



National Institute for Public Health  
and the Environment  
*Ministry of Health, Welfare and Sport*

**Sixteenth EURL-*Salmonella*  
interlaboratory comparison study  
(2011) on typing of *Salmonella* spp.**

RIVM report 330604027/2012

W.F. Jacobs-Reitsma et al.



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## Colophon

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## Abstract

### **Sixteenth EURL-*Salmonella* interlaboratory comparison study (2011) on typing of *Salmonella* spp.**

The 28 National Reference Laboratories (NRLs) of all 27 European Union (EU) Member States performed well on the 2011 quality control test on *Salmonella* typing. Two laboratories were found to require a follow-up study on their first test. Altogether, the EU-NRLs were able to assign the correct name to 97% of the strains tested.

### **Other participants interlaboratory comparison study *Salmonella***

Since 1992, the NRLs of the EU Member States have been required to participate in annual quality control tests, which consist of interlaboratory comparison studies on *Salmonella*. Laboratories from countries outside the European Union, like EU-candidate countries, occasionally participate in these tests on a voluntary basis. Eight additional laboratories participated in the current study. Two EU-candidate countries amongst these eight additional participants did not meet the criteria for good performance in the first round. One of them did not reach this goal in the follow-up study either. The other was not able to participate in the follow-up study; a follow-up study is not compulsory for non-EU laboratories.

Each EU Member State designates a specific laboratory within their national boundaries to be responsible for the detection and identification of *Salmonella* strains from animals and/or food products. These laboratories are then referred to as the National Reference Laboratories. The performance of these NRLs on *Salmonella* typing is assessed annually, based on their capability to correctly identify 20 *Salmonella* strains.

### **Phage typing**

Nine NRLs not only serotyped the 20 *Salmonella* strains of the quality control test, but also subtyped 20 additional strains by phage typing. For this, the laboratories received ten strains of *Salmonella* Enteritidis and ten strains of *Salmonella* Typhimurium. These NRLs typed 98% of the *S. Typhimurium* strains correctly. Of the *S. Enteritidis* strains, 88% were phage typed correctly.

The European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organises this annual interlaboratory comparison study on typing of *Salmonella* in cooperation with the Health Protection Agency in London, UK. The EURL-*Salmonella* is situated at the National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands.

**Keywords:** EURL-*Salmonella*, *Salmonella*, serotyping, phage typing, interlaboratory comparison study



## Rapport in het kort

### **Zestiende EURL-*Salmonella* ringonderzoek (2011) voor de typering van *Salmonella* spp.**

De 28 Nationale Referentie Laboratoria (NRL's) van de 27 Europese lidstaten scoorden in 2011 goed bij de kwaliteitscontrole om *Salmonella* te typeren. Twee laboratoria hadden hiervoor een herkansing nodig. Alle NRL's samen konden gemiddeld genomen aan 97 procent van de geteste stammen de juiste naam geven.

#### **Overige deelnemers ringonderzoek *Salmonella***

Sinds 1992 zijn de NRL's van de Europese lidstaten verplicht om deel te nemen aan jaarlijkse kwaliteitstoetsen, de zogeheten ringonderzoeken voor *Salmonella*. Soms doen ook landen buiten de Europese Unie vrijwillig mee, zoals kandidaat-lidstaten. Dit jaar deden er acht niet-lidstaten mee. Twee EU-kandidaat-lidstaten onder hen scoorden in de eerste ronde onvoldoende. Eén van hen behaalde ook in de herkansing niet het gewenste resultaat. De ander heeft de herkansing niet kunnen uitvoeren; voor niet-lidstaten is de herkansing niet verplicht.

Voor de ringonderzoeken wijst elke lidstaat een laboratorium aan, het Nationale Referentie Laboratorium (NRL), dat binnen dat land verantwoordelijk is om *Salmonella* uit monsters van levensmiddelen of dieren aan te tonen en te typeren. Om te controleren of de laboratoria hun werk goed uitvoeren moeten zij onder andere 20 *Salmonella*-stammen op juiste wijze identificeren.

#### **Faagtyperingen**

Van de NRL's zijn er negen laboratoria die, behalve de standaardtoets (serotypering) op *Salmonella*, preciezere typering uitvoeren, de zogeheten faagtypering. Voor deze kwaliteitstoets moeten zij 20 extra stammen met deze methode typeren. De laboratoria ontvingen hiervoor tien *Salmonella* Enteritidis-stammen en tien *Salmonella* Typhimurium-stammen. Deze NRL's typeerden 98 procent van de *S. Typhimurium*-stammen en 88 procent van de *S. Enteritidis*-stammen op de juiste wijze.

De organisatie van het typeringsringonderzoek is in handen van het Europese Unie Referentie Laboratorium (EURL) voor *Salmonella* (EURL-*Salmonella*). Het EURL-*Salmonella* is ondergebracht bij het Nationaal Instituut voor Volksgezondheid en Milieu (RIVM) in Bilthoven, Nederland. De organisatie van dit ringonderzoek is uitgevoerd in samenwerking met de Health Protection Agency (HPA) in Londen, Engeland.

Trefwoorden: EURL-*Salmonella*, *Salmonella*, serotypering, faagtypering, vergelijkend laboratoriumonderzoek



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## Summary

In November 2011, the 16th interlaboratory comparison study on typing of *Salmonella* was organised by the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*, Bilthoven, the Netherlands) in collaboration with the Health Protection Agency (HPA, London, United Kingdom). The main objective of the study was to evaluate whether typing of *Salmonella* strains by the National Reference Laboratories (NRLs-*Salmonella*) within the European Union was being carried out uniformly and whether comparable results were obtained.

A total of 28 NRLs-*Salmonella* of the 27 Member States of the European Union participated, supplemented by eight participants from non-EU countries, like e.g. EU-candidate countries.

All 36 laboratories performed serotyping. A total of 20 *Salmonella* strains were selected for serotyping by the EURL-*Salmonella*. The strains had to be typed with the method routinely used in each laboratory, following the White-Kauffman-Le Minor scheme. The laboratories were allowed to send strains for serotyping to another specialised laboratory in their country if this was part of their usual procedure.

