RIVM report 284500020/2002

Test results of *Salmonella* typing by the NRLs-*Salmonella* in the Member States of the EU and the EnterNet Laboratories

Collaborative study VI (2001) on typing of *Salmonella*

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

This investigation has been performed by order and for the account of the European Commission, LegislationVeterinaire 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: 32-2-2959928; telefax: 32-2-2953144

Abstract

Test results of *Salmonella* sero- and phage typing and antimicrobial susceptibility testing by the National Reference Laboratories for *Salmonella* in the Member States of the European Union and the EnterNet Laboratories: Collaborative study VI (2001) for Salmonella

The sixth collaborative typing study for *Salmonella* was organised by the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*, Bilthoven, The Netherlands) in collaboration with the Public Health Laboratory Services (PHLS), London, UK. Seventeen National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) and 15 EnterNet laboratories (ENLs) participated in the study. Three of the NRLs for *Salmonella* are also ENLs. The results of these three NRL-ENL laboratories will only be evaluated with the NRLs for *Salmonella*. In total, 19 strains of the species *Salmonella enterica* subsp. *enterica* and one strain of the species *Salmonella enterica* subsp. *arizonae* were selected for serotyping and antimicrobial susceptibility testing, 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 with typing the H antigens. Antimicrobial susceptibility testing 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, Nederland) heeft een zesde ringonderzoek voor de typering van *Salmonella* georganiseerd in samenwerking met het Public Health Laboratory Services (PHLS, Colindale) in Londen. Voor de geïnteresseerde laboratoria bestond de mogelijkheid om ook faagtypering en antimicrobiële gevoeligheidsbepalingen uit te voeren. Het doel van dit ringonderzoek was het onderling vergelijken van de testresultaten van de Nationale Referentie Laboratoria voor *Salmonella* (NRLs-*Salmonella*) en tussen de EnterNet Laboratoria (ENLs) onderling.

Alle NRLs-*Salmonella* van de Lidstaten van de Europese Unie (16) en NRL Noorwegen namen deel aan het ringonderzoek. Van deze 17 laboratoria voerden er 7 ook faagtypering uit. Tevens namen 15 ENLs deel waarvan er 12 faagtypering uitvoerden. Van de 17 NRLs-*Salmonella* zijn drie tevens ENL. De resultaten van deze NRL/ENLs worden alleen vermeld bij de NRLs-*Salmonella*. Alle drie deze laboratoria voerden faagtypering uit. Antimicrobiële gevoeligheidsbepalingen werden uitgevoerd door 17 NRLs-*Salmonella* en 10 ENLs.

In totaal werden 20 stammen van het species *Salmonella enterica* door het CRL-*Salmonella* geselecteerd. Hiervan waren er 19 van het subspecies *enterica* en één van het subspecies *arizonae.* 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.

De resultaten van de antimicrobiële gevoeligheidsbepalingen bevestigden dat het belangrijk is om een gestandaardiseerde methode te gebruiken om vergelijkingen te kunnen maken tussen laboratoria. Bij dezelfde stammen wordt door de meeste laboratoria resistentie aangetoond tegen één of meerdere van de gebruikte antibiotica. De verscheidenheid van de verschillende gebruikte antibiotica in dit ringonderzoek maakt het moeilijk om de resultaten met elkaar te vergelijken. Voor de overzichtelijkheid is daarom gekozen om het aantal antibiotica in de toekomst te verminderen tot twaalf. De selectie hiervan is gebaseerd op hetgeen is afgesproken tijdens de 6e Workshop georganiseerd door het CRL-*Salmonella* in 2001.

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). Er traden geen grote problemen op bij het bepalen van het faagtype van de geselecteerde stammen.

Summary

A sixth collaborative study on serotyping of *Salmonella* was organised by the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*, Bilthoven, The Netherlands) in collaboration with the Public Health Laboratory Service (PHLS, Colindale) in London. Laboratories that were interested had the possibility to perform phage typing and antimicrobial susceptibility testing. The main goal of this collaborative study was to compare the results among the National Reference Laboratories (NRLs-*Salmonella*) and among the EnterNet Laboratories (ENLs).
All NRLs-*Salmonella* of the Member States of the European Union (16) and NRL-Norway participated in the collaborative study. Seven 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. The results of these NRL/ENLs will only be mentioned with the NRLs-*Salmonella*. All three of these laboratories performed phage typing. Antimicrobial susceptibility testing was performed by 16 NRLs-*Salmonella* and by 10 ENLs.
A total of 20 strains of the species *Salmonella enterica* were selected by the CRL-*Salmonella*. Nineteen of these strains were of the subspecies *Salmonella enterica* and one was from the subspecies *arizonae*. The strains had to be typed with the method routinely used in their own laboratory. The laboratories were allowed to send strains for serotyping to another specialised laboratory in their country. Most problems were encountered when typing the H-antigens.
The results of the anti-microbial susceptibility testing confirmed that standardisation of the method is necessary for comparison of the results between laboratories. Most laboratories found resistance in the same strains with one or more of the antibiotics used. The diversity of the various antibiotics used in this collaborative study made it difficult to compare the results. For convenience the number of antibiotics used for comparison was decreased to twelve. The selection of these twelve antibiotics is based on discussions held at the Sixth Workshop organised by the CRL-*Salmonella* in 2001.
The PHLS selected 20 strains for phage typing, 10 were of the serovar *Salmonella* Enteritidis (SE) and 10 of the serovar *Salmonella* Typhimurium (STM). No major problems occurred by assigning the correct phage type to the strains.

1. Introduction

In this report the sixth collaborative typing study of *Salmonella* strains is described. This study was organised by the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*, Bilthoven, The Netherlands) in accordance with the Council Directive 92/117/EEC. It is one of the tasks of the CRL-*Salmonella* to organise this kind of studies 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 (Voogt et al. 1996) one strain of *Salmonella enterica* subspecies *salamae* and one strain of subspecies *houtenae* were included among the 20 strains to be tested. In the second, third and fourth collaborative study only strains belonging to subspecies *enterica* were included (Voogt et al. 1997,1999; Raes et al. 2000). The 20 strains for the second and third study were selected from the more frequently found serovars. In the fifth study (Raes et al. 2001) among the 20 selected serovars, 1 was of the subspecies *salamae* and 1 was of the subspecies *houtenae.* In the sixth study, described in this report, 19 strains were of the subspecies *enterica* and 1 was of the subspecies *arizonae.*
Seventeen NRLs-*Salmonella* and fifteen 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* and among the ENLs. All participants performed serotyping of the strains. In cooperation with the Public Health Laboratory Services (PHLS), London, phage typing was included in this study. Seven 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 antimicrobial susceptibility testing on the strains used for serotyping. All of the NRLs-*Salmonella* and 10 ENLs tested the antimicrobial susceptibility of the strains using their own methods.
The diversity of the various antibiotics used in this collaborative study makes it very difficult to interpret the results. For convenience CRL-*Salmonella* has chosen to decrease the number of antibiotics for comparison to twelve. The selection of these twelve antibiotics is based on discussions held at the Sixth Workshop organised by the CRL-*Salmonella* in 2001.
All participating laboratories were asked to fill in a questionnaire with general and more specific questions about methods, storage, subculturing, number of typings, etc. The outcome of this questionnaire is discussed in a separate chapter in this report.

2. Participants

Country	Institute/City	National Reference Laboratory for *Salmonella* (NRL) or EnterNet Laboratory (ENL)	
Austria	Bundesstaatliche bakteriologisch-serologische Untersuchungsanstalt Graz	NRL	ENL
Belgium	Veterinary and Agrochemical Research Center (VAR) Bruxelles	NRL	
Belgium	Institute Scientifique de Santé Publique – Louis Pasteur Brussels		ENL
Czech Republic	National Reference Laboratory for Salmonella National Institute of Public Health Prague		ENL
Denmark	Danish Veterinary Laboratory Copenhagen	NRL	
Denmark	Statens Serum Institut 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, Helsinki		ENL
France	Centre National d'Etudes Vétérinaires et Alimentaires, Centre National, Laboratoire central de recherches avicole et porcine. Ploufragan	NRL	
France	Unite des Enterobacteries Institute Pasteur Paris		ENL

Country	Institute/City	National Reference Laboratory for *Salmonella* (NRL) or EnterNet Laboratory (ENL)	
Germany	Bundesinstitut für Gesundheitlichen Verbraucherschutz und Veterinärmedizin Berlin	NRL	
Germany	Robert-Koch Institut Bereich Wernigerode Harz		ENL
Greece	Veterinary Laboratory of Halkis Halkis	NRL	
Greece	National School of Public Health, Department of Public & Administrative Health (Serotyping) and Department of Microbiology, Medical School, University of Athens (Phage typing) Athens		ENL
Ireland	Department of Agriculture and Food Central Veterinary Research Laboratory Dublin	NRL	
Ireland	National Salmonella Reference Laboratory University College Hospital Galway		ENL
Italy	Istituto Zooprofilattico Sperimentale delle Venezie Legnaro	NRL	
Italy	Istituto Superiore di Sanita Lab. Of Medical Bacteriology & Mycology Rome		ENL
Japan	Department of Bacteriology National Institute of Infectious Diseases Tokyo		ENL
Luxembourg	Laboratoire de Médecine Vétérinaire de l'Etat Animal Zoonosis Luxembourg	NRL	
Luxembourg	Laboratoire National de Santé Luxembourg		ENL

Country	Institute/City	National Reference Laboratory for *Salmonella* (NRL) or EnterNet Laboratory (ENL)	
The Netherlands	Rijksinstituut voor Volksgezondheid en Milieu (RIVM) – Laboratorium voor Infectieziektendiagnostiek en Screening (LIS) Bilthoven	NRL	ENL
Northern Ireland (UK)	Department of Agriculture for Northern Ireland Veterinary Sciences Division, Bact. Department Belfast	NRL	
Norway	National Institute of Public Health Oslo	NRL	ENL
Portugal	Laboratório Nacional de Investigaçã Veterinária Lisboa	NRL	
Scotland (UK)	Scottish Salmonella Reference Laboratory Department of Bacteriology Glasgow		ENL
Spain	Laboratorio Central de Veterinaria de Algete Madrid	NRL	
Spain	Laboratorio de Enterobacterias, CNM Instituto de Salud Carlos III 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 New Haw, Addlestone	NRL	

3. Materials and Methods

3.1 *Salmonella* strains for serotyping and antimicrobial susceptibility testing

Twenty 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 most recent Kauffmann-White scheme (Popoff, 2001) of the 20 serovars are shown in Table 1.

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

No	Serovar	O antigens	H antigens	Origin of strains
1	*S.* Blockley	6, 8	k : 1, 5	Human faeces
2	*S.* Agona	1, 4, [5], 12	f, g, s : [1, 2]	Human faeces
3	*S.* Rissen	6, 7, 14	f, g : -	Human faeces
4	*S.* Brazzaville	6, 7	b : 1, 2	Human faeces
5	*S.* Kiambu	1, 4, 12	z : 1, 5	Chicken
6	*S.* Typhimurium	1, 4, [5], 12	i : 1, 2	Human faeces
7	*S.* Goldcoast	6, 8	r : l, w	Human faeces
8	*S.* Kottbus	6, 8	e, h : 1, 5	Human faeces
9	*S.* Blockley	6, 8	k : 1, 5	Human faeces
10	*S.* Yoruba	16	c : l, w	Animal feed
11	*S.* Grumpensis	1, 13, 23	d : 1, 7	Human faeces
12	*S.* Heidelberg	1, 4, [5], 12	r : 1, 2	Human faeces
13	*S. spp.arizonae* 41 : z4, z23 : -	41	z4, z23 : -	Human faeces
14	*S.* Enteritidis	1, 9, 12	g, m : -	Human faeces
15	*S.* Newport	6, 8, 20	e, h : 1, 2 : [z67]	Human faeces
16	*S.* Dublin	1, 9, 12	g, p : -	Human faeces
17	*S.* Muenchen	6, 8	d : 1, 2 : [z67]	Human faeces
18	*S.* Lexington	3, 10 [15][15, 34]	z10 : 1, 5	Environmental sample
19	*S.* Waycross	41	z4, z23 : [e, n, z15]	Human faeces
20	*S.* Llandoff	1, 3, 19	z29 : [z6]	Animal feed

3.2 Antibiotics for antimicrobial susceptibility testing

The number of different antibiotics that were used by the NRLs-*Salmonella* and the EnterNet Laboratories are mentioned in Table 2.

