parcel containing 20 *Salmonella* cultures (numbered 1 to 20) for sero- and optionally resistance pattern typing. On arrival the cultures must be subcultured on agar plates. Optionally the laboratories will receive a parcel containing 20 *Salmonella* cultures (numbered M1 to M10 and E1 to E10) for phage typing.
The performance of the study will be in week 10 (starting on 6 March 2000) or one week earlier or later. All data will be reported in the test report to the CRL-*Salmonella* and will be used for analysis. The data on phage typing will be sent to PHLS.

Timetable of the collaborative typing study on of *Salmonella* strains (5)

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

31 Jan - 4 Feb Mailing the protocol and test report to the participating laboratories.

21-25 February Mailing the strains to the participants.
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 participant.

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

If the parcel did not arrive at the airport before or on 25 February 2000, do contact the CRL immediately.

28 Feb - 3 March Checking the presence of all necessary reagents and materials for the performance of the study.

6 - 10 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.

20-24 March Completion of the test report and faxing it to the CRL. The original test report will be send to the CRL. Results of phage typing will also be send to PHLS.

27 - 31 March Checking the results by the NRLs-*Salmonella*.

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

Maurice Raes
(research assistant CRL-*Salmonella*)
P.O. Box 1
3720 BA Bilthoven
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:

Linda 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

Salmonella phage typing protocol from PHLS (London)

1. Media

1.1 Double strength nutrient broth

Bacto dehydrated nutrient broth (Difco laboratories)	20 grams
NaCl	8.5 grams
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 grams
NaCl	8.5 grams
Bacto agar dehydrated (Difco laboratories)	13 grams
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 aseptically 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 3 Test Report

COLLABORATIVE STUDY
ON TYPING OF *SALMONELLA* STRAINS (5)
ORGANISED BY CRL *SALMONELLA*

TEST REPORT

FIFTH COLLABORATIVE TYPING STUDY
OF *SALMONELLA* STRAINS

Laboratory code	:
Laboratory name	:
Date of collecting the parcel	: ..... - ..... - 2000
Starting date for serotyping	: ..... - ..... - 2000

GENERAL QUESTIONS

Shipment:

Parcel damaged } YES
} NO

date of receipt at the laboratory : ..... - 2000
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 : ..... - 2000

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 10 OF THE TEST REPORT!

1. What was the frequency of serotyping at <u>your</u> laboratory in **1999**?
   - ☐ once a week
   - ☐ twice a month
   - ☐ once a month
   - ☐ more frequent, namely .....
   - ☐ less frequent, namely .....

2. How many strains did your laboratory serotype in **1999**?

   .....

3. What kind of sera do you use?
   - ☐ commercial available sera
   - ☐ manufacturer: ….....
   .....
   .....
   - ☐ prepared in own laboratory

4. Is your laboratory the reference laboratory for serotyping veterinary or human *Salmonella* strains in your country?
   - ☐ YES, Veterinary / Human (Mark the correct answer)
   - ☐ 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 10 OF THE TEST REPORT!

Questions 6, 7 and 8 only when your laboratory does phage typing:

6. Does your laboratory perform phage typing of
   - *Salmonella* Typhimurium
   - *Salmonella* Enteritidis

7. Which typing system is used for
   - *Salmonella* Typhimurium

     .....

     .....
   - *Salmonella* Enteritidis

     .....

     .....

8. How many strains did your laboratory phage type in **1999**?

   .....

PLEASE WRITE YOUR REMARKS AND COMMENTS ON PAGE 10 OF THE TEST REPORT!

Questions 9 and 10 only when your laboratory does resistance pattern typing:

9. How many resistance patterns did your laboratory type in **1999**?

.....

10. What kind of antibiotics do you use?

Manufacturer: ….....

.....

.....