Overall, 98% of the strains were typed correctly for the O-antigens, 96% of the strains were typed correctly for the H-antigens and 96% of the strains were correctly named by the participants.

At the CRL-*Salmonella* workshop in 2007, the CRL-*Salmonella* proposed a definition for good performance of the NRLs regarding the serotyping. Using this definition, 32 participants achieved good performance. The four laboratories that did not achieve the level of good performance were offered a follow-up study including ten additional strains for serotyping. This follow-up study is obligatory for EU-NRLs and the two EU-NRLs concerned obtained good scores in this follow-up study. Of the other two laboratories, both EU-candidate countries, one voluntarily performed the follow-up study, but did not obtain a good performance. The other was not able to participate in the follow-up study.

Nine of the participating NRLs-*Salmonella* also performed phage typing. Eight NRLs participated in the phage typing of both *S. Enteritidis* and *S. Typhimurium*. One NRL participated only in the phage typing of *S. Enteritidis*. The HPA selected 20 strains for phage typing. Ten were of the serovar *Salmonella* Enteritidis (SE) and ten of the serovar *Salmonella* Typhimurium (STM). The phage typing results of the majority of the laboratories were good. The nine NRLs phage typed 88% of the *Salmonella* Enteritidis strains correctly and eight NRLs correctly phage typed 98% of the *Salmonella* Typhimurium strains.



## List of abbreviations

CRL- <i>Salmonella</i>	Community Reference Laboratory for <i>Salmonella</i> (nowadays EURL- <i>Salmonella</i> )
DT	Definitive type
EFTA	European Free Trade Association
EU	European Union
EURL- <i>Salmonella</i>	European Union Reference Laboratory for <i>Salmonella</i>
HPA	Health Protection Agency
LGP	Laboratory of Gastrointestinal Pathogens
NL	The Netherlands
NRLs- <i>Salmonella</i>	National Reference Laboratories for <i>Salmonella</i>
Nt	Not typable
PT	Phage Type
REF	Reference
RIVM	National Institute for Public Health and the Environment
RNDC	Reacts with the phages but does not confirm to a recognised pattern
SE	<i>Salmonella</i> Enteritidis
STM	<i>Salmonella</i> Typhimurium
UK	United Kingdom



# 1 Introduction

This report describes the 16th interlaboratory comparison study on the typing of *Salmonella* spp. organised by the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*, Bilthoven, the Netherlands) in November 2011.

According to Regulation (EC) no 882/2004, it is one of the tasks of the EURL-*Salmonella* to organise interlaboratory comparison studies for the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) of the European Union. The main objective is for typing of *Salmonella* strains in the Member States to be carried out uniformly and comparable results to be obtained. The organisation of the typing studies started in 1995.

A total of 36 laboratories participated in this study. These included 28 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) in the 27 EU Member States, 3 NRLs of EU-candidate countries, 2 NRLs of EFTA countries and 3 other participants. The main objective of this study was to check the performance of the NRLs for typing of *Salmonella* spp. and to compare the results of typing of *Salmonella* spp. among the NRLs-*Salmonella*. All NRLs performed serotyping of the strains. NRLs of the EU Member States which did not achieve the defined level of good performance for serotyping had to participate in a follow-up study in which ten additional strains were serotyped.

Nine of the NRLs-*Salmonella* performed phage typing on ten *Salmonella* Enteritidis strains and eight of the NRLs-*Salmonella* performed phage typing on ten *Salmonella* Typhimurium strains. The selection of the strains and interpretation of the results of the phage typing were performed in close cooperation with the Health Protection Agency, London, UK.



## 2 Participants

Country	Institute/City
<b>Austria</b>	Austrian Agency for Health and Food Safety (AGES) NRC <i>Salmonella</i> Graz
<b>Belgium</b>	Veterinary and Agrochemical Research Centre (VAR-CODA-CERVA) Brussels
<b>Belgium</b>	Institute of Public Health Brussels
<b>Bulgaria</b>	National Reference Centre of Food Safety Sofia
<b>Croatia</b>	Croatian Veterinary Institute Zagreb
<b>Cyprus</b>	Laboratory for the Control of Foods of Animal Origin (LCFAO) Cyprus Veterinary Services Nicosia
<b>Czech Republic</b>	State Veterinary Institute National Reference Laboratory for Salmonellosis Prague
<b>Denmark</b>	National Food Institute, Technical University of Denmark Department of Microbiology and Risk Assessment Copenhagen
<b>Estonia</b>	Estonian Veterinary and Food Laboratory Tartu
<b>Finland</b>	Finnish Food Safety Authority EVIRA Research Department, Veterinary Bacteriology, Kuopio Laboratory Section Kuopio
<b>France</b>	ANSES, Laboratoire de Sécurité des Aliments, Unité CEB Maisons Alfort
<b>Germany</b>	Federal Institute for Risk Assessment (BfR) National Veterinary Salmonella Reference Laboratory Berlin
<b>Greece</b>	Veterinary Laboratory of Chalkis Chalkis
<b>Hungary</b>	Central Agricultural Office, Food and Feed Directorate Department Food Microbiology Budapest
<b>Ireland</b>	Central Veterinary Research Laboratory Dublin
<b>Italy</b>	Istituto Zooprofilattico Sperimentale delle Venezie Legnaro
<b>Latvia</b>	Institute of Food Safety, Animal Health and Environment 'BIOR' Animal Disease Diagnostic Laboratory Riga