Table 2. Total number of antibiotics used for antimicrobial susceptibility testing

Antibiotics	NRLs (n=17)	ENLs (n=10)	Antibiotics	NRLs (n=17)	ENLs (n=10)
Amikacin	2	1	**GENTAMYCIN (GEN)**	**14**	**10**
Amoxicillin	1	0	Imipenem	0	1
Amox+Clavulanate	11	1	**KANAMYCIN (KAN)**	**7**	**9**
AMPICILLIN (AMP)	**16**	**10**	Marbofloxacin	1	0
Ampicillin+Sulbactam	0	1	Mecillinam	0	2
Apramycin	5	1	Mezlocillin	0	1
Cefalotin	4	2	Mezloc.+Sulfalactam	0	1
Cefoperazone	2	0	Minocyclin	0	1
Cefotaxim	4	9	**NALADIXIC ACID (NAL)**	**14**	**10**
Cefotiam	0	1	**NEOMYCIN (NEO)**	**11**	**0**
Cefoxitine	1	1	Netilmicin	0	1
Ceftazidine	2	3	Nitrofurantoin	1	2
Ceftiofur	4	1	Nourseothricin	0	1
Cefuroxim	2	0	Oxolinic Acid	1	0
Cephazolin	1	0	Oxytetracyclin	0	1
CHLORAMPHENICOL (CHL)	**17**	**9**	Polymyxin	0	1
CIPROFLOXACIN (CIP)	**10**	**10**	Spectinomycin	3	5
Colistin	6	0	**STREPTOMYCIN (STR)**	**16**	**8**
Co-sulphonamides	3	2	Sulfamerazin	0	1
Co-trimoxazole	0	1	Sulfamethoxazole	3	2
Doxycyclin	2	0	**SULPHAM.+TMP (SXT)**	**14**	**4**
Enrofloxacin	8	0	Sulfisoxazole	1	1
FLORFENICOL (FFN)	**4**	**0**	Sulfonamides	4	4
Flumequin	3	0	**TETRACYCLIN (TET)**	**17**	**9**
Framycetin	1	0	**TRIMETHOPRIM (TMP)**	**7**	**7**
Furazolidone	4	1	Triple sulphonamides	1	0

In Table 2 a list of 52 different antibiotics used by the participating laboratories is shown. For convenience of comparison a choice has been made to only describe the results of a panel of twelve antibiotics. The choice of these antibiotics was discussed during the Sixth Workshop organised by CRL-*Salmonella* in 2001 and are marked in the table in green capitals with their respective abbreviation. The participants used either the agar disc diffusion test or the more quantitative method of Minimal Inhibitory Concentration (MIC) testing. MIC is defined as the lowest concentration of antibiotic required to inhibit growth of the organism.

3.3 *Salmonella* 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*

		Phages at Routine Test Dilution															
QA No.	**Phage type**	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
E1	**6**	-	scl	-	scl	-	scl	-	scl	ol	ol	-	-	-	-	-	-
E2	**1**	ol	scl	cl	scl	cl	scl	cl	ol	ol	ol	cl	cl	cl	cl	-	-
E3	**21**	cl	scl	-	scl	-	scl	-	ol	ol	ol	-	-	-	cl	-	-
E4	**4b**	-	scl	ol	scl	cl	scl	cl	ol	ol	ol	cl	cl	cl	-	-	scl
E5	**14b**	-	-	-	-	-	scl	-	-	±	-	-	-	-	-	-	-
E6	**4**	-	scl	cl	scl	cl	scl	cl	ol	ol	ol	cl	cl	cl	-	-	-
E7	**25**	-	-	-	-	-	-	-	+	-	ol	-	-	-	-	-	-
E8	**8**	-	-	scl	scl	cl	scl	scl	ol	ol	ol	scl	cl	-	-	-	-
E9	**6a**	-	scl	-	scl	-	scl	-	-	ol	-	-	-	-	-	-	-
E10	**11**	-	-	scl	-	cl	-	+	ol	-	ol	+	cl	±	-	-	+++

Table 4. Phage reactions of the Salmonella *Typhimurium strains*

		Phages at Routine Test Dilution																	
QA No.	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
M11	36	ol	scl	ol	ol	ol	ol	ol	ol	ol	ol	ol	ol	ol	ol	ol	ol	cl	cl
M12	8	-	-	-	-	-	-	-	scl	scl	scl	-	-	-	-	3+	-	-	-
M13	18	-	-	-	-	-	-	-	-	-	ol	-	-	-	ol	-	scl	scl	scl
M14	41	cl	scl	cl	ol	cl	ol	cl	-	cl	cl	-	cl	cl	cl	cl	cl	cl	cl
M15	U302	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M16	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M17	12	-	-	-	-	-	-	-	-	-	-	ol	ol	-	-	-	-	-	-
M18	104	-	-	-	-	-	-	-	-	-	-	++ <<	scl	-	-	-	-	3+	-
M19	208	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M20	170	-	-	-	-	-	-	-	-	-	-	ol	cl	3+	-	-	±	-	-

		Phages at Routine Test Dilution													Additional phages				
QA No.	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	O*	1	2	3	10	18
M11	36	ol	ol	ol	ol	ol	ol	ol	ol	ol	ol	ol	ol	cl					
M12	8	scl	-	scl	scl	-	±	±	-	-	cl	scl	-	cl					
M13	18	scl	-	-	-	-	-	-	+	±	-	scl	+	3+					
M14	41	cl	cl	ol	cl	cl	cl	cl	cl	-	cl	cl	ol	ol					
M15	U302	-	-	-	-	-	-	-	-	-	-	-	-	cl	-	-	-	ol	-
M16	193	-	-	-	-	-	-	-	-	-	-	-	-	cl	3+	3+	3+	-	-
M17	12	-	-	-	-	-	-	-	-	-	-	-	-	cl	3+	3+	3+	ol	-
M18	104	-	-	-	-	-	-	-	-	-	-	-	±	cl	-	-	-	ol	-
M19	208	-	-	-	-	-	-	-	-	-	-	-	-	cl	-	-	-	scl	scl
M20	170	-	±	-	-	-	-	-	++ <<	-	-	-	scl	cl					

O*: O pooled
(<)CL: clear lysis
(<)OL: opaque lysis
SCL: semi confluent lysis
<< : Merging plaques towards semi-confluent lysis

3.4 Laboratory codes

The NRLs were assigned a laboratory code (labcode) from one to seventeen (1-17) which differs from the previous typing studies. The alfabetical labcodes for the ENLs were given by PHLS, Colindale (London, UK).

3.5 Guidelines for evaluation of serotyping results

Table 5. Evaluation of serotyping results

Results of serotyping	Evaluation
Auto agglutination or incomplete set of antisera (outside the range of antisera)	**nt = not typable**
Partly typable due to incomplete set of antisera or part of the formula (for the name of the serovar)	**+/- = partly correct**
Wrong serovar or mixed sera formula	**- = incorrect**

3.6 List of abbreviations

BAB	Blood Agar Base
BGA	Brilliant Green Agar
CRL-*Salmonella*	Community Reference Laboratory – *Salmonella*
ENL	EnterNet Laboratory
EU	European Union
NRL-*Salmonella*	National Reference Laboratory – *Salmonella*
PHLS	Public Health Laboratory Service
PT	Phage Type
RIVM	Rijksinstituut voor Volksgezondheid en Milieu
S.	*Salmonella*
SE	*Salmonella* Enteritidis
STM	*Salmonella* Typhimurium
XLD	Xylose Lysine Desoxycholate

The abbreviations of the antibiotics used in this study are mentioned in Table 2 on page 11.

4. Questionnaire

4.1 General questions

Question A: Was the package damaged at arrival ?

One laboratory (labcode 17) mentioned that they received the package in a severely damaged state. For this study the laboratory used the strains from the package. No new parcel was sent.

Question B: What was the date of receipt at the laboratory ?

All NRLs except for one lab (labcode 16) received their parcels within the same week as the samples were sent. The laboratory with labcode 16 received the parcel after seven days.

Question C: Did you store the strains before subculturing ? At what temperature ?

Eighteen laboratories (10 NRLs and 8 ENLs) kept the strains at a temperature between 3°C and 8°C (Figure 1). Several laboratories kept them at 18°C-20°C (1 NRL and 4 ENLs). Three NRLs and three ENLs directly subcultured the *Salmonella* strains at arrival. One laboratory used a storage temperature of minus 20°C and from two NRLs data were not available.

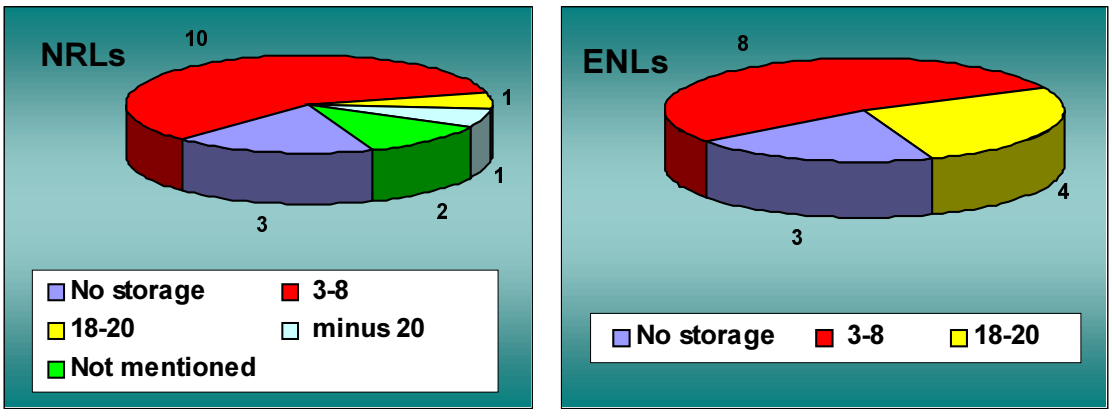

Figure 1. Storage of the strains before subculturing in degrees Centigrade

Question D: What was the date the strains were subcultured ?

In this report only the time between arrival at the laboratory and the date of subculturing is mentioned.
Three NRLs (1, 15 and 16) subcultured their strains directly after arrival. NRLs 2, 3, 6, 7, 8, 9, 10, 13, 14 and 17 subcultured the *Salmonella* strains from one to ten days after arrival. All stored the strains at a temperature of 3-8°C. Laboratory 4 subcultured the strains 17 days after arrival of the parcel and stored them to that time at minus 20°C. From three labs (5, 11 and 12) data are incomplete or not available.
The distribution of the parcels to the EnterNet Laboratories (ENLs) was done by PHLS, at Colindale Hospital (London, UK). The ENLs needed to subculture the strains received from CRL-*Salmonella* (via PHLS) as soon as possible after arrival (end of February 2001).
Three laboratories (F, P and W) received their parcel at the end of February 2001, eight (A, B, C, E, H, J, K and R) in March 2001 and one (T) in April 2001. Three labs (D, L and V) did not mention the date of receiving the parcel. The six ENLs (A, B, J, R, T, W) that subcultured the strains within ten days after receiving the parcel all stored them at a temperature between 3 and 8°C. Three labs (F, H and P) stored the strains at room temperature (18-20°C) but kept the strains at that temperature from ten days till two months before subculturing. From six labs (C, D, E, K, L and V) the data were not available or incomplete.

Question E: What kind of medium did you use for subculturing the strains ?

A variety of media from various manufacturers were used for the subculturing of the *Salmonella* strains. TSA was used by 2 NRLs and 4 ENLs. Two NRLs and two ENLs subcultured the strains on a nutrient agar, two NRLs on trypcase-soya medium, two NRLs on BAB, three ENLs on XLD and two ENLs on MacConkey medium.
Furthermore the NRLs (8) mentioned agar, BGA, Columbia, Brolac, Gassner agar, Agar Tryptose, Bouillon agar and Brom cresol agar as their medium of choice.
The other ENLs (2) used Endo agar or casitone as the subculturing medium.
One NRL and two ENLs did not answer this question.

Question F: Did you store the strains after subculturing ? And at what temperature ?

Fifteen (15) NRLs and twelve (12) ENLs stored the original strains after subculturing. One NRL and two ENLs did not store the strains at all and from one NRL and one ENL no data are available. Eleven (11) NRLs and seven (7) ENLs stored the strains at a temperature between 3°C and 8°C. Four NRLs and three ENLs kept the strains at room temperature (18-20°C), one ENL at minus 20°C and another ENL at minus 70°C.

4.2 Questions regarding content.

Question 1: What was the frequency of serotyping at your laboratory in 2000 ?
Question 2: How many strains did your lab serotype in 2000 ?

