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

Collaborative study VII (2002) on typing of *Salmonella*

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Abstract

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

A seventh 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, United Kingdom. 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 ENL. The results obtained by these three NRL-ENL laboratories will only be evaluated in conjunction with the results of the 17 NRLs for *Salmonella*. In total, 20 strains of the species *Salmonella enterica* subspecies *enterica* were selected for serotyping 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. Almost all laboratories faced only minor differences in phage typing. Some ENLs had considerable problems with the phage typing of STM as well as SE strains. A questionnaire held among the NRLs revealed that for comparison between laboratories, standardisation of the antimicrobial susceptibility testing would have to be a requirement.

Contents

Samenvatting

Het Communautair Referentie Laboratorium voor *Salmonella* (CRL-*Salmonella*, Bilthoven, Nederland) heeft een zevende ringonderzoek voor de typering van *Salmonella* georganiseerd in samenwerking met de Public Health Laboratory Services (PHLS, Colindale) in Londen. Voor de geïnteresseerde laboratoria bestond de mogelijkheid om ook faagtypering 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 11 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.

In totaal werden 20 stammen van het species *Salmonella enterica* subspecies *enterica* door het CRL-*Salmonella* geselecteerd. Deze stammen moesten door elk laboratorium getypeerd worden met de methode die zij routinematig toepassen. Ook mochten de laboratoria de stammen voor serotypering opsturen naar een ander gespecialiseerd laboratorium in hun land. De meeste problemen werden gevonden bij het typeren van de H-antigenen.

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). Drie EnterNet Labs typeerden 60% of minder van zowel de SE als de STM stammen correct. De overige laboratoria hadden minder problemen met het typeren van het faag type.

In dit rapport worden ook de resultaten van een questionnaire over antimicrobiële gevoeligheidsbepalingen behandeld. Alle NRLs hebben deze questionnaire ingevuld. De vragen hadden betrekking op kwaliteitscontrole, dichtheid en grootte van het inoculum, incubatie periodes en temperaturen, media en het aflezen en interpreteren van de resultaten. Voor de kwantitatieve Minimal Inhibition Concentration (MIC) testmethode werden minimale verschillen aangetoond. Belangrijke verschillen werden gevonden voor de kwalitatieve of semi-kwantitatieve disk diffusie methode. Deze verschillen betreffen zaken als dichtheid van de cultuur, het aantal disks per plaat en de interpretatie van de resultaten. De uitkomsten van deze questionnaire laat zien dat verdere standaardisatie van de methode van antimicrobiële gevoeligheidsbepalingen vereist is alvorens resultaten tussen labs te kunnen vergelijken.

Summary

A seventh 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 Services (PHLS, Colindale) in London.
Laboratories that were interested had the possibility to perform phage typing. 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 11 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. A total of 20 strains of the species *Salmonella enterica* subspecies *enterica* were selected by the CRL-*Salmonella*. 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 PHLS selected 20 strains for phage typing, 10 were of the serovar *Salmonella* Enteritidis (SE) and 10 of the serovar *Salmonella* Typhimurium (STM). Three EnterNet Labs typed 60% or less of both the SE and STM strains correctly. The other laboratories faced less problems with the typing of the phage type.
In this report also the results of a questionnaire about antimicrobial susceptibility testing are included. All NRLs willingly contributed by responding to this questionnaire. Questions related to: quality control, inoculum density and size, incubation periods and temperatures, media and reading and interpretation of the results. For the quantitative Minimal Inhibition Concentration (MIC) testing minor differences were noticed. Considerable differences were obtained for the qualitative or semi-quantitative disk diffusion testing. These differences occured in density of the inoculum, the number of disks on one plate and the interpretation of the results. The outcome of the questionnaire revealed data showing that further standardisation of the technique is required before being able to compare results between laboratories.

1. Introduction

In this report the seventh 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. The history of the various collaborative typing studies starting in 1995 is shown in Table 1.

Table 1. History of collaborative typing studies

Study NRLs	Study ENLs	Year	Serotyping of *Salmonella enterica* strains	Phage typing	Antibiotic resistance testing	Reference
I		1995	*spp. enterica* 18 *spp. salamae* 1 *spp. houtenae* 1			Voogt et al., 1996 (report 284500004)
II		1996/ 1997	*spp. enterica* 20			Voogt et al., 1997 (report 284500008)
III		1998	*spp. enterica* 20	SE 4 STM 5		Voogt et al., 1998 (report 284500010)
IV	I	1999	*spp. enterica* 16	SE 10 STM 10		Raes et al., 2000 (report 284500013)
V	II	2000	*spp. enterica* 18 *spp. salamae* 1 *spp. houtenae* 1	SE 10 STM 10	YES	Raes et al., 2001 (report 284500016)
VI	III	2001	*spp. enterica* 19 *spp. arizonae* 1	SE 10 STM 10	YES	Korver et al., 2002 (report 284500020)
VII	IV	2002	*spp. enterica* 20	SE 10 STM 10		This report

Seventeen NRLs-*Salmonella* and fifteen EnterNet Laboratories (ENLs) participated in the seventh 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 11 ENLs performed phage typing on 10 *Salmonella* Enteritidis and 10 *Salmonella* Typhimurium strains.

For reasons of standardisation in typing 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 (chapter 4). Another questionnaire, about antimicrobial susceptibility testing, is also included in this report (chapter 6).

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) Brussels	NRL	
Belgium	Institute Scientifique de Santé Publique – Louis Pasteur Brussels		ENL
Canada	National Laboratory for Enteric Pathogens Canadian Science Centre for Human and Animal Health - Winnipeg		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 Kuopio Department Kuopio	NRL	
Finland	National Public Health Institute (KTL) Laboratory of Enteric Pathogens, Helsinki		ENL
France	Agence française de sécurité sanitaire des aliments (AFSSA), Laboratoire d'études et de recherches avicoles et porcines (LERAP), Ploufragan	NRL	
France	Unité 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
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) Bilthoven	NRL	ENL

Country	Institute/City	National Reference Laboratory for *Salmonella* (NRL) or EnterNet Laboratory (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 de Sanidad Y Produccion Animal 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
United Kingdom	Veterinary Laboratories Agency Weybridge Department of Bacterial Diseases New Haw, Addlestone	NRL	
United Kingdom	Laboratory of Enteric Pathogens Public Health Laboratory Service (PHLS) London		ENL

3. Materials and Methods

3.1 *Salmonella* strains for serotyping

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 2.

Table 2. Antigenic formulas of the 20 Salmonella *strains according to the Kauffmann-White scheme*

No	Serovar	O antigens	H antigens	Origin of strains
1	*S.* Typhimurium	1, 4, [5], 12	i : 1,2	Human
2	*S.* Jangwani	17	a : 1, 5	Human
3	*S.* Kapemba	9, 12	l, v : 1, 7	Human
4	*S.* Brandenburg	4, [5], 12	l, v : e, n, z15	Human
5	*S.* Enteritidis	1, 9, 12	g, m : -	Human
6	*S.* Paratyphi B var. Java	1, 4, [5], 12	b : 1, 2	Chicken
7	*S.* Bareilly	6, 7, 14	y : 1, 5	Chicken
8	*S.* Manhattan	6, 8	d : 1, 5	Human
9	*S.* Give	3, 10, [15] [15, 34]	[d], l, v : 1, 7	Chicken
10	*S.* Paratyphi B var. Java	1, 4, [5], 12	b : 1, 2	Chicken
11	*S.* Vinorahdy	28	m, t : [e, n, z15]	Environment
12	*S.* Derby	1, 4, [5], 12	f, g : [1, 2]	Pig
13	*S.* London	3, 10, [15]	l, v : 1, 6	Pig
14	*S.* Adelaide	35	f, g : -	Pig
15	*S.* Bovismorbificans	6, 8, 20	r, [i] : 1,5	Human
16	*S.* Oranienburg	6, 7, 14	m, t : [z57]	Soy
17	*S.* Llandoff	1, 3, 19	z29 : [z6]	Pig
18	*S.* Stanley	1, 4, [5], 12, 27	d : 1,2	Human
19	*S.* Agona	1, 4, [5], 12	f, g, s : [1, 2]	Pig
20	*S.* Kedougou	1, 13, 23	i : l, w	Pig

3.2 *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 Services (PHLS, London, UK). 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	**11**	-	-	scl	-	cl	-	+	ol	-	ol	+	cl	±	-	-	++ +
E2	**9a**	-	-	cl	-	cl	±	cl	ol	-	ol	cl	cl	cl	-	±	cl
E3	**34**	-	-	-	-	-	-	-	ol	-	ol	-	-	-	-	-	-
E4	**4**	-	scl	cl	scl	cl	scl	cl	ol	ol	ol	cl	cl	cl	-	-	-
E5	**1**	ol	scl	cl	scl	cl	scl	cl	ol	ol	ol	cl	cl	cl	cl	-	-
E6	**6**	-	scl	-	scl	-	scl	-	ol	ol	ol	-	-	-	-	-	-
E7	**5c**	-	scl	±	scl	scl	scl	+	ol	ol	ol	±	scl	scl	-	-	-
E8	**1b**	ol	scl	cl	scl	cl	scl	cl	ol	ol	ol	cl	cl	cl	cl	cl	cl
E9	**6a**	-	scl	-	scl	-	scl	-	-	ol	-	-	-	-	-	-	-
E10	**5a**	-	scl	-	scl	cl	scl	±	-	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	66	-	-	-	-	-	-	-	-	cl	ol	-	-	-	-	scl	-	-	-
M12	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M13	10	-	-	-	-	-	-	-	-	cl	ol	cl	cl	-	-	scl	-	-	-
M14	141	-	-	-	++	++	+	-	-	scl	+++	+	+	-	scl	scl	-	-	scl
M15	104(L)	-	-	-	-	-	-	-	-	-	-	++	scl	-	-	-	-	+++	-
M16	1	ol	scl	ol	ol	cl	cl	cl	-	cl	ol	ol	ol	cl	cl	cl	cl	cl	cl
M17	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M18	104(H)	-	-	-	-	-	-	-	-	-	-	cl	cl	-	-	-	-	scl	-
M19	U310	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M20	124	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

		Phages at Routine Test Dilution												Additional phages					
QA No.	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10 var	18
M11	66	cl	-	ol	cl	-	±	±	-	-	cl	cl	-	±	±	+	ol	ol	-
M12	22	-	-	ol	-	-	-	-	-	-	-	-	-	+++	+++	+++	-	-	-
M13	10	cl	-	ol	cl	-	-	±	-	-	cl	cl	-	+	+	+	ol	ol	-
M14	141	scl	-	ol	cl	-	+++	+	cl	-	cl	scl	ol	+++	+++	++	ol	ol	+++
M15	104(L)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ol	ol	-
M16	1	cl	ol	ol	cl	cl	cl	cl	cl	±	cl	cl	ol	++	++	++	ol	ol	ol
M17	193	-	-	-	-	-	-	-	-	-	-	-	-	+++	+++	+++	-	-	-
M18	104(H)	-	-	-	-	-	-	-	±	-	-	-	±	-	-	-	ol	ol	-
M19	U310	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ol	-
M20	124	-	-	-	-	-	-	-	-	ol	-	-	-	-	-	+	±	±	-

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

3.3 Laboratory codes

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

3.4 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**

3.5 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 Services
PT	Phage Type
RIVM	Rijksinstituut voor Volksgezondheid en Milieu
S.	*Salmonella*
SE	*Salmonella* Enteritidis
STM	*Salmonella* Typhimurium
TSI	Triple Sugar Iron
XLD	Xylose Lysine Desoxycholate
var.	variant

4. Questionnaire sero- and phage typing

4.1 General questions

Question A: Was the package damaged at arrival ?

All packages were received in a perfect state and no damage occurred during transport.

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

All NRLs except for one laboratory (labcode 11) received their parcel within the same week (week 16) as the samples were sent. The laboratory with labcode 11 received the parcel in the beginning of the week following the shipment of the parcels. The phage typing strains were sent directly from PHLS, London to all participating NRLs in week 17.
Two EnterNet Laboratories received their samples for sero- and phage typing in week 16, three in week 22, seven in week 23, one in week 25, one in week 27 and another in week 29.

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

Seventeen laboratories (10 NRLs and 7 ENLs) kept the strains at a temperature between 3°C and 8°C (Figure 1). Several laboratories kept them between 20°C and 22°C (1 NRL and 2 ENLs). Six NRLs and six ENLs directly subcultured the *Salmonella* strains upon arrival.

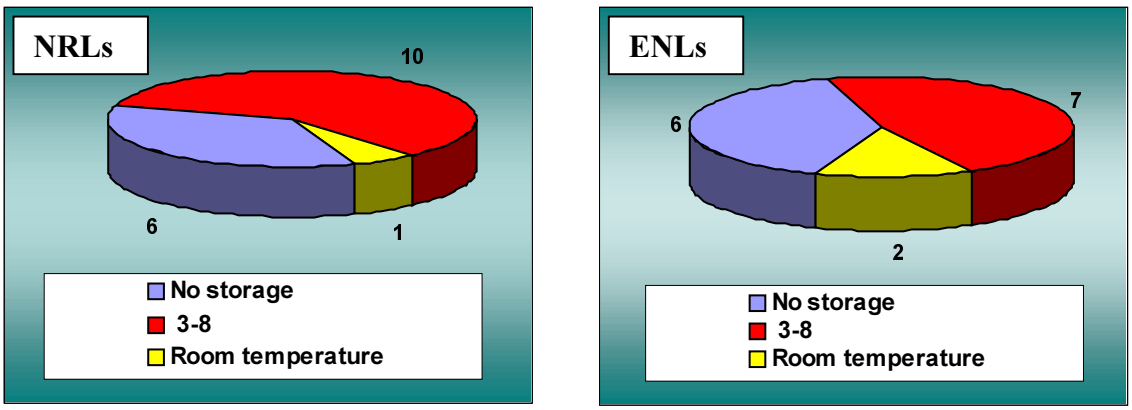

Figure 1. Storage of the strains before subculturing in degrees Centigrade

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

In this report only the time-interval between arrival at the laboratory and the date of subculturing is mentioned.
Six NRLs (1, 5, 6, 10, 13 and 14) subcultured their strains directly after arrival. NRLs 2, 3, 4, 7, 8, 9, 11, 12, 15, 16 and 17 subcultured the *Salmonella* strains from one to thirteen days after arrival.
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 (April, May, June and July).
One laboratory received the parcel in April (W), two laboratories (A and H) at the end of May 2002, nine (B, C, D, F, J, K, P, T and Z) in June 2002 and two (R and Y) in July 2002. One lab (E) did not mention the date of receipt of the parcel and two ENLs did not mention the date of subculturing (K and P).

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. Two NRLs and five ENLs subcultured the strains on a nutrient agar, three NRLs on blood agar, one NRL and two ENLs on tryptic-soy medium and two NRLs and three ENLs on MacConkey medium.
Furthermore a total of 16 different media were mentioned by the NRLs and ENLs. Among these media they mentioned agar, BAB, BGA, Brolac, Bromcresol agar, Gassner agar, Tryptose agar, Plate count agar, TSI, Lactose agar, Swarm agar, Drigalski, Conrad agar, Endo agar, XLD and Dorset Egg medium as their media of choice.
One NRL and one ENL did not answer this question.

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

Sixteen (16) NRLs and eleven (11) ENLs stored the original strains after subculturing. Two ENLs did not store the strains at all and from one NRL and two ENL no data are available. Thirteen (13) NRLs and four (4) ENLs stored the strains at a temperature between 3^{o}C and 8^{o}C. Three NRLs and four ENLs kept the strains at room temperature (18-20^{o}C), one ENL at minus 20^{o}C and two ENLs at minus 70^{o}C.

4.2 Questions regarding content.

Question 1: What was the frequency of serotyping at your laboratory in 2001 ?
Question 2: How many strains did your lab serotype in 2001 ?

Table 6. Frequency and number of strains serotyped in 2000

Labcode NRLs	Typing frequency	Number of strains typed in 2001	Labcode ENLs	Typing frequency	Number of strains typed in 2001
1	Daily	3,650	A	Daily	10,783
2	Daily	1,500	B	Daily	2,500
3	Weekly	1,700	C	Daily	10,062
4	Daily	14,264	D	Twice a week	990
5	Daily	5,500	E	Daily	1,032
6	Weekly	7,000	F	Monthly	200
7	Daily	1,063	H	Daily	4,500
8	Daily	10,700	J	Daily	9,640
9	Weekly	859	K	Thrice a week	5,000
10	At arrival	???	P	Daily	> 8,000
11	Daily	700	R	Daily	412
12	Daily	3,500	T	Daily	980
13	Weekly	29	W	Daily	2,600
14	Daily	400	Y	Daily	1,000
15	Daily	1,199	Z	Daily	6,909
16	Daily	2,797			
17	Daily	1,249			

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	7	6
From 2 manufacturers	4	2
From 3 manufacturers	4	2
From 4 manufacturers	2	1
From 5 manufacturers	0	1
Preparation own laboratory	5	5

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

Name Manufacturer	Number of NRLs (n=17)	Number ENLs (n=12)
Biorad (= Sanofi = Pasteur)	7	5
Biostat	1	0
Dade Behring	3	2
Denka Seiken	0	1
Difco	3	0
Eurobio	1	1
Imuna (Slovakia)	0	1
MAST	0	1
Murex-Abbott	3	3
Prolab Diagnostic	3	1
Reagensia AB (Sweden)	1	2
Sevapharma (Czech Republic)	0	1
SIFIN (Germany)	4	2
SSIC (Staten Serum Institute Copenhagen)	9	5

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

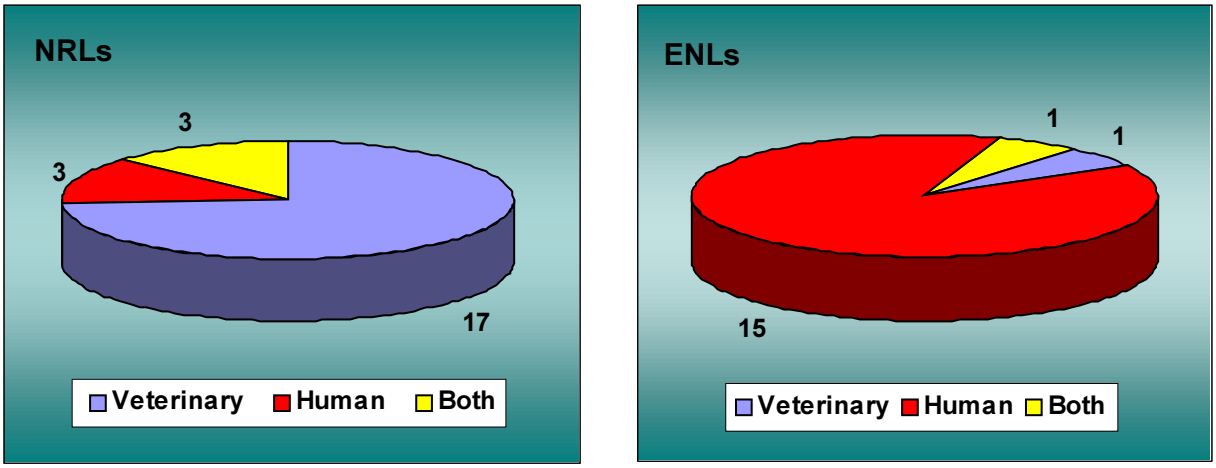

Figure 2. Number of NRLs and ENLs being reference lab for veterinary and/or human Salmonella *strains*

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

Two NRLs-*Salmonella* (labcodes 4 and 12) sent respectively one and four 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 of other strains ?