<b>Country</b>	<b>Institute/City</b>
<b>Lithuania</b>	National Food and Veterinary Risk Assessment Institute Vilnius
<b>Luxembourg</b>	Laboratoire National de Santé Luxembourg
<b>Macedonia, FYR of</b>	Food Institute Skopje
<b>Malta</b>	Public Health Laboratory Valletta
<b>the Netherlands</b>	National Institute for Public Health and the Environment Laboratory for Infectious Diseases and Perinatal Screening Bilthoven
<b>Northern Ireland (UK)</b>	Agri-Food and Biosciences Institute (AFBI) Veterinary Sciences Division, Bacteriological Department Belfast
<b>Norway</b>	Norwegian Veterinary Institute Section of Bacteriology Oslo
<b>Norway</b>	Norwegian Institute of Public Health Oslo
<b>Poland</b>	National Veterinary Research Institute Microbiological Department Pulawy
<b>Portugal</b>	Laboratório Nacional de Veterinária Lisboa
<b>Romania</b>	Institute of Diagnosis and Animal Health Bucharest
<b>Serbia</b>	Institute of Veterinary Medicine of Serbia Belgrade
<b>Slovak Republic</b>	State Veterinary and Food Institute Reference laboratory for <i>Salmonella</i> Bratislava
<b>Slovenia</b>	National Veterinary Institute Veterinary Faculty Ljubljana
<b>Spain</b>	Laboratorio Central de Veterinaria Madrid
<b>Sweden</b>	National Veterinary Institute (SVA) Uppsala
<b>Switzerland</b>	Institute of Veterinary Bacteriology National Centre for Zoonoses, Bacterial Animal Diseases and Antimicrobial Resistance (ZOBA) Bern
<b>United Kingdom</b>	Animal Health and Veterinary Laboratories Agency (AHVLA) Addlestone
<b>United Kingdom</b>	Health Protection Agency London

### 3 Materials and Methods

#### 3.1 *Salmonella* strains for serotyping

A total of 20 different *Salmonella* strains (coded S1 - S20) had to be serotyped by the participants. As discussed at the 16th EURL-*Salmonella* Workshop in Zandvoort (Mooijman, 2011), one additional strain (S21) from an uncommon source (flax seeds) and subspecies was included in the study and serotyping of this strain was optional.

The *Salmonella* strains used for the study on serotyping originated from the collection of the National *Salmonella* Centre in the Netherlands. The strains were typed once again by this Centre before mailing. The complete antigenic formulas, according to the most recent White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007), of the 21 serovars are shown in Table 1.

Table 1 Antigenic formulas of the 21 *Salmonella* strains according to the White-Kauffmann-Le Minor scheme used in the 16th EURL-*Salmonella* typing study

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
S1	13,23	d	l,w	Putten
S2	6,7	c	1,5	Choleraesuis
S3	6,7,14	r	1,5	Infantis
S4	1,4,[5],12	i	-	1,4,[5],12:i:-
S5	6,8	b	1,6	Stourbridge
S6	1,13,23	g,t	-	1,13,23:g,t:-
S7	6,8	z <sub>10</sub>	e,n,x	Hadar
S8	6,7,14	r	1,2	Virchow
S9	1,9,12	g,m	-	Enteritidis
S10	1,3,19	y	l,w	Krefeld
S11	1,4,[5],12	e,h	1,2	Saintpaul
S12	1,4,[5],12	z <sub>10</sub>	1,2	Haifa
S13	1,9,12	a	e,n,z <sub>15</sub>	Durban
S14	1,13,23	f,g,[s]	-	Havana
S15	6,8,20	r,[i]	1,5	Bovismorbificans
S16	6,7,14	e,h	e,n,z <sub>15</sub>	Braenderup
S17	11	k	1,5	Abaetetuba
S18	3,{10}{15}{15,34}	g,m,s	-	Amsterdam
S19	1,4,[5],12	i	1,2	Typhimurium
S20	8,20	i	z <sub>6</sub>	Kentucky
S21	38	r	z	38:r:z

S4: Typhimurium, monophasic variant as determined by PCR (EFSA Journal, 2010; 8(10):1826).

S21: *Salmonella enterica* subspecies *diarizonae*

#### 3.2 *Salmonella* strains for phage typing

The *Salmonella* strains for phage typing were obtained from the collection of the *Salmonella* Reference Unit of the Laboratory of Gastrointestinal Pathogens (LGP), Health Protection Agency (HPA), London, UK. Ten strains of *Salmonella* Enteritidis and ten strains of *Salmonella* Typhimurium were selected.

The explanation of the various notations in Tables 2 and 3 are as follows:

-	=	no reaction
±	=	5–20 plaques
+	=	21–40 plaques
++	=	41–80 plaques
+++	=	81–100 plaques
SCL	=	semi-confluent lysis
CL	=	confluent clear lysis
OL	=	confluent opaque lysis
<<	=	merging plaques towards semi-confluent lysis

*Table 2 Phage reactions of the Salmonella Enteritidis strains used in the 16th EURL-Salmonella typing study*

<b>Phage reactions at Routine Test Dilution (<i>S. Enteritidis</i>)</b>																		
Strain number	Phage type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
E1	21c	CL	SCL	-	OL	-	SCL	-	OL	OL	SCL	-	-	-	CL	CL	CL	+++
E2	35	-	SCL	-	OL	-	-	-	-	OL	-	-	-	-	-	-	-	SCL
E3	21	OL	SCL	-	OL	-	SCL	-	OL	OL	OL	-	-	-	CL	-	-	+++
E4	14b	-	-	-	±	-	SCL	-	-	±	-	-	-	-	-	-	-	+++
E5	9b	-	-	CL	-	CL	-	-	-	-	-	-	CL	-	+++	-	-	-
E6	8	-	-	SCL	OL	CL	SCL	CL	OL	OL	OL	CL	CL	-	-	-	-	+++
E7	4b	-	SCL	CL	OL	CL	SCL	CL	OL	OL	OL	CL	CL	CL	-	+	CL	+++
E8	4	-	SCL	CL	OL	CL	SCL	CL	OL	OL	SCL	CL	CL	CL	-	-	-	+++
E9	2	OL	-	CL	OL	CL	SCL	CL	OL	OL	OL	CL	CL	-	SCL	-	-	+++
E10	1b	OL	SCL	CL	OL	CL	SCL	CL	OL	OL	SCL	CL	CL	CL	CL	++	CL	+++

Table 3 Phage reactions of the *Salmonella Typhimurium* strains used in the 16th EURL-Salmonella typing study