Antibiotics used for resistance patterns

	Antibiotic	**Concentration**	**Code**	**Manufacturer**
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				

PLEASE WRITE YOUR REMARKS AND COMMENTS ON PAGE 10 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 typing: ..... - ..... - 2000

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 PHAGETYPING

Salmonella Enteritidis phage typing QA Strains March 2000
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	11	12	13	14	15	16
E1																	
E2																	
E3																	
E4																	
E5																	
E6																	
E7																	
E8																	
E9																	
E10																	

Salmonella Typhimurium phage typing QA Strains March 2000

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																																									

TEST RESULTS OF THE COLLABORATIVE STUDY ON RESISTANCE PATTERN TYPING

strain no.	Serotype	Resistance pattern
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 Media used for subculturing

Table 1: Media used for subculturing the strains resulting in correct typing of strain nr. 5

Medium used	Components
Agar, L11 Oxoid	Lemco bouillon, agar nr. 1
Tryptose Agar, Biogenetics BM 347	Tryptose, dextrose, Sodium chloride, agar
MacConkey agar without salt	Peptone, lactose, bile salts, neutral red, agar
No subculturing, directly from agar	-
Beef broth agar, schworm agar	Meat extract, peptone, sodium chloride, glucose, disodiumhydrogenphosphate, fatty acids, agar

Table 2: Media used for subculturing the strains resulting serotype S. *Chomedey for strain nr. 5*

Medium used	Components
Plate Count Agar	
Nutrient Agar (3x)	Beef extract, peptone, agar
Lactose agar + nutrient agar + swarm agar	Lab-lemco powder, yeast extract, peptone, sodium chloride, agar (for O antigens) Bacto agar (for H antigens)
Blood agar base + lactose + neutral red	Proteose peptone, liver digest, yeast extract, sodium chloride, agar + lactose + neutral red
Blood agar base + MacConkey + Kohns	Proteose peptone, liver digest, liver digest, yeast extract, sodium chloride, agar + peptone, lactose, bile salts, sodium chloride, neutral red, crystal violet, agar + meat extract, peptone, mannitol, agar, yeast extract, dextrose, phenol red

Table 3: Media used for subculturing the strains resulting in rough or autoagglutinable strain nr. 5

Medium used	Components
Columbia agar	Special peptone, starch, sodium chloride, agar
Brilliant green agar	
Gassner agar	Peptone, sodium chloride, lactose, water blue, methachrome yellow, agar
Bromcresol-purpur-lactose agar	Pepton, sodium chloride, sodiumammoniumphosphate, lab-lemco powder, agar
Xylose lysine desoxycholaat agar	
Swarm gard agar	Lab-lemco, pancreatic digest of meat, sodium chloride, yeast extract, D-mannitol, agar
Drigalski-Conrad's agar	Lab-lemco, peptone, yeast extract, sodium chloride, agar, laxtose, bromcresol purple (1,6%)
Endo agar + nutrient agar + meat extract + yeast extract + lactose	Nutrient broth, sodium chloride
Tryptic soya agar	Tryptic casein, soy peptone, sodium chloride, phenol red
Unknown (ENL Sweden)	
Triple sugar iron agar	Beef extract, yeast extract, peptone, dextrose, lactose, sucrose, ferrous sulphate, sodium chloride, sodium thiosulfate, phenol red, agar
Dorsetts' egg	

Table 4: Media used for subculturing the strains resulting in serotype other then mentioned above for strain nr. 5

Medium used	Components
Brolac	Peptone from meat and casein, sodium chloride, lactose, bromothymol blue, agar
Triple sugar iron agar (2x)	Beef extract, yeast extract, peptone, dextrose, lactose, sucrose, ferrous sulfate, sodium chloride, sodium thiosulfate, agar, phenol red
SMID	Peptone, bile salts, brilliant green, chromogenic substrate, sodium glucuronate, sorbitol, agar
+ trypcase soya	Peptone, sodium chloride, di-sodium phosphate, potassium di-hydrogenphosphate
Xylose lysine desoxycholaat agar	
Tryptone soya agar	
Nutrient agar	Peptone from meat, meat extract, agar
Tryptic aey agar	
Kligler Iron agar (first subculturing XLD)	Lactose, glucose, sodium chloride, ammonium citrate, sodium thiosulphate, phenol red, agar

Appendix 5 Antibiotics used per NRL-*Salmonella*

Labcode 1

	Name	Concentration (µg)	manufacturer
1	Ampicillin	10	Oxoid
2	Chloramphenicol	30	Oxoid
3	Streptomycin	10	Oxoid
4	Compound Sulphonamides	300	Oxoid
5	Tetracycline	30	Oxoid
6	Trimethoprim	5	Oxoid
7	Ciprofloxacin	5	Oxoid
8	Gentamicin	10	Oxoid
9	Kanamycin	30	Oxoid
10	Nalidixic acid	30	Oxoid
11	Cefotaxime sodium	30	Oxoid