Table 6. Frequency and number of strains serotyped in 2000

Labcode NRLs	Typing frequency	Number of strains typed in 2000	Labcode ENLs	Typing frequency	Number of strains typed in 2000
1	Daily	11,191	A	Daily	14,000
2	Weekly	1,750	B	Daily	2,300
3	Daily	15,070	C	Daily	9,123
4	80 per month	800	D	Twice a week	980
5	Weekly	1,000	E	Daily	850
6	Daily	5,000	F	Monthly	70
7	Twice a week	289	H	Daily	3,580
8	Daily	1,036	J	Daily	9,015
9	Daily	1,337	K	Thrice a week	5,526
10	Twice a month	??	L	Daily	2,767
11	Weekly	6,000	P	Daily	8,000
12	??	??	R	Daily	479
13	At arrival	437	T	Daily	1,214
14	Daily	900	V	Daily	200
15	Twice a week	950	W	Daily	2,600
16	Daily	9,000			
17	Daily	2,050			

Question 3: What kind of sera do you use ? (commercially available sera or prepared in own laboratory)

Table 7. Number of laboratories using serotyping sera from one or more manufacturers or in-house prepared sera

Number of manufacturers	Number of NRLs	Number of ENLs
From 1 manufacturer	2	6
From 2 manufacturers	8	1
From 3 manufacturers	2	4
From 4 manufacturers	3	1
Not mentioned	2	3
Preparation own laboratory	4	4

Table 8. Number of laboratories using sera from the following manufacturers

Name Manufacturer	Number of NRLs (n=15)	Number ENLs (n=12)
Biorad (= Sanofi = Pasteur)	7	6
Biotec	1	0
Dade Behring	4	2
Difco	3	1
Eurobio	1	0
Murex-Abbott	5	2
Prolab Diagnostic	4	2
Reagensia (Sweden)	1	2
Seiken	1	0
SIFIN (Germany)	2	2
SMI (Sweden)	1	0
SSIC (Staten Serum Institute Copenhagen)	5	6

Question 4: Is your laboratory the reference laboratory for serotyping veterinary or human *Salmonella* strains in your country ?

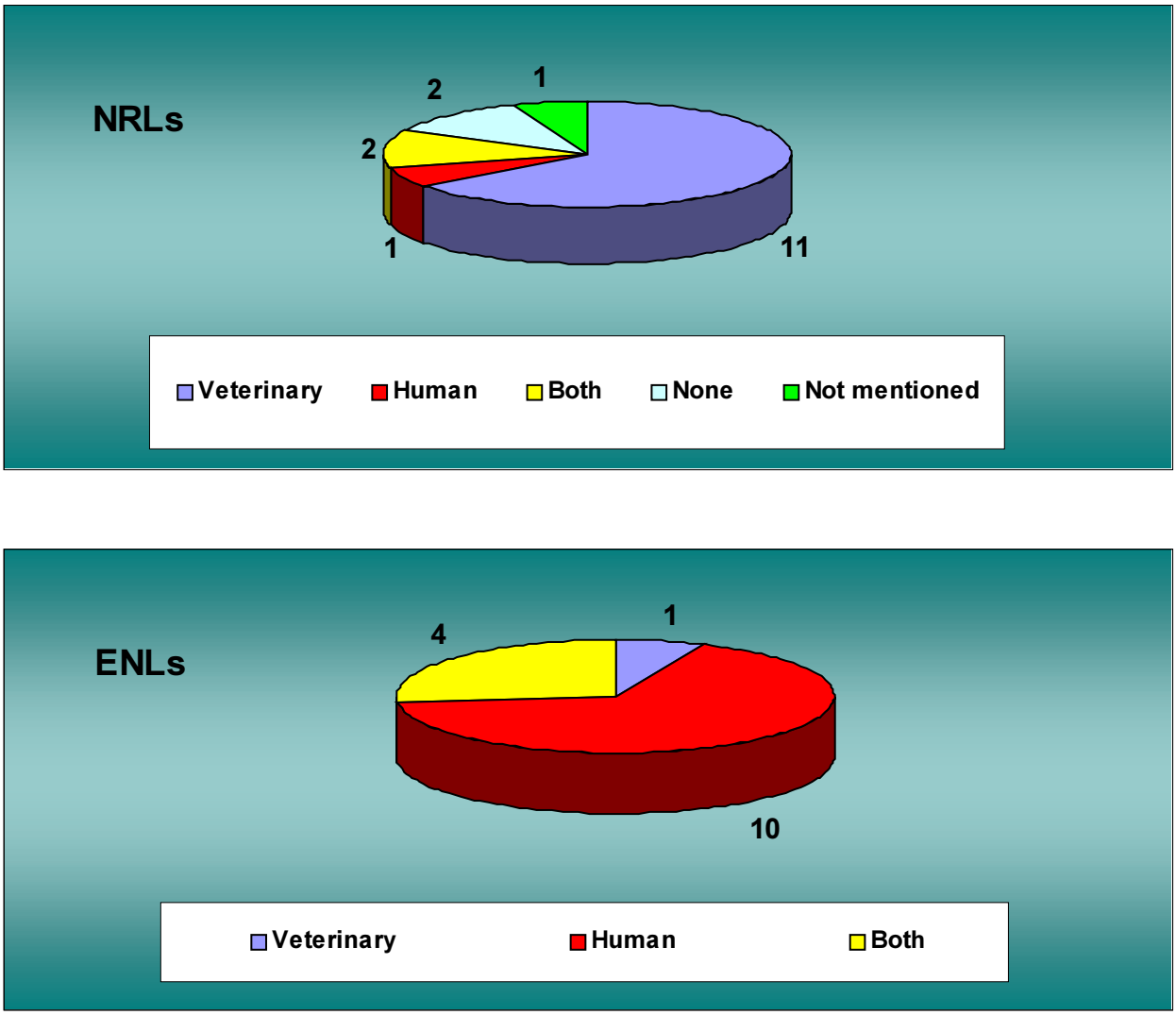

Figure 2. Number of NRLs and ENLs being reference lab for veterinary or human Salmonella *strains*

Question 5: Were the strains in the collaborative study typed in your own laboratory?

One NRL-Salmonella (labcode 5) sent six strains to another laboratory. All laboratories that were interested in performing phage typing typed the strains in their own laboratory.

Question 6: Does your laboratory perform phage typing of *Salmonella* Typhimurium, *Salmonella* Enteritidis and/or others ?

Eight NRLs and seven ENLs performed phage typing of Typhimurium and Enteritidis strains. One ENL has sent the strains to another laboratory. Three NRLs and three ENLs also phage typed other strains like *S.* Typhi, *S.* Paratyphi B, *S.* Virchow, *S.* Heidelberg and *S.* Hadar.

Question 7: Which typing system is used for phage typing of Typhimurium and Enteritidis strains ?

Four NRLs (1, 9, 13, 16) and six ENLs (B, E, H, J, K, V) use for both kind of strains the Colindale system.
Two NRLs (3, 6) and one ENL (C) mentioned Anderson for the Typhimurium system and Ward for the Enteritidis system, respectively. The Anderson and Ward systems are the same as the Colindale system. One NRL (labcode 11) used their own system for *S.* Typhimurium and the Colindale system for *S.* Enteritidis.

Question 8: How many strains did your laboratory phage type in 2000 ?

Table 9. Number of phage typings and their relationship to the serotyping in 2000

Laboratory codes	Sero typing	Phage typing
1	11,191	9,000
3	15,070	4,540
6	5,000	2,406
9	1,337	350
11	6,000	3,000
13	437	317
16	9,000	2,500
B	2,300	1,200
C	9,123	7,245
E	850	589
H	3,580	2,162
J	9,015	7,390
K	5,526	3,160
V	200	2,000

Question 9: How many resistance patterns did your laboratory type in 2000 ?

Table 10. Number of resistance pattern typings in 2000

Labcode NRLs	Number	Labcode ENLs	Number
1	11,191	A	500
2	1,700	B	2,300
3	6,000	C	9,123
4	300	E	850
5	150	H	5,249
6	4,623	J	1,000
7	25	R	479
11	2,500	T	3,500
13	434	V	300
14	100	W	1,206
15	67		
16	6,000		
17	1,100		

Question 10. What kind of antibiotics do you use ?

All data about NRLs and ENLs using various antibiotics are mentioned in Appendix 3.

5. Results

5.1 Serotyping by the NRLs

5.1.1. Evaluation per laboratory

The evaluation of the detection of O- and H-antigens and identification of the strains per laboratory are shown in Figures 3, 4 and 5. Nine laboratories (labcode 1, 3, 6, 9, 11, 12, 14, 15 and 16) typed all O antigens correctly. Seven laboratories (labcode 1, 3, 6, 7, 11, 12 and 16) identified all H antigens correctly and 6 laboratories (labcode 1, 3, 6, 11, 12 and 16) identified all serovar names correctly. The correct results per laboratory are not shown in the tables.