Seven NRLs and eleven ENLs performed phage typing of *S.* Typhimurium and/or *S.* Enteritidis strains. Four NRLs and six ENLs also phage typed other strains like *S.* Bovismorbificans, *S.* Derby, *S.* Hadar, *S.* Heidelberg, *S.* Infantis, *S.* Newport, *S.* Panama, *S.* Paratyphi B, *S.* Thompson, *S.* Typhi, *S.* Virchow.

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

Four NRLs (2, 5, 8, 16) and eight ENLs (B, E, H, J, K, P, T, Y) used for both kind of strains the Colindale system.
Two NRLs (1, 4) and one ENL (C) mentioned Anderson for the Typhimurium system and Ward for the Enteritidis system, respectively. Two ENLs mentioned PHLS for both kind of strains. The Anderson and Ward systems are the same as the Colindale or PHLS system. One NRL (labcode 6) 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 2001 ?

Table 9. Number of phage typings and their relationship to the serotyping in 2001

Laboratory codes	Sero typing	Phage typing
1	3,650	2,056
2	1,500	500
4	14,264	4,393
5	5,500	750
6	7,000	2,500
8	10,700	8,700
16	2,797	296
A	10,783	830
B	2,500	1,136
C	10,062	7,994
E	1,032	581
F	200	200
H	4,500	1,984
J	9,640	???
K	5,000	3,177
P	> 8,000	1,259
T	980	600
Y	1,000	2,008

5. Results

5.1 Serotyping by the NRLs-*Salmonella*

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. Thirteen laboratories (labcode 1, 2, 4, 5, 6, 8, 9, 10, 11, 12, 14, 16 and 17) typed all O antigens correctly. Six laboratories (labcode 1, 4, 7, 10, 11 and 13) identified all H antigens correctly and 5 laboratories (labcode 1, 4, 5, 6 and 11) identified all serovar names correctly.

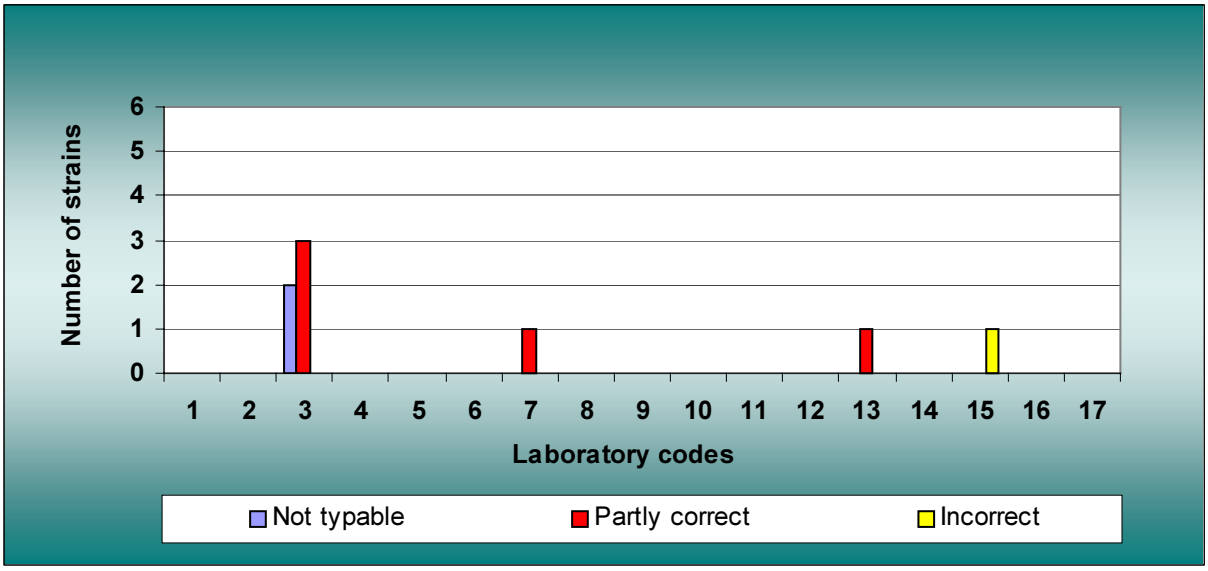

Figure 3. Evaluation of serotyping of O-antigens per NRL

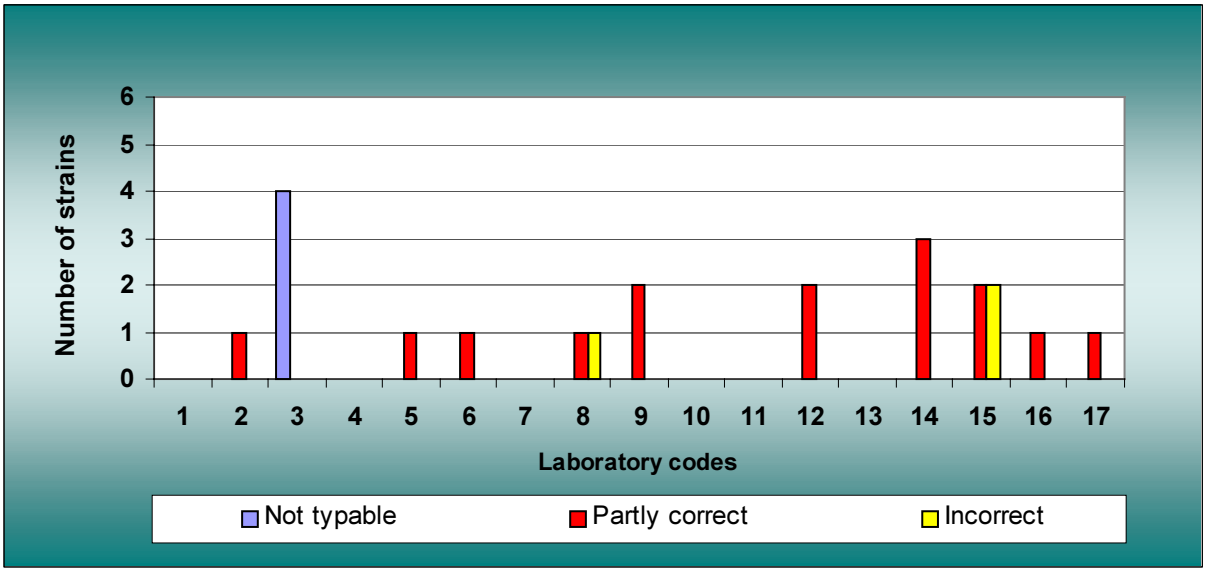

Figure 4. Evaluation of serotyping of H-antigens per NRL

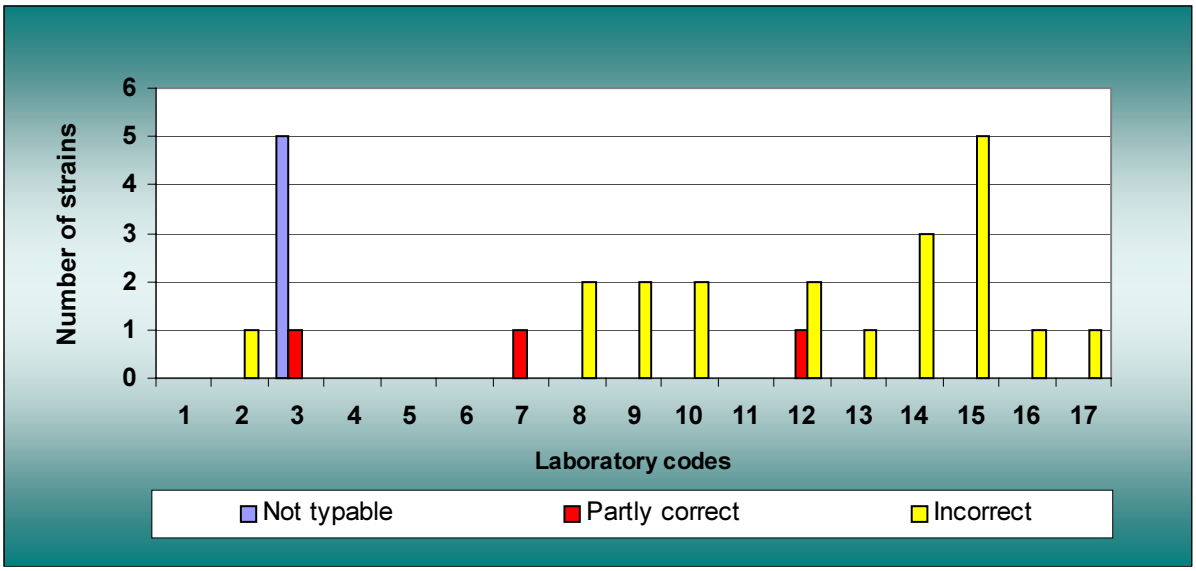

Figure 5. Evaluation of the correctness of serovar names per NRL

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 10. The O-antigens of 13 strains were typed correctly by all participants. The H-antigens were typed correctly for 10 strains by all participants. Problems arose with strains *S.* Paratyphi B (strains 6 and 10), *S.* Vinohrady (strain 11) and *S.* Oranienburg (strain 16). These four strains were given the correct serovar name by respectively 14, 13, 10 and 10 participants. A total correct identification by all participants was obtained for 9 strains (*S.* Typhimurium, *S.* Bareilly, *S.* Manhattan, *S.* Give, *S.* Derby, *S.* London, *S.* Bovismorbificans, *S.* Llandoff and *S.* Agona). Therefore these strains are not mentioned in Table 10. Two identical strains (strain 6 and strain 10) were typed slightly different by a number of laboratories.

Table 10. Evaluation of serotyping per strain for NRLs

Strain		O antigen detected				H antigen detected				Name serovar			
No.	Serotype	+	nt	+/-	-	+	nt	+/-	-	+	nt	+/-	-
2	*S.* Jangwani	16	-	1	-	16	1	-	-	16	1	-	-
3	*S.* Kapemba	17	-	-	-	16	-	1	-	17	-	-	-
4	*S.* Brandenburg	17	-	-	-	16	-	-	1	16	-	-	1
5	*S.* Enteritidis	16	-	1	-	17	-	-	-	16	-	-	1
6	*S.* Paratyphi B	16	1	-	-	14	1	1	1	14	1	-	2
10	*S.* Paratyphi B	16	1	-	-	13	1	3	-	13	1	-	3
11	*S.* Vinohrady	16	-	1	-	12	-	4	1	10	1	2	4
14	*S.* Adelaide	16	-	1	-	16	1	-	-	16	1	-	-
16	*S.* Oranienburg	17	-	-	-	13	-	4	-	10	-	1	6
18	*S.* Stanley	17	-	-	-	15	-	2	-	15	-	-	2
20	*S.* Kedougou	16	-	-	1	16	-	1	-	16	-	-	1

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

The characterisations that caused major problems in serotyping by the NRLs are shown in Table 11. 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. Laboratories having problems with the detection of the H antigen: g, which should be absent in strains 11 (*S.* Vinohrady) and/or 16 (*S.* Oranienburg) were asked, in a separate mail sent to their

institute, to answer some important questions about the use of monovalent (i.e. g-monovalent) or polyvalent (i.e.G-complex) antisera, the manufacturer of these sera and quality control measures.

Table 11. Identifications per strain that caused major problems in serotyping by NRLs

	Strain 6	Strain 10	Strain 11	Strain 16
Correct typing	*S.* Paratyphi B 1, 4, [5], 12 ; b: 1, 2	*S.* Paratyphi B 1, 4, [5], 12 ; b: 1, 2	*S.* Vinohrady 28 ; m, t : [e, n, z15]	*S.* Oranienburg 6, 7, 14 ; m, t : [z57]
Labcode 2				*S.* Winston 6, 7 ; m, t : 1, 6
Labcode 3	Auto agglutination	Auto agglutination	??? OMC : m, t	O 6, 7 ; m, t 6, 7 ; m, t
Labcode 5			*S.* Vinohrady 28 ; g, m, t : -	
Labcode 7			OMC : m, t : -	
Labcode 8	*S.* Kingston 1, 4 ; s, t ; -	*S.* Abony 1, 4 ; b : x		
Labcode 9			*S.* 28 : g, m, t: - 28 ; g, m, t : -	*S.* Othmarschen 6, 7 ; g, m, t : -
Labcode 10			??? 28 ; m, t : -	??? 6, 7 ; m, t : -
Labcode 12	*S.* Uppsala 4, 5, 12 ; b : 1, 7		*S.* Vinohrady/ Morillons 28 ; m, t : -	*S.* Montevideo 6, 7 ; g, m, t : -
Labcode 14			??? 28 ; g, m, t : -	*S.* Othmarschen 6, 7 ; g, m, t : -
Labcode 15			*S.* Techimani 28 ; c, z6	*S.* ??? 6, 7 : m, t
Labcode 16		*S.* Heidelberg 1, 4, 12 ; r : 1, 2		
Labcode 17		*S.* Limete 4, 12, 27 : b : 1,5		

Strains 6 and 10 were named *S.* Paratyphi B by respectively seven and nine NRLs, *S.* Paratyphi B var. Java by resp. five and three NRLs and *S.* Java by resp. two and one laboratory.

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.
Eleven ENLs (A, B, C, D, E, H, J, K, T, W and Z) typed all O-antigens correctly. Two laboratories with labcodes F and Y detected the O-antigens from respectively two and one strain partly correct. Laboratory P had difficulties in characterising the O-antigens from two strains caused by auto-agglutination.
Nine ENLs (A, B, C, H, K, R, W, Y and Z) typed all H-antigens correctly. Four laboratories (D, E, J and T) typed the H-antigens for one or more strains partly correct. One incorrect identification was obtained by ENL with labcode F.
Seven laboratories namely D, E, F, J, R, T and Y used an incorrect serovar name for one or more serovars.

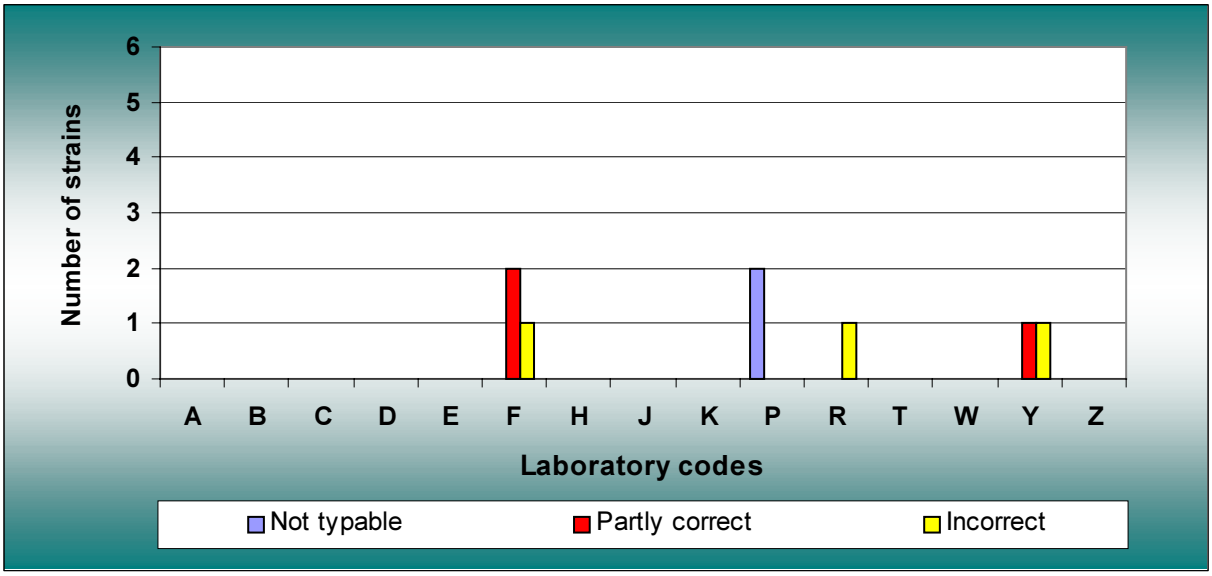

Figure 6. Evaluation of serotyping of O-antigens per ENL

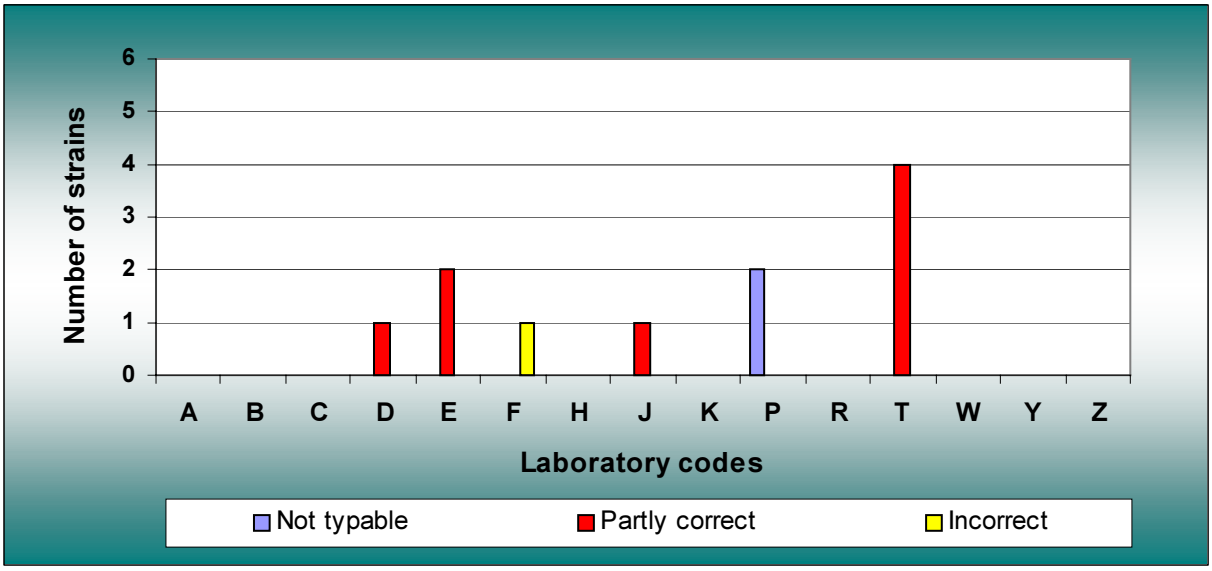

Figure 7. Evaluation of serotyping of H-antigens per ENL

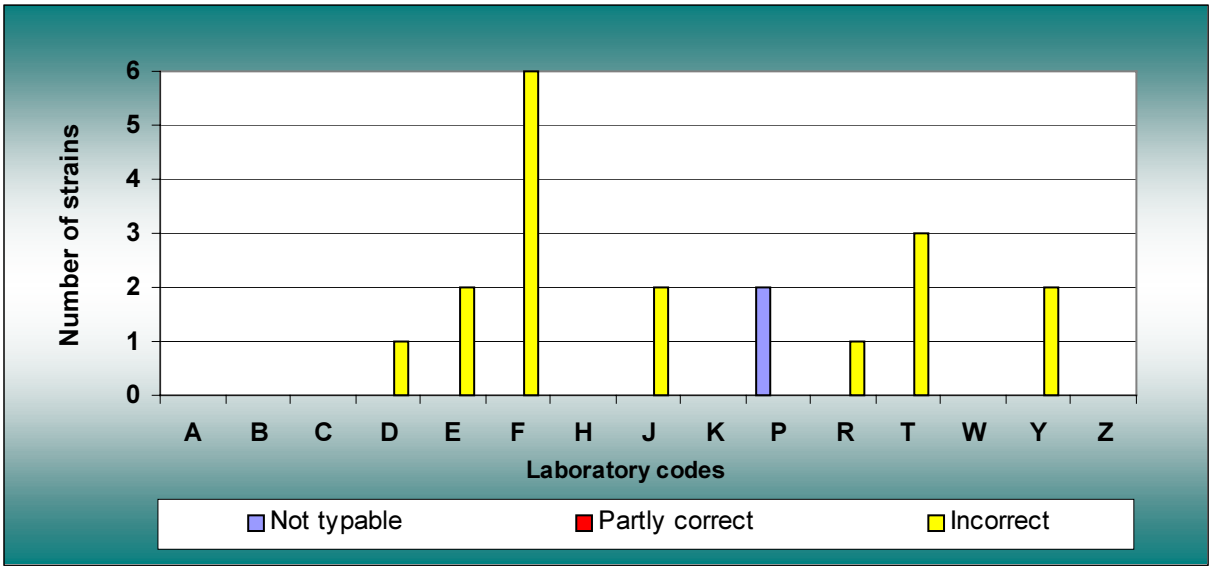

Figure 8. Evaluation of the correctness of serovar names per ENL

5.2.2 Evaluation per strain

Strains *S.* Typhimurium (strain 1), *S.* Jangwani (strain 2), *S.* Kapemba (strain 3), *S.* Brandenburg (strain 4), *S.* Enteritidus (strain 5), *S.* Bareilly (strain 7), *S.* Manhattan (strain 8), *S.* Give (strain 9), *S.* Derby (strain 12), *S.* Stanley (strain 18), *S.* Agona (strain 19) and *S.* Kedougou (strain 20) were all typed correctly by all ENLs and are therefore not mentioned in Table 12.
Concerning the typing of the O-antigens incorrect identification was obtained for three strains (one strain of *S.* Paratyphi B var. Java, one strain of *S.* Vinohrady and one strain of *S.* Adelaide). Two strains of the same serovar *S.* Paratyphi B var. Java were not typable by one laboratory due to auto-agglutination. An incorrect identification of the H-antigens only occurred for one of the two strains belonging to *S.* Paratyphi B var. Java. Seven strains were typed partly correct (see table 12). Strains that caused major problems (strains 6, 10, 11 and 16) for some laboratories are shown in Table 14. Incorrect identification is shown in red.