Labcode 2

	Name	Concentration	manufacturer
1	Polymyxine B	300 UI	Sanofi
2	Spectinomycine	100µg	Sanofi
3	Tetracycline	30 UI	Sanofi
4	Minocycline	30 UI	Sanofi
5	Chloramphenicol	30µg	Sanofi
6	Kanamycine	30 UI	Sanofi
7	Ampicilline	10µg	Sanofi
8	Cefalotine	30µg	Sanofi
9	Trimethoprim + sulfamethaoxazole	1,25µg +23,75µg	Sanofi
10	Gentamycine	10 UI-10µg	Sanofi
11	Cefotaxime	30µg	Sanofi
12	Amoxicilline + Ac	20µg +10µg	Sanofi
13	Ac Nalidixique	30µg	Sanofi
14	Enrofloxacine	5µg	Bayer
15	Apramycine	40µg	Rosco

Labcode 3

	Name	Concentration (ng/ml)	manufacturer
1	Amoxicillin + clavulanat	2-32	Trek
2	Ampicillin	1-32	Trek
3	Apramycin	4-64	Trek
4	Ceftiofur	0.5-8	Trek
5	Chloramphenicol	2-64	Trek
6	Ciprofloxacin	0.03-4	Trek
7	Colistin	4-64	Trek
8	Florfenicol	2-64	Trek
9	Gentamycin	1-32	Trek
10	Nalidixic acid	4-128	Trek

Laboratory 3 continued

11	Neomycin	2-32	Trek
12	Spectinomycin	2-128	Trek
13	Streptomycin	4-64	Trek
14	Sulphamethoxozole	32-512	Trek
15	Tetracycline	2-32	Trek
16	Trimethoprim	4-32	Trek
17	Trimethoprim + sulpha	1-8	Trek

Labcode 4

	Name	Concentration (µg)	manufacturer
1	Ampicillin	10	oxoid
2	Chloramphenicol	30	oxoid
3	Cefotaxime sodium	30	oxoid
4	Enrofloxacin	5	oxoid
5	nalidixic acid	30	oxoid
6	Streptomycin	10	oxoid
7	Trimethoprim + sulfa	25	oxoid
8	Tetracycline	30	oxoid

Labcode 5

	Name	Concentration	manufacturer
1	Amoxicilline	25µg	Sanofi-Pasteur
2	amoxi+acide clavulinique	20µg +10µg	Sanofi-Pasteur
3	Ceftazidime	30µg	Sanofi-Pasteur
4	Cefalotine	30µg	Sanofi-Pasteur
5	Cefoxitine	30µg	Sanofi-Pasteur
6	Cefixime	10µg	Sanofi-Pasteur
7	Streptomycine	10ui/10µg	Sanofi-Pasteur
8	Kanamycine	30ui	Sanofi-Pasteur
9	Apramycine	15µg	Elanco
10	Gentamycine	10ui/15µg	Sanofi-Pasteur
11	Tetracycline	30ui	Sanofi-Pasteur
12	Chloramphenicol	30µg	Sanofi-Pasteur
13	Erythromycine	15ui/15µg	Sanofi-Pasteur
14	Trimethoprim+sulfa	1,25µg /23,75µg	Sanofi-Pasteur
15	Trimethoprime	5µg	Sanofi-Pasteur
16	acide naladique	30µg	Sanofi-Pasteur
17	acide oxolinique	10µg	Sanofi-Pasteur
18	Enrofloxacine	5µg	Bayer

Labcode 6

	Name	Concentration	manufacturer
1	Amikacin	30 µg/ disc	Oxoid
2	Ampicillin	10 µg/ disc	Oxoid
3	Cefuroxim	30 µg/ disc	Oxoid
4	Chloramphenicol	30 µg/ disc	Oxoid
5	Colistinsulfate	10 µg/ disc	Oxoid
6	Enrofloxacin	5 µg/ disc	Bayer