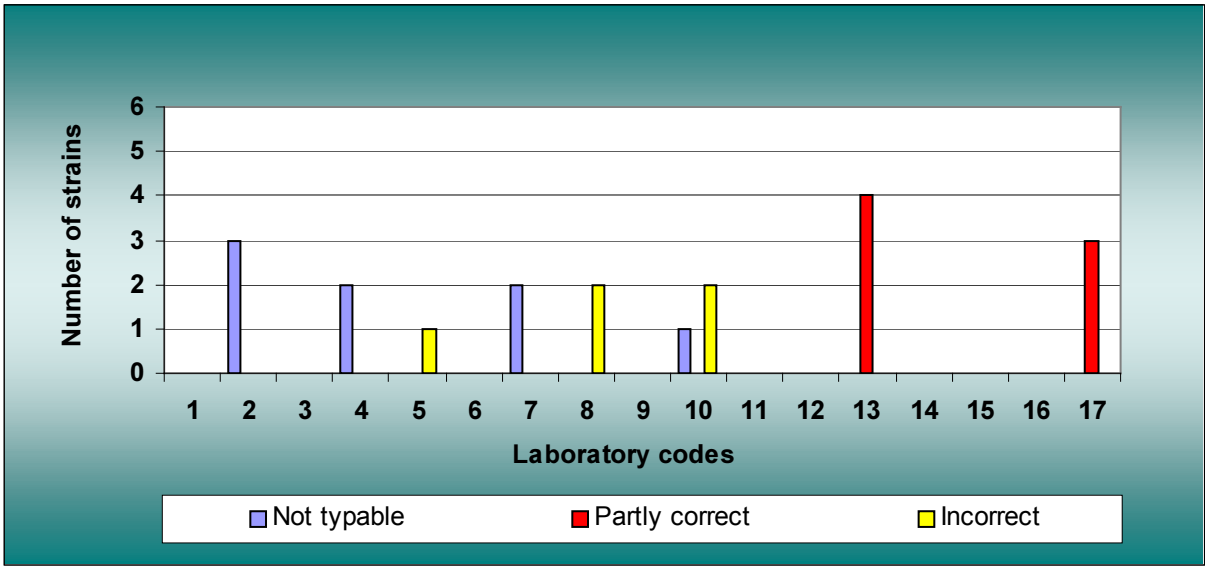

Figure 3. Evaluation of serotyping of O-antigens per NRL

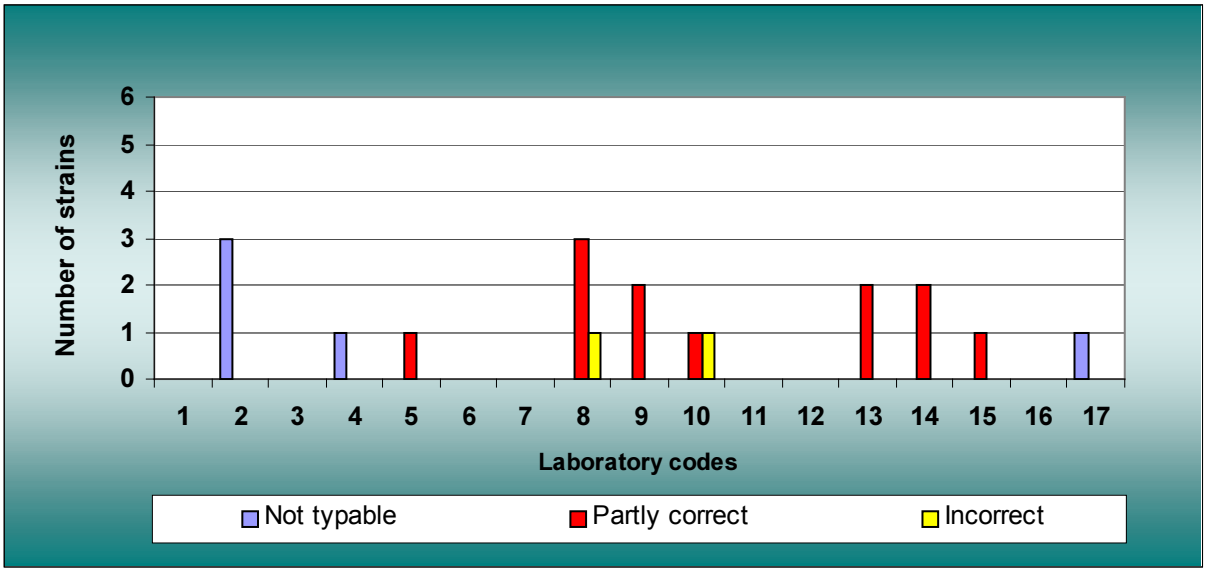

Figure 4. Evaluation of serotyping of H-antigens per NRL

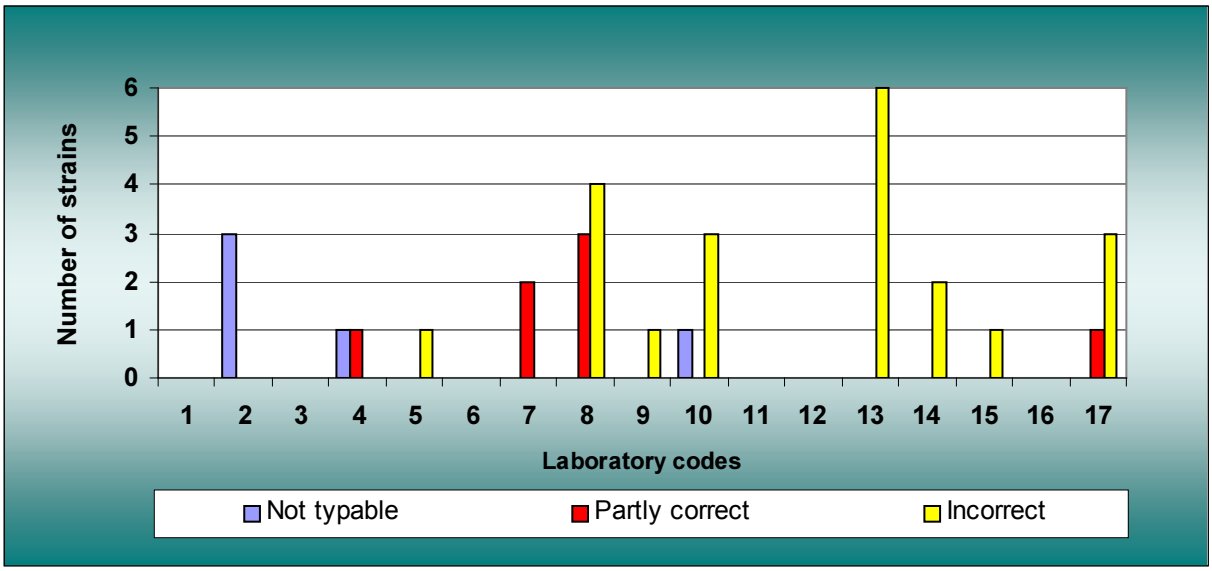

Figure 5. Evaluation of the correctness of serovar names per NRL

5.1.2 Evaluation per strain

The evaluation of the detection of O- and H-antigens and identification of the serovar names per strain are shown in Table 11. The O antigens of 10 strains were typed correctly by all participants. Most problems arose with strain 13 from subspecies *arizonae*. Four participants could not type the O-antigens of this strain. The H-antigens were typed correctly for 10 strains by all participants. The identified antigen structure was assigned correctly for 6 strains by all participants. Problems arose with strains *S.* Waycross and *S.* Kottbus. All three strains (*spp. arizonae*, *S.* Waycross and *S.* Kottbus) were given the correct serovar name by 12 participants. A total correct identification by all participants was obtained for 5 strains (*S.* Agona, *S.* Blockley, *S.* Enteritidis, *S.* Lexington and *S.* Typhimurium). Therefore these strains are not mentioned in Table 11. One laboratory typed the O- and H-antigens of a particular strain correctly but made a mistake in assigning the correct serovar name. Two identical strains (strain 1 and strain 9) were typed differently by two laboratories.

Table 11. Evaluation of serotyping per strain for NRLs

Strain		O antigen detected				H antigen detected				Name serovar			
No.	Serotype	+	nt	+/-	-	+	nt	+/-	-	+	Nt	+/-	-
3	*S.* Rissen	17				16		1		16			1
4	*S.* Brazzaville	17				16		1		16			1
5	*S.* Kiambu	17				17				16			1
7	*S.* Goldcoast	16		1		17				16			1
8	*S.* Kottbus	16		1		14		3		12			5
9	*S.* Blockley	15		2		17				15			2
10	*S.* Yoruba	15	1		1	14	1	2		14	1		2
11	*S.* Grumpensis	15			2	17				17			
12	*S.* Heidelberg	17				16		1		16			1
13	*S. spp.arizonae* 41:z4,z23:-	13	4			13	2	2		12	3	2	
15	*S.* Newport	15		2		17				15			2
16	*S.* Dublin	17				16		1		16			1
17	*S.* Muenchen	16		1		16		1		15		1	1
19	*S.* Waycross	13	3		1	16	1			12	1	3	1
20	*S.* Llandoff	16			1	14	1		2	14		1	2

+ = correctly; nt = not typable ; +/- = partly correct ; - = incorrect

The characterisations that caused major problems in serotyping by the NRLs is shown in Table 12. The empty cells in the table indicate that strains were typed correctly by the laboratories mentioned. Incorrect identification is shown in red in this table.

Table 12. Identifications per strain that caused major problems in serotyping by NRLs

	Strain 8	**Strain 10**	**Strain 13**	**Strain 19**	**Strain 20**
Correct typing	***S.* Kottbus 6,8: e,h: 1,5**	***S.* Yoruba 16: c: l,w**	***S. spp. arizonae* 41: z4, z23:-**	***S.* Waycross 41: z4,z23 : [e,n,z15]**	***S.* Llandoff 1,3,19: z29: [z6]**
Labcode 2		??	??	??	
Labcode 4			*spp. arizonae* Polyvalent II+: ------	?? Polyvalent II+: z4,z23	
Labcode 7			*spp.* IIIb: z4z23:-	*spp. enterica*: z4z23:-	
Labcode 8	*S.* Cremieu 6,8: e,h:6	II 39: c	?? 41: z4,z23:-	?? 41: z4,z23:-	S. Cannstatt 3,10,19: m, t
Labcode 9		*S.* Vancouver 16: c: 1,5			
Labcode 10	*S.* Manhattan 6,8: e,h: 1,5		?? ??: z4	*S.* Parera 11: z4,z23:-	*S.* Simsbury 1,3,19: z27
Labcode 13	*S.* Tshiogwe 6,8: e,h:e,n,z15				
Labcode 15	*S.* Tshiogwe 6,8: e,h:e,n,z15				
Labcode 17	*S.* Lomita 6,7: e,h: 1,5				*spp. entericaI* 3,19:Poly Hph1+2

5.2 Serotyping by the ENLs

5.2.1 Evaluation per laboratory

The evaluation of the detection of O- and H-antigens and the correctness of the serovar names are shown in Figures 6, 7 and 8. In these Figures and Tables 13 and 14 the results of the laboratory with labcode V are not mentioned. In the test report this laboratory did not mention anything about the detected O- and H-antigens and furthermore ten from the twenty serovar names were incorrect.
Twelve ENLs (A, B, C, D, E, H, J, K, L, P, R and T) typed all O-antigens correctly. One laboratory with labcode F detected the O-antigens from one strain partly correct and laboratory T from one strain incorrect.
Nine ENLs (B, C, E, H, J, K, P, R and W) typed all H-antigens correctly. Four laboratories (A, D, L and T) typed the H-antigens for one strain partly correct and laboratory F typed the H-antigens from two strains partly correct.
Seven laboratories namely A, D, E, F, L, T and W used an incorrect serovar name for one or more serovars.