Table 12. Evaluation of serotyping per strain for ENLs

		O antigen detected				H antigen detected				Name serovar			
No	**Serovar**	**+**	**nt**	**+/-**	**-**	**+**	**nt**	**+/-**	**-**	**+**	**nt**	**+/-**	**-**
6	*S.* Paratyphi B var. Java	13	1		1	13	1	1		12	1		2
10	*S.* Paratyphi B var. Java	14	1			11	1	2	1	12	1		2
11	*S.* Vinohrady	14			1	13		2		10			5
13	*S.* London	15				14		1		14			1
14	*S.* Adelaide	14			1	15				14			1
15	*S.* Bovismorbificans	15				14		1		14			1
16	*S.* Oranienburg	15				13		2		12			3
17	*S.* Llandoff	15				13		2		13			2

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

The characterisations that caused major problems in serotyping by the NRLs are shown in Table 13. The empty cells in this table indicate that strains were typed correctly by the laboratories mentioned. Incorrect identification is shown in red in this table. Laboratories having problems with the detection of the H antigen: g, which should be absent in strains 11 (*S.* Vinohrady) and/or 16 (*S.* Oranienburg) were asked, in a separate e-mail sent to their institute, to answer some important questions about the use of monovalent (i.e. g-

monovalent) or polyvalent (i.e.G-complex) antisera, the manufacturer of these sera and quality control measures.
Strains 6 and 10 were named S. Paratyphi B by respectively two and one ENLs. S. Paratyphi B var. Java by resp. seven and seven ENLs and S. Java by resp. three and three laboratories.

Table 13. Identifications per strain that caused major problems in serotyping by ENLs

	Strain 6	Strain 10	Strain 11	Strain 16
Correct typing	*S.* Paratyphi B 1, 4, [5], 12 ; b: 1, 2	*S.* Paratyphi B 1, 4, [5], 12 ; b: 1, 2	*S.* Vinohrady 28 ; m, t : [e, n, z15]	*S.* Oranienburg 6, 7, 14 ; m, t : [z57]
Labcode D		*S.* Haifa 4, 12 ; z10: 1, 2		
Labcode E			*S.* ?? 28 ; g, m, t : -	*S.* Othmarschen 6, 7 ; g, m, t : -
Labcode F	*S.* Paratyphi 2 ; b: 1, 2	*S.* Kimuenza 4, 27; lv, e, n, x	*S.* ?? 28 ; m, t : -	*S.* ?? 6, 7 ; m, t : -
Labcode J	*Spp. II* 4, 12 ; b : ---		*Spp. II* 28 ; m, t : -	
Labcode P	*Spp. enteritica* Auto-aggl.	*Spp. enteritica* Auto-aggl.		
Labcode T			*S.* ?? 28 ; g, m, t : -	*S.* Othmarschen 6, 7 ; g, m, t : -
Labcode Y			*S.* Bama 17 ; m, t : -	

5.3 Results phage typing

5.3.1 Results phage typing by the NRLs-*Salmonella*

The phage typing results of the NRLs were evaluated per strain and by laboratory and are shown in Tables 14 and 15. Seven laboratories peformed phage typing. None of the laboratories assigned the correct phage type for all *S*. Enteritidis (SE) strains. One laboratory (labcode 16) assigned all the *S*. Typhimurium (STM) strains correctly. Four strains of SE (PT 4, 6, 1b and 6a) and four strains of STM (PT66, 104L, 1 and 124) were typed correctly by all laboratories. Separate notations per phage and per laboratory are given in Appendix 4.

Table 14. Results of Salmonella *Enteritidis phage typing by the NRLs*

		Laboratory codes						
Strain	**PT**	**1**	**2**	**4**	**5**	**6**	**8**	**16**
E1	11	11	11	11	9a	11	11	11
E2	9a	9a	9a	9a	31	9a	9a	9a
E3	34	34	34b	34	34	34	34	34b
E4	4	4	4	4	4	4	4	4
E5	1	1	1	1	1	1	1	1c
E6	6	6	6	6	6	6	6	6
E7	5c	5c	5c	5b	5b	ARS	RDNC	5b
E8	1b	1b	1b	1b	1b	1b	1b	1b
E9	6a	6a	6a	6a	6a	6a	6a	6a
E10	5a	6b	5a	6b	6b	5a	5a	5a

PT = Phage type

Table 15. Results of Salmonella *Typhimurium phage typing by the NRLs*

		Laboratory codes						
Strain	**PT**	**1**	**2**	**4**	**5**	**6**	**8**	**16**
M11	66	66	66	66	66	NT	66	66
M12	22	193	22	22	22	NT	107	22
M13	10	10	10	10	10	NT	67	10
M14	141	141	141	68	4	NT	141	141
M15	104(L)	104L	104L	104	104	NT	104L	104L
M16	1	1	1	1	1	NT	1	1
M17	193	193	194	193	193	NT	193	193
M18	104(H)	104H	104H	104	U308	NT	104H	104H
M19	U310	193	U310	U310	U310	NT	U310	U310
M20	124	124	124	124	124	NT	124	124

PT = Phage Type NT = Not Tested

5.3.2 Results phage typing by the ENLs

The phage typing results of the ENLs are summarized in Tables 16 and 17. Eleven laboratories performed phage typing. Four of the ENLs (labcode C, E, H and Y) assigned all the *S*. Enteritidis strains the correct phage type and two laboratories (labcode E and H) also assigned all the *S*. Typhimurium strains correctly. Two strains of SE (PT6 and 1b) and two strains of STM (PT10 and 104L) were correctly identified by all ENLs. Three ENLs (F, P and T) had a relatively low correct score for all twenty strains of respectively 50%, 55% and 35%.

Table 16. Results of Salmonella *Enteritidis phage typing by the ENLs*

		Laboratory codes										
Strain	**PT**	**A**	**B**	**C**	**E**	**F**	**H**	**J**	**K**	**P**	**T**	**Y**
E1	11	11	11	11	11	9a	11	11	11	9a	8	11
E2	9a	9a	9a	9a	9a	41	9a	RDNC	9a	41	1c	9a
E3	34	34	34	34	34	25	34	34	34	13a	34b	34
E4	4	4	4	4	4	4	4	4	4	4	4b	4
E5	1	1	1	1	1	1b	1	1	1	1	31	1
E6	6	6	6	6	6	6	6	6	6	6	6	6
E7	5c	5b	5b	5c	5c	27	5c	5b	5b	5b	5	5c
E8	1b	1b	1b	1b	1b	1b	1b	1b	1b	1b	1b	1b
E9	6a	6a	6a	6a	6a	6a	6a	6a	6a	6a	35	6a
E10	5a	4a	5a	5a	5a	6b	5a	5a	5a	5a	6b	5a

Table 17. Results of Salmonella *Typhimurium phage typing by the ENLs*

		Laboratory codes										
Strain	**PT**	**A**	**B**	**C**	**E**	**F**	**H**	**J**	**K**	**P**	**T**	**Y**
M11	66	66	66	66	66	66	66	66	69	66	8	66
M12	22	193	193	22	22	193	22	22	193	U302	RDNC	22
M13	10	10	10	10	10	10	10	10	10	10	10	10
M14	141	U296	141	68	141	141A	141	4	141	68	141	4
M15	104(L)	104L	104	104L	104L	104L	104L	104L	104	104H	104	104
M16	1	1	1	1	1	36	1	1	1	1	1	1
M17	193	193	193a	193	193	193	193	193	193	193	208	193
M18	104 (H)	104H	104	104H	104H	104H	104H	104H	104	104C	104	104
M19	U310	U310	U310	U310	U310	208	U310	195	U310	208	U302	U310
M20	124	124	124	195	124	124	124	NT	NT	NT	U	124

6. Questionnaire antimicrobial susceptibility testing (AST)

6.1 Introduction

In Newsletter Vol.5 No.4 (December 1999) the NRLs-*Salmonella* were asked to fill in a postal survey. One of the items of this survey was antibiotic resistance typing. Questions were:

1. Would you be interested in typing of the resistance patterns of the strains of the next typing study ?
2. How many resistance patterns did your laboratory type in 1998 and 1999 ?
3. Which antibiotics do you use for resistance pattern typing and from which manufacturer are they ?

Most NRLs were interested in performing antibiotic resistance pattern typing
(Newsletter Vol.6 No.1 / March 2000).

In the 5th collaborative study (2000) CRL-*Salmonella* offered the possibility to perform antibiotic resistance pattern typing on the strains for serotyping. All NRLs-*Salmonella* and 10 EnterNet Laboratories (ENLs) typed the resistance patterns of the strains using their own method. Two NRLs and two ENLs used a quantitative method and all other laboratories used a disc diffusion method. The number of antibiotics used per NRL varied from 7 to 18 and per ENL from 11 to 23 (Raes et al., 2001).

During the preparation of the report (Korver et al., 2002) on the 6th collaborative study (2001) CRL-*Salmonella* decided to decrease the number of antibiotics in the tables of the report. The diversity of the various antibiotics (52 in total) 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. Furthermore, antimicrobial susceptibility testing revealed data showing that standardisation and harmonisation of the technique would be required to allow for comparison between laboratories.

At the Seventh Workshop in Ploufragan (28 May 2002) it was decided to include a questionnaire on antimicrobial susceptibility testing in one of the next newsletters. A summary of the answers to the questionnaire is given in chapters 6.1 and 6.2. After analysing the data of the questionnaire and carefull discussion with experts in the field, CRL-*Salmonella* will present a new plan for the eighth collaborative study of 2003.

6.2 Minimal Inhibitory Concentration testing (MIC)

A total of five NRLs-*Salmonella* (labcode 1, 4, 6, 10 and 11) are performing the MIC testing on a regular basis in their laboratories. One laboratory (labcode 13) did not fill in the questionnaire because they wanted to switch from disk diffusion to MIC testing this year, i.e. 2002.

General information

Question 1: Why did your laboratory chose for the MIC testing instead of the disk diffusion method ? (advantages of the test, disadvantages of the test, flexibility, costs, time to perform, etc.)

Among others MIC testing was chosen to get quantitative results. The introduction of this kind of test improves the reproducibility of the results in different member states significantly. The method is relatively cheap and robust, is not laborious and does not need expensive equipment. The MIC testing reduces methodological problems, can easily be standardised and the time needed for the test is of no importance.
Further advantages over the disk diffusion are that complex quality control and control of stock solutions of antibiotics used in agar dilution are not necessary and the quality of the Muller Hinton Broth in the diffusion method may greatly affect the results of some antibiotics. The ready-to-use trays for MIC testing can be stored at room temperature and have a long shelf life. The test needs specific expertise, which is easily built up during practice. One laboratory mentioned that the MIC values are the basis for further, molecular investigations to the resistance problem (resistance mechanisms, resistance genes, expression of the genes, etc).
Three NRLs (labcode 1, 4 and 6) received their trays for MIC testing from Trek Diagnostics LTD (UK) and another two (labcode 10 and 11) from VetMIC™ (Sweden).

Quality control

Question 2: According to what guidelines (system) do you read the MIC ?

All five NRLs are using the NCCLS (National Committee for Clinical Laboratory Standards) guidelines but sometimes national guidelines are being used.

Question 3: Do you use (a) control strain(s) ?

Each of the five NRLs performing MIC testing uses one or more control strains.

Question 4: What is/are the name(s) of your control strain(s) ?

Strain E.coli (ATCC 25922) is being used by all five NRLs. Two of them (labcode 4 and 6) are using three additional control strains. These strains are E.faecalis (ATCC 29212), S.aureus (ATCC 29213) and P.aeruginosa (ATCC 27853). One laboratory (labcode 11) tests besides E.coli ((ATCC 25922) another E.coli strain namely ATCC 35218.

Question 5: Do you include the control strain(s) each time you test susceptibility ?

Three NRLs (labcodes 1, 6 and 11) are including control strains each time of testing.

Question 6: How do you store your control strain(s) ?

Three laboratories (labcode 1, 4 and 6) store their working batches of control strains at minus 80^{o}C and the others at minus 70^{o}C. The laboratory with labcode 6 lyophilises their control strains and after lyophilisation store these strains at –20^{o}C. Laboratory 11 stores the original batch of the control strain in liquid nitrogen.

Inoculum

Question 7: What is the size of your inoculum in organisms per ml ?

Among the five laboratories the size of the inoculum differs from 2.5 x 10^4 till 5.5 x 10^5 cfu/ml.

Question 8: How do you measure the density of your inoculum ? What method ?

All laboratories use the McFarland scale, but three laboratories (labcode 1, 4 and 6) calibrate their nephelometer against a 0.5 McFarland scale unit.

Incubation

Question 9: What is the incubation time of your plates ?

All NRLs incubate their plates/trays overnight, i.e. 16-22 hours

Questions 10: What is the incubation temperature of your plates ?

All laboratories incubate their plates at a temperature of 37°C.

Reading of the results

Question 11: In what way do you read the results ?
(by eye or with an automatic reader). Please describe your method.

Two laboratories (labcode 4 and 6) read their results with a semi-automatic reader (Sensi-touch). The laboratory with labcode 1 transfers a fluorogenic substance to the medium. Its counts are determined after 18 hours of incubation and automatically transferred to a MIC-value. Two laboratories (labcode 10 and 11) read the plates by eye with the aid of a magnifying mirror.

Interpretation of results

All NRLs were asked to fill in tables in which they could indicate which MIC value in µg/ml for each antibiotic belongs to so-called resistant, intermediate or sensitive test results.
Furthermore each laboratory could indicate the breakpoint in MIC-value for each of the antibiotics. The laboratory with labcode 10 only mentioned the test range without mentioning anything about resistant, intermediate or sensitive test results. The MIC is registered as the lowest concentration of the antimicrobial that inhibits visible bacterial growth.
In figure 9. an example is given for ampicillin (AMP). The figures with the MIC values, breakpoints and notations resistant, intermediate or sensitive of all other antibiotics are given in Appendix 5.

	MIC in ug/ml for ampicillin (AMP)														
Labcode	.03	.06	.12	.25	.50	1	2	4	8	16	32	64	128	256	512
1											B				
4											B				
6															
10															
11										B					

Sensitive | Resistant | Test range
Intermediate | Not tested | B = Breakpoint

Figure 9. MIC-values and breakpoints for ampicillin used by several NRLs

6.3 Disc Diffusion testing

Eleven NRLs (labcodes 2, 5, 7, 8, 9, 12, 13, 14, 15, 16 and 17) perform the disk diffusion test to measure the inhibition against a variety of antimicrobials. They all filled in the questionnaire which was sent to them by e-mail.

General information

Question 1: Why did your laboratory chose for the disk diffusion method instead of the MIC testing? (advantages of the test, disadvantages of the test, flexibility, costs, time to perform, etc.)

Several NRLs are performing the disk diffusion test for historical or traditional reasons and mention that most technicians are familiar with the method and well trained in performing the test. One NRL (labcode 7) would be open to change to MIC if the CRL-*Salmonella* could provide them with a standardised protocol. Some laboratories never compared disk diffusion with MIC testing. The relative simplicity of disk diffusion testing was mentioned by four laboratories (labcode 9, 13, 14 and 16). The cost effectiveness and the short time for the performance of the test was mentioned by respectively five and four laboratories. It seems that the choice for the disk diffusion is also influenced by national regulations.

Question 2: The NRLs were asked to include the manufacturer of the separate antibiotics in Table 2 of the questionnaire.

The names of the manufacturers of the antibiotics and the number of NRLs using these antibiotics are shown in Table 18.

Table 18. NRLs using antibiotic discs or tablets from the following manufacturers

Name manufacturer	Number of NRLs	Labcodes
AB Biodisc	1	16
Becton Dickinson	1	2
BioMérieux	2	7 and 14
Biorad	2	9 and 12
Oxoid	3	5, 8 and 17
Rosco	1	13
Unknown	1	15

Quality control

Question 2: According to what guidelines (system) do you read the inhibition zones ?

Eight NRLs are reading their inhibition zones according to the NCCLS (National Committee for Clinical Laboratory Standards) guidelines. Several laboratories are using national standards like VLA Internal Guidelines, CASFM (French Committee for antibiograms from the french society of microbiology) or AFA (Norwegian Working Group on Antibiotics).

Question 3: Do you use (a) control strain(s) ?

Eight NRLs utilise control strains when testing inhibition zones. Three laboratories do not use any control strain for their quality control.

Question 4: What is/are the name(s) of your control strain(s) ?

Seven NRLs use E.coli ATCC 25922 as their control strain. The NRL with labcode 2 also applies S.aureus ATCC 25923 and the laboratory with labcode 5 uses E.coli NCTC 10418.

Question 5: Do you include the control strain(s) each time you test susceptibility ?

Two NRLs (labcode 2 and 16) include control strains at least once a week. Five NRLs (labcode 5, 8, 14, 15 and 17) include control strains each time they test susceptibility. One laboratory (labcode 9) does not include a control strain each time they test antimicrobial susceptibility.