Laboratory 6 continued

7	Furazolidone	100 µg/ disc	Oxoid
8	Gentamicin	10 µg/ disc	Oxoid
9	Kanamycin	30 µg/ disc	Oxoid
10	Nalidixic acid	30 µg/ disc	Oxoid
11	Neomycin	10 µg/ disc	Oxoid
12	Polymyxin B	300 I.U/ disc	Oxoid
13	Streptomycine	10 µg/ disc	Oxoid
14	Trimethoprim+sulfa	25 µg/ disc	Oxoid
15	Sulfonamides	300 µg/ disc	Oxoid
16	Tetracycline	30 µg/ disc	Oxoid
17	Trimethoprim	2.5 µg/ disc	Oxoid

Labcode 7

	Name	Concentration	manufacturer
1	Ampicilline	10µg	Sanofi Pasteur
2	Streptomycine	10UI	Sanofi Pasteur
3	Chloramphenicol	30µg	Sanofi Pasteur
4	Tetracycline	30UI	Sanofi Pasteur
5	Sulfamides forte	200µg	Sanofi Pasteur
6	Amoxicilline + clavul.	20µg /10µg	Sanofi Pasteur
7	Trimpethoprim+sulfa	1,25µg /23,75µg	Sanofi Pasteur
8	Nalidixic acid	30µg	Sanofi Pasteur
9	Amikacin	30µg	Sanofi Pasteur
10	Neomycine	30UI	Sanofi Pasteur
11	Gentamycine	10µg	Sanofi Pasteur
12	Enrofloxacin	5µg	Bayer

Labcode 8

	Name	Concentration (µg)	manufacturer
1	Nalidixic acid	30	Oxide
2	Tetracycline	10	Oxide
3	Neomycin	10	Oxide
4	Ampicillin	10	Oxide
5	Furazolidone	15	Oxide
6	Cefuroxime	30	Oxide
7	Trimethoprim+sulfa	25	Oxide
8	Chloramphenicol	10	Oxide
9	Amikacin	30	Oxide
10	Amoxycillin/clavulanic acid	30	Oxide
11	Gentamicin	10	Oxide
12	Streptomycin	25	Oxide
13	Compound/sulphonamides	300	Oxide
14	Cefoperazone	30	Oxide
15	Apramycin	15	Oxide
16	Colistin Sulphate	25	Oxide
17	Ciprofloxacin	1	Oxide
18	Enrofloxacin	5	Oxide

Labcode 9

	Name	Concentration (µg)	Manufacturer
1	Colistin	10	Becton Dickinson
2	Trimethoprim+sulfa	25	Becton Dickinson
3	Amikacin	30	Becton Dickinson
4	Gentamycin	10	Becton Dickinson
5	Neomycin	30	Becton Dickinson
6	Cefuroxine	30	Becton Dickinson
7	Amoxycillin clavulanic acid	30	Becton Dickinson
8	nalidixic acid	30	Becton Dickinson
9	Tetracycline	30	Becton Dickinson
10	Ampicillin	10	Becton Dickinson
11	Streptomycin	10	Becton Dickinson
12	Sulphonamides		Becton Dickinson
13	chloramphenicol	30	Becton Dickinson
14	cefropepazone	75	Becton Dickinson
15	enrofloxacin	5	Bayer

Labcode 10

	Name	Concentration (µg)	manufacturer
1	Ampicilline	33	Rosco
2	Cephazoline	60	Rosco
3	Neomycine	120	Rosco
4	Gentamycine	40	Rosco
5	Tetracycline	80	Rosco
6	Colistine	150	Rosco
7	Trimethoprim + sulfa	5,2+240	Rosco
8	Sulphonamides	240	Rosco
9	Flumequine	30	Rosco
10	Enrofloxacine	10	Rosco
11	Streptomycine	100	Rosco
12	Chloramphenicol	60	Rosco

Labcode 11

	Name	Concentration (µg)	manufacturer
1	Ampicilline	33	Rosco
2	Chloramphenicol	60	Rosco
3	Flumequine	30	Rosco
4	Furazolidon	50	Rosco
5	Neomycin	120	Rosco
6	Tetracycline	80	Rosco
7	Trimethoprim+sulfa	5,2+240	Rosco