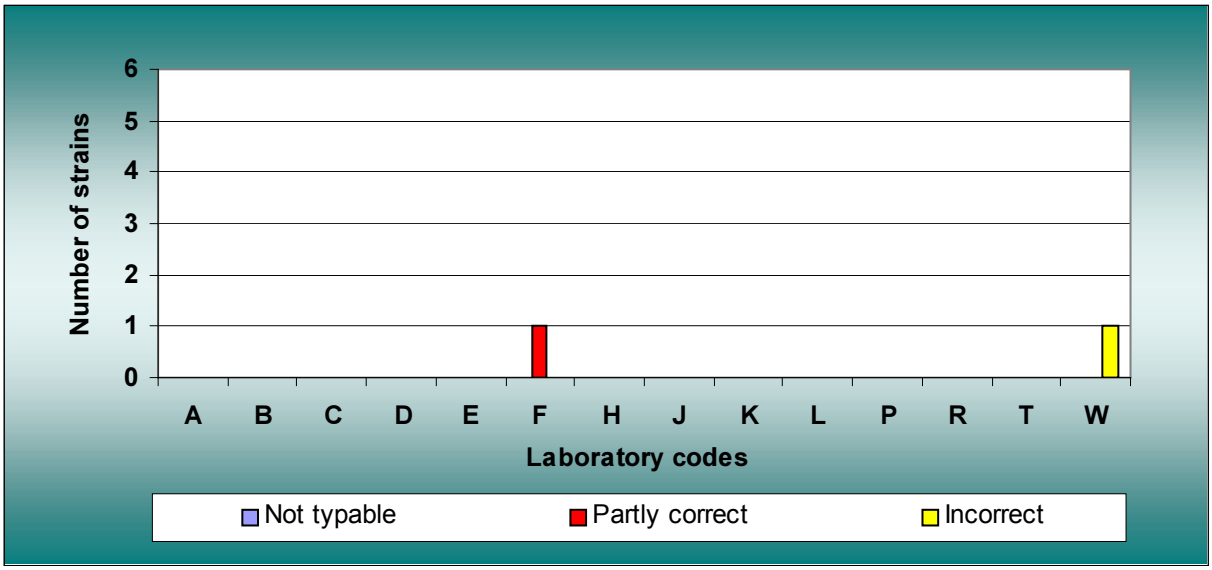

Figure 6. Evaluation of serotyping of O-antigens per ENL

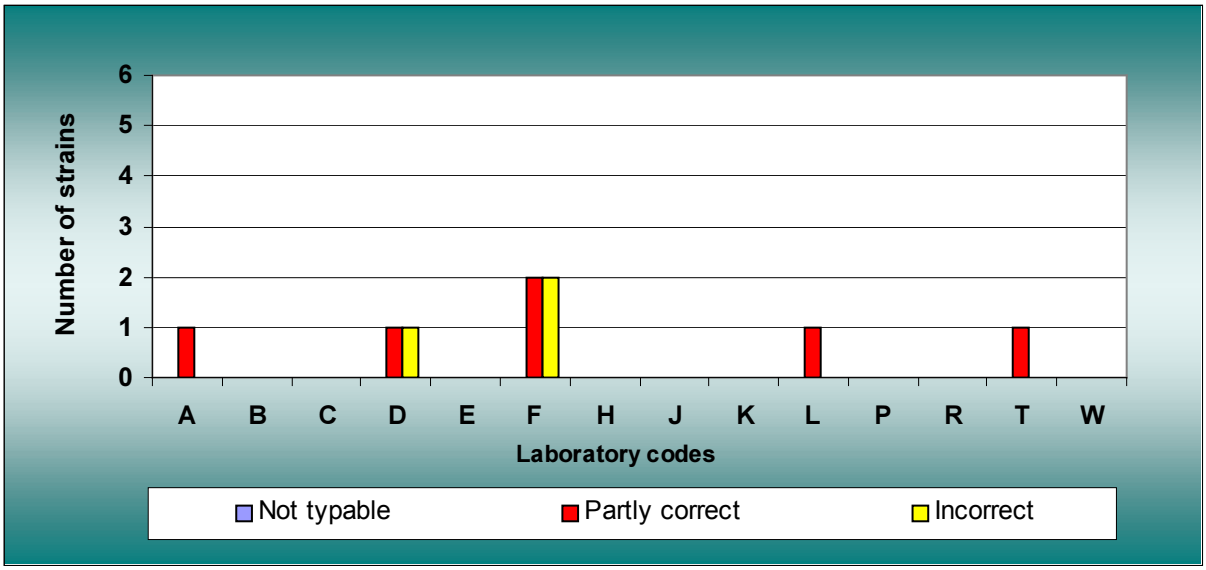

Figure 7. Evaluation of serotyping of H-antigens per ENL

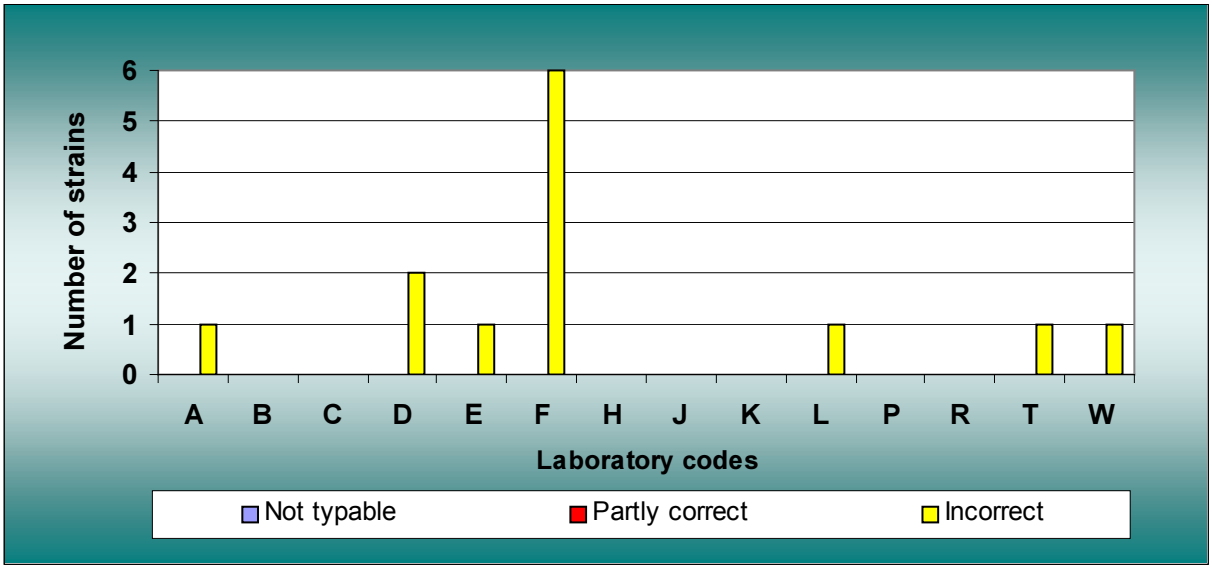

Figure 8. Evaluation of the correctness of serovar names per ENL

5.2.2 Evaluation per strain

Strains *S.* Blockley (strains 1+9), *S.* Agona (strain 2), *S.* Rissen (strain 3), *S.* Typhimurium (strain 6), *S.* Goldcoast (strain 7), *S.* Kottbus (strain 8), *S.* Yoruba (strain 10), *S.* Heidelberg (strain 12), *S.* Enteritidis (strain 14) and *S.* Lexington (strain 18) were all typed correctly by all ENLs and are therefore not mentioned in Table 13. The results of laboratory V are not mentioned in the table either (see 5.2.1.). Concerning the typing of the O-antigens for only one strain (*S.* Llandoff) one laboratory typed this strain incorrect and another partly correct. Each of the following strains (*S.* Brazzaville, *S.* Kiambu, *spp. arizonae*, *S.* Newport, *S.* Dublin and *S.* Muenchen) were typed partly correct by one laboratory. Incorrect identification of the H-antigens only occurred for strains *S.* Brazzaville, *S.* Grumpensis and *S.* Llandoff. Serovars *S.* Brazzaville, *S.* Kiambu, *S.* Grumpensis, *spp. arizonae*, *S.* Newport, *S.* Dublin, *S.* Waycross and *S.* Llandoff were characterised incorrectly by one to three laboratories. Strains that caused major problems for some laboratories are shown in Table 14. Incorrect identification is shown in red.

Table 13. Evaluation of serotyping per strain for ENLs

		O antigen detected				H antigen detected				Name serovar			
No	**Serovar**	**+**	**nt**	**+/-**	**-**	**+**	**nt**	**+/-**	**-**	**+**	**nt**	**+/-**	**-**
4	*S.* Brazzaville	14				12		1	1	12			2
5	*S.* Kiambu	14				13		1		13			1
11	*S.* Grumpensis	14				13			1	13			1
13	*S. spp arizonae* 41:z4,z23:-	14				13		1		12			2
15	*S.* Newport	14				13		1		13			1
16	*S.* Dublin	14				13		1		13			1
17	*S.* Muenchen	14				13		1		14			
19	*S.* Waycross	14				14				13			1
20	*S.* Llandoff	12		1	1	13			1	11			3

Table 14. Identifications per strain that caused major problems in serotyping by ENLs

	Strain 4	**Strain 13**	**Strain 20**
Correct identification	***S*.Brazzaville 6,7: b:1,2**	***S. spp. arizonae* 41: z4,z23:-**	***S*.Llandoff 1,3,19: z29:[z26]**
Labcode A		S. IIIa 41:z4,z23,z32 41: z4,z23,z32:-	
Labcode D			*S.* Dallgow 3,19: z10:e,n,z15
Labcode E		??? 41: z4,z23	
Labcode F	*S.* Infantis 7: r:1,5		*S.* Jetburgh 3,10: z29:-
Labcode L	*S.* Edinburg 6,7: b:1,5		
Labcode W			*S.* Brancaster 1,4,12: z29:-

5.3 Antimicrobial susceptibility testing by NRLs and ENLs

5.3.1 Minimal Inhibitory Concentration testing by NRLs and ENLs

Three NRLs and three ENLs tested the susceptibility of the same twenty strains as used for serotyping against the 12 antibiotics mentioned before. For names and abbreviations of the antibiotics see Table 2.
Strains 5, 7, 9, 10, 11, 13, 15, 17, 19 and 20 are not mentioned in the Table 15 because all these strains were sensitive for all twelve antibiotics as far as tested.
Strain number 2 (*S*. Agona), resistant to many antibiotics (see Table 15) shows similar results with all laboratories except for laboratory 6. Strain 3 (*S*. Rissen) revealed resistance to tetracycline by five laboratories. One laboratory did not test this antibiotic. Strain 6 (*S*. Typhimurium) showed overall agreement in resistance with antibiotics ampicillin, chloramphenicol, florfenicol, streptomycin and tetracycline.
All six laboratories found resistance of strain 8 (*S*. Kottbus) with naladixic acid and of strain 16 (S. Dublin) with chloramphenicol and streptomycin.

5.3.2 Agar disc diffusion testing by the NRLs

Thirteen NRLs tested the susceptibility of the same twenty strains against the antibiotics. The antibiotics that were not tested are indicated in Tables 16 (I) and 16 (II). Most laboratories found resistance with strains 2 (*S*. Agona), 3 (*S*. Rissen), 6 (*S*. Typhimurium), 8 (*S*. Kottbus) and 16 (*S*. Dublin) for one or more antibiotics. Laboratory with labcode 12 found intermediate resistance to almost all strains with chloramphenicol (30 μg/ml). These results are not shown in the table. The results of laboratory 13 are also not shown in the table for reasons of complexity. This laboratory found with almost all strains intermediate resistance and/or resistance to many antibiotics.

5.3.3. Agar disc diffusion testing by the ENLs

Seven ENLs were interested in testing the resistance of the twenty strains against the panel of antibiotics. Results are shown in Table 17. Most laboratories showed resistance in strain 2 (*S*. Agona) to streptomycin, sulfamethoxazole/ trimethoprim, tetracycline, and trimethoprim. Tetracycline in concentrations of more than 30 μg/ml was not able to prevent the growth of strain 3 (*S*. Rissen). As with the NRLs six laboratories found strain 6 (*S*. Typhimurium) not susceptible to ampicillin, chloramphenicol, streptomycin and tetracycline. All laboratories showed resistance of naladixic acid to strain number 8 (*S*. Kottbus) and of chloramphenicol and streptomycin to strain number 16 (*S*. Dublin).

Table 15. MIC testing of Salmonella *strains against antibiotics in µg/ml*

Strains/ Antibiotics	Laboratory codes					
	3	6	15	C	H*	T
1/ STR		STR 32		STR >64		
2/ STR	STR >64		STR 64	STR >64	STR >20	STR >64
2/ SXT	SXT >8		n.t.	SXT >128	n.t.	n.t.
2/ TET	TET >32		TET >64	n.t.	TET >10	TET >32
2/ TMP	TMP >32		TMP >16	n.t.	TMP >2	TMP >32
3/CHL				CHL >32		
3/ STR				STR >64		
3/ TET	TET >32	TET 8	TET >64	n.t.	TET >10	TET >32
4/ GEN						GEN >32
4/ KAN	n.t.		n.t.			KAN >64
4/ NAL				NAL >32		
4/ STR	STR >64	STR 32		STR >64		STR >64
6/ AMP	AMP >32	AMP >32	AMP >32	AMP >16	AMP >50	AMP >32
6/ CHL	CHL >64	CHL >64	CHL >16	CHL >32	CHL >20	CHL >32
6/ FFN	FFN >64	FFN 64	FFN 16	n.t.	n.t.	n.t.
6/ STR	STR >64	STR >64	STR 128	STR >64	STR >20	STR >64
6/ TET	TET >32	TET >32	TET 64	n.t.	TET >10	TET >32
8/ CIP			n.t.			CIP >4
8/ NAL	NAL >128	NAL >128	NAL >128	NAL >32	NAL >40	NAL >64
12/ STR				STR >64		
14/ STR	STR 4-64					
16/ CHL	CHL >64	CHL >64	CHL >16	CHL >32	CHL >20	CHL >32
16/ STR	STR >64	STR 64	STR 64	STR >64	STR >20	STR >64
18/ STR				STR >64		

Antibiotics not tested						
Antibiotic	Lab 3	Lab 6	Lab 15	Lab C	Lab H	Lab T
CIP			CIP			
FFN				FFN	FFN	FFN
KAN	KAN		KAN			
NEO				NEO	NEO	NEO
SXT			SXT		SXT	SXT
TET				TET		
TMP				TMP		

* = Breakpoint testing with in-house prepared solutions

n.t. = not tested, see also in lower part of the table.

Table 16. Disc diffusion testing by NRLs

Strains/ Antibiotics	Laboratory codes					
	Lab 2	Lab 4	Lab 5	Lab 7	Lab 8	Lab 9
2/ AMP			n.t.			
2/ GEN		n.t.				
2/ STR	STR/10 IU	STR/10	STR/10	STR/30 IU	STR/10*	STR/10
2/ SXT	SXT/25	SXT/25	SXT25	SXT/25	SXT/25	SXT/100
2/ TET	TET/30 IU	TET/30	TET/30 IU	TET/30	TET/30	TET/30
2/ TMP	n.t.	n.t.	TMP/5	TMP/5	n.t.	n.t.
3/ TET	TET/30 IU	TET/30	TET/30 IU	TET/30	TET/30	TET/30
4/ STR		STR/10			STR/10*	
6/ AMP	AMP/10	AMP/10	n.t.	AMP/10	AMP/10	AMP/10
6/ CHL	CHL/30	CHL/30	CHL/30	CHL/30	CHL/30	CHL/30
6/ FFN	FFN/30	n.t.	n.t.	n.t.	n.t.	n.t.
6/ STR	STR/10 IU	STR/10	STR/10	STR/30 IU	STR/10	STR/10
6/ TET	TET/30 IU	TET/30	TET/30 IU	TET/30	TET/30	TET/30
7/ STR						
8/ NAL	NAL/30	NAL/30	NAL/30			NAL/30
12/ STR					STR/10*	
12/ TET					TET/30*	
14/ STR				STR/30*		
16/ CHL	CHL/30	CHL/30	CHL/30	CHL/30	CHL/30	CHL/30
16/ STR	STR/10 IU	STR/10	STR/10	STR/30 IU	STR/10*	STR/10
16/ TET						TET/30
20/ STR					STR/10*	
Antibiotics not tested						
Antibiotic	**Lab 2**	**Lab 4**	**Lab 5**	**Lab 7**	**Lab 8**	**Lab 9**
AMP			AMP			
CIP	CIP		CIP			
FFN		FFN	FFN	FFN	FFN	FFN
GEN		GEN				
KAN	KAN	KAN		KAN		
NAL						
NEO		NEO	NEO	NEO	NEO	
STR						
SXT						
TMP	TMP	TMP			TMP	TMP

* = Intermediate
SXT/25 = Sulpham. 23.75/TMP 1.25 µg/ml
SXT/100 = Sulpham. 75/TMP 25 µg/ml
SXT/245,2 = Sulpham. 240/TMP 5.2 µg/ml
n.t. = not tested, see also in lower part of the table.

Table 16. Disc diffusion testing by NRLs (continued)

Strains/	Laboratory codes					
Antibiotics	Lab 10	Lab 11	Lab 12	Lab 14	Lab 16	Lab 17
2/ AMP				AMP/10		
2/ GEN		n.t.	n.t.	GEN/10		
2/ STR	STR/100*	n.t.		STR/10*		STR/10
2/ SXT	SXT/245.2	SXT/245.2	SXT/25	SXT/25	SXT/25	
2/ TET	TET/80	TET/80	TET/30	TET/30	TET/10	TET/10
2/ TMP	n.t.	n.t.	n.t.	n.t.	n.t.	TMP/5
3/ TET	TET/80	TET/80	TET/30	TET/30	TET/10	
4/ STR		n.t.				
6/ AMP	AMP/33	AMP/33	AMP/10	AMP/10	AMP/10	AMP/10
6/ CHL	CHL/60	CHL/60	CHL/30	CHL/30	CHL/10	CHL/30
6/ FFN	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
6/ STR	STR/100*	n.t.	STR/30	STR/10	STR/25	STR/10
6/ TET	TET/80	TET/80	TET/30	TET/30	TET/10	TET/10
7/ STR		n.t.		STR/10*		
8/ NAL	n.t.	n.t.	NAL/30	n.t.	NAL/30	NAL/30
12/ STR		n.t.				
12/ TET						
14/ STR	STR/100*	n.t.		CHL/30*		
16/ CHL	CHL/60	CHL/60		CHL/30	CHL/10	CHL/30
16/ STR	STR/100*	n.t.		STR/10	STR/25	STR/10
16/ TET						
20/ STR		n.t.				
Antibiotics not tested						
Antibiotic	Lab 10	Lab 11	Lab 12	Lab 14	Lab 16	Lab 17
AMP						
CIP	CIP	CIP			CIP	
FFN	FFN	FFN	FFN	FFN	FFN	FFN
GEN		GEN	GEN			
KAN	KAN	KAN	KAN	KAN	KAN	
NAL	NAL	NAL		NAL		
NEO			NEO			
STR		STR				
SXT						SXT
TMP	TMP	TMP	TMP	TMP	TMP	

* = Intermediate
SXT/25 = Sulpham. 23.75/TMP 1.25 µg/ml
SXT/100 = Sulpham. 75/TMP 25 µg/ml
SXT/245,2 = Sulpham. 240/TMP 5.2 µg/ml
n.t. = not tested, see in lower part of the table.