Question 6: How do you store your control strain(s) ?

Most laboratories store their control strain(s) at a temperature between minus 70^{o}C - 80^{o}C. Some of the NRLs store the control strain(s) on Dorset Egg Slopes at room temperature or at +4^{o}C.

Medium

Question 7: What is the name of the agar medium you use in your laboratory ?
Question 8: What is the name of the manufacturer of your medium ?

Mueller Hinton Agar medium (from Becton Dickinson, Merck, Oxoid, Biorad, BioMérieux or Difco) is used by eight NRLs-*Salmonella.* Two laboratories mention Iso-Sensitest Agar from Oxoid as their medium of choice. One laboratory mentions PDM II-agar Antibiotic Sensitivity Medium from AB-Biodisk.

Question 9: Do you receive together with the medium a certificate of analysis from the manufacturer ?

Eight NRLs receive a certificate of analysis together with their medium. Two laboratories answered this question with a negative answer.

Question 10: Is the agar medium you use prepared in your own laboratory ?

Only three NRLs (labcode 8, 12 and 14) prepare the medium in their own laboratory.

Question 11: What is the diameter of your plates in mm ?
Question 12: How many milliliters of medium does one plate contain ?
Question 13: What is the thickness/depth of the agar in the plate ?
Question 14: What is the maximum number of discs you transfer to one plate ?

Ten NRLs (see Table 19) are using round plates. Nine of them use plates with a diameter of 85-90 mm. The volume in the 85-90 mm plates varies from 15 – 25 ml medium.
Another difference is the number of disks on these plates which varies from 3 till 9 antibiotic disks.

Table 19. Variables of disks diffusion testing

	Round plates				Squarred plates			
Labcode	**Size in mm**	**Volume in ml**	**Depth of agar**	**Number of disks**	**Size in mm**	**Volume in ml**	**Depth of agar**	**Number of disks**
2					**120x120**	**???**	**4 mm**	**16**
5	**85**	**15 (?)**	**5 mm**	**8**				
7	**150**	**40**	**~4 mm**	**12**				
8	**90**	**~20**	**~4 mm**	**6**				
9	**90**	**25**	**~4 mm**	**6**				
12	**90**	**17**	**4 mm**	**3**	**140x140**	**30**	**4 mm**	**15**
13	**90**	**???**	**5 mm**	**7**	**125x125**	**???**	**7 mm**	**12**
14	**90**	**20**	**~5 mm**	**9**				
15	**90**	**16**	**4 mm**	**6**				
16	**90**	**25**	**3.5-4.5**	**6**				
17	**90**	**20**	**3.0-3.5**	**6**				

Inoculum

Question 15: Describe the way you transfer the inoculum to the plate ?

Most NRLs (labcode 2, 7, 8, 9, 14, 15 and 17) transfer the inoculum to the plate with a sterile swab which is dipped into an adjusted suspension and remove superfluous inoculum by pressing the swab to the edge of the plate. Others rub the swab against the agar surface by rotating the plate three times 60 degrees. In three laboratories (labcode 5, 12 and 16) the medium is flooded with a certain inoculum of the bacterial suspension and immediately pipetting of the excess of the supernatant. Sometimes drying time (which is absolutely necessary) is employed before the discs are transferred to the plates.

Question 16: What is the size of your inoculum in bacteria per ml ?

The size of the inoculum varies considerably among the eleven NRLs performing disc diffusion testing. Two laboratories (labcode 2 and 9) mentioned that they never calculated the density of their inoculum. Two NRLs (labcode 5 and 12) took a certain amount of a 2-3 hours incubated culture of *Salmonella* in diluent or pepton salt broth. The density of other NRLs are as follows: laboratory 7: 1.5 x 10^8 cfu/ml; laboratory 8: 10^5 – 10^6 cfu/ml; laboratory 13: 1.5 x 10^8 cfu/ml; laboratory 14: 10^8 cfu/ml; laboratory 16: 10^5 cfu/ml and laboratory 16: 1-2 x 10^8 cfu/ml.

Question 17: How do you measure the density of your inoculum ? What method ? (McFarland scale, nephelometric, spectrophotometric or otherwise)

Six NRLs (labcode 7, 9, 13, 15, 16 and 17) measure the density of the Salmonella culture by using the McFarland scale. One laboratory (labcode 8) did not answer this question, while another laboratory (labcode 12) does not measure the density of the inoculum. The laboratory with labcode 5 does the measurement by eye, laboratory 2 by using a nephelometer and laboratory 14 by using a densitometer.

Incubation

Question 18: What is the incubation time of your plates ?
Question 19: What is the incubation temperature of your plates ?

The time necessary for the incubation of the plates is in most cases between 18-24 hours. Some NRLs mention overnight as their incubation time. Ten laboratories have an incubation temperature of 37^{o}C. One laboratory (labcode 16) incubates the plates at a temperature of 35^{o}C.

Reading of the results

Question 20: In what way do you read the results ? (by eye or with an automatic reader). Please describe your method.

The NRLs with labcodes 5, 7, 8, 9, 13, 14, 16 and 17 read the size of the inhibition zones by eye using a ruler. Only three laboratories (labcode 2, 12 and 15) are reading their results with an automated device.

Interpretation of results

NRLs were asked to enter data in the tables in the questionnaire in which they could indicate the inhibition zones in mm for each of the twelve antibiotics and the breakpoints used in their country. In Figure 10. An example is shown for streptomycin (STR). The figures of all other antibiotics are given in Appendix 6.

		Inhibition zones in mm for streptomycin (STR)																			
Labcode	ug/ml	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2	10					B	B	B	B	B											
5	25							B													
7	10	?	?	?	?	?															
8	10					B	B	B	B	B											
9	10	?	?	?	?	?															
12	10						B	B	B	B											
13	100																				B
14	10					B	B	B	B	B											
15	10																				
16	30							B	B	B	B	B	B								
17	10					B															

Sensitive | Resistant | B = Breakpoint

Intermediate | Not tested

Figure 10. Inhibition zones in mm and breakpoints for streptomycin used by NRLs

7. Discussion

Serotyping

For the NRLs as well as ENLs most of the O-antigens were typed correctly. Two strains (strain 6 and 10) were subcultured from the same tube at CRL-*Salmonella* and were typed differently by 4 NRLs and by three ENLs. Both strains belong to serovar *S.* Paratyphi B var. Java. Strains 11 and 16 belonging to *S.* Vinohrady and *S.* Oranienburg respectively, could only be named after a serovar if biochemical tests were carried out. For *S.* Vinohrady five NRLs and four ENLs did not perform these tests indicating they could not assign serovar names to this strain. For *S.* Oranienburg the number of laboratories were three NRLs and one ENL. From the reactions of the separate mail sent to those laboratories having problems in serotyping *S.* Vinohrady and *S.* Oranienburg it may be concluded that these problems are attributed to unfamiliarity with the separate parts of polyvalent antisera (i.e. G, 1, L and Z4), non-specific reactions of monovalent antisera (i.e. g-antiserum) and the discriminating capacity of mixed antisera (i.e. gm-antiserum or gp-antiserum).
For some NRLs the detection of the H-antigens is still the most frequently occurring problem. The achievements in percentages correctness of the serotyping of each of the NRLs and CRLs are shown in Appendix 7.

Phage typing

The strains selected for this study were representative of those occurring in the European Union.
The *S.* Enteritidis strain giving most difficulty was PT5c being confused mainly with PT5b. Only two NRLs and four ENLs correctly identified this type. These types may be combined when the scheme is revised as there are only subtle differences between the two types. *S.* Enteritidis PT5c is a fairly new phage type, being first reported in England & Wales in 2001 and in Scotland in 2000. The type is linked to foreign travel particularly to Teneriffe and was implicated in three egg related outbreaks in England during 2001.
Problems were also encountered with strain E10, PT5a being identified as PT6b. In this case the majority of laboratories had the correct readings for PT5a obtaining a high reading with phage 5.
In the collaborative study VI (2001) , *S.* Enteritidis PT11 was the problem strain being incorrectly identified as PT9a by a number of participants. To aid in the differentiation between PT9a and PT11 both types were included in the 2002 collaborative study. The results were encouraging with seven of the ENLs and all but one of the NRLs identifying both types correctly.
S. Typhimurium strains M12 (PT22) and M14 (PT141) produced most problems. PT22 is dependant on the lysis of phage 22. However a number of laboratories obtained no lysis of this phage when typing strain M12. This result is difficult to explain as the phage had behaved correctly producing lysis when typing other strains of the test panel (a replacement phage will be sent where necessary). For strain M14 (PT141) a number of laboratories

obtained the correct pattern of lysis with the phages but had incorrectly interpreted the data. Some laboratories had missed the readings with phages 4 and 5 that require magnification to be seen clearly.
A new provisional type of *S.* Typhimurium PTU310 was included for the first time. The type requires a new extra typing phage (10 variant) for its identification. Although the majority of laboratories would not have typed strains of PTU310 before, the results were very encouraging. Five NRLs and seven ENLs typed this strain (M19) correctly. *S.* Typhimurium PTU310 is predominately a pig strain and is usually resistant to tetracyclines.
The achievements in percentages correctness of the phage typing of each of the NRLs and CRLs are shown in Appendix 7.

Questionnaire antimicrobial susceptibility testing

In collaborative studies V and VI the susceptibility of strains against a panel of antibiotics was tested. In both studies 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.
The NRLs were asked to fill in a questionnaire with all kind of questions about a variety of subjects. The MIC testing did not encounter important differences. This quantitative method can easily be standardised. For the qualitative or semi-quantitative disk diffusion method considerably more differences were found in a number of variables in the test procedure. The most important variables for which standardisation is needed are the use of control strains, the volume of medium per plate (differed from 15 to 25 ml per 85-90 mm plate), the number of disks per plate (differed from 3 to 9 per plate), the way in which the inoculum was transferred to the plate and the size of the inoculum.

8. 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. Having experience by typing on a regular basis is apparently essential to get better results.

Antisera for the use of serotyping *Salmonella* strains should be ordered from manufacturers producing certified products. CRL-*Salmonella* will investigate whether the manufacturers mentioned in this report meet this criterium.

Phage typing

Overall the results were satisfactory. The problems identified were partly due to the interpretation of the patterns of lysis. This will improve with experience, as was shown with the correct typing of *S.* Enteritidis PT11 when compared with PT9a. The introduction of the new phage type PTU310 was successful, with twelve laboratories getting the correct results. It is essential that all the laboratories continue to use identical phage preparations with the standardised methods. This will ensure correct and consistent results.

Antimicrobial 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. 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. Standardisation is needed for the following variables:

- Use of control strains;
- Use of Mueller Hinton medium as medium of choice;
- Volume of medium per plate;
- Maximum number of disks per plate to avoid overlapping inhibition zones;
- The way in which the inoculum should be transferred to the plate;
- The time of pre-diffusion;
- Size of inoculum and the way this should be measured.

Acknowledgments

We are grateful to all the contact persons from the National Reference Laboratories for Salmonella in the Member States of the European Union and the EnterNet laboratories. We are also very grateful to Dr. A.J. de Neeling for his helpful comments on the antimicrobial susceptibility testing part of this manuscript. Drs. Kirsten Mooijman is thanked for her critical reading of the manuscript.

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Appendix 1 Protocol of the collaborative study

PROTOCOL OF THE COLLABORATIVE STUDY (VII, 2002) ON SERO- AND PHAGE TYPING OF SALMONELLA STRAINS ORGANISED BY CRL-SALMONELLA

Introduction

The Community Reference Laboratory (CRL)-Salmonella organises the seventh collaborative typing study of *Salmonella* strains amongst the National Reference Laboratories for Salmonella (NRLs-Salmonella) and EnterNet laboratories (ENLs).

The main objective of this typing study is to compare the test results of sero- and phage typing of the participating laboratories with the results obtained by the CRL-Salmonella.

The performance of the study will take place in week 18 (starting on 29 April 2002) or one week earlier or later.

All data will be reported in the study report, send to the CRL-Salmonella and will be used for analysis. The data on phage typing will be send to CRL-Salmonella and to Linda Ward, PHLS, London.

The CRL-Salmonella has decided not to include the antibiotic resistance pattern typing in this study. This antibiotic resistance pattern typing will be discussed with several experts into detail and the CRL will present a new plan for the Collaborative Study of next year.

Transportation of the *Salmonella* strains

CRL will mail the parcels with the strains by cargo freight from Schiphol Airport (The Netherlands) to the airport of destination. The participants have to collect the parcels at their airport. To be able to collect the parcel from the airport you need the airway bill number. This number and other important information will be mentioned in a fax which will be send to you one week (=week 15) before mailing the parcels.

The transport costs from the airport of destination to the laboratory can not be paid by the CRL, so this will be at the expense of the participant.

Serotyping

In this study a total number of 20 *Salmonella* strains, supplied by the CRL, are tested. The method routinely performed in your laboratory will be used in this study. A NRL is allowed to send strains for serotyping to another reference laboratory in their country.

Phagetyping

Optionally the laboratories will receive a parcel containing 20 *Salmonella* cultures (supplied by PHLS, London) for phage typing:

- 10 strains of *S.* Enteritidis numbered E1-E10
- 10 strains of *S.* Typhimurium numbered M1-M10

Evaluation

The results will be evaluated by the CRL. 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 serotyping results will be performed according to Table 1.

Table 1: Guidelines for evaluation

Results	Evaluation	Abbreviation
Autoagglutination or Incomplete set of antisera (outside range of antisera)	Not typable	NT
Partly typable due to incomplete set of antisera or Part of the formula (for the name of the serovar) or No name serovar	Partly correct	+/-
Wrong serovar or mixed sera formula	Incorrect	-

The evaluation of the phage typing results will be done in collaboration with Linda Ward, PHLS, London.

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

Linda R.Ward
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Laboratory of Enteric Pathogens
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Timetable of the collaborative study on sero- and phage typing(VII, 2002)

Week	Date	Topic
11	11-15 March	Mailing of the protocol and test report 2002
15	8-12 April	The airway bill number and other important information will be mentioned in a fax which will be send to you in this week. Checking the presence of all necessary reagents and materials for the performance of the study
16	15-19 April	Mailing the strains to the participants. After arrival at the laboratory the strains need to be subcultured and stored until the performance of the typing. If the parcel has not arrived at the airport on 19 April, please do contact the CRL immediately.
18	29 April-3 May	Starting with the identification of the strains.
20	13-17 May	Completion of the test report. Sending of the complete report to the CRL by fax or e-mail. The original test report will be send to CRL by mail. Send the results of the phage typing also to PHLS, London. Keep a copy for your own information
21	21-25 May	A printed version of the individual results will be send to all NRLs. Checking of the results on this printed version will be done by the NRLs-Salmonella.
	June-September	Analysis and reporting of the results by CRL.

As an example the phage typing protocol from PHLS is included

Salmonella phage typing protocol from PHLS (London).

1. Media

1.1 Double strength nutrient broth

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

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

1.2 Nutrient agar

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

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 a-sceptically 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 (VII, 2002)
ORGANISED BY CRL SALMONELLA

TEST REPORT

COLLABORATIVE TYPING STUDY OF *SALMONELLA* STRAINS

(SEVENTH FOR THE NATIONAL REFERENCE LABORATORIES AND FOURTH FOR THE ENTERNET LABORATORIES)

Laboratory code	
Laboratory name	
Address	
Country	
Date of collecting the parcel	…… - …… - 2002
Starting date typing	…… - …… - 2002

PLEASE WRITE YOUR REMARKS AND COMMENTS ON PAGE 7 OF THE TEST REPORT!

GENERAL QUESTIONS

Shipment	
Was your parcel damaged at arrival ?	❑ NO ❑ YES
Date of receipt at your laboratory	…… - …… - 2002
Time of receipt at your laboratory	…… h …… - min
Did you store the strains before subculturing?	❑ NO ❑ YES, temperature:……^{o}C

Subculturing	
Date the strains were subcultured	…… - …… - 2002
Medium used for subculturing the strains	Name…….. Manufacturer……..
Did you store the strains after subculturing ?	❑ NO ❑ YES, temperature:……^{o}C

QUESTIONS SEROTYPING

What was the frequency of serotyping of *Salmonella* at your laboratory in 2001 ?	❑ Daily ❑ Once a week ❑ Twice a week ❑ Thrice a week ❑ Weekly ❑ Monthly
How many *Salmonella* strains did your laboratory serotype in 2001 ?	Number of strains:……..
What kind of sera do you use ?	❑ Prepared in own laboratory ❑ Commercial sera Manufacturer(s): ……
Is your laboratory the veterinary or human reference laboratory for typing *Salmonella* strains in your country ?	❑ YES, Veterinary ❑ YES, Human ❑ NO, the name and address of the reference laboratory is:…… …… ……
The strains in this collaborative study were serotyped by:	❑ Own laboratory, Strain……... ❑ Other laboratory, namely…… …… …… Strains:…… ……

TEST RESULTS OF THE COLLABORATIVE STUDY ON SEROTYPING

LABCODE	……
Starting date of typing	…… - …… - 2002
Finishing date of typing	…… - …… - 2002

Strain no.	O-antigens detected	H-antigens detected	Serovar
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			

QUESTIONS PHAGE TYPING

Does your laboratory perform phage typing of the following strains ?	❑ *Salmonella* Typhimurium ❑ *Salmonella* Enteritidis ❑ Other(s)…… ……
Which typing system is used for:	❑ *Salmonella* Typhimurium …… ❑ *Salmonella* Enteritidis ……
How many strains did your laboratory phage type in 2001 ?	Number of strains……...

TEST RESULTS OF THE COLLABORATIVE STUDY ON PHAGETYPING

LABCODE	……
Starting date of typing	…… - …… - 2002
Finishing date of typing	…… - …… - 2002

		Phages reactions at Routine Test Dilution (*S.* Enteritidis)															
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																	

TEST RESULTS OF THE COLLABORATIVE STUDY ON PHAGETYPING

LABCODE	……
Starting date of typing	…… - …… - 2002
Finishing date of typing	…… - …… - 2002

		Phages at Routine Test Dilution (*S.* Typhimurium)																
QA number	Phage type	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
M11																			
M12																			
M13																			
M14																			
M15																			
M16																			
M17																			
M18																			
M19																			
M20																			

		Phages at Routine Test Dilution (*S.* Typhimurium)													Additional phages				
QA number	Phage type	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**32**	**35**	**O***	**1**	**2**	**3**	**10**	**18**
M11																			
M12																			
M13																			
M14																			
M15																			
M16																			
M17																			
M18																			
M19																			
M20																			

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

REMARKS AND COMMENTS

Name of person carring out the serotyping	
Date and signature	

Name of person in charge	
Date and signature	

Appendix 3. Questionnaire antimicrobial susceptibility testing (AST)

QUESTIONNAIRE ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST)

INTRODUCTION

In Newsletter Vol.5 No.4 (December 1999) the NRLs-*Salmonella* were asked to fill in a postal survey. One of the items of this survey was antibiotic resistance typing. Questions were:

1. Would you be interested in typing of the resistance patterns of the strains of the next typing study ?
2. How many resistance patterns did your laboratory type in 1998 and 1999 ?
3. Which antibiotics do you use for resistance pattern typing and from which manufacturer are they ?

Most NRLs were interested in performing antibiotic resistance pattern typing (Newsletter Vol.6 No.1 / March 2000).