Phage reactions at Routine Test Dilution ( <i>S. Typhimurium</i> )																			
Strain number	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
T1	36	CL	OL	CL	OL	OL	OL	OL	CL	CL	OL	CL	CL	CL	OL	OL	OL	CL	OL
T2	136	-	-	-	OL	OL	OL	-	-	-	OL	OL	OL	-	OL	OL	-	-	OL
T3	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T4	104	-	-	-	-	-	-	-	-	-	-	SCL	SCL	-	-	-	-	++	-
T5	99	-	-	-	-	-	-	-	-	-	<CL	-	-	-	-	-	-	-	-
T6	66a	-	-	-	-	-	-	-	-	OL	CL	-	-	-	-	++	-	-	-
T7	66	-	-	-	-	-	-	-	-	OL	OL	++	++	-	-	SCL	-	-	-
T8	10	-	-	-	-	-	-	-	-	CL	OL	OL	OL	-	-	CL	-	-	-
T9	8	-	-	-	-	-	-	-	SCL	SCL	CL	-	-	-	-	+++	-	-	-
T10	1	OL	OL	CL	OL	CL	CL	SCL	-	CL	OL	CL	CL	OL	OL	OL	OL	CL	CL

Phage reactions at Routine Test Dilution ( <i>S. Typhimurium</i> )														Additional phages						
Strain number	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10 var 2	10 var 3	18
T1	36	OL	OL	OL	CL	OL	OL	CL	OL	OL	OL	CL	OL	+++	+++	+++	SCL	OL	OL	OL
T2	136	±	-	-	-	-	CL	-	-	-	-	-	-	+	±	±	SCL	SCL	SCL	-
T3	193	-	-	-	-	-	-	-	-	-	-	-	-	+++	+++	+++	-	-	-	-
T4	104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	OL	OL	-
T5	99	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	SCL	OL	OL	-
T6	66a	SCL	-	SCL	OL	-	±	±	-	-	CL	-	-	-	-	-	-	-	-	-
T7	66	SCL	-	OL	OL	-	±	±	-	-	CL	OL	-	++	++	++	OL	OL	OL	-
T8	10	SCL	-	SCL	SCL	-	±	±	-	-	CL	CL	-	++	+	++	SCL	OL	OL	-
T9	8	++	-	SCL	SCL	-	±	±	-	-	CL	CL	-	+++	+++	+++	SCL	OL	OL	-
T10	1	OL	OL	OL	OL	CL	CL	CL	CL	-	CL	CL	OL	+++	++	++	SCL	OL	OL	CL

### 3.3 Laboratory codes

The NRLs-*Salmonella* were assigned a laboratory code 1–36, which differed from the previous typing studies.

### 3.4 Protocol and test report

Two weeks before the start of the study, the NRLs received the protocol and a test report via e-mail. The protocol and test report can be found in Annex 1 and Annex 2, respectively.

### 3.5 Transport

All samples were packed and transported as Biological Substance Category B (UN 3373) and transported by door-to-door courier service. The parcels containing the strains for serotyping and phage typing were sent by the EURL-*Salmonella* on 7 November 2011 (week 45).

### 3.6 Guidelines for evaluation

The evaluation of the various serotyping results as mentioned in this report is described in Table 4.

*Table 4 Evaluation of serotyping results*

Results	Evaluation	Abbreviation
Auto-agglutination 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	-

At the EURL-*Salmonella* workshop in Bilthoven in May 2007 (Mooijman, 2007), the EURL-*Salmonella* made a proposal for the level of 'good performance' that the NRLs need to achieve during an interlaboratory comparison study on serotyping. Penalty points are given for strains that are typed incorrectly. A distinction is made between the five most important *Salmonella* serovars (as indicated in EU legislation) and all other strains:

- **4 penalty points:** Incorrect typing of *S. Enteritidis*, *S. Typhimurium* (including the monophasic one), *S. Hadar*, *S. Infantis* or *S. Virchow* or assigning the name of one of these five serovars to another strain.
- **1 penalty point:** Incorrect typing of all other *Salmonella* serovars.

For each NRL-*Salmonella* the total number of penalty points is determined. The NRL meets the criterion of 'good performance' if it has fewer than four penalty points.

A follow-up study is organised for NRLs with four penalty points or more. All NRLs of the EU Member States not meeting the criterion of 'good performance' have to participate in this follow-up study.

### 3.7 Follow-up study

The follow-up study for serotyping consisted of typing an additional set of ten *Salmonella* strains. The strains for the follow-up study are shown in Table 5. All EU-NRLs with four penalty points or more had to participate in this follow-up study. The protocol and test report for the follow-up study can be found in Annex 3 and Annex 4, respectively.

*Table 5 Antigenic formulas of the ten Salmonella strains according to the White-Kauffmann-Le Minor scheme used in the follow-up part of the 16th EURL-Salmonella typing study*

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
SF1	6,7, <u>14</u>	r	1,2	Virchow
SF2	<u>1</u> ,4,[5],12	f,g,s	-	Agona
SF3	3,{10}{ <u>15</u> }{ <u>15</u> ,34}	e,h	1,6	Anatum
SF4	<u>1</u> ,9,12	g,m	-	Enteritidis
SF5	<u>1</u> ,4,[5],12	e,h	e,n,x	Chester
SF6	6,8	Z <sub>10</sub>	e,n,x	Hadar
SF7	<u>1</u> ,4,[5],12	i	1,2	Typhimurium
SF8	6,8	k	1,5	Blockley
SF9	<u>1</u> ,4,12	z	1,7	Indiana
SF10	6,7, <u>14</u>	r	1,5	Infantis



## 4 Questionnaire

### 4.1 General

A questionnaire was incorporated in the test report of the interlaboratory comparison study (Annex 2). Below is a list of the questions and a summary of the answers received.

### 4.2 General questions

#### **Question 1: Was your parcel damaged on arrival?**

All packages were received in good condition and no damage occurred during transport.

#### **Question 2: What was the date of receipt of the parcel at the laboratory?**

All but four NRLs received their package in the same week as it was sent (week 45 of 2011). The remaining NRLs received their package 7, 9, 11 and 14 days after shipment of the parcels on 7 November 2011.