Table 17. Disc diffusion testing by ENLs

Strains/ Antibiotics	Laboratory codes						
	Lab A	Lab B	Lab E	Lab J	Lab R	Lab V	Lab W
1/ STR							STR/100 (*)
2/ STR	STR/10	STR/10 (*)	STR/10	STR/10	STR/10	STR/10	STR/100
2/ SXT	SXT/25	n.t.	n.t.	SXT/25	SXT/25	n.t.	n.t.
2/ TET	TET/30	TET/30	TET/30	TET/30	TET/30	TET/30	TET/80
2/ TMP	TMP/5	TMP/5	TMP/5	n.t.	n.t.	TMP/5	TMP/5,2
3/ TET	TET/30	TET/30	TET/30	TET/30	TET/30	TET/30	TET/80
4/ STR					STR/10		STR/100 (*)
5/ STR							STR/100 (*)
6/ AMP	AMP/10	AMP/10	AMP/10	AMP/10	AMP/10		AMP/33
6/ CHL	CHL/30	CHL/30	CHL/30	CHL/30	CHL/30		CHL/60
6/ STR	STR/10	STR/10	STR/10	STR/10	STR/10		STR/100
6/ TET	TET/30	TET/30	TET/30	TET/30	TET/30		TET/80
7/ STR							STR/100 (*)
8/ NAL	NAL/30	NAL/30	NAL/30	NAL/30	NAL/30	NAL/30	NAL/130
8/ STR					STR/10 (*)		
10/ TET					TET/30 (*)		
11/ STR							STR/100 (*)
12/ STR							STR/100 (*)
14/ STR	STR/10				STR/10 (*)		STR/100 (*)
15/ STR							STR/100 (*)
16/ CHL	CHL/30	CHL/30	CHL/30	CHL/30	CHL/30	CHL/30	CHL/60
16/ STR	STR/10	STR/10	STR/10	STR/10 (*)	STR/10	STR/10	STR/100
19/ STR							STR/100 (*)
19/ TET					TET/30 (*)		
20/ TET					TET/30 (*)		
Antibiotics not tested							
Antibiotic	Lab A	Lab B	Lab E	Lab J	Lab R	Lab V	Lab W
FFN		FFN	FFN	FFN	FFN	FFN	FFN
KAN		KAN					
NEO	NEO	NEO	NEO	NEO	NEO	NEO	NEO
SXT		SXT	SXT			SXT	SXT
TMP	TMP			TMP	TMP		

(*) Intermediate

n.t. = not tested, see in lower part of the table.

5.4 Results phage typing

5.4.1 Results phage typing by the NRLs

The phage typing results of the NRLs are shown in Tables 18 and 19. The correct phage type for all *S.* Enteritidis (SE) strains was assigned by only one laboratory (labcode 6). Four laboratories assigned all the *S.* Typhimurium (STM) strains correctly. Four strains of SE (PT 6, 4b, 4 and 6a) and five strains of STM (PT36, 18, U302, 104 and 208) were typed correctly by all laboratories.

Table 18. Results of Salmonella *Enteritidis phage typing by the NRLs*

		Phage type of each laboratory						
Strain	**PT**	**1**	**3**	**6**	**9**	**11**	**13**	**16**
E1	6	6	6	6	6	6	6	6
E2	1	1	1	1	**1b**	1	**1b**	1
E3	21	21	21	21	21	21	**21b**	21
E4	4b	4b	4b	4b	4b	4b	4b	4b
E5	14b	14b	14b	14b	14b	14b	14b	**24**
E6	4	4	4	4	4	4	4	4
E7	25	25	25	25	25	25	25	**11**
E8	8	8	8	8	8	**28**	**29a**	8
E9	6a	6a	6a	6a	6a	6a	6a	6a
E10	11	**9a**	**9a**	11	11	11	**9a**	**9a**

PT = Phage type

Table 19. Results of Salmonella *Typhimurium phage typing by the NRLs*

		Phage type of each laboratory						
Strain	**PT**	**1**	**3**	**6**	**9**	**11**	**13**	**16**
M11	36	36	36	36	36	NT	36	36
M12	8	8	8	8	**9**	NT	**115**	8
M13	18	18	18	18	18	NT	18	18
M14	41	41	41	41	41	NT	**41A**	41
M15	U302	U302	U302	U302	U302	NT	U302	U302
M16	193	193	193	193	193	NT	193	193
M17	12	12	12	12	12	NT	**104A**	12
M18	104(L)	104L	104	104L	104	NT	104L	104L
M19	208	208	208	208	208	NT	208	208
M20	170	170	170	170	170	NT	**104A**	170

PT = Phage Type

NT = Not Tested

5.4.2 Results phage typing by the ENLs

The phage typing results were evaluated per strain and by laboratory. Tables 20 and 21 show the result of phage typing as stated in the test report. Four laboratories (labcode C, E, H and V) assigned all the *S.* Enteritidis strains the correct phage type and five laboratories (labcode B, C, H, J and K) assigned all the *S.* Typhimurium strains correctly. Eight laboratories (labcode A, B, C, E, H, J, K and V) achieved at least 90% correct identification for all the phage typable strains. Four strains of SE (PT4, 6, 8 and 21) and two strains of STM (PT18 and 104) were assigned correctly by all laboratories.

Table 20. Results of Salmonella *Enteritidis phage typing by the ENLs*

		Phage types of each laboratory											
Strain	**PT**	**A**	**B**	**C**	**E**	**F**	**H**	**J**	**K**	**P**	**S**	**T**	**V**
E1	6	6	6	6	6	6	6	6	6	6	6	6	6
E2	1	1	1	1	1	**1a**	1	1	1	1	**1b**	1	1
E3	21	21	21	21	21	21	21	21	21	21	21	21	21
E4	4b	4b	4b	4b	4b	4b	4b	4b	4b	4b	4b	**4**	4b
E5	14b	14b	14b	14b	14b	14b	14b	14b	14b	14b	**13**	**9**	14b
E6	4	4	4	4	4	4	4	4	4	4	4	4	4
E7	25	25	**34**	25	25	25	25	**34**	25	25	25	25	25
E8	8	8	8	8	8	8	8	8	8	8	8	8	8
E9	6a	6a	6a	6a	6a	6a	6a	6a	6a	**35**	6a	6a	6a
E10	11	**20**	11	11	11	**1b**	11	**9a**	**9a**	11	**9a**	**9a**	11

Table 21. Results of Salmonella *Typhimurium phage typing by the ENLs*

		Phage types of each laboratory											
Strain	**PT**	**A**	**B**	**C**	**E**	**F**	**H**	**J**	**K**	**P**	**S**	**T**	**V**
M11	36	36	36	36	36	36	36	36	36	**1**	36	36	36
M12	8	8	8	8	8	8	8	8	8	**66**	8	8	8
M13	18	18	18	18	18	18	18	18	18	18	18	18	18
M14	41	**U298**	41	41	41	41	41	41	41	**3**	41	41	41
M15	U302	U302	U302	U302	U302	U302	U302	U302	U302	U302	U302	**U**	U302
M16	193	193	193	193	193	193	193	193	193	**NS**	193	**194**	193
M17	12	12	12	12	12	12	12	12	12	12	12	**U**	12
M18	104	104	104	104	104	104	104	104	104	104	104	104	104
M19	208	208	208	208	208	208	208	208	208	**U302**	**108**	208	208
M20	170	170	170	170	**12 var**	**108**	170	170	170	**104a**	**108 var**	**108**	**RD NC**

6. Discussion

Serotyping

For the NRLs as well as ENLs most of the O-antigens were typed correctly except for strain 13 (*spp. arizonae*). Strain S. Waycross (strain 19) was typed incorrectly by three NRLs. Strains 13 and 19 both possess the same O-antigen 41 and H-antigens z4 and z23. The differentiation between these two strains can only be made by biochemical tests.

For some NRLs the detection of the second phase of the H-antigens is still the most occurring problem. For the ENLs minor problems occurred with the typing of the H-antigens.

A remark should be made about the typing of two identical strains. Strains 1 and 9 (both belonging to serovar *S*. Blockley) were typed differently by two of the NRLs and none of the ENLs although these two strains were subcultured from the same tube.

Antimicrobial susceptibility testing

This is the second time that the susceptibility of strains against a panel of antibiotics is tested. Like in the first year of testing (2000) the number and kind of antibiotics per laboratory was very diverse. This diversity of the various antibiotics made it very difficult to interpret all results given by the NRLs-*Salmonella* and ENLs. For the convenience of comparison a panel of twelve antibiotics was chosen. The selection of this panel was based on discussions held at the Sixth Workshop organised by the CRL-*Salmonella* in 2001.

Six laboratories (three NRLs and three ENLs) used a quantitative method (MIC or breakpoint testing with in-house prepared solutions). The other laboratories tested the susceptibility for the antibiotics with an agar disc diffusion test. Quantitative as well as qualitative methods gave comparable results.

Phage typing

The strains selected represented types that are occurring in the European Union.*S*. Enteritidis phage type 21, a type common in Belgium, was identified correctly by all the ENL and all but one of the NRLs. All NRLs and ENLs typed phage types 6 and 4 correctly. The most difficult strain of *S*. Enteritidis was phage type 11, a type associated with carriage in hedgehogs. Four NRLs and six ENLs had incorrect identifications mainly due to the interpretation of the degrees of lysis.

The *S*. Typhimurium results were encouraging, only one type (phage type 8) was incorrectly identified by single NRLs and all but three ENLs had at least 90% *S*. Typhimurium types correct. The problem type was again phage type 170 with six ENLs giving incorrect identification. However, all but one of the NRLs correctly identified this type.

7. Conclusions and recommendations

Serotyping
In general, problems with the typing of the O-antigens were of minor importance. 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 better results.

Microbial Susceptibility Testing
Susceptibility testing of *Salmonella* strains with a variety of antibiotics revealed data which show that a certain standardisation in the technique is required for comparison between laboratories. The type of antibiotic as well as the number of antibiotics should also be standardised. In the near future the microbial susceptibility testing will be discussed into detail with several experts in the field and the CRL-*Salmonella* will present a new plan for a next collaborative study.

Phage typing
Overall the results were good. Two laboratories had some problems with the *S*. Typhimurium strains but this was the first time for one laboratory in the *S*. Typhimurium collaborative study and the first time for the other in the complete study.
The majority of laboratories again achieved over 90% correct identifications. The continuation of the standardisation of the methods used and the use of identical phage preparations is essential for each laboratory to obtain consistent results.

References

Popoff Michel Y and Leon Le Minor, Institut Pasteur, Paris. Antigenic formulas of the Salmonella serovars, 1997 (7th edition). WHO Collaborating Centre for Reference and Research on *Salmonella*.

Popoff Michel Y, Institut Pasteur, Paris. Antigenic formulas of the *Salmonella* serovars, 2001 (8th edition). WHO Collaborating Centre for Reference and Research on *Salmonella*;

Raes M, Ward LR, Maas HME, Leeuwen WJ van and Henken AM. 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. Bilthoven: National Institute of Public Health and the Environment (RIVM). 2000. Report no 284500013.

Raes M, Ward LR, Maas HME, Wannet WJB and Henken AM. Test results of *Salmonella* sero-, phage and antibiotic resistance pattern 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. Bilthoven: National Institute of Public Health and the Environment (RIVM). 2001. Report no 284500016.

Voogt N, Maas HME, Leeuwen WJ van and Henken AM. A collaborative study on serotyping of *Salmonella* amongst the National Reference Laboratories for *Salmonella.* Bilthoven: National Institute of Public Health and the Environment (RIVM). 1996. Report no 284500004.