In the 5th collaborative study (2000) CRL-*Salmonella* offered the possibility to perform antibiotic resistance pattern typing on the strains for serotyping. All NRLs-*Salmonella* and 10 EnterNet Laboratories (ENLs) typed the resistance patterns of the strains using their own method. Two NRLs and two ENLs used a quantitative method and all other laboratories used a disc diffusion method. The number of antibiotics used per NRL varied from 7 to 18 and per ENL from 11 to 23 (RIVM report 284500 016).

During the preparation of the report (RIVM report 284500 020, in press) on the 6th collaborative study (2001) CRL-*Salmonella* decided to decrease the number of antibiotics in the tables of the report. The diversity of the various antibiotics (52 in total) 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. Furthermore, antimicrobial susceptibility testing revealed data showing that standardisation and harmonisation of the technique would be required to allow for comparison between laboratories.

At the Seventh Workshop in Ploufragan (28 May 2002) it was decided to include a questionnaire on antimicrobial susceptibility testing in one of the next newsletters. After analysing the data of the questionnaire and carefull discussion with experts in the field, CRL-*Salmonella* will present a new plan for the collaborative study of next year (2003).

GENERAL INFORMATION

Table 1. List of abbreviations used in this questionnaire

Number	Antibiotic	Abbreviation
1	AMPICILLIN	AMP
2	CHLORAMPHENICOL	CHL
3	CEFOTAXIME	CEF
4	CIPROFLOXACIN	CIP
5	GENTAMICIN	GEN
6	KANAMYCIN	KAN
7	NALIDIXIC ACID	NAL
8	NEOMYCIN	NEO
9	STREPTOMYCIN	STR
10	SULFAMETHOXAZOLE+TRIMETHOPRIM	SXT
11	TETRACYCLIN	TET
12	TRIMETHOPRIM	TMP

Why did your laboratory chose for the disc diffusion method instead of MIC testing ? (advantages of the test, disadvantages of the test, flexibility, costs, time to perform, etc.)	
Why did your laboratory chose for the MIC testing instead of the disc diffusion method ? (advantages of the test, disadvantages of the test, flexibility, costs, time to perform, etc.)	

DISC DIFFUSION TESTING

Please fill in the load in µg/ml and the manufacturer of the twelve antibiotics if used in your laboratory. Use table 2 for this purpose. If you do not use one or more of the antibiotics mentioned below leave the cells of the table open.

Table 2. Load and manufacturer of antibiotics

Number	Antibiotic	Load in µg/ml	Manufacturer
1	AMP		
2	CHL		
3	CEF		
4	CIP		
5	GEN		
6	KAN		
7	NAL		
8	NEO		
9	STR		
10	SXT		
11	TET		
12	TMP		

QUALITY CONTROL

According to what guidelines (system) do you read the inhibition zones ?	
Do you use (a) control strain(s) ?	
What is/are the name(s) of your control strain(s) ?	
Do you include the control strain(s) each time you test susceptibility ?	
How do you store your control strain(s) ?	

MEDIUM	
What is the name of the agar medium you use in your laboratory ?	
What is the name of the manufacturer of your medium ?	
What is the product number of this medium ?	
Do the plates with medium arrive at your laboratory in a ready-to-use state ?	
Do you receive together with the medium a certificate of analysis from the manufacturer ?	
Is the agar medium you use prepared in your own laboratory ?	
What is the composition of this home-made medium ?	
What is the diameter of your plates in mm ?	
How many milliliters of medium does one plate contain ?	
What is the thickness/depth of the agar in the plate ?	

INOCULUM

Describe the way you transfer the inoculum to the plate ?	
What is the maximum number of discs you transfer to one plate ?	
What is the size of your inoculum in bacteria per ml ?	
How do you measure the density of your inoculum ? What method ? (McFarland scale, nephelometric, spectrophotometric or otherwise)	

INCUBATION

What is the incubation time of your plates ?	
What is the incubation temperature of your plates ?	

READING OF THE RESULTS

In what way do you read the results ? (by eye or with an automatic reader). Please describe your method.	

INTERPRETATION OF RESULTS
To be able to compare results in the future CRL-*Salmonella* asks you to fill in each cell of table 3. Use the capitals R (for resistant), I (for intermediate) and S (for sensitive) to indicate what the size of the inhibition zones is in relation with the above mentioned notations.

Table 3. Inhibition zones in mm for each antibiotic in relation to resistant, intermediate or sensitive test results

	Inhibition zones (diameter in mm)														
	12	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**
AMP															
CHL															
CEF															
CIP															
GEN															
KAN															
NAL															
NEO															
STR															
SXT															
TET															
TMP															

Indicate in table 4 what breakpoints are being used in your country for the use of antibiotics.

Table 4. Breakpoint per antibiotic in diameter of the inhibition zones.

Number	Antibiotic	Breakpoint in mm inhibition zones (diameter)
1	AMP	
2	CHL	
3	CEF	
4	CIP	
5	GEN	
6	KAN	
7	NAL	
8	NEO	
9	STR	
10	SXT	
11	TET	
12	TMP	

MINIMAL INHIBITORY CONCENTRATION (MIC) TESTING

Please fill in (in table 5) the range in µg/ml and the manufacturer of the twelve antibiotics if used in your laboratory. If you do not use one or more of the below mentioned antibiotics leave the cells of the table open.

Table 5. Range and manufacturer of MIC antibiotics.

Number	Antibiotic	Range in µg/ml	Manufacturer
1	AMP		
2	CHL		
3	CEF		
4	CIP		
5	GEN		
6	KAN		
7	NAL		
8	NEO		
9	STR		
10	SXT		
11	TET		
12	TMP		

QUALITY CONTROL

According to what guidelines (system) do you read the MIC ?	
Do you use (a) control strain (s) ?	
What is/are the name(s) of your control strain(s) ?	
Do you include the control strain(s) each time you test susceptibility ?	
How do you store your control strain(s) ?	

INOCULUM	
What is the size of your inoculum in organisms per ml ?	
How do you measure the density of your inoculum ? What method ? (McFarland scale, nephelometric, spectrophotometric or otherwise)	

INCUBATION	
What is the incubation time of your plates ?	
What is the incubation temperature of your plates ?	

READING OF THE RESULTS	
In what way do you read the results ? (by eye or with an automatic reader). Please describe your method.	

INTERPRETATION OF RESULTS
To be able to compare results in the future CRL-*Salmonella* asks you to fill in each cell of table 6. Use the capitals R (for resistant), I (for intermediate) and S (for sensitive) to indicate what the MICis in relation with the above mentioned notations.

Table 6. MIC in µg/ml for each antibiotic in relation to resistant, intermediate or sensitive test results

	MIC in µg/ml														
	.03	**.06**	**.12**	**.25**	**.50**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**
AMP															
CHL															
CEF															
CIP															
GEN															
KAN															
NAL															
NEO															
STR															
SXT															
TET															
TMP															

Indicate in table 7. what breakpoints are being used in your country for the use of antibiotics.

Table 7. Breakpoint per antibiotic in µg/ml.

Number	Antibiotic	Breakpoint in µg/ml
1	AMP	
2	CHL	
3	CEF	
4	CIP	
5	GEN	
6	KAN	
7	NAL	
8	NEO	
9	STR	
10	SXT	
11	TET	
12	TMP	

Appendix 4 Test results of phage typing per strain

Strain E 1

		Phages reactions at Routine Test Dilution (*S.* Enteritidis)															
Lab code	Phage type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PHLS	11	-	-	scl	-	cl	-	+	ol	-	ol	+	cl	±	-	-	++ +
1	11	- 1	-	<cl	-	cl	-	±L <c	ol	-	ol	<cl	cl	±	-	-	±
2	11	-	-	<< scl	-	cl	-	+	<ol	-	ol	±	cl	±	-	-	++ +
4	11	-	-	scl	-	ol	-	3	ol	-	scl	±	ol	±	2	-	++ +
5	9a	-	-	cl	-	cl	-	scl	cl	-	cl	cl	cl	scl	-	±	cl
6	11	-	-	++ +n	-	cl	-	+ n	ol	-	ol	+ n	cl	-	-	-	++ + sm
8	11	-	-	<cl	-	cl	-	+ ln	ol	-	<ol	+ln	cl	± sm	-	4 ns	++ ns
16	11	-	-	++ +	-	scl	-	±	++	-	++ +	±	cl	++	±	-	scl
A	11	+	-	scl	-	cl	-	++	scl	-	scl	++	cl	+	-	+	++ +
B	11	-	-	+	-	cl	-1	-2	< cl	-	scl	±	cl	+	-	-	++ +
C	11	-	-	scl	-	cl	-	2n	scl	-	scl	++ n	cl	-	-	-	±s
E	11	-	-	++ +	-	cl	-	++ +	< ol	-	< ol	±±	cl	++ µ	-	-	< scl
F	9a	-	-	ol	-	scl	-	++	ol	-	ol	++	cl	ol	-	-	scl
H	11	-	-	< cl	-	cl	-	±	ol	-	ol	±	cl	-	Rough	-	scl
J	11	4n	-	< cl	-	cl	-	++ +	cl	-	ol	±n	cl	+	4s	4s	++ s
K	11	-	-	scl	-	ol	-	+2	ol	-	ol	+µ	cl	+5	-	-	++ s
P	9a	-	-	cl	-	cl	-	-	cl	-	cl	cl	cl	cl	-	-	cl
T	8	-	-	scl	scl	cl	?	scl	ol	ol	scl	scl	cl	-	-	-	-
Y	11	-	-	scl	-	< cl	-	±L	ol	-	ol	±L	cl	±µ	2P	-	+s

Strain E 2

		Phages reactions at Routine Test Dilution (*S.* Enteritidis)															
Lab code	Phage type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PHLS	9a	-	-	cl	-	cl	±	cl	ol	-	ol	cl	cl	cl	-	±	cl
1	9a	- 1L	- 2	cl	+/-	cl	+/-	cl	ol	-	ol	cl	cl	cl	-	-	cl
2	9a	±	-	scl	-	cl	-	scl	<ol	-	<ol	scl	cl	cl	-	±	ol
4	9a	-	+	cl	±	cl	+	<cl	ol	±	ol	cl	cl	cl	8	-	ol
5	31	-	ol	cl	++ +	ol	-	cl	ol	++ +	cl	cl	cl	cl	-	-	cl
6	9a	-	-	cl	-	cl	-	cl	ol	-	ol	cl	cl	cl	-	-	ol
8	9a	-	-	<cl	-	cl	-	<cl	ol	-	<ol	<cl	cl	cl	1s	5 1	<ol
16	9a	-	±	scl	-	cl/ ol	+±	cl	++ + scl	±	++ + scl	cl	cl/ ol	cl/ ol	±	-	cl/ ol
A	9a	++	+	scl	-	scl	-	cl	ol	-	ol	scl	cl	cl	-	+	scl
B	9a	±	-	scl	-	cl	-	scl	ol	-	ol	cl	cl	cl	-1	-6	cl
C	9a	-	-	< cl	-	< cl	-	scl	ol	-	scl	< cl	< cl	< cl	-	3 1	scl
E	9a	+	++ µ	cl	-	cl	-	cl	cl	-	< cl	< cl	cl	cl	++ µ	-	scl
F	41	++	++	scl	-	scl	++ +	scl	ol	-	ol	scl	scl	scl	-	-	scl
H	9a	-	-	cl	-	cl	-	cl	ol	-	ol	cl	cl	cl	-	±	cl
J	RDNC	±	++ +	cl	++ +-	cl	++ +-	cl	ol	-	ol	cl	cl	cl	±n	±< ol	< cl
K	9a	-	++ +s	cl	+8	cl	+8	cl	ol	+8	ol	cl	cl	cl	-	-	ol
P	41	cl	scl	cl	-	cl	cl	ol	ol	-	ol	cl	cl	cl	-	-	scl
T	1c	ol	scl	scl	ol	cl	?	++ +	scl	scl	scl	+	cl	scl	cl	-	-
Y	9a	3P	±µ	< cl	-	< cl	±µ	< cl	ol	< ol	ol	< cl	< cl	< cl	4P	-	Scl

Strain E 3

		Phages reactions at Routine Test Dilution (*S.* Enteritidis)															
Lab code	Phage type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PHLS	34	-	-	-	-	-	-	-	**ol**	-	**ol**	-	-	-	-	-	-
1	34	-	-	-	-	-	-	-	**ol**	-	**ol**	-	-	-	**- L**	-	-
2	34b	±	-	-	-	-	-	-	**ol**	-	**ol**	-	-	-	±	-	±±
4	34	-	-	-	-	-	-	-	**ol**	-	**ol**	-	-	-	-	-	-
5	34	-	-	-	-	-	-	-	**cl**	-	**cl**	-	-	-	-	-	-
6	34	-	-	-	-	-	-	**+s**	**ol**	-	**ol**	-	-	-	-	-	-
8	34	-	-	-	-	-	-	-	**ol**	-	**<ol**	-	-	-	-	-	-
16	34b	-	-	-	-	-	-	-	**++ + scl**	-	**++ + scl**	-	-	-	±	-	**++ ±**
A	34	++	-	-	-	-	-	-	**scl**	-	**scl**	-	-	-	-	-	++
B	34	**-8**	-	-	-	-	-	-	**scl**	-	**scl**	-	-	-	-	-	**cl**
C	34	-	-	-	-	-	-	-	**scl**	-	**scl**	-	-	-	-	-	-
E	34	-	-	-	-	-	-	-	**ol**	-	**ol**	-	-	-	**5**	-	-
F	25	-	-	-	-	-	-	-	**ol**	-	**ol**	-	**<ol**	-	-	-	-
H	34	-	-	-	-	-	-	-	**ol**	-	**ol**	-	-	-	-	-	-
J	34	-	-	-	-	-	-	-	**ol**	-	**ol**	-	-	-	**8s**	-	-
K	34	-	-	-	-	-	-	-	**cl**	-	**cl**	-	-	-	-	-	-
P	13a	-	-	-	**scl**	-	**scl**	-	**ol**	**cl**	**ol**	-	-	-	-	-	-
T	34b	-	-	-	-	-	**?**	+	**scl**	-	**scl**	-	-	-	-	-	-
Y	34	-	-	-	-	-	-	-	**ol**	-	**ol**	-	-	-	-	-	-

Strain E 4

		Phages reactions at Routine Test Dilution (*S.* Enteritidis)															
Lab code	Phage type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PHLS	4	-	scl	cl	scl	cl	scl	cl	ol	ol	ol	cl	cl	cl	-	-	-
1	4	-	scl	cl	ol	cl	+	cl	ol	<ol	ol	cl	cl	cl	-	-	-
2	4	±	scl	cl	++ +	cl	++ +	cl	<ol	<ol	ol	scl	cl	cl	-	-	-
4	4	-	<cl	<cl	<ol	cl	sol	cl	ol	ol	ol	<cl	cl	cl	-	-	-
5	4	-	cl	cl	ol	cl	scl	cl	ol	ol	ol	cl	cl	cl	-	-	-
6	4	-	scl	cl	scl	cl	scl	cl	ol	<ol	ol	cl	cl	cl	-	-	-
8	4	-	+++ ns <<	cl	sol	cl	++ ns< <	cl	ol	<ol	<ol	cl	cl	cl	-	-	-
16	4	-	++ + scl	scl	++ scl	scl	++ +	scl	++ +	++ <ol	++ + scl	scl	scl	scl	-	-	±
A	4	-	scl	scl	scl	cl	++	cl	scl	++ +	scl	scl	cl	cl	-	-	++
B	4	-	scl	scl	ol	cl	++ +	cl	ol	ol	ol	cl	cl	cl	-4	-	-
C	4	-	< scl	< cl	++ +n	cl	++ n	< cl	ol	++ + nc	scl	cl	cl	cl	-	-	-
E	4	-	scl	cl	< ol	cl	++ +	cl	ol	< ol	ol	cl	cl	cl	-	-	-
F	4	-	cl	scl	< ol	cl	ol	scl	ol	ol	ol	scl	cl	cl	-	-	-
H	4	-	< cl	cl	ol	cl	scl	cl	ol	ol	ol	cl	cl	cl	-	-	-
J	4	-	cl	cl	ol	cl	scl	cl	ol	scl	ol	cl	cl	cl	1s	-	-
K	4	-	cl	cl	ol	cl	scl	cl	ol	ol	ol	cl	cl	cl	-	-	-
P	4	-	cl	cl	scl	cl	scl	cl	cl	cl	ol	cl	cl	cl	-	-	-
T	4b	-	scl	scl	scl	cl	?	scl	ol	scl	scl	scl	scl	scl	-	-	scl
Y	4	-	scl	cl	ol	cl	< scl	< cl	ol	ol	ol	< cl	cl	cl	-	-	-

Strain E 5

		Phages reactions at Routine Test Dilution (*S.* Enteritidis)															
Lab code	Phage type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PHLS	1	**ol**	**scl**	**cl**	**scl**	**cl**	**scl**	**cl**	**ol**	**ol**	**ol**	**cl**	**cl**	**cl**	**cl**	**-**	**-**
1	1	**ol**	**scl**	**cl**	**ol**	**cl**	**++ -**	**cl**	**ol**	**<ol**	**ol**	**cl**	**cl**	**cl**	**cl**	**- 4**	**-**
2	1	**ol**	**++ +**	**cl**	**<ol**	**cl**	**++ +**	**cl**	**<ol**	**ol**	**ol**	**scl**	**cl**	**<cl**	**scl**	**-**	**+**
4	1	**ol**	**<cl**	**cl**	**sol**	**cl**	**sol**	**<cl**	**ol**	**<ol**	**ol**	**<cl**	**cl**	**cl**	**cl**	**-**	**-**
5	1	**ol**	**cl**	**cl**	**ol**	**cl**	**scl**	**cl**	**ol**	**ol**	**ol**	**cl**	**cl**	**cl**	**cl**	**-**	**-**
6	1	**ol**	**scl**	**cl**	**<ol**	**cl**	**scl**	**cl**	**ol**	**ol**	**ol**	**cl**	**cl**	**cl**	**cl**	**-**	**-**
8	1	**ol**	**++ + ns**	**cl**	**sol**	**<cl**	**sol**	**cl**	**ol**	**sol**	**<ol**	**cl**	**cl**	**cl**	**cl**	**-**	**-**
16	1c	**ol**	**++ +**	**cl**	**++ <ol**	**cl**	**++ +**	**scl**	**++ +**	**++ <ol**	**++ +**	**scl**	**cl**	**scl**	**cl**	**+**	**++**
A	1	**ol**	**++ +**	**cl**	**++ +**	**cl**	**++**	**cl**	**scl**	**++**	**scl**	**cl**	**cl**	**cl**	**scl**	**++ +**	**++ +**
B	1	**ol**	**scl**	**scl**	**ol**	**cl**	**++ +**	**scl**	**ol**	**ol**	**ol**	**cl**	**cl**	**cl**	**cl**	**++ µ**	**++ µ**
C	1	**ol**	**scl**	**cl**	**< scl**	**< cl**	**++ n**	**< cl**	**ol**	**++ +n**	**ol**	**< cl**	**< cl**	**< cl**	**< scl**	**-**	**-**
E	1	**ol**	**scl**	**cl**	**< ol**	**cl**	**++ +**	**cl**	**ol**	**< ol**	**ol**	**< cl**	**cl**	**cl**	**cl**	**-**	**-**
F	1b	**ol**	**scl**	**scl**	**ol**	**cl**	**ol**	**scl**	**ol**	**ol**	**ol**	**scl**	**cl**	**cl**	**scl**	**++**	**++ +**
H	1	**ol**	**< cl**	**cl**	**ol**	**cl**	**scl**	**cl**	**ol**	**ol**	**ol**	**cl**	**cl**	**cl**	**cl**	**-**	**-**
J	1	**ol**	**cl**	**cl**	**ol**	**cl**	**++ + scl**	**cl**	**<ol**	**++ + scl**	**ol**	**cl**	**cl**	**cl**	**cl**	**-**	**-**
K	1	**++ + scl**	**cl**	**cl**	**ol**	**cl**	**scl**	**cl**	**ol**	**ol**	**ol**	**cl**	**cl**	**cl**	**cl**	**+ m**	**-**
P	1	**ol**	**cl**	**cl**	**< ol**	**cl**	**ol**	**cl**	**ol**	**ol**	**ol**	**cl**	**cl**	**cl**	**cl**	**-**	**-**
T	31 ?	**-**	**scl**	**cl**	**scl**	**cl**	**?**	**cl**	**ol**	**scl**	**scl**	**cl**	**cl**	**scl**	**-**	**-**	**+**
Y	1	**ol**	**scl**	**cl**	**<< ol**	**cl**	**<< ol**	**cl**	**ol**	**ol**	**ol**	**cl**	**cl**	**cl**	**cl**	**±s**	**±s**