#### **Question 3: What kind of medium was used for sub-culturing the strains?**

The NRLs used a variety of media from various manufacturers for the sub-culturing of the *Salmonella* strains. Non-selective nutrient agar was most commonly used.

### 4.3 Questions regarding serotyping

#### **Question 4: What was the frequency of serotyping of *Salmonella* at your laboratory in 2010?**

#### **Question 5: How many *Salmonella* strains (approximately) did your laboratory serotype in 2010?**

Replies to questions 4 and 5 are summarised in Table 6.



*Table 6 Frequency and number of strains serotyped in 2010 (n=35\*)*

<b>Laboratory code</b>	<b>Typing frequency</b>	<b>Number of strains serotyped in 2010</b>	<b>Laboratory code</b>	<b>Typing frequency</b>	<b>Number of strains serotyped in 2010</b>
25	Other	12	1	Daily	1002
23	Twice a week	58	27	Daily	1114
19	Once a week	100	22	Daily	1200
11	Twice a week	159	2	Daily	1700
21	Twice a week	159	20	Daily	1790
12	Thrice a week	164	9	Daily	1900
10	Thrice a week	200	26	Daily	2000
16	Daily	200	34	Once a week	3500
4	Daily	204	15	Daily	4100
30	Daily	249	17	Daily	4400
29	Daily	332	31	Daily	4740
32	Daily	440	7	Daily	5000
35	Daily	500	3	Daily	5200
14	Daily	552	18	Daily	5960
24	Weekly	581	5	Daily	6058
28	Daily	800	8	Thrice a week	no info
33	Daily	800	13	Daily	no info
6	Daily	1000			

\*no info from 1 laboratory

**Question 6: What kind of sera do you use (commercially available or prepared in own laboratory)?**

The replies to question 6 are summarised in Table 7 and Table 8.

*Table 7 Number of laboratories using sera from one or more manufacturers and/or in-house prepared sera*

<b>Number of manufacturers from which sera are obtained</b>	<b>Number of NRLs (n=32*)</b>
From 1 manufacturer	11
From 2 manufacturers	8
From 3 manufacturers	9
From 4 manufacturers	3
From 5 manufacturers or more	5
Preparation in own laboratory	6

\*no information from four laboratories

*Table 8 Number of laboratories using sera from different manufacturers*

<b>Manufacturer</b>	<b>Number of NRLs (n=32*)</b>
BD-Difco	2
Biomed	1
Biorad	14
BUL-BIO	1
Dade Behring	1
Denka Seiken	3
Difco	1
Immunolab	1
Imuna	1
Institute of Public Health of Serbia	1
Mast Assure	2
Own preparation	6
Pro-Lab	6
Reagensia	3
Remel	1
Sifin	18
Statens Serum Institute (SSI)	27

\*no information from four laboratories

**Question 7: Were the strains in this study typed in your own laboratory?**

One NRL-*Salmonella* (laboratory code 10) sent two strains (S13 and S21) to another laboratory for further serotyping or confirmation. All other laboratories tested all strains in their own laboratory.

#### **4.4 Questions regarding phage typing**

**Question 8: Does your laboratory perform phage typing of *S. Enteritidis*, *S. Typhimurium* and/or other strains?**

Eight NRLs performed phage typing of *S. Typhimurium* and *S. Enteritidis* strains and one NRL performed phage typing only of *S. Enteritidis*. For routine purposes, two NRLs also phage typed other strains, including *S. Hadar*, *S. Virchow*, *S. Paratyphi B* and *S. Typhi*.

**Question 9: Which typing system is used for *S. Enteritidis* and *S. Typhimurium*?**

All phage typing laboratories used the HPA/Colindale system.

**Question 10: How many strains did your laboratory phage type in 2010?**

Replies to question 10 are summarised in Table 9.

*Table 9 Number of strains phage typed in 2010*

<b>Laboratory code</b>	<b>Number of strains phage typed in 2010</b>
18	200
34	700
26	1000
1	1050
5	1537
31	1563
3	2063
15	2300
17	2500

#### 4.5 Questions regarding the use of PCR

Several labs reported using PCR for confirmation of the monophasic strain of *S. Typhimurium* (or others).

The references given included:

- EFSA Journal, 2010; 8(10):1826;
- Tennant et al., PLoS Negl Trop Dis 2010;4:e621;
- Barco et al., Foodborne Pathog Dis. 2011;8(6):741–743;
- Presentation of Lisa Barco at the XVIth Workshop (Mooijman, 2011).

In fact, these references all relate to more or less the same method (Barco et al.).

One laboratory reported using PCR for *Salmonella* subspecies differentiation (Lee et al., J Appl Microbiol 2009;107(3):805–811).

So far, only two laboratories reported more extended typing results based on PCR. Their results are given in Annex 8.

## 5 Results

### 5.1 Serotyping results

#### 5.1.1 *General comments on this year's evaluation*

**Strain S2** was evaluated only on the O-antigens and H-antigens results, and not on the biochemical reactions concerning serovar 6,7:c:1,5 which finally result in the name. Biochemical differentiation of serovars with formula 6,7:c:1,5 (page 10 in the Antigenic formulae of the *Salmonella* serovars, 9th ed., Grimont and Weill, 2007) are given below:

	<b>Dulcitol</b>	<b>H<sub>2</sub>S</b>	<b>Mucate</b>	<b>d-Tartrate</b>
Paratyphi C	+	+	-	+
Choleraesuis	-	-	-	+
Choleraesuis var. Kunzendorf	-	+	-	+
Choleraesuis var. Decatur	+	+	+	+
Typhisuis	-	-	-	-

**Strain S6** has a deviation in its biochemical reactions: Malonate negative, Gelatinase positive, d-tartrate negative, Galacturonate positive. It is a monophasic strain, as confirmed by PCR (EFSA Journal, 2010; 8(10):1826). Because of the deviations in the biochemical reactions, strain S6 was evaluated only on the O-antigens and H-antigens results.

**Strain S16** was excluded from the evaluation, since it showed too many rough colonies.

As decided at the 16th EURL-*Salmonella* Workshop (Mooijman, 2011), **strain S21** was an additional strain to the study. Testing of this strain was optional and results were not included in the evaluation.

#### 5.1.2 *Serotyping results per laboratory*

The evaluation of the detection of O- and H-antigens and identification of the strains per laboratory are shown in Figures 1, 2 and 3 and the percentages of correct results in Figure 4.

The O-antigens were typed correctly by 31 of the 36 participants (88%). This corresponds to 98% of the total number of strains. The H-antigens were typed correctly by 25 of the 36 participants (69%), corresponding to 96% of the total number of strains. A total of 25 participants (69%) gave the correct serovar names, corresponding to 96% of all strains evaluated.

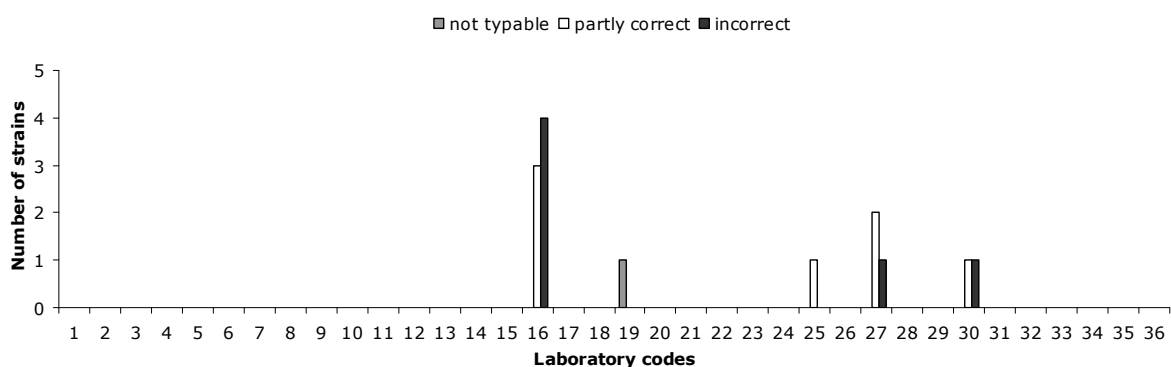


Figure 1 Evaluation of serotyping of O-antigens per NRL

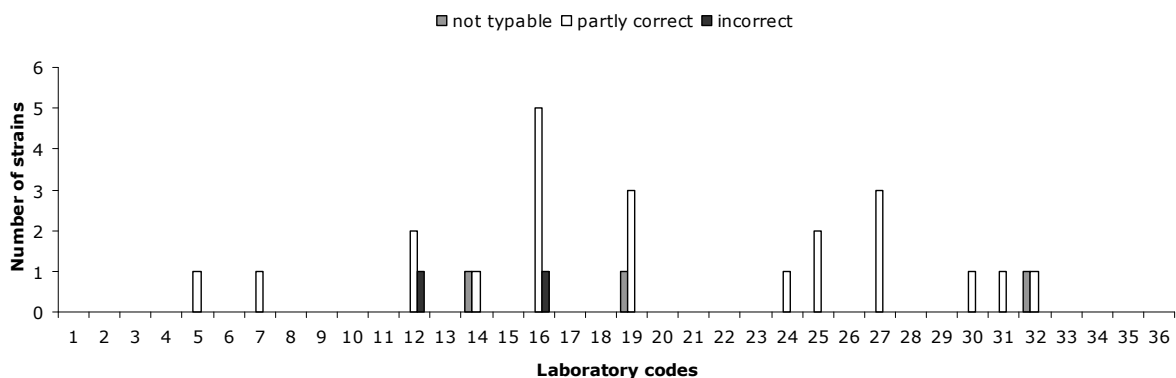


Figure 2 Evaluation of serotyping of H-antigens per NRL

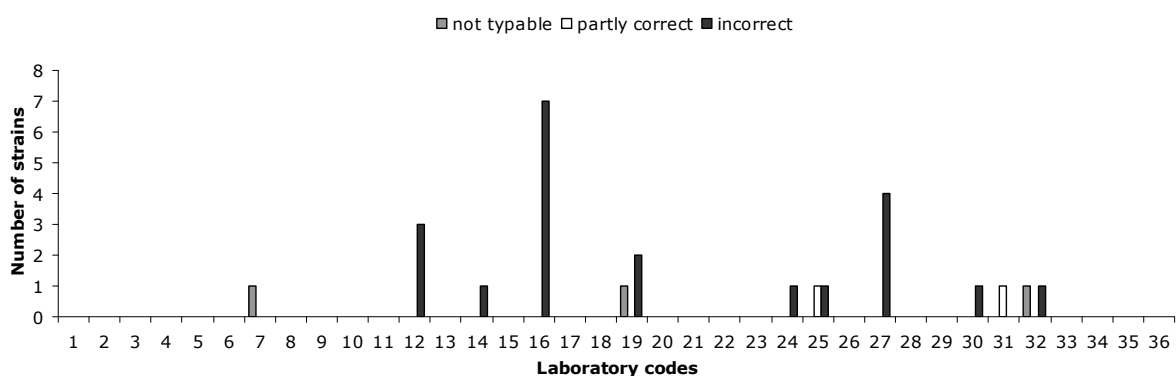


Figure 3 Evaluation of the correct serovar names per NRL

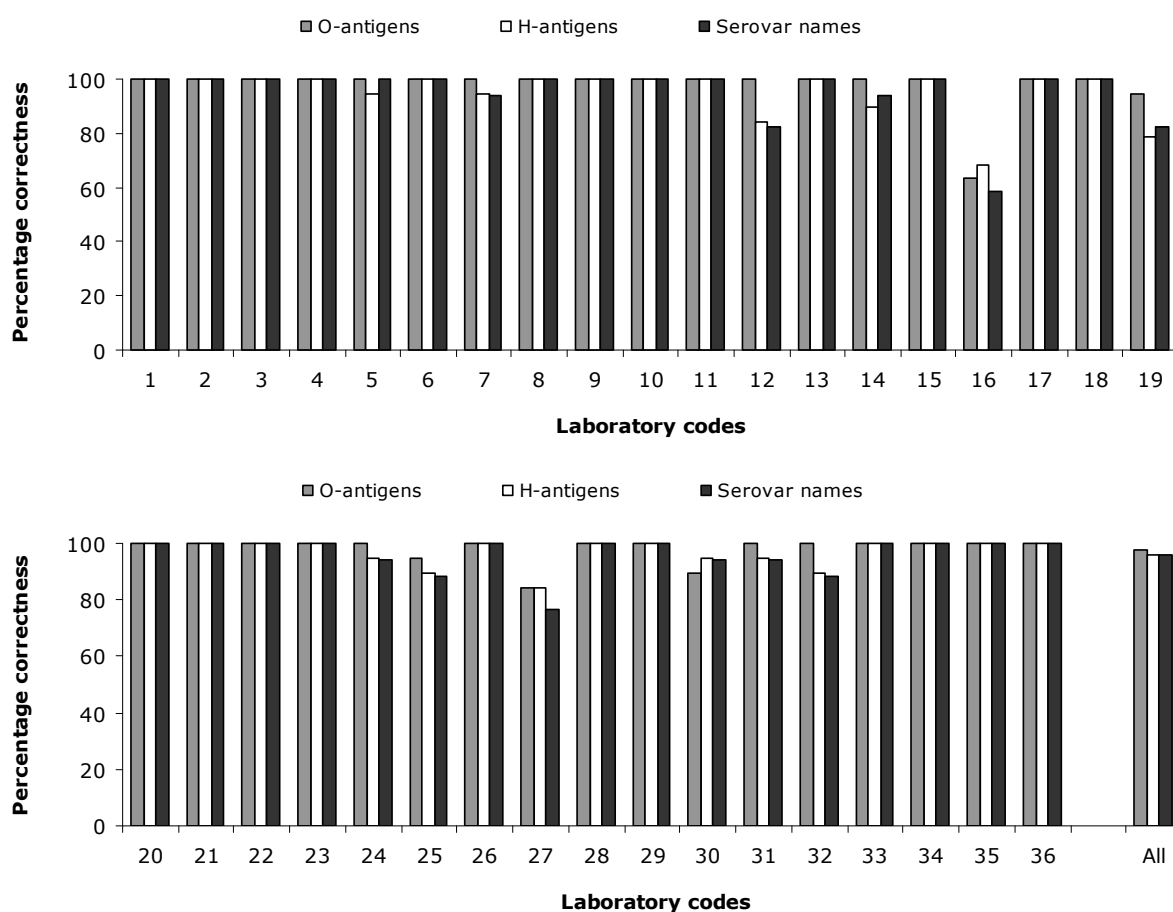


Figure 4 Percentage correctness of serotyping

For each NRL the number of penalty points was determined using the guidelines in section 3.6. Table 5 shows the number of penalty points for each NRL and, in the second column, whether the level of good performance was achieved. Four NRLs did not meet the level of good performance at this stage of the study and for these laboratories a follow-up study was organised, in which an additional ten strains had to be serotyped.

Table 5 Evaluation of serotyping results per NRL

Lab code	Penalty points	Good performance	Lab code	Penalty points	Good performance
1	0	yes	19	2	yes
2	0	yes	20	0	yes
3	0	yes	21	0	yes
4	0	yes	22	0	yes
5	0	yes	23	0	yes
6	0	yes	24	1	yes
7	0	yes	25	<b>5</b>	<b>no</b>
8	0	yes	26	0	yes
9	0	yes	27	<b>11</b>	<b>no</b>
10	0	yes	28	0	yes
11	0	yes	29	0	yes
12	<b>6</b>	<b>no</b>	30	2	yes
13	0	yes	31	1	yes
14	1	yes	32	1	yes
15	0	yes	33	0	yes
16	<b>11</b>	<b>no</b>	34	0	yes
17	0	yes	35	0	yes
18	0	yes	36	0	yes

### 5.1.3 Serotyping results per strain

Results found per strain and per laboratory are given in Annex 5, except for the more complicated strains S2, S4, S6, S16 and S21.

A completely correct identification by all participants was obtained for four strains: *S. Hadar* (S7), *S. Enteritidis* (S9), *S. Abaetetuba* (S17) and *S. Typhimurium* (S19).

Most problems occurred with the serovar *S. Krefeld* (S10). Five laboratories had difficulties correctly assigning the correct serovar name to this strain. The characterisations of strains that caused problems in serotyping are shown in Annex 6.

The reported serovar name for strain S4 again showed a large variation of 'Typhimurium-like' names. Therefore the reported serovar names are summarised for all participants in Annex 6. These results confirm the findings published in the EFSA opinion of September 2010. In this opinion a proposal is made to harmonise reportings of this serovar by reporting the antigenic formula in as much detail as possible. However, it seems that it will take some time before this will be common practice.

Details on the strains that were (partly) excluded from the evaluation (S2, S6, S16) as well as the additional strain from the uncommon source and type (S21) are also given in Annex 6 for all participants.

All but two participants did serotype this additional strain, being a *Salmonella enterica* subspecies *diarizonae* 38:r:z. The majority of the participants were able to serotype this strain correctly, though also in this case the exact naming might need some more harmonisation.

### 5.1.4 Follow-up

Four participants (two EU-NRLs and two EU-candidate countries) did not achieve the level of good performance (Table 5; Labcodes 12, 16, 25 and 27) and were offered a follow-up study. This follow-up study is obligatory for laboratories from EU Member States, but optional for other laboratories. Three laboratories

participated in the follow-up study and received ten additional strains in week 12, 2012.

The evaluation of the detection of O- and H-antigens and identification of the strains per laboratory of the follow-up study are shown in Figure 5.

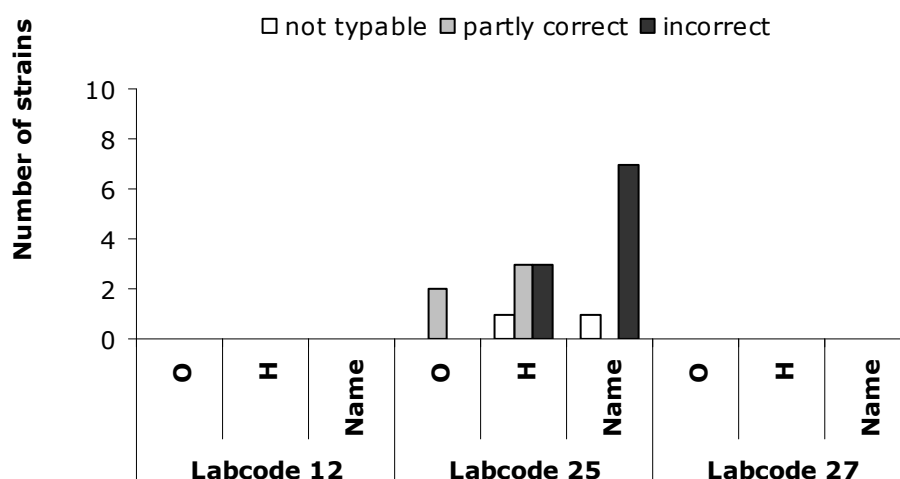


Figure 5 Evaluation of serotyping O- and H-antigens and of the serovar names by the NRLs during the follow-up study

Results found per serovar and per laboratory are given in Table 6. For each participant the number of penalty points was determined using the guidelines in section 3.6. Table 7 shows the number of penalty points for each participant and whether or not the level of good performance was achieved. The two EU-NRLs achieved the level of good performance in this follow-up study. One EU-candidate country did not achieve the level of good performance. The other EU-candidate country was not able to participate in the follow-up study.

Table 6 Serotyping results per Salmonella strain and per NRL, in the follow-up study

Lab	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
REF	Virchow	Agona	Anatum	Enteritidis	Chester	Hadar	Typhimurium	Blockley	Indiana	Infantis
12	Virchow	Agona	Anatum	Enteritidis	Chester	Hadar	Typhimurium	Blockley	Indiana	Infantis
25	Infantis	Derby	Amsterdam	Enteritidis	Saintpaul	Bovismobificans	Enterica (O4)	Braenderup	Typhimurium	Infantis
27	Virchow	Agona	Anatum	Enteritidis	Chester	Hadar	Typhimurium	Blockley	Indiana	Infantis
X	1	1	1	0	1	1	1	1	1	0

X = number of deviating laboratories per strain

Table 7 Evaluation of serotyping results per NRL in the follow-up study

Lab code	Penalty points	Good performance
12	0	yes
25	16	no
27	0	yes



## 5.2 Phage typing results

Eight NRLs performed phage typing of both *S. Enteritidis* and *S. Typhimurium*.

One NRL performed phage typing of *S. Enteritidis* only.

The results for *S. Enteritidis* are shown in Table 8 and the results for *S. Typhimurium* in Table 9. The percentages of strains correctly phage typed for each laboratory for both *S. Enteritidis* and *S. Typhimurium* are shown in Figure 6.

Separate notations per phage and per laboratory are given in Annex 7.

Five laboratories assigned the correct phage type to all ten of the *S. Enteritidis* strains. The laboratory with labcode 1 assigned the wrong phage type to one of the strains (E2) and laboratory 18 assigned the wrong phage type to two of the strains (E5 and E10). Two laboratories assigned the wrong phage type to four of the *S. Enteritidis* strains. Strains E2, E3, E9 and E10 were incorrectly phage typed by laboratory 5 and strains E2, E3, E5 and E8 were incorrectly phage typed by laboratory 34.

Six laboratories assigned the correct phage type to all ten strains of *S. Typhimurium*. Two laboratories assigned the correct phage type to nine of the *S. Typhimurium* strains. Strain T4 was incorrectly phage typed by laboratory 3 and strain T9 was incorrectly phage typed by laboratory 26.

Overall, 86% of the *S. Enteritidis* strains and 97% of the *S. Typhimurium* strains were correctly phage typed.

**Table 8 Results of Salmonella Enteritidis phage typing**

S. Enteritidis strain numbers											
Lab code	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	Y
HPA	21c	35	21	14b	9b	8	4b	4	2	1b	
1	21c	RDNC/21	21/21c	14b	9b	8	4b	4	2	1b	1
3	21c	35	21	14b	9b	8	4b	4	2	1b	0
5	21c	not given	not given	14b	9b	8	4b	4	43	1d	4
15	21c	35	21	14b	9b	8	4b	4	2	1b	0
17	21c	35	21	14b	9b	8	4b	4	2	1b	0
18	21c	35	21	14b	11b	8	4b	4	2	1	2
26	21c	35	21	14b	9b	8	4b	4	2	1b	0
31	21c	35	21	14b	9b	8	4b	4	2	1b	0
34	21c	7	21b	14b	11b	8	4b	53	2	1b	4
X	0	3	2	0	2	0	0	1	1	2	11

Grey cells = deviating results, X = number of deviating laboratories per strain,

Y = number of deviating strains per laboratory

**Table 9 Results of Salmonella Typhimurium phage typing**

S. Typhimurium strain numbers											
Lab code	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	Y
HPA	36	136	193	104	99	66a	66	10	8	1	
1	36	136	193	104	99	66a	66	10	8	1	0
3	36	136	193	104b	99	66a	66	10	8	1	1
5	36	136	193	104	99	U283 (66a)	66	10	8	1	0
15	36	136	193	104	99	66a	66	10	8	1	0
17	36	136	193	104	99	66a	66	10	8	1	0
18	36	136	193	104	99	66a	66	10	8	1	0
26	36	136	193	104	99	66a	66	10	9	1	1
31	36	136	193	104	99	66a	66	10	8	1	0
X	0	0	0	1	0	0	0	0	1	0	2

Grey cells = deviating results, X = number of deviating laboratories per strain,

Y = number of deviating strains per laboratory