Voogt N, Maas HME, Leeuwen WJ van and Henken AM. Test results of *Salmonella* serotyping in the Member States of the European Union. A collaborative study amongst the National Reference Laboratories for *Salmonella.* Bilthoven: National Institute of Public Health and the Environment (RIVM). 1997. Report no 284500008.

Voogt N, Maas HME, Leeuwen WJ van and Henken AM. Test results of *Salmonella* serotyping in the Member States of the European Union. Collaborative study III amongst the National Reference Laboratories for *Salmonella.* . Bilthoven: National Institute of Public Health and the Environment (RIVM). 1998. Report no 284500010.

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	SBC/Communicatie	
54	Registration agency for Scientific Reports	
55-56	Library RIVM	
57-71	Sales department of RIVM Reports	
72-80	Spare copies	

Appendix 1 Protocol

PROTOCOL OF THE COLLABORATIVE STUDY ON TYPING OF *SALMONELLA* STRAINS (6) ORGANISED BY CRL *SALMONELLA*

Introduction:
The Community Reference Laboratory (CRL) *Salmonella* organises a sixth collaborative typing study of *Salmonella* strains amongst the National Reference Laboratories for *Salmonella* (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 can also perform resistance pattern typing with the method routinely used by the laboratory.
For serotyping, 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. The evaluation of the results by the CRL will be performed according to Table 1.
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).

Table 1: Guidelines for evaluation

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 (-)

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 5 March 2001) 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 also be sent to PHLS.

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 please contact:

Linda R. Ward
Public Health Laboratory Service
Laboratory of Enteric Pathogens
61 Colindale Avenue, London NW9 5HT
tel. Number: ..- 44-20-8200 4400
fax number: ..- 44-20-8905 9929
e-mail: LWard@PHLS.nhs.uk or lward@phls.org.uk

Time table of the collaborative typing study on of *Salmonella* strains (6)

The identification of the *Salmonella* cultures must take place in week 10 (starting on March 5th) or one week earlier or later.

29 Jan - 2 Feb Mailing the protocol and test report to the participating laboratories.

19-23 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 an e-mail 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 at the time mentioned in your flight details, please contact the CRL.

26 Feb - 2 March Checking the presence of all necessary reagents and materials for the performance of the study.

5 - 9 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.

19-23 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.

26 - 30 March Checking the results by the NRLs.

Salmonella Phage typing protocol from PHLS (London).

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 2 Test Report

COLLABORATIVE STUDY
ON TYPING OF *SALMONELLA* STRAINS (6)
ORGANISED BY CRL *SALMONELLA*

TEST REPORT
COLLABORATIVE TYPING STUDY OF *SALMONELLA* STRAINS

SIXTH FOR THE NATIONAL REFERENCE LABORATORIES
AND THIRD FOR THE ENTERNET LABORATORIES

Laboratory code	:
Laboratory name	:
Date of collecting the parcel	: ..... - ..... – 2001
Starting date for serotyping	: ..... - ..... – 2001

GENERAL QUESTIONS

Shipment:

Parcel damaged } YES
} NO

date of receipt at the laboratory : ..... - 2001
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 : ..... - 2001

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 of *Salmonella* at <u>your</u> laboratory in **2000**?
   - } once a week
   - } twice a month
   - } once a month
   - } more frequent, namely .....
   - } less frequent, namely .....

2. How many *Salmonella* strains did your laboratory serotype in **2000**?
   .....

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

4. Is your laboratory the veterinary or human reference laboratory for typing *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
   - ☐ Other: ……..

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

   .....
   .....
   - ☐ *Salmonella* Enteritidis

   .....
   .....

8. How many strains did your laboratory phage type in **2000**?

   .....

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

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

9. For how many strains was the resistance typed in your laboratory **2000**?
   Salmonella: ..... Other: .....

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: ..... - ..... - 2001

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 2001

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 2001

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	O pooled	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 3 Antibiotics used per laboratory

Labcode 1			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Ampicillin	10 µg	Oxoid
2	Cefotaxime sodium	30 µg	Oxoid
3	Chloramphenicol	30 µg	Oxoid
4	Ciprofloxacin	5 µg	Oxoid
5	Gentamicin	10 µg	Oxoid
6	Kanamycin	30 µg	Oxoid
7	Nalidixic Acid	30 µg	Oxoid
8	Streptomycin	10 µg	Oxoid
9	Sulphonamides Compound	300 µg	Oxoid
10	Tetracycline	30 µg	Oxoid
11	Trimethoprim	5 µg	Oxoid

Labcode 2			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Ampicillin	10 µg	Biorad
2	Ceftiofur	30 µg	Biorad
3	Chloramphenicol	30 µg	Biorad
4	Enrofloxacin	5 µg	Bayer
5	Florfenicol	30 µg	Mast Diagnostics
6	Gentamicin	10 IU	Biorad
7	Nalidixic Acid	30 µg	Biorad
8	Neomycin	30 IU	Biorad
9	Streptomycin	10 IU	Biorad
10	Sulphonamides	200 µg	Biorad
11	Tetracycline	30 IU	Biorad
12	Trimethoprim/Sulphonamides	1.25+23.75 µg	Biorad

Labcode 3			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Amoxicillin + Clavulanate	2-32 µg/ml	Trek Diagnostic
2	Ampicillin	1-32 µg/ml	Trek Diagnostic
3	Apramycin	4-64 µg/ml	Trek Diagnostic
4	Ceftiofur	0,5-8 µg/ml	Trek Diagnostic
5	Chloramphenicol	2-64 µg/ml	Trek Diagnostic
6	Ciprofloxacin	0.03-4 µg/ml	Trek Diagnostic
7	Colistin	4-64 µg/ml	Trek Diagnostic
8	Florfenicol	2-64 µg/ml	Trek Diagnostic
9	Gentamicin	1-32 µg/ml	Trek Diagnostic
10	Nalidixic Acid	4-128 µg/ml	Trek Diagnostic
11	Neomycin	2-32 µg/ml	Trek Diagnostic
12	Spectinomycin	2-128 µg/ml	Trek Diagnostic
13	Streptomycin	4-64 µg/ml	Trek Diagnostic
14	Sulphamethoxazole	32-512 µg/ml	Trek Diagnostic
15	Tetracycline	2-32 µg/ml	Trek Diagnostic
16	Trimethoprim	4-32 µg/ml	Trek Diagnostic
17	Trimethoprim/Sulpha	1-8 µg/ml	Trek Diagnostic

Labcode 4			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Ampicillin	10 µg	Oxoid
2	Cefotaxime Sodium	30 µg	Oxoid
3	Chloramphenicol	30 µg	Oxoid
4	Ciprofloxacin	5 µg	Oxoid
5	Nalidixic Acid	30 µg	Oxoid
6	Streptomycin	10 µg	Oxoid
7	Sulphamethoxazole/Trimethoprim	25 µg	Oxoid
8	Tetracycline	30 µg	Oxoid

Labcode 5			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Amoxicilline	25 µg	Biorad
2	Amoxicillin + Clavulanate	20+10 µg	Biorad
3	Apramycine	15 µg	Elanco
4	Cefalotine	30 µg	Biorad
5	Cefoxitine	30 µg	Biorad
6	Ceftazidime	30 µg	Biorad
7	Chloramphenicol	30 µg	Biorad
8	Enrofloxacine	5 µg	Bayer
9	Gentamicin	10 UI/15µg	Biorad
10	Kanamycin	30 UI	Biorad
11	Nalidixic Acid	30 µg	Biorad
12	Oxolinic Acid	30 µg	Biorad
13	Streptomycine	10 UI/10 µg	Biorad
14	Tetracycline	30 UI	Biorad
15	Trimethoprim	5 µg	Biorad
16	Trimethoprim+Sulfa	1.25+23.75 µg	Biorad

Labcode 6			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Amoxicillin + Clavulanate	2/1-32/16 µg/ml	Trek Diagnostic
2	Ampicillin	1-32 µg/ml	Trek Diagnostic
3	Ceftiofur	0.5-8 µg/ml	Trek Diagnostic
4	Chloramphenicol	2-64 µg/ml	Trek Diagnostic
5	Ciprofloxacin	0.03-4 µg/ml	Trek Diagnostic
6	Colistin	4-64 µg/ml	Trek Diagnostic
7	Florfenicol	2-64 µg/ml	Trek Diagnostic
8	Gentamicin	1-32 µg/ml	Trek Diagnostic
9	Kanamycin	4-64 µg/ml	Trek Diagnostic
10	Nalidixic Acid	4-128 µg/ml	Trek Diagnostic
11	Neomycin	1-32 µg/ml	Trek Diagnostic
12	Spectinomycin	2-128 µg/ml	Trek Diagnostic
13	Streptomycine	4-64 µg/ml	Trek Diagnostic
14	Sulfamethoxazole	32-512 µg/ml	Trek Diagnostic
15	Sulfamethoxazole/Trimethoprim	19/1-152/8 µg/ml	Trek Diagnostic
16	Tetracycline	2-32 µg/ml	Trek Diagnostic
17	Trimethoprim	4-32 µg/ml	Trek Diagnostic

Labcode 7			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Amoxicillin + Clavulanate	20+10 µg	Biorad
2	Ampicillin	10 µg	Biorad
3	Chloramphenicol	30 µg	Biorad
4	Ciprofloxacin	5 µg	Biorad
5	Enrofloxacin	5 µg	Bayer
6	Gentamicin	10 µg	Biorad
7	Nalidixic Acid	30 µg	Biorad
8	Streptomycine	30 IU	Biorad
9	Sulphonamides	300 µg	Biorad
10	Tetracycline	30 µg	Biorad
11	Trimethoprim	5 µg	Biorad
12	Trimethoprim/Sulfamethoxazole	1.25-23.75 µg	Biorad

Labcode 8			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Amikacin	30 µg	Oxoid
2	Amoxycillin / Clavulanate	30 µg	Oxoid
3	Ampicillin	10 µg	Oxoid
4	Apramycin	15 µg	Oxoid
5	Cefoperazone	75 µg	Oxoid
6	Cefuroxime	30 µg	Oxoid
7	Chloramphenicol	30 µg	Oxoid
8	Ciprofloxacin	1 µg	Oxoid
9	Colistin Sulphate	25 µg	Oxoid
10	Enrofloxacin	5 µg	Oxoid
11	Furazolidone	15 µg	Oxoid
12	Gentamicin	10 µg	Oxoid
13	Kanamycin	30 µg	Oxoid
14	Nalidixic Acid	30 µg	Oxoid
15	Streptomycin	10 µg	Oxoid
16	Sulfamethoxazole/Trimethoprim	25 µg	Oxoid
17	Sulphonamides/Compound	300 µg	Oxoid
18	Tetracycline	30 µg	Oxoid

Labcode 9			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Amoxicillin + Clavulanate	20+10 µg	Becton Dickinson
2	Ampicillin	10 µg	Becton Dickinson
3	Cefalotin	30 µg	Becton Dickinson
4	Cefotaxine	30 µg	Becton Dickinson
5	Chloramphenicol	30 µg	Becton Dickinson
6	Ciprofloxacin	5 µg	Becton Dickinson
7	Colistin	10 µg	Becton Dickinson
8	Enrofloxacin	5 µg	Becton Dickinson
9	Gentamicin	10 µg	Becton Dickinson
10	Kanamycin	30 µg	Becton Dickinson
11	Nalidixic Acid	30 µg	Becton Dickinson
12	Neomycin	30 µg	Becton Dickinson
13	Streptomycin	10 µg	Becton Dickinson
14	Sulphanomides Triple	25 µg	Becton Dickinson
15	Tetracyclin	30 µg	Becton Dickinson
16	Trimethoprim/Sulfa	25+75 µg	Becton Dickinson

Labcode 10			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Ampicillin	33 µg	Rosco
2	Cephazolin	60 µg	Rosco
3	Chloramphenicol	60 µg	Rosco
4	Colistin	150 µg	Rosco
5	Enrofloxacin	10 µg	Rosco
6	Gentamicin	40 µg	Rosco
7	Marbofloxacin	5 µg	Rosco
8	Neomycin	120 µg	Rosco
9	Streptomycin	100 µg	Rosco
10	Sulphonamide	240 µg	Rosco
11	Tetracycline	80 µg	Rosco
12	Trimethoprim/Sulfa	5.2+240 µg	Rosco

Labcode 11			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Ampicillin	33 µg	Rosco
2	Chloramphenicol	60 µg	Rosco
3	Flumequine	30 µg	Rosco
4	Furazolidon	50 µg	Rosco
5	Neomycin	120 µg	Rosco
6	Tetracycline	80 µg	Rosco
7	Trimethoprim/Sulfa	5.2+240 µg	Rosco

Labcode 12			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Ampicillin	10 µg	AB Biodisk
2	Chloramphenicol	30 µg	AB Biodisk
3	Ciprofloxacin	10 µg	AB Biodisk
4	Doxycycline	30 µg	AB Biodisk
5	Nalidixic Acid	30 µg	AB Biodisk
6	Streptomycin	30 µg	AB Biodisk
7	Sulfisoxazole	250 µg	AB Biodisk
8	Tetracyclin	30 µg	AB Biodisk
9	Trimethoprim/Sulfa	1.2+23.8 µg	AB Biodisk