Strain E 6

		Phages reactions at Routine Test Dilution (*S.* Enteritidis)															
Lab code	Phage type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PHLS	6	-	**scl**	-	**scl**	-	**scl**	-	**ol**	**ol**	**ol**	-	-	-	-	-	-
1	6	-	++	-	**ol**	-	+±	-	**ol**	**<ol**	**ol**	-	±	-	-	-	-
2	6	-	**scl**	-	++ +	-	++ +	-	++ +	**<ol**	**<ol**	-	-	-	-	-	-
4	6	-	**<cl**	-	**<ol**	-	**sol**	-	**<ol**	**<ol**	**<ol**	-	-	-	-	-	-
5	6	-	**cl**	-	**scl**	-	**scl**	-	**ol**	**ol**	**ol**	-	-	-	-	-	-
6	6	-	**scl**	-	**scl**	-	**scl**	-	**ol**	**<ol**	**ol**	-	-	-	-	-	-
8	6	-	++ + **ns**	-	**sol**	-	**sol**	-	**<ol**	**<ol**	**<ol**	-	-	-	-	-	-
16	6	-	++ +	-	++ +< **ol**	-	++ ±	-	++ +< **ol**	++ +< **ol**	++ + **scl**	-	-	-	-	-	-
A	6	-	++ +	-	++ +	-	++	-	**scl**	++	**scl**	-	-	-	-	-	-
B	6	-	**scl**	-	**scl**	-	++ +	-	**ol**	**ol**	**ol**	-	-	-	-	-	-
C	6	-	< **scl**	-	< **scl**	-	**±s**	-	**scl**	< **scl**	**scl**	-	-	-	-	-	-
E	6	-	++ +	-	±± ±	-	++ +	-	**ol**	< **ol**	**ol**	-	-	-	**5**	-	-
F	6	-	**scl**	-	++ +	-	**scl**	-	**ol**	**ol**	**ol**	-	-	-	-	-	-
H	6	-	< **cl**	-	< **ol**	-	**scl**	-	**ol**	**ol**	**ol**	-	-	-	-	-	-
J	6	-	**<cl**	-	**ol**	-	**scl**	-	**<ol**	++ + **scl**	**ol**	-	-	-	**1s**	-	-
K	6	-	**cl**	-	**ol**	-	**scl**	-	**ol**	**ol**	**ol**	-	-	-	-	-	-
P	6	-	**scl**	-	**scl**	-	**sol**	-	**ol**	**ol**	**ol**	-	-	-	-	-	-
T	6	-	**scl**	-	**scl**	-	**?**	-	**ol**	**scl**	**scl**	-	-	-	-	-	-
Y	6	-	**scl**	-	< **ol**	-	< **ol**	-	**ol**	**ol**	**ol**	-	-	-	-	**1P**	**±s**

Strain E 7

		Phages reactions at Routine Test Dilution (*S.* Enteritidis)															
Lab code	Phage type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PHLS	5c	-	scl	±	scl	scl	scl	+	ol	ol	ol	±	scl	scl	-	-	-
1	5c	-	scl	-1	ol	scl	<scl	+-LL	ol	<ol	ol	<cl	<cl	<cl	-	-	-
2	5c	-	+++	±	<ol	<ol	<ol	++	scl	scl	scl	±	scl	+	-	-	-
4	5b	1	<cl	2	<ol	±	sol	±	ol	ol	ol	±	+++	++	-	-	-
5	5b	-	cl	+	ol	cl	cl	scl	ol	ol	cl	cl	cl	scl	-	-	-
6	ARS	-	scl	-	scl	+	scl	+	ol	ol	ol	+	-	2	-	-	-
8	RDNC	-	++ +s <<	-	sol	-	sol	± ln	ol	ol	<ol	±l	-	++ n <<	-	-	-
16	5b	-	++ ±	-	<ol	+	++ +ol	±	++	<ol	++ +	±	++	+±	-	-	±
A	5b	++	scl	-	scl	++	++	++	scl	++ +	scl	++	++ +	++ +	-	-	++ +
B	5b	-	scl	±	<cl	scl	++ +	++	ol	ol	ol	-6	<scl	++ +	-	-	-
C	5c	-	scl	± ns	<scl	<scl	++ s	±n	scl	<scl	<scl	++1	<scl	++ n	-	-	-
E	5c	+	+++	++ +	<ol	ol	++ +	scl	<ol	ol	<ol	<scl	ol	<cl	-	-	-
F	27	-	cl	-	ol	-	scl	-	ol	ol	ol	++	-	<ol	-	-	-
H	5c	-	<cl	±	ol	scl	scl	±	ol	ol	ol	2	ol	<cl	-	-	-
J	5b	-	cl	-	ol	++ s	scl	+1 << scl	cl	++ + scl	ol	++1	++ +<ol	++ +<ol	5n	-	-
K	5b	-	cl	+8	ol	+5	scl	++ 2	ol	ol	ol	++ 2	scl	++ +	-	-	-
P	5b	-	scl	-	scl	scl	scl	+	ol	ol	ol	+	scl	scl	-	-	-
T	5	-	scl	+	scl	scl	?	-	++	scl	++	+	scl	scl	-	-	scl
Y	5c	-	scl	-	<ol	++ s	<ol	-	ol	ol	ol	1L	<scl	<scl	-	-	±µ

Strain E 8

		Phages reactions at Routine Test Dilution (*S.* Enteritidis)															
Lab Code	Phage type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PHLS	1b	**ol**	**scl**	**cl**	**scl**	**cl**	**scl**	**cl**	**ol**	**ol**	**ol**	**cl**	**cl**	**cl**	**cl**	**cl**	**cl**
1	1b	**ol**	**scl**	**cl**	**ol**	**cl**	+	**cl**	**<ol**	**<< ol**	**<ol**	**cl**	**cl**	**cl**	**cl**	**cl**	**cl**
2	1b	**< cl**	**++ +**	**cl**	**±±**	**cl**	**<cl**	**cl**	**<ol**	**ol**	**<ol**	**scl**	**cl**	**cl**	**cl**	**scl**	**<cl**
4	1b	**< ol**	**< cl**	**cl**	**<cl**	**cl**	**sol**	**<cl**	**<ol**	**<ol**	**sol**	**<cl**	**cl**	**< cl**	**cl**	**<cl**	**cl**
5	1b	**scl**	**cl**	**cl**	**ol**	**cl**	**scl**	**cl**	**cl**	**ol**	**scl**	**cl**	**cl**	**cl**	**cl**	**cl**	**cl**
6	1b	**ol**	**scl**	**cl**	**<ol**	**cl**	**scl**	**cl**	**ol**	**<ol**	**ol**	**cl**	**cl**	**cl**	**cl**	**ol**	**ol**
8	1b	**ol**	**++ +s**	**cl**	**sol**	**cl**	**sol**	**cl**	**ol**	**<ol**	**<ol**	**<cl**	**cl**	**cl**	**cl**	**<ol**	**<ol**
16	1b	**ol**	**++ ±**	**cl**	**<ol**	**scl**	**++ +**	**scl**	+	**<ol**	++	**scl**	**cl**	**cl**	**cl**	**scl**	**scl**
A	1b	**ol**	**++ +**	**cl**	++	**cl**	++	**cl**	**scl**	++	**scl**	**scl**	**cl**	**cl**	**scl**	**scl**	**scl**
B	1b	**scl**	**scl**	**cl**	**ol**	**cl**	**++ +**	**cl**	**scl**	**ol**	**scl**	**cl**	**cl**	**cl**	**cl**	**scl**	**cl**
C	1b	**scl**	**< scl**	**< cl**	**< scl**	**< cl**	**< scl**	**scl**	**scl**	**< scl**	**< scl**	**< cl**	**< cl**	**< cl**	**< scl**	**scl**	**scl**
E	1b	**ol**	**scl**	**cl**	**< ol**	**cl**	**scl**	**cl**	**ol**	**ol**	**< ol**	**< cl**	**cl**	**cl**	**< cl**	**scl**	**scl**
F	1b	**ol**	**ol**	**cl**	**ol**	**cl**	**scl**	**scl**	**ol**	**ol**	**scl**	**scl**	**cl**	**cl**	**cl**	**scl**	**scl**
H	1b	**ol**	**<cl**	**cl**	**ol**	**cl**	**scl**	**cl**	**ol**	**ol**	**ol**	**cl**	**cl**	**cl**	**cl**	**cl**	**cl**
J	1b	**cl**	**<cl**	**cl**	**ol**	**cl**	**scl**	**cl**	**scl**	**++ +-**	**cl**	**<cl**	**cl**	**cl**	**cl**	**<cl**	**<cl**
K	1b	**++ + scl**	**cl**	**cl**	**ol**	**cl**	**scl**	**cl**	**ol**	**ol**	**ol**	**cl**	**cl**	**cl**	**cl**	**cl**	**ol**
P	1b	**ol**	**scl**	**cl**	**scl**	**cl**	**scl**	**cl**	**ol**	**ol**	**ol**	**cl**	**cl**	**cl**	**cl**	**cl**	**cl**
T	1b	**ol**	**scl**	**scl**	**scl**	**cl**	**?**	**cl**	**ol**	**scl**	**scl**	**cl**	**scl**	**scl**	**scl**	**ol**	**ol**
Y	1b	**ol**	**scl**	**cl**	**ol**	**cl**	**< scl**	**< cl**	**ol**	**ol**	**ol**	**< cl**	**cl**	**cl**	**cl**	**scl**	**scl**

Strain E 9

		Phages reactions at Routine Test Dilution (*S.* Enteritidis)															
Lab Code	Phage type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PHLS	6a	-	**scl**	-	**scl**	-	**scl**	-	-	**ol**	-	-	-	-	-	-	-
1	6a	-	**scl**	-	**ol**	-	**scl**	-	-	**<ol**	-	-	-	-	-	-	-
2	6a	-	**scl**	-	**< ol**	-	**scl**	-	-	**<ol**	-	-	-	-	-	-	-
4	6a	-	**< cl**	-	**< ol**	-	**sol**	-	-	**<ol**	-	-	-	-	-	-	-
5	6a	-	**cl**	-	**cl**	-	**scl**	-	-	**cl**	-	-	-	-	-	-	-
6	6a	-	**scl**	-	**scl**	-	**scl**	-	-	**<ol**	-	-	-	-	-	-	-
8	6a	-	**++ +s**	-	**sol**	-	**sol**	-	-	**<ol**	-	-	-	-	-	-	-
16	6a	-	**++ ±**	-	**<ol**	-	**++**	-	-	**<ol**	-	-	-	-	-	-	-
A	6a	-	**scl**	-	**scl**	-	**++**	-	-	**++**	-	-	-	-	-	-	-
B	6a	-	**scl**	-	**ol**	-	**++ +**	-	-	**ol**	-	-	-	-	-	-	-
C	6a	-	**< scl**	-	**++n ++s**	-	**+s**	-	-	**< scl**	-	-	-	-	-	-	-
E	6a	-	**scl**	-	**scl**	-	**scl**	-	-	**ol**	-	-	-	-	-	-	-
F	6a	-	**cl**	-	**ol**	-	**ol**	-	-	**ol**	-	-	-	-	-	-	-
H	6a	-	**< cl**	-	**ol**	-	**scl**	-	-	**ol**	-	-	-	-	-	-	-
J	6a	-	**cl**	-	**ol**	-	**scl**	-	-	**++ + scl**	-	-	-	-	-	-	-
K	6a	-	**cl**	-	**ol**	-	**scl**	-	-	**ol**	-	-	-	-	-	-	-
P	6a	-	**scl**	-	**scl**	-	**scl**	-	-	**sol**	-	-	-	-	-	-	-
T	35	-	**scl**	-	**scl**	-	**?**	-	-	**++ +**	-	-	-	-	-	-	-
Y	6a	-	**scl**	-	**< scl**	-	**< scl**	-	-	**ol**	-	-	-	-	-	-	-

Strain E 10

		Phages reactions at Routine Test Dilution (*S.* Enteritidis)															
Lab Code	Phage type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PHLS	5a	-	scl	-	scl	cl	scl	±	-	ol	-	+	cl	-	-	-	-
1	6b	-	scl	-	ol	cl	++ -	-	-	<ol	-	-	cl	-	-	-	-
2	5a	-	scl	-	++ +	scl	scl	-	-	<ol	-	-	cl	2	-	-	-
4	6b	-	<cl	-	sol	<ol	++ +	-	-	<ol	-	-	cl	-	-	-	-
5	6b	-	cl	-	ol	cl	scl	-	-	cl	-	-	ol	-	-	-	-
6	5a	-	scl	-	scl	cl	scl	-	-	<ol	-	-	cl	-	-	-	-
8	5a	-	++ +s	-	sol	<cl	sol	+ sm	2m	<ol	-	± mu	cl	-	-	-	-
16	5a	-	++ ±	±	<ol	cl	++ ±	±	-	<ol	-	±	cl	-	-	-	-
A	4a	-	scl	scl	++ +	cl	++	++ +	-	++	-	++	cl	++ +			
B	5a	-	scl	-	scl	cl	++ +	±	±	ol	-	±	cl	+	-	-	-
C	5a	-	< scl	-	++ +n	scl	< scl	-	-	++ +n	-	-	scl	-	-	-	-
E	5a	-	scl	-	< ol	cl	scl	-	-	ol	-	-	cl	-	-	-	-
F	6b	-	cl	-	ol	cl	scl	-	-	ol	-	-	-	scl	-	-	-
H	5a	-	< cl	-	ol	cl	scl	-	-	ol	-	-	cl	-	-	-	-
J	5a	-	cl	±s	ol	cl	scl	+	-	++ +< scl	++	±s	cl	-	-	-	-
K	5a	-	cl	++ m	cl	++ 2	scl	++	-	ol	-	+	cl	-	-	-	-
P	5a	-	scl	-	+	cl	< ol	+	-	ol	-	+	cl	-	-	-	-
T	6b	-	scl	-	scl	-	?	-	-	ol	-	-	+	-	-	-	-
Y	5a	-	scl	-	ol	scl	< scl	-	-	ol	-	2P	< cl	-	-	-	-

Strain M 11 (A)

		Phages at Routine Test Dilution (*S.* Typhimurium)																	
Labcode	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
PHLS	**66**	-	-	-	-	-	-	-	-	cl	ol	-	-	-	-	scl	-	-	-
1	**66**	-	-	-	-	-	-	-	-	< cl	ol	-	-	-	-	< cl	-	-	-
2	**66**	-	-	-	-	-	-	-	-	scl	ol	-	-	-	-	scl	-	-	-
4	**66**	-	-	-	-	-	-	-	-	< cl	ol	-	-	-	-	< cl			
5	**66**	-	-	-	-	-	-	-	-	cl	cl	-	-	-	-	cl	-	-	-
8	**66**	-	-	-	-	-	-	-	-	< cl	±n	-	-	-	-	++ ns <<			
16	**66**	-	-	-	-	-	-	-	-	++ +	++ +	-	-	-	-	-	-	-	-
A	**66**	-	-	-	-	-	-	-	-	scl	+	-	-	-	-	scl	-	-	-
B	**66**	-	-	-	-	-	-	-	-	scl	scl	-	-	-	-	++	-	-	-
C	**66**	-	-	-	-	-	-	-	-	scl	< scl	-	-	-	-	< scl	-	-	-
E	**66**	-	-	-	-	-	-	-	-	< cl	scl	-	-	-	-	scl	-	-	-
F	**66**	-	-	-	-	-	-	-	-	cl	++ +	-	-	-	-	scl	-	-	-
H	**66**	-	-	-	-	-	-	-	-	cl	< ol	-	-	-	-	< cl			
J	**66**	-	-	-	-	-	-	-	-	< ol	ol	-	-	-	-	< cl	-	-	-
K	**69**	-	-	-	-	-	-	-	-	scl	++ 2	-	-	-	-	scl	-	-	-
P	**66**	-	-	-	-	-	-	-	-	scl	cl	-	-	-	-	scl	-	-	-
T	**8**	-	-	-	-	-	-	-	++ +	scl	++ +	-	-	-	-	scl	-	-	-
Y	**66**	-	-	-	-	-	-	-	-	cl	< ol	-	-	-	-	< cl	-	-	-

Strain M 11 (B)

		Phages at Routine Test Dilution (*S.* Typhimurium)												Additional phages					
Labcode	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10 var	18
PHLS	**66**	cl	-	ol	cl	-	±	±	-	-	cl	cl	-	±	±	+	ol	ol	-
1	**66**	±L scl	-	ol	cl	-	-	-	-4	-	cl	cl	-						
2	**66**	scl	-	cl	scl	-	±	±	-	-	cl	scl	-						
4	**66**	scl	-	cl	scl	-	[4]	++	-	-	cl	scl	-	-	-	-	< ol	< ol	-
5	**66**	cl	-	cl	cl	-	±	±	-	-	cl	cl	-						
8	**66**	++ ns <<	-	< ol	++ ns <<	-	1s	+ ns	-	-	cl	< cl	-						
16	**66**	+++	-	scl	cl	-	±	±	-	-	cl	+	-	±	+±	±	ol	ol	-
A	**66**	scl	-	scl	scl	-	+	+	-	-	scl	scl	-	+	+++	++	scl		-
B	**66**	scl	-	scl	< cl	-	±	++	-	-	cl	+++	-	+	++	++	ol	ol	-
C	**66**	< scl	-	++ 1	++ +n	-	±n	±n	-	-	< cl	scl	-						
E	**66**	+++	-	< cl	+++	-	+	+	-	-	cl	scl	-	-	-	-	ol	ol	-
F	**66**	scl	-	cl	cl	-	-	++	-	-	cl	scl	-	+	+++	++	-		-
H	**66**	cl	-	cl	cl	-	±	±	-	-	cl	< cl	-						
J	**66**	< cl	1n	cl	< cl	-	6n	4n	-	-	cl	cl	-						
K	**69**	+++ scl	-	scl	scl	-	-	+	-	-	cl	++ scl	-	-	+	+	ol	ol	-
P	**66**	cl	-	cl	cl	-	-	-	-	-	cl	scl	-	-	-	-	-	ol	ol
T	**8**	scl	-	scl	scl	-	-	-	-	-	scl	+++	-						
Y	**66**	< cl	-	ol	< cl	-	-	±s	-	-	ol	scl	-						