Labcode 12

	Name	Concentration	manufacturer
1	Ampicillin	10µg/disc	AB Biodisk
2	Ciprofloxacin	10µg/disc	AB Biodisk
3	Tetracycline	30µg/disc	AB Biodisk
4	Chloramphenicol	30µg/disc	AB Biodisk
5	Nalidixic acid	30µg/disc	AB Biodisk
6	Trimethoprim+sulfa	1,2+23,8µg/disc	AB Biodisk
7	Doxycycline	30µg/disc	AB Biodisk
8	Streptomycin	30µg/disc	AB Biodisk
9	Sulfisoxazole	250µg/disc	AB Biodisk

Labcode 13

	Name	Concentration (µg)	manufacturer
1	Ampicillin	10	Biomerieux
2	Chloramphenicol	30	Biomerieux
3	Doxycyclin	30	Biomerieux
4	Erythromycin	15	Oxoid
5	Flumequin	30	Biomerieux
6	Gentamycin	10	Biomerieux
7	Kanamycin	30	Oxoid
8	Nalidixic acid	30	Biomerieux
9	Neomycin	30	Oxoid
10	Streptomycin	10	Biomerieux
11	Trimethoprim+sulfa	25	Oxoid
12	Tetracycline	30	Oxoid

Labcode 14

	Name	Concentration (µg)	manufacturer
1	Amoxicillin + clavulanic acid	30	biomerieux
2	Ampicillin	10	biomerieux
3	Cephalotin	30	biomerieux
4	Enrofloxacin	5	bayer
5	Chloramfenicol	30	biomerieux
6	flumequine	30	biomerieux
7	Gentamycin	10	biomerieux
8	Neomycin	30	biomerieux
9	Nitrofurantoin	300	biomerieux
10	Streptomycin	10	biomerieux
11	Tetracycline	30	biomerieux
12	Trimetoprim + sulfametoxazol	1,25 + 32,75	biomerieux

Labcode 15

	Name	Concentration	manufacturer
1	Ampicillin	Variable amount	VetMIC™
2	Cephalotin	Variable amount	VetMIC™
3	Enrofloxacin	Variable amount	VetMIC™
4	Tetracycline	Variable amount	VetMIC™

Laboratory 15 continued

5	Chloramphenicol	Variable amount	VetMIC™
6	Streptomycin	Variable amount	VetMIC™
7	Neomycin	Variable amount	VetMIC™
8	Gentamycin	Variable amount	VetMIC™
9	Sulfamethoxazole	Variable amount	VetMIC™
10	Trimethoprim+sulfamethoxazole	Variable amount	VetMIC™

Labcode 16

	Name	Concentration (µg)	manufacturer
1	Nalidixic acid	30	Oxoid
2	Tetracycline	10	Oxoid
3	Neomycin	10	Oxoid
4	Ampicillin	10	Oxoid
5	Furazolidone	15	Oxoid
6	Cefuroxime	30	Oxoid
7	Trimethoprom+sulfa	25	Oxoid
8	Chloramphenicol	10	Oxoid
9	Amikacin	30	Oxoid
10	Amoxycillin+clavul.	30	Oxoid
11	Gentamicin	10	Oxoid
12	Streptomycin	25	Oxoid
13	Compound Sulphonamides	500	Oxoid
14	Cefoperazone	30	Oxoid
15	Apramycin	15	Oxoid
16	Colistin sulphate	25	Oxoid

Labcode 17

	Name	Concentration (µg)	manufacturer
1	Ampicillin	10	oxoid
2	Chloramphenicol	30	oxoid
3	Gentamycin	10	oxoid
4	Kanamycin	30	oxoid
5	Streptomycin	10	oxoid
6	Sulphonomides	300	oxoid
7	Tetracyclines	10	oxoid
8	Trimethprim	5	oxoid
9	Furazolidine	50	oxoid
10	Nalidixic acid	30	oxoid
11	Ciprofloxacin	5	oxoid
12	Amoxycillin/ Clavulanic acid	30	oxoid
13	Enrofloxacin	5	oxoid
14	Framycetin	100	oxoid
15	Apramycin	15	oxoid
16	Spectinomycin	25	oxoid
17	Neomycin	10	oxoid

Appendix 6 Antibiotics used per ENL

Labcode B

	Name	Concentration (µg)	manufacturer
1	Ampicillin	10	Oxoid
2	Chloramphenicol	30	Oxoid
3	Streptomycin	10	Oxoid
4	Sulfonamides	3	Oxoid
5	Tetracycline	30	Oxoid
6	Trimethoprim	5	Oxoid
7	Ciprofloxacin	5	Oxoid
8	Gentamicin	10	Oxoid
9	Nalidixic acid	30	Oxoid
10	Cefotaxime	30	Oxoid
11	Mecillinam	10	Oxoid
12	Imipenem	10	Oxoid

Labcode C

	Name	Concentration (µg/ml)	manufacturer
1	Ampicillin	1-16	Bayer AG
2	Amikazin	2-32	Bristle Myers Sguibb GmbH
3	Cefotaxim	1-16	Hoechst AG
4	Cefotiam	0.5-8	Grimenthal GmbH
5	Cefoxitim	0.5-32	MSD Sharp Dolme
6	Ceftazidim	2-32	Cascan GmbH
7	Chloramphenicol	4-32	Cephasaar GmbH
8	Ciprofloxacin	0.063-64	Bayer AG
9	Gentamicin	0.5-8	Ratiopharm GmbH
10	Kanamizin	2-32	Ursapharm GmbH
11	Mezlocillin	2-32	Bayer AG
12	Mezlocillin+sulfalactam	2-32	Pfizer GmbH
13	Nalidixic saure	4-32	Sigma
14	Nourseothricin	2, 16	H-Knoll Inst Jena
15	Oxytetracyclin	0.5-8	Basotherm GmbH
16	Streptomycin	4-64	Grimenthal GmbH
17	Sulfamerazin	32-512	Berlin GmbH
18	Trimethoprim+sulfa	4-128	Berlin GmbH

Labcode D

	Name	Concentration	manufacturer
1	Ampicillin	Unknown	Unknown
2	Cefamandole	Unknown	Unknown
3	Amoxycillin/clavulanate	Unknown	Unknown
4	Cefoxitin	Unknown	Unknown
5	Cefotaxime	Unknown	Unknown
6	Gentamicin	Unknown	Unknown
7	Kanamycin	Unknown	Unknown

Laboratory D continued

8	Netilmicin	Unknown	Unknown
9	Amikacin	Unknown	Unknown
10	Sulphamethoxazole/trimethoprim	Unknown	Unknown
11	Tetracylcine	Unknown	Unknown
12	Chloramphenicol	Unknown	Unknown

Labcode E

	Name	Concentration (µg)	manufacturer
1	Ampicillin	10	Oxoid
2	Chloramphenicol	30	Oxoid
3	Streptomycin	10	Oxoid
4	Sulphonmides compound	300	Oxoid
5	Tetracycline	30	Oxoid
6	Trimethoprim	5	Oxoid
7	Nalidixic acid	30	Oxoid
8	Kanmycin	30	Oxoid
9	Ciprofloxacin	5	Oxoid
10	Nitrofurantoin	300	Oxoid
11	Ceftazidime	30	Oxoid
12	Gentamycin	10	Oxoid
13	Minocycine	30	Oxoid
14	Spectinomycin	25	Oxoid

Labcode H

	Name	Concentration (µg/ml)	manufacturer
1	Ampicillin	50	Sigma
2	Cefotaxime	1	Sigma
3	Chloramphenicol	20	Sigma
4	Ciprofloxacin	0,5	supplied by hospital pharmacy
5	Ciprofloxacin	0,125	supplied by hospital pharmacy
6	Furazolidone	20	Sigma
7	Gentamicin	20	Sigma
8	Kanamycin	20	Sigma
9	Nalidixic acid	40	Sigma
10	Netilmicin	20	supplied by hospital pharmacy
11	Spectinomycin	100	Sigma
12	Streptomycin	20	Sigma
13	Sulphamethoxazole	100	Sigma
14	Tetracycline	10	Sigma
15	Trimethoprim	2	Sigma

Labcode J

	Name	Concentration (µg)	manufacturer
1	Ampicillin	10	Oxoid
2	Chloramphenicol	30	Oxoid
3	Sulphonamides compounds	300	Oxoid
4	Gentamicin	10	Oxoid
5	Ciprofloxacin	5	Oxoid
6	Kanamycin	30	Oxoid
7	Streptomycin	10	Oxoid
8	Cephalotin	30	Oxoid
9	Nalidixic acid	30	Oxoid
10	Cefotaxime	30	Oxoid
11	Tetracycline	30	Oxoid
12	trimethoprim+sulfa	25	Oxoid

Labcode P

	Name	Concentration	manufacturer
1	Amoxicilline	25µg	Biorad/sanofi diag pasteur
2	Streptomycine	10 UI	Biorad/sanofi diag pasteur
3	Cefalotine	30µg	Biorad/sanofi diag pasteur
4	Imipenem	10µg	Biorad/sanofi diag pasteur
5	Kanamycin	30 UI	Biorad/sanofi diag pasteur
6	Tobramycine	10µg	Biorad/sanofi diag pasteur
7	Amikacine	30µg	Biorad/sanofi diag pasteur
8	Gentamycine	10 UI	Biorad/sanofi diag pasteur
9	Netilmicine	30µg	Biorad/sanofi diag pasteur
10	Tetracycline	30 UI	Biorad/sanofi diag pasteur
11	Chloramphenicol	30µg	Biorad/sanofi diag pasteur
12	Sulfamides	200µg	Biorad/sanofi diag pasteur
13	Ciprofloxacin	5µg	Biorad/sanofi diag pasteur
14	Ofloxacine	5µg	Biorad/sanofi diag pasteur
15	Nalidixic acid	30µg	Biorad/sanofi diag pasteur
16	Trimethoprime	5µg	Biorad/sanofi diag pasteur
17	Cefotaxime	30µg	Biorad/sanofi diag pasteur
18	Ceftriaxone	30µg	Biorad/sanofi diag pasteur
19	Ceftazidime	30µg	Biorad/sanofi diag pasteur
20	Amocixillin+clavulanic acid	20 mg + 10µg	Biorad/sanofi diag pasteur
21	Aztreonam	30µg	Biorad/sanofi diag pasteur
22	Furanes	300µg	Biorad/sanofi diag pasteur
23	Colistine	50µg	Biorad/sanofi diag pasteur

Labcode R

	Name	Concentration (µg)	manufacturer
1	Ampicillin	10	Becton-Dickinson
2	Chloramphenicol	30	Becton-Dickinson
3	Streptomycin	10	Becton-Dickinson
4	Sulfonamides	0,25 mg	Becton-Dickinson
5	Tetracycline	30	Becton-Dickinson

Laboratory R continued

6	Trimethoprim+sulfa.	1,25-23,75	Becton-Dickinson
7	Ciprofloxacin	5	Becton-Dickinson
8	Gentamycin	10	Becton-Dickinson
9	Kanamycin	30	Becton-Dickinson
10	Nalidixic acid	30	Becton-Dickinson
11	Cefotaxim	30	Becton-Dickinson

Labcode T

	Name	Concentration (µg)	manufacturer
1	Streptomycin	100	Rosco
2	Gentamycin	40	Rosco
3	Tetracyclines	80	Rosco
4	Ampicillin	33	Rosco
5	Sulphonamides	240	Rosco
6	Mecillinam	33	Rosco
7	Nalidixic acid	130	Rosco
8	Chloramphenicol	60	Rosco
9	Spectomycin	200	Rosco
10	Ciprofloxacin	10	Rosco
11	Apramycin	40	Rosco
12	Ceftiofur	30	Rosco
13	Kanamycin	100	Rosco
14	Nitrofurantoin	260	Rosco
15	Trimethoprim	5,2	Rosco
16	Phosphomycin	70+40	Rosco

Labcode Z

	Name	Concentration	manufacturer
1	Ampicillin	Various	Sigma
2	Chloramphenicol	Various	Parke Davis
3	Colomycin	Various	Pharmay
4	Neomycin	Various	Oxoid
5	Kanamycin	Various	Winthrup
6	Gentamicin	Various	Sigma
7	Streptomycin	Various	Ejans Medical
8	Sulphonomides	Various	Sigma
9	Spectinomycin	Various	Sigma
10	Tetracyclines	Various	Sigma
11	Trimethoprim	Various	Sigma
12	Furazoldone	Various	Sigma
13	Nalidixic acid	Various	aldrich
14	Ciprofloxacin	Various	Sigma