Labcode 13			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Amoxicillin + Clavulanate	30 µg	BioMerieux
2	Ampicillin	10 µg	Oxoid
3	Cefotaxim	30 µg	BioMerieux
4	Cefuroxim	30 µg	BioMerieux
5	Cephalotin	30 µg	BioMerieux
6	Chloramphenicol	30 µg	BioMerieux
7	Doxycyclin	30 µg	BioMerieux
8	Enrofloxacin	5 µg	Bayer
9	Flumequin	30 µg	Oxoid
10	Gentamicin	10 µg	BioMerieux
11	Kanamycin	30 µg	BioMerieux
12	Nalidixic Acid	30 µg	Oxoid
13	Neomycin	30 µg	BioMerieux
14	Streptomycin	10 µg	Oxoid
15	Tetracycline	30 µg	Oxoid
16	Trimethoprim+Sulfametoxazole	1.25+23.75 µg	BioMerieux

Labcode 14			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Amoxicillin + Clavulanate	30 µg	BioMerieux
2	Ampicillin	10 µg	BioMerieux
3	Cefalotin	30 µg	BioMerieux
4	Chloramphenicol	30 µg	BioMerieux
5	Ciprofloxacin	5 µg	BioMerieux
6	Flumequin	30 µg	BioMerieux
7	Gentamicin	10 µg	BioMerieux
8	Neomycin	30 µg	BioMerieux
9	Nitrofurantoin	300 µg	BioMerieux
10	Streptomycin	10 µg	BioMerieux
11	Tetracyclin	30 µg	BioMerieux
12	Trimethoprim/Sulfamethoxazole	1.25+23.75 µg	BioMerieux

Labcode 15			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Amoxicillin + Clavulanate	2/1+16/8 µg/ml	VetMIC™
2	Ampicillin	0.25-32 µg/ml	VetMIC™
3	Ceftiofur	0.25-2 µg/ml	VetMIC™
4	Chloramphenicol	2-16 µg/ml	VetMIC™
5	Enrofloxacin	0.03-4 µg/ml	VetMIC™
6	Florfenicol	2-16 µg/ml	VetMIC™
7	Gentamicin	0.25-32 µg/ml	VetMIC™
8	Nalidixic Acid	1-128 µg/ml	VetMIC™
9	Neomycin	1-128 µg/ml	VetMIC™
10	Streptomycin	2-256 µg/ml	VetMIC™
11	Sulfamethoxazole	64-512 µg/ml	VetMIC™
12	Tetracycline	0.5-64 µg/ml	VetMIC™
13	Trimethoprim	0.12-16 µg/ml	VetMIC™

Labcode 16			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Amoxicillin + Clavulanate	30 µg	Oxoid
2	Amikacin	30 µg	Oxoid
3	Ampicillin	10 µg	Oxoid
4	Apramycin	15 µg	Oxoid
5	Cefoperazone	30 µg	Oxoid
6	Ceftazidine	30 µg	Oxoid
7	Chloramphenicol	10 µg	Oxoid
8	Colistin Sulphate	25 µg	Oxoid
9	Furazolidone	15 µg	Oxoid
10	Gentamicin	10 µg	Oxoid
11	Nalidixic Acid	30 µg	Oxoid
12	Neomycin	10 µg	Oxoid
13	Streptomycin	25 µg	Oxoid
14	Sulphonamides Compound	300 µg	Oxoid
15	Tetracycline	10 µg	Oxoid
16	Trimethoprim+Sulfa	25 µg	Oxoid

Labcode 17			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Amoxicillin + Clavulanate	30 µg	Oxoid
2	Ampicillin	10 µg	Oxoid
3	Apramycin	15 µg	Oxoid
4	Ciprofloxacin	5 µg	Oxoid
5	Chloramphenicol	30 µg	Oxoid
6	Enrofloxacin	5 µg	Oxoid
7	Framycetin	100 µg	Oxoid
8	Furazolidone	50 µg	Oxoid
9	Gentamicin	10 µg	Oxoid
10	Kanamycin	30 µg	Oxoid
11	Nalidixic Acid	30 µg	Oxoid
12	Neomycin	10 µg	Oxoid
13	Spectinomycin	25 µg	Oxoid
14	Streptomycin	10 µg	Oxoid
15	Sulphonamides	300 µg	Oxoid
16	Tetracycline	10 µg	Oxoid
17	Trimethoprim	5 µg	Oxoid

Labcode A			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Amoxicillin + Clavulanate	20+10 µg	Biorad
2	Ampicillin	10 µg	Biorad
3	Cefalothin	30 µg	Biorad
4	Cefotaxim	30 µg	Biorad
5	Chloramphenicol	30 µg	Biorad
6	Ciprofloxacin	5 µg	Biorad
7	Gentamicin	10 µg	Biorad
8	Kanamycin	30 µg	Biorad
9	Nalidixic Acid	30 µg	Biorad
10	Streptomycin	10 µg	Biorad
11	Sulfonamides	300 µg	Biorad
12	Tetracycline	30 µg	Biorad
13	Trimethoprim	5 µg	Biorad
14	Trimethoprim/Sulfamethoxazole	1.25-23.75 µg	Biorad

Labcode B			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Ampicillin	10 µg	Oxoid
2	Cefotaxime	30 µg	Oxoid
3	Chloramphenicol	30 µg	Oxoid
4	Ciprofloxacin	5 µg	Oxoid
5	Gentamicin	10 µg	Oxoid
6	Imipenem	10 µg	Oxoid
7	Mecillinam	10 µg	Oxoid
8	Nalidixic Acid	30 µg	Oxoid
9	Streptomycin	10 µg	Oxoid
10	Sulfonamides	3 µg	Oxoid
11	Tetracycline	30 µg	Oxoid
12	Trimethoprim	5 µg	Oxoid

Labcode C			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Ampicillin	1-16 µg/ml	Bayer
2	Amikazin	2-32 µg/ml	Bristle Myers
3	Cefotaxim	1-16 µg/ml	Hoechst
4	Cefotiam	0.5-8 µg/ml	Grünenthal
5	Cefoxitin	0.5-32 µg/ml	MSD Sharpe Dolme
6	Ceftazidim	2-32 µg/ml	Cascan
7	Chloramphenicol	4-32 µg/ml	Cephasaas
8	Ciprofloxacin	0.063-64 µg/ml	Bayer
9	Gentamicin	0.5-8 µg/ml	Ratiopharm
10	Kanamycin	2-32 µg/ml	Ursapharm
11	Mezlocillin	2-32 µg/ml	Bayer
12	Mezlocillin/Sulfalactam	2-32 µg/ml	Pfizer
13	Nalidixic Acid	4-32 µg/ml	Sigma
14	Nourseothricin	2-16 µg/ml	H-Knöll Inst.Jena
15	Oxytetracycline	0.5-8 µg/ml	Basotherm
16	Streptomycin	4-64 µg/ml	Grünenthal
17	Sulfamerazin	32-512 µg/ml	Berlin
18	Trimethoprim/Sulfamerazin	4-128 µg/ml	Berlin

Labcode E			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Ampicillin	10 µg	Oxoid
2	Cefotaxime	30 µg	Oxoid
3	Ceftazidime	30 µg	Oxoid
4	Chloramphenicol	30 µg	Oxoid
5	Ciprofloxacin	5 µg	Oxoid
6	Gentamicin	10 µg	Oxoid
7	Kanamycin	30 µg	Oxoid
8	Minocycline	30 µg	Oxoid
9	Nalidixic Acid	30 µg	Oxoid
10	Nitrofurantoin	300 µg	Oxoid
11	Spectinomycin	100 µg	Oxoid
12	Streptomycin	10 µg	Oxoid
13	Sulphonamides Compound	300 µg	Oxoid
14	Tetracycline	30 µg	Oxoid
15	Trimethoprim	5 µg	Oxoid

Labcode H			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Ampicillin	50 µg/ml	Sigma
2	Cefotaxime	1 µg/ml	Sigma
3	Chloramphenicol	20 µg/ml	Sigma
4	Ciprofloxacin	0.5 µg/ml	Hospital Pharmacy
5	Ciprofloxacin	0.125 µg/ml	Hospital Pharmacy
6	Furazolidone	20 µg/ml	Sigma
7	Gentamicin	20 µg/ml	Sigma
8	Kanamycin	20 µg/ml	Sigma
9	Nalidixic Acid	40 µg/ml	Sigma
10	Netilmicin	20 µg/ml	Hospital Pharmacy
11	Spectinomycin	100 µg/ml	Sigma
12	Streptomycin	20 µg/ml	Sigma
13	Sulphamethoxazole	100 µg/ml	Sigma
14	Tetracycline	10 µg/ml	Sigma
15	Trimethoprim	2 µg/ml	Sigma

Labcode J			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Ampicillin	10 µg	Oxoid
2	Cefotaxime	30 µg	Oxoid
3	Cephalotine	30 µg	Oxoid
4	Chloramphenicol	30 µg	Oxoid
5	Ciprofloxacin	5 µg	Oxoid
6	Gentamicin	10 µg	Oxoid
7	Kanamycin	30 µg	Oxoid
8	Nalidixic Acid	30 µg	Oxoid
9	Streptomycin	10 µg	Oxoid
10	Sulphonamides Compound	300 µg	Oxoid
11	Tetracycline	30 µg	Oxoid
12	Trimethoprim/Sulfa	25 µg	Oxoid

Labcode R			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Ampicillin	10 µg	Becton-Dickinson
2	Cefotaxim	30 µg	Becton-Dickinson
3	Chloramphenicol	30 µg	Becton-Dickinson
4	Ciprofloxacin	5 µg	Becton-Dickinson
5	Gentamicin	10 µg	Becton-Dickinson
6	Kanamycin	30 µg	Becton-Dickinson
7	Nalidixic Acid	30 µg	Becton-Dickinson
8	Streptomycin	10 µg	Becton-Dickinson
9	Sulfonamides	0.25 mg	Becton-Dickinson
10	Tetracycline	30 µg	Becton-Dickinson
11	Trimethoprim/Sulfamides	1.25-23.75 µg	Becton-Dickinson

Labcode T			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Ampicillin	0.25-32 µg/ml	MAST
2	Ampicillin/Sulbactam	0.5-64 µg/ml	MAST
3	Cefotaxim	0.125-16 µg/ml	MAST
4	Ceftazidime	0.125-16 µg/ml	MAST
5	Chloramphenicol	0.25-32 µg/ml	MAST
6	Ciprofloxacin	0.032-4 µg/ml	MAST
7	Co-trimoxazole	1-128 µg/ml	MAST
8	Gentamicin	0.25-32 µg/ml	MAST
9	Kanamycin	0.5-64 µg/ml	Sigma
10	Nalidixic Acid	1-64 µg/ml	MAST
11	Streptomycin	0.5-64 µg/ml	Sigma
12	Sulfamethoxazol	4-256 µg/ml	MAST
13	Tetracycline	0.25-32 µg/ml	MAST
14	Trimethoprim	0.25-32 µg/ml	MAST

Labcode V			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Ampicillin	10 µg	Becton-Dickinson
2	Cefotaxim	30 µg	Becton-Dickinson
3	Chloramphenicol	30 µg	Becton-Dickinson
4	Ciprofloxacin	5 µg	Becton-Dickinson
5	Gentamicin	10 µg	Becton-Dickinson
6	Kanamycin	30 µg	Becton-Dickinson
7	Nalidixic Acid	30 µg	Becton-Dickinson
8	Streptomycin	10 µg	Becton-Dickinson
9	Sulfonamides	250 µg	Becton-Dickinson
10	Tetracycline	30 µg	Becton-Dickinson
11	Trimethoprim	5 µg	Becton-Dickinson

Labcode W			
Nrs.	Names antibiotics	Concentration	Manufacturer
1	Ampicillin	33 µg	Rosco
2	Apramycin	40 µg	Rosco
3	Ceftiofur	30 µg	Rosco
4	Chloramphenicol	60 µg	Rosco
5	Ciprofloxacin	10 µg	Rosco
6	Fosfomycin	70+40 µg	Rosco
7	Gentamicin	40 µg	Rosco
8	Kanamycin	100 µg	Rosco
9	Mecillinam	33 µg	Rosco
10	Nalidixic Acid	130 µg	Rosco
11	Nitrofurantoin	260 µg	Rosco
12	Polymyxin	150 µg	Rosco
13	Spectinomycin	200 µg	Rosco
14	Streptomycin	100 µg	Rosco
15	Sulphonamides	240 µg	Rosco
16	Tetracycline	80 µg	Rosco
17	Trimethoprim	5.2 µg	Rosco