Strain M 12 (A)

		Phages at Routine Test Dilution (*S.* Typhimurium)																	
Labcode	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
PHLS	**22**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	**22**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	**22**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	**22**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	**107**	-	-	-	-	-	-	-	-	-	-	++ ns <<	scl	-	-	-	-	-	-
16	**22**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	**22**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E	**22**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H	**22**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
J	**22**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
K	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P	**U302**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T	**RDNC**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Y	**22**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Strain M 12 (B)

		Phages at Routine Test Dilution (*S.* Typhimurium)												Additional phages					
Labcode	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10 var	18
PHLS	**22**	-	-	ol	-	-	-	-	-	-	-	-	-	+++	+++	+++	-	-	-
1	**193**	-	-	-	-	-	-	-	-	-	-	-	-	++	scl	scl	-		-
2	**22**	-	-	ol	-	-	-	-	-	-	-	-	-						
4	**22**	-	-	sol	-	-	-	-	-	-	-	-	-	+++	+++	+++	-	-	-
5	**22**	-	-	ol	-	-	-	-	-	-	-	-	-						
8	**107**	-	-	< ol	-	-	-	-	-	-	-	-	-						
16	**22**	-	-	scl	-	-	-	-	-	-	-	-	-	+	+±	+	-	-	-
A	**193**	-	-	-	-	-	-	-	-	-	-	-	-	+	scl	scl	-		-
B	**193**	-	-	-	-	-	-	-	-	-	-	-	-	++	+++	++	-	-	-
C	**22**	-	-	< ol	-	-	-	-	-	-	-	-	-						
E	**22**	-	-	±±±	-	-	-	-	-	-	-	-	-						
F	**193**	-	-	-	-	-	-	-	-	-	-	-	-						
H	**22**	-	2	ol	-	-	-	-	-	-	-	-	-						
J	**22**	-	-	< ol	-	-	-	-	-	-	-	-	-						
K	**193**	-	-	-	-	-	-	-	-	-	-	-	-	+++	+++	+++	-	-	-
P	**U302**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ol	ol	-
T	**RDNC**	-	-	-	-	-	-	-	-	-	-	-	-	+	scl	+	-		-
Y	**22**	-	-	< ol	-	-	-	-	-	-	-	-	-						

Strain M 13 (A)

		Phages at Routine Test Dilution (*S.* Typhimurium)																	
Labcode	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
PHLS	**10**	-	-	-	-	-	-	-	-	cl	ol	cl	cl	-	-	scl	-	-	-
1	**10**	-	-	-	-	-	-	-	-	< cl	ol	cl	cl	-	-	< cl	-	-	-
2	**10**	-	-	-	-		-	-	-	scl	scl	scl	scl	-	-	++ +	-	-	-
4	**10**	-	-	-	-	-	-	-	-	scl	ol	cl	cl	-	-	< cl	-	-	-
5	**10**	-	-	-	-	-	-	-	-	scl	scl	scl	scl	-	-	scl	-	-	-
8	**67**	-	-	-	-	-	-	-	-	< cl	± l	< cl	cl	-	-	+l ns	-	-	-
16	**10**	-	-	-	-	-	-	-	-	+	scl	scl	scl	-	-	-	-	-	-
A	**10**	-	-	-	-	-	-	-	-	scl	++	scl	scl	-	-	scl	-	-	-
B	**10**	-	-	-	-	-	-	-	-	scl	cl	scl	cl	-	-	++	-	-	-
C	**10**	-	-	-	-	-	-	-	-	< scl	scl	scl	< cl	-	-	++ +1			
E	**10**	-	-	-	-	-	-	-	-	scl	< cl	cl	cl	-	-	scl	-	-	-
F	**10**	-	-	-	-	-	-	-	-	cl	scl	scl	scl	-	-	++ +	-	-	-
H	**10**	-	-	-	-	-	-	-	-	cl	ol	cl	cl	-	-	< cl	-	-	-
J	**10**	-	-	-	-	-	-	-	-	< scl	ol	cl	cl	-	-	scl	-	-	-
K	**10**	-	-	-	-	-	-	-	-	++ + scl	+2	scl	scl	-	-	scl	-	-	-
P	**10**	-	-	-	-	-	-	-	-	scl	cl	ol	ol	-	-	scl	-	-	-
T	**10**	-	-	-	-	-	-	-	-	cl	scl	scl	scl	-	-	++ +	-	-	-
Y	**10**	-	-	-	-	-	-	-	-	cl	< ol	< cl	< cl	-	-	< cl	-	-	-

Strain M 13 (B)

		Phages at Routine Test Dilution (*S.* Typhimurium)												Additional phages					
Labcode	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10 var	18
PHLS	**10**	cl	-	ol	cl	-	-	±	-	-	cl	cl	-	+	+	+	ol	ol	-
1	**10**	-	-	cl	cl	-	-	-	-	-	cl	cl	-						
2	**10**	scl	-	scl	++ +	-	1	±	-	-	cl	scl	-						
4	**10**	scl	-	cl	< cl	-	4	++	-	-	cl	scl	-	-	-	-	ol	< ol	-
5	**10**	scl	-	cl	cl	-	±	±	-	-	cl	cl	-						
8	**67**	+ ns	-	< ol	++ ns <<	-	1s	± ns	-	-	cl	< cl	-						
16	**10**	++ +	-	ol	scl	-	-	±	-	-	cl	++	-	±	+	-	ol	ol	-
A	**10**	scl	+	scl	scl	-	+	++	-	-	cl	scl	++	+	++ +	++ +	ol		-
B	**10**	scl	-	scl	cl	-	-3	±	-	-	cl	scl	-	±	+	+	ol	ol	-
C	**10**	< scl	1 1	< scl	scl	-	-	-	-	-	ol	scl	-						
E	**10**	++ +	-	cl	++ +	-	3	3	-	-	cl	< cl	-	-	-	-	ol	ol	-
F	**10**	cl	-	ol	cl	-	-	-	-	-	cl	cl	-						
H	**10**	< cl	-	cl	< cl	-	±	2	-	-	cl	scl	-						
J	**10**	< cl	-	< cl	< scl	-	4s	5n	-	-	cl	< cl	-						
K	**10**	++ + scl	-	++ 2	scl	-	-	-	-	-	cl	-	-	+	++	++	ol	ol	-
P	**10**	cl	-	cl	cl	-	-	-	-	-	cl	cl	-	-	-	-	-	ol	ol
T	**10**	++ +	-	cl	scl	-	+	+	-	-	scl	++ +	-						
Y	**10**	< cl	-	ol	< cl	-	-	±s	-	-	cl	scl	-						

Strain M 14 (A)

		Phages at Routine Test Dilution (*S.* Typhimurium)																	
Labcode	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
PHLS	**141**	-	-	-	++	++	+	-	-	scl	+++	+	+	-	scl	scl	-	-	scl
1	**141**	-	-	-	±	±ų	-	-	-	scl	+	±	±	- 4	scl	scl	-	-	±
2	**141**	-	-	-	±±	±± ±	-	-	-	scl	++	±	+	-	++ +	++ +	-	-	±
4	**68**	-	-	-	±	-	-	-	-	< cl	±	5	±	-	±	++ +	-	-	-
5	**4**	-	-	-	±±	±±	±±	-	-	scl	scl	±	±	-	cl	cl	-	-	++
8	**141**	-	-	-	++ +m µ	+ m µ	-	-	-	scl	-	+ ns m	+ sm µ	-	++ + m	++ sm µ	-	-	± m µ
16	**141**	-	-	-	-	+	-	-	-	+±	+±	+±	+	-	++	+	-	-	-
A	**U296**	+	+	-	++ +	++ +	++	++	-	scl	++	++	scl	scl	scl	cl	+	+	++
B	**141**	-	-	-	+	±	-	-	-	++ +	++ +	+	+	-	±	+	-1	-	-
C	**68**	-	-	-	-	-	-	-	-	< scl	-	-	-	-	-	++ ns	-	-	-
E	**141**	-	-	-	++ +	±± ±	-	-	-	scl	±± ±	++	++	-	< scl	< scl	-	-	++
F	**141A**	-	-	-	ol	ol	-	-	-	scl	ol	++ +	++ +	-	cl	scl	-	-	ol
H	**141**	-	-	-	±± ±	++ +	-	-	-	cl	+	+	+	-	< cl	cl	-	-	±±
J	**4**	-	-	-	++ +	++ +	++	-	-	++ + scl	++ + scl	+ +-	+ +-	-	++ + scl	scl	2n	-	+s
K	**141**	-	-	-	++ +	++	-	-	-	++ +	++	+	+	-	++ +	++ +	-	-	-
P	**68**	-	-	-	-	-	-	-	-	cl	-	-	-	-	-	cl	-	-	-
T	**141**	-	-	-	++ +	++ +	-	-	-	++ +	-	+	-	-	++ +	scl	-	-	scl
Y	**4**	-	-	-	< scl	< scl	++ +	-	-	< cl	< scl	< scl	< scl	-	< scl	scl	-	-	++ s

Strain M 14 (B)

		Phages at Routine Test Dilution (*S.* Typhimurium)												Additional phages					
Labcode	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10 var	18
PHLS	**141**	scl	-	ol	cl	-	++ +	+	cl	-	cl	scl	ol	++ +	++ +	++	ol	ol	++ +
1	**141**	±	-	ol	< cl	-	±	± -1	cl	- 1	cl	scl	cl						
2	**141**	++	-	scl	++ +	-	++	+	cl	±	cl	<< scl	cl						
4	**68**	++ +	-	< cl	< cl	-	4	+	cl	±	cl	scl	ol	+	+	+	ol	< ol	±
5	**4**	scl	-	cl	scl	-	scl	±	cl	-	cl	scl	cl						
8	**141**	++ sm	-	sol	++ ns	-	± m µ	+ sm	cl	+ ns	cl	scl	cl						
16	**141**	+	-	scl	scl	-	+±	-	scl	±	cl	±	ol	+±	+±	+±	ol	ol	-
A	**U296**	scl	++	scl	scl	++	++	++	scl	-	ol	scl	ol	+	scl	++ +	scl		++ +
B	**141**	++ +	-	scl	scl	-	++ µ	+	cl	-2	< cl	scl	ol	++	++ +	++	ol	ol	-5
C	**68**	< scl	2n	<< scl	< scl	-	1n	++ 1	< scl	-	ol	scl	scl						
E	**141**	++ +	-	scl	++ +	-	++ +	++	cl	-	cl	++ +	cl	-	+	++	ol	-	-
F	**141A**	++ +	-	scl	scl	-	++ +	+	cl	-	cl	++ +	ol						
H	**141**	< cl	-	cl	< cl	-	±±	+	cl	±	cl	scl	ol						
J	**4**	scl	-	cl	scl	-	++ +-	±n	cl	-	cl	++ + scl	ol						
K	**141**	+	-	++ + scl	++ + scl	-	+	+	++ + scl	-	cl	??	cl	++	++	++	ol	ol	+
P	**68**	cl	-	cl	cl	-	-	-	cl	-	cl	cl	cl	-	-	-	cl	ol	ol
T	**141**	scl	-	scl	scl	-	+	+	scl	-	scl	scl	scl						
Y	**4**	< scl	-	< ol	scl	-	++ s	-	cl	-	cl	scl	ol						

Strain M 15 (A)

		Phages at Routine Test Dilution (*S.* Typhimurium)																	
Labcode	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
PHLS	**104(L)**	-	-	-	-	-	-	-	-	-	-	++	scl	-	-	-	-	+++	-
1	**104L**	-	-	-	-	-	-	-	-1	-	-	± scl	±L scl	-	-	-	-	±	-
2	**104L**	-	-	-	-	-	-	-	-	-	-	++	+++	-	-	-	-	±	-
4	**104**	-	-	-	-	-	-	-	-	-	-	++	scl	-	-	-	-	-	
5	**104**	-	-	-	-	-	-	-	-	-	-	cl	cl	-	-	-	-	scl	-
8	**104L**	-	-	-	-	-	-	-	-	-	-	++ n	< scl	-	-	-	-	++ ns	-
16	**104L**	-	-	-	-	-	-	-	-	-	-	+±	+++	-	-	-	-	++	-
A	**104L**	-	-	-	-	-	-	-	+	-	-	++	+++	-	-	-	-	+++	-
B	**104**	-	-	-	-	-	-	-	-	-	-	++	+++	-	-	-	-	++	
C	**104L**	-	-	-	-	-	-	-	-	-	-	++ 1	+ 1	-	-	-	-	+s	-
E	**104L**	-	-	-	-	-	-	-	-	-	-	scl	scl	-	-	-	-	++	-
F	**104L**	-	-	-	-	-	-	-	-	-	-	scl	scl	-	-	-	-	cl	-
H	**104L**	-	-	-	-	-	-	-	3	-	-	< +	scl	-	-	-	-	±± ±	-
J	**104L**	-	-	-	-	-	-	-	-	-	-	< scl	scl	-	-	-	-	+s	-
K	**104**	-	-	-	-	-	-	-	-	-	-	++ + scl	scl	-	-	-	-	++	-
P	**104H**	-	-	-	-	-	-	-	-	-	-	scl	cl	-	-	-	-	cl	-
T	**104**	-	-	-	-	-	-	-	-	-	-	scl	scl	-	-	-	-	scl	-
Y	**104**	-	-	-	-	-	-	-	-	-	-	< scl	< scl	-	-	-	-	< scl	-

Strain M 15 (B)

		Phages at Routine Test Dilution (*S.* Typhimurium)												Additional phages					
Labcode	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10 var	18
PHLS	**104(L)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ol	ol	-
1	**104L**	-	-	-	-	-	-	-	-	-	-	-	-						
2	**104L**	-	-	-	-	-	-	-	-	-	-	-	-						
4	**104**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ol	ol	-
5	**104**	±	-	-	-	-	-	-	±	-	-	±	±	-	-	-	ol		-
8	**104L**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< ol	< ol	-
16	**104L**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ol	ol	-
A	**104L**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ol		-
B	**104**	-	-	-	-	-	-	-	±	-	-	-	-	-	-	-	ol	ol	-
C	**104L**	-	-	-	-	-	-	-	-	-	-	-	-						
E	**104L**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ol	ol	-
F	**104L**	-	-	-	-	-	-	-	-	-	-	-	-						
H	**104L**	-	-	-	-	-	-	-	±	-	-	-	-				-	-	-
J	**104L**	-	-	-	-	-	-	-	-	-	-	-	-						
K	**104**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	scl	ol	-
P	**104H**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ol	ol
T	**104**	-	-	-	-	-	-	-	-	-	-	-	-						
Y 104	**104**	-	-	-	-	-	-	-	-	-	-	-	-						

Strain M 16 (A)

		Phages at Routine Test Dilution (*S.* Typhimurium)																	
Labcode	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
PHLS	**1**	ol	scl	ol	ol	cl	ol	cl	-	cl	ol	ol	ol	cl	cl	cl	cl	cl	cl
1	**1**	cl	< cl	cl	ol	cl	< cl	cl	-	++ LL scl	cl	cl	cl	cl	cl	cl	cl	< cl	cl
2	**1**	++ +	scl	++ +	ol	ol	ol	ol	-	< ol	ol	scl	scl	cl	cl	cl	cl	++ +	scl
4	**1**	< ol	+	< ol	ol	cl	cl	cl	-	scl	ol	< cl	ol	cl	cl	cl	scl	< cl	< cl
5	**1**	cl	scl	cl	cl	cl	cl	cl	-	cl	cl	scl	cl	cl	cl	cl	cl	cl	cl
8	**1**	< cl	< cl	++ n <<	ol	< cl	cl	cl	-	< cl	< scl	< cl	< cl	cl	cl	cl	< cl	< cl	< cl
16	**1**	-	±	cl	+	++	++	cl	-	±	scl	cl/ ol	cl/ ol	cl	cl	++ +	++ +	cl	±
A	**1**	scl	scl	scl	scl	scl	scl	cl	+	scl	scl	scl	scl	cl	scl	cl	scl	ol	scl
B	**1**	cl	++	cl	ol	cl	cl	cl	-3	scl	cl	cl	cl	cl	cl	scl	cl	cl	scl
C	**1**	scl	< scl	scl	< cl	scl	< scl	scl	-	< scl	< scl	scl	< cl	ol	ol	< cl	++ 1	< scl	++ 1
E	**1**	cl	< cl	cl	cl	cl	cl	cl	-	scl	cl	cl	cl	cl	cl	cl	cl	< cl	< cl
F	**36**	cl	cl	cl	ol	cl	cl	cl	++	cl	scl	scl	scl	ol	ol	cl	cl	cl	scl
H	**1**	cl	< cl	cl	ol	cl	cl	cl	-	cl	cl	cl	cl	cl	cl	cl	< cl	< cl	cl
J	**1**	cl	< cl	< cl	ol	cl	cl	cl	±s	++ < scl	cl	ol	ol	cl	cl	< cl	cl	cl	scl
K	**1**	++ + scl	++ + scl	++ + scl	ol	cl	++ + scl	scl	-	scl	cl	cl	cl	ol	ol	cl	cl	cl	++ + scl
P	**1**	scl	-	scl	scl	cl	cl	cl	-	scl	scl	ol	cl	scl	cl	cl	cl	cl	cl
T	**1**	scl	scl	scl	scl	scl	++ +	cl	-	++ +	scl	scl	scl	++ +	cl	scl	scl	++ +	scl
Y	**1**	< cl	++ L	< scl	< ol	> cl	cl	scl	-	cl	ol	cl	cl	cl	ol	ol	ol	cl	cl

Strain M 16 (B)

		Phages at Routine Test Dilution (*S.* Typhimurium)												Additional phages					
Labcode	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10 var	18
PHLS	**1**	**cl**	**ol**	**ol**	**cl**	**cl**	**cl**	**cl**	**cl**	**±**	**cl**	**cl**	**ol**	**++**	**++**	**++**	**ol**	**ol**	**ol**
1	**1**	++ LL <cl	**cl**	**ol**	**cl**	**cl**	- 1 LL <cl	± L	**cl**	- 2L	**cl**	**cl**	**ol**						
2	**1**	++ +	++ +	**ol**	++ +	++ +	++ +	**scl**	**cl**	**±**	**cl**	**scl**	**ol**						
4	**1**	**scl**	< cl	**ol**	**scl**	**scl**	**scl**	**cl**	**ol**	**±**	**cl**	< cl	**ol**	**+**	**+**	**+**	**ol**	**ol**	**cl**
5	**1**	**scl**	**cl**	**cl**	**cl**	**cl**	**cl**	**cl**	**cl**	**-**	**cl**	**cl**	**cl**						
8	**1**	++ µ <<	< cl	< cl	**scl**	< cl	< scl	< cl	**cl**	+ 1	**cl**	< cl	**ol**						
16	**1**	++ +	**ol**	**ol**	**ol/ scl**	+ ±	+ +±	**cl**	**cl/ ol**	**±**	**cl**	++±	**ol**	**±**	**+**	**±**	**ol**	**ol**	**ol**
A	**1**	**cl**	**cl**	**scl**	**scl**	**cl**	**scl**	**scl**	**scl**	**+**	**cl**	scl	**ol**	**+**	scl	scl	scl		**-**
B	**1**	**scl**	< cl	**scl**	**cl**	< cl	**scl**	**scl**	**cl**	**-3**	**cl**	++ +	**ol**	**+**	**++**	**+**	**ol**	**ol**	**ol**
C	**1**	**scl**	**scl**	< scl	**scl**	**scl**	< scl	< scl	**ol**	**-**	**ol**	< cl	**ol**						
E	**1**	< scl	< cl	**cl**	**scl**	< cl	**scl**	**cl**	**cl**	**-**	**cl**	< cl	**cl**	**-**	**+**	**+**	**ol**	**ol**	**cl**
F	**36**	**cl**	**scl**	**ol**	**cl**	**cl**	**cl**	**ol**	**scl**	++	**cl**	**cl**	**ol**						
H	**1**	< cl	**cl**	**cl**	**cl**	**cl**	**cl**	**cl**	**cl**	**±**	**cl**	**scl**	**ol**						
J	**1**	< cl	< cl	**ol**	**cl**	**cl**	**scl**	**scl**	**cl**	**-**	**cl**	< cl	**ol**						
K	**1**	++ + scl	++ + scl	**scl**	**scl**	**ol**	++ + scl	**scl**	**scl**	**-**	**cl**	++ + scl	**ol**	**++**	++ +	**++**	**ol**	**ol**	**ol**
P	**1**	**cl**	**cl**	**cl**	**cl**	**cl**	**scl**	**scl**	**cl**	**-**	**cl**	**cl**	**cl**	**-**	**-**	**-**	**ol**	**ol**	**ol**
T	**1**	++	**scl**	**cl**	++ +	**cl**	++ +	**cl**	**cl**	**-**	**scl**	++ +	**scl**						
Y	**1**	cl	**cl**	**ol**	cl	**cl**	< scl	< scl	**cl**	**-**	**cl**	< cl	**ol**						

Strain M 17 (A)

		Phages at Routine Test Dilution (*S.* Typhimurium)																	
Labcode	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
PHLS	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	**194**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B	**193a**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
J	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
K	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T	**208**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Y	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Strain M 17 (B)

		Phages at Routine Test Dilution (*S.* Typhimurium)												Additional phages					
Labcode	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10 var	18
PHLS	**193**	-	-	-	-	-	-	-	-	-	-	-	-	++ +	++ +	++ +	-	-	-
1	**193**	-	-	-	-	-	-	-	-	-	-	-	-	++- ++ +	scl	scl	++ +ol		-
2	**194**	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
4	**193**	-	-	-	-	-	-	-	-	-	-	-	-	++ +	scl	++ +	-	-	-
5	**193**	-	-	-	-	-	-	-	-	-	-	-	-	scl	scl	scl	-		-
8	**193**	-	-	-	-	-	-	-	-	-	-	-	-	++ ns	++ ns	++ ns	-	-	-
16	**193**	-	-	-	-	-	-	-	-	-	-	-	-	++	++ +	++	< ol	+±	-
A	**193**	-	-	-	-	-	-	-	-	-	-	-	-	+	scl	scl	scl		-
B	**193a**	-	-	-	-	-	-	-	-	-	-	-	-	++ +	++ +	++ +	++	±	±
C	**193**	-	-	-	-	-	-	-	-	-	-	-	-	++ sn	++ sn	++ sn	-	ol	-
E	**193**	-	-	-	-	-	-	-	-	-	-	-	-	++	++ +	++ +	++ +	+ +	-
F	**193a**	-	-	-	-	-	-	-	-	-	-	-	-	++ +	scl	++ +	++		< ol
H	**193**	-	-	-	-	-	-	-	-	-	-	-	-	scl	scl	scl	-	-	-
J	**193**	-	-	-	-	-	-	-	-	-	-	-	-	++ +	< scl	< scl	ol		-
K	**193**	-	-	-	-	-	-	-	-	-	-	-	-	++	++	++ +	ol	++ +5	-
P	**193**	-	-	-	-	-	-	-	-	-	-	-	-	scl	scl	scl	-	-	-
T	**208**	-	-	-	-	-	-	-	-	-	-	-	-	+	scl	+	scl		++
Y	**193**	-	-	-	-	-	-	-	-	-	-	-	-	< scl	< scl	scl	-		-

Strain M 18 (A)

		Phages at Routine Test Dilution (*S.* Typhimurium)																	
Labcode	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
PHLS	**104(H)**	-	-	-	-	-	-	-	-	-	-	cl	cl	-	-	-	-	scl	-
1	**104H**	-	-	-	-	-	-	-	-1	-	-	cl	cl	-	-	-	-	scl	-
2	**104H**	-	-	-	-	-	-	-	-	-	-	< cl	cl-	-	-	-	±	±±	-
4	**104H**	-	-	-	-	-	-	-	-	-	-	cl	cl	-	-	-	-	scl	-
5	**U308**	-	-	-	-	-	-	-	-	-	-	ol	ol	-	±±	-	±	±	±
8	**104H**	-	-	-	-	-	-	-	-	-	-	< cl	cl	-	-	-	-	scl	-
16	**104H**	-	-	-	-	-	-	-	-	-	-	cl	cl	-	-	-	-	++ +	-
A	**104H**	-	-	-	-	-	-	-	+	-	-	scl	cl	-	-	-	-	cl	-
B	**104**	-	-	-	-	-	-	-	-5	-	-	cl	cl	-	-	-	-3	scl	-
C	**104H**	-	-	-	-	-	-	-	-	-	-	scl	scl	-	-	-	-	< scl	
E	**104H**	-	-	-	-	-	-	-	-	-	-	cl	cl	-	-	-	-	++ +	-
F	**104H**	-	-	-	-	-	-	-	-	-	-	scl	scl	-	-	-	-	-	-
H	**104H**	-	-	-	-	-	-	-	3	-	-	cl	cl	-	-	-	-	< cl	-
J	**104H**	-	-	-	-	-	-	-	±s	-	-	cl	cl	-	-	-	1n	cl	-
K	**104**	-	-	-	-	-	-	-	-	-	-	++ + scl	scl	-	-	-	-	scl	-
P	**104C**	-	-	-	-	-	-	-	scl	-	-	cl	ol	-	-	-	-	cl	-
T	**104**	-	-	-	-	-	-	-	-	-	-	scl	scl	-	+	-	-	++	-
Y	**104**	-	-	-	-	-	-	-	±s	-	-	< cl	< cl	-	-	-	-	< cl	-

Strain M 18 (B)

		Phages at Routine Test Dilution (*S.* Typhimurium)												Additional phages					
Labcode	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10 var	18
PHLS	**104(H)**	-	-	-	-	-	-	-	±	-	-	-	±	-	-	-	ol	ol	-
1	**104H**	-	-	-	-	-	-	-	-	-	-	-	-						
2	**104H**	-	-	-	-	-	-	-	±	-	-	-	±						
4	**104H**	-	-	-	-	-	-	-	5	-	-	-	3	1	-	-	ol	ol	-
5	**U308**	scl	-	-	-	-	-	-	±	2L	-	+	+						
8	**104H**	-	-	-	-	-	-	-	± ns	1 n	-	1s	3 ns	-	-	-	< ol	< ol	-
16	**104H**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ol	ol	-
A	**104H**	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	ol		-
B	**104**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ol	ol	-
C	**104H**	-	-	-	-	-	-	-	-	-	-	-	-						
E	**104H**	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	ol	ol	-
F	**104H**	-	-	-	-	-	-	-	+	-	-	-	+						
H	**104H**	±	-	-	-	-	-	-	±	-	-	-	±						
J	**104H**	3l	-	-	-	-	1l	-	2s	-	-	1l	6n						
K	**104**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	scl	scl	-
P	**104C**	-	-	-	-	-	-	-	scl	-	-	-	-	-	-	-	ol	ol	-
T	**104**	-	-	-	-	-	-	-	-	-	-	-	-						
Y	**104**	-	-	-	-	-	-	-	-	-	-	-	-						

Strain M 19 (A)

		Phages at Routine Test Dilution (*S.* Typhimurium)																	
Labcode	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
PHLS	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	**193**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F	**208**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
J	**195**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
K	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P	**208**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T	**U302**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Y	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Strain M 19 (B)

		Phages at Routine Test Dilution (*S.* Typhimurium)												Additional phages					
Labcode	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10 var	18
PHLS	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ol	-
1	**193**	-	-	-	-	-	-	-	-	-	-	-	-	±	±	+	< cl		-
2	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+ L	ol	-
4	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	scl	-
5	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	±	±	±			
8	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±s	ol	-
16	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ol	-
A	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	ol	-
B	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	ol	-
C	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++ l	ol	-
F	**208**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		ol
E	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< scl	ol	-
H	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		ol	-
J	**195**	-	-	-	-	-	-	-	-	-	-	-	-	±	±	+±	< ol		-
K	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+2	ol	-
P	**208**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ol	ol	ol
T	**U302**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++ +		-
Y	**U310**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++ L		-

Strain M 20 (A)

		Phages at Routine Test Dilution (*S.* Typhimurium)																	
Labcode	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
PHLS	**124**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	**124**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	**124**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	**124**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	**124**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	**124**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	**124**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A	**124**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B	**124**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	**195**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E	**124**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F	**124**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H	**124**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
J	**NT**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
K	**NT**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P																			
T	**U**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Y	**124**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Strain M 20 (B)

		Phages at Routine Test Dilution (*S.* Typhimurium)												Additional phages					
Labcode	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10	18
PHLS	**124**	-	-	-	-	-	-	-	-	ol	-	-	-	-	-	+	±	±	-
1	**124**	-	-	-	-	-	-	-	-	ol	-	-	-						
2	**124**	-	-	-	-	-	-	-	-	ol	-	-	-						
4	**124**	-	-	-	-	-	-	-	-	sol	-	-	-	-	-	+	[2]	[2]	-
5	**124**	-	-	-	-	-	-	-	-	ol	-	-	-						
8	**124**	-	-	-	-	-	-	-	-	ol	-	-	-						
16	**124**	-	-	-	-	-	-	-	-	ol	-	-	-	-	-	+	-	-	-
A	**124**	-	-	-	-	-	-	-	-	scl	-	-	-	-	-	++	-		-
B	**124**	-	-	-	-	-	-	-	-	++ +	-	-	-	-	-	++	±	±	-
C	**195**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++ ns	-	ol	-
E	**124**	-	-	-	-	-	-	-	-	ol	-	-	-	-	-	-	-	-	-
F	**124**	-	-	-	-	-	-	-	-	ol	-	-	-						
H	**124**	-	-	-	-	-	-	-	-	ol	-	-	-						
J	**NT**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-
K	**NT**	-	-	-	-	-	-	-	-	++ 2	-	-	-	-	-	+	-	-	-
P																			
T	**U**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-
Y	**124**	-	-	-	-	-	-	-	-	ol	-	-	-						

Appendix 5 Results questionnaire AST per antibiotic (MIC testing)

	MIC in ug/ml for ampicillin (AMP)														
Labcode	.03	.06	.12	.25	.50	1	2	4	8	16	32	64	128	256	512
1											B				
4											B				
6															
10															
11										B					

	MIC in ug/ml for chloramphenicol (CHL)														
Labcode	.03	.06	.12	.25	.50	1	2	4	8	16	32	64	128	256	512
1											B				
4											B				
6										B					
10															
11										B					

	MIC in ug/ml for cefotaxime (CEF)														
Labcode	.03	.06	.12	.25	.50	1	2	4	8	16	32	64	128	256	512
1															
4															
6											B				
10															
11															

	MIC in ug/ml for ciprofloxacin (CIP)														
Labcode	.03	.06	.12	.25	.50	1	2	4	8	16	32	64	128	256	512
1							B								
4								B							
6								B							
10															
11															

	MIC in ug/ml for gentamicin (GEN)														
Labcode	.03	.06	.12	.25	.50	1	2	4	8	16	32	64	128	256	512
1										B					
4										B					
6									B						
10															
11										B					

	MIC in ug/ml for kanamycin (KAN)														
Labcode	.03	.06	.12	.25	.50	1	2	4	8	16	32	64	128	256	512
1												B			
4															
6															
10															
11															

Sensitive	Resistant	Test range
Intermediate	Not tested	B = Breakpoint

	MIC in ug/ml for nalidixic acid (NAL)														
Labcode	.03	.06	.12	.25	.50	1	2	4	8	16	32	64	128	256	512
1											B				
4											B				
6															
10															
11											B				

	MIC in ug/ml for neomycin (NEO)														
Labcode	.03	.06	.12	.25	.50	1	2	4	8	16	32	64	128	256	512
1										B					
4										B					
6											B				
10															
11												B			

	MIC in ug/ml for streptomycin (STR)														
Labcode	.03	.06	.12	.25	.50	1	2	4	8	16	32	64	128	256	512
1											B				
4											B				
6															
10															
11												B			

	MIC in ug/ml for sulfamethoxazole+trimethoprim (SXT)														
Labcode	.03	.06	.12	.25	.50	1	2	4	8	16	32	64	128	256	512
1								B							
4															
6								B							
10															
11															

	MIC in ug/ml for tetracyclin (TET)														
Labcode	.03	.06	.12	.25	.50	1	2	4	8	16	32	64	128	256	512
1										B					
4										B					
6															
10															
11										B					

	MIC in ug/ml for trimethoprim (TMP)														
Labcode	.03	.06	.12	.25	.50	1	2	4	8	16	32	64	128	256	512
1										B					
4										B					
6								B							
10															
11										B					

Sensitive	Resistant	Test range
Intermediate	Not tested	B = Breakpoint

Appendix 6 Results questionnaire AST per antibiotic (Disk diffusion)

		Inhibition zones in mm for ampicillin (AMP)																			
Labcode	ug/ml	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2	10							B	B	B	B	B									
5	10							B													
7	10	?	?	?	?	?															
8	10							B	B	B	B	B									
9	10																				
12	10								B	B	B	B	B	B							
13	33														B						
14	10							B	B	B	B	B									
15	10																				
16	10		B	B	B	B	B	B	B	B	B	B	B	B	B	B	B				
17	10							B													

		Inhibition zones in mm for chloramphenicol (CHL)																			
Labcode	ug/ml	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
2	30	B	B	B	B	B	B	B													
5	10		B																		
7	30																				
8	30	B	B	B	B	B	B	B													
9	30																				
12	30								B	B	B	B	B								
13	60														B						
14	30	B	B	B	B	B	B	B													
15	30																				
16	30													B	B	B	B	B	B	B	B
17	30	B																			

		Inhibition zones in mm for cefotaxime (CEF)																			
Labcode	ug/ml	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2	30								B	B	B	B	B	B	B	B	B	B			
5																					
7																					
8	30								B	B	B	B	B	B	B	B	B	B			
9																					
12	30										B	B	B	B	B						
13	60																	B			
14	30								B	B	B	B	B	B	B	B	B	B			
15																					
16																					
17	30								B												

Sensitive | Resistant | B = Breakpoint

Intermediate | Not tested

		Inhibition zones in mm for ciprofloxacin (CIP)																			
Labcode	ug/ml	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
2	5						B	B	B	B	B	B	B								
5																					
7	5																				
8	5						B	B	B	B	B	B	B								
9	5																				
12																					
13	5											B									
14																					
15	5																				
16	10									B	B	B	B	B	B	B	B	B	B		
17	5						B														

		Inhibition zones in mm for gentamicin (GEN)																			
Labcode	ug/ml	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2	10						B	B	B	B											
5	10							B													
7	10																				
8	10						B	B	B	B											
9	10																				
12	10								B	B	B										
13	40																	B			
14	10						B	B	B	B											
15	10																				
16																					
17	10						B														

		Inhibition zones in mm for kanamycin (KAN)																			
Labcode	ug/ml	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2	30							B	B	B	B	B	B								
5																					
7																					
8	30							B	B	B	B	B	B								
9	30																				
12	30									B	B	B									
13																					
14	30						B	B	B	B	B	B	B	B							
15	30																				
16																					
17	30							B													

Sensitive	Resistant	B = Breakpoint
Intermediate	Not tested	

		Inhibition zones in mm for nalidixic acid (NAL)																			
Labcode	ug/ml	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2	30							B	B	B	B	B	B	B							
5	30							B													
7																					
8	30							B	B	B	B	B	B	B							
9	30																				
12	30								B	B	B	B	B	B	B						
13																					
14	30							B	B	B	B	B	B	B							
15	30																				
16	30								B												
17	30							B													

		Inhibition zones in mm for neomycin (NEO)																			
Labcode	ug/ml	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2	30						B	B	B	B	B	B									
5	10							B													
7	30																				
8																					
9																					
12																					
13	120																			B	
14	30						B	B	B	B											
15																					
16																					
17	10					B															

		Inhibition zones in mm for streptomycin (STR)																			
Labcode	ug/ml	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2	10					B	B	B	B	B											
5	25							B													
7	10	?	?	?	?	?															
8	10					B	B	B	B	B											
9	10	?	?	?	?	?															
12	10						B	B	B	B											
13	100																				B
14	10					B	B	B	B	B											
15	10																				
16	30							B	B	B	B	B	B								
17	10					B															

Sensitive	Resistant	B = Breakpoint
Intermediate	Not tested	

		Inhibition zones in mm for sulfamethoxazole+trimethoprim (SXT)																			
Labcode	ug/ml	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
2	25		B	B	B	B	B	B	B												
5	25					B															
7	25	?	?	?																	
8																					
9	25	?	?	?																	
12	25		B	B	B	B	B	B	B												
13	245																				B
14	25		B	B	B	B	B	B	B												
15	25																				
16	25					B	B	B	B	B	B	B	B	B	B	B	B	B	B		
17	25		B																		

		Inhibition zones in mm for tetracyclin (TET)																			
Labcode	ug/ml	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
2	30						B	B	B	B	B	B									
5	10					B															
7	30																				
8	30						B	B	B	B	B	B									
9	30																				
12	30									B	B	B									
13	80															B					
14	30						B	B	B	B	B	B									
15	30																				
16	30											B	B	B	B	B	B	B			
17	30						B														

		Inhibition zones in mm for trimethoprim (TMP)																			
Labcode	ug/ml	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2																					
5																					
7																					
8	5				B	B	B	B	B	B	B										
9	5	?	?	?	?	?															
12	5				B	B	B	B	B	B	B										
13																					
14																					
15																					
16																					
17	5				B																

Sensitive	Resistant	B = Breakpoint
Intermediate	Not tested	

Appendix 7 Achievements in % correctness by NRLs and ENLs

Labcodes NRLs	O-antigens n = 20	H-antigens n = 20	Serovar names n = 20	SE Phage n = 10	STM Phage n = 10
1	100	100	100	90	80
2	95	100	95	90	90
3	70	75	80		
4	100	100	100	80	90
5	100	100	95	60	80
6	100	100	95	90	
7	95	95	100		
8	90	100	90	90	80
9	90	100	90		
10	90	100	100		
11	100	100	100		
12	85	100	90		
13	95	95	100		
14	85	100	85		
15	75	95	80		
16	95	100	95	80	100
17	95	100	95		

Labcodes ENLs	O-antigens n = 20	H-antigens n = 20	Serovar names n = 20	SE Phage n = 10	STM Phage n = 10
A	100	100	100	80	80
B	100	100	100	90	80
C	100	100	100	100	80
D	95	100	95		
E	90	100	90	100	100
F	70	85	95	40	60
H	100	100	100	100	100
J	90	100	95	80	70
K	100	100	100	90	70
P	90	90	90	60	50
R	95	95	100		
T	85	100	80	20	50
W	100	100	100		
Y	90	90	100	100	90
Z	100	100	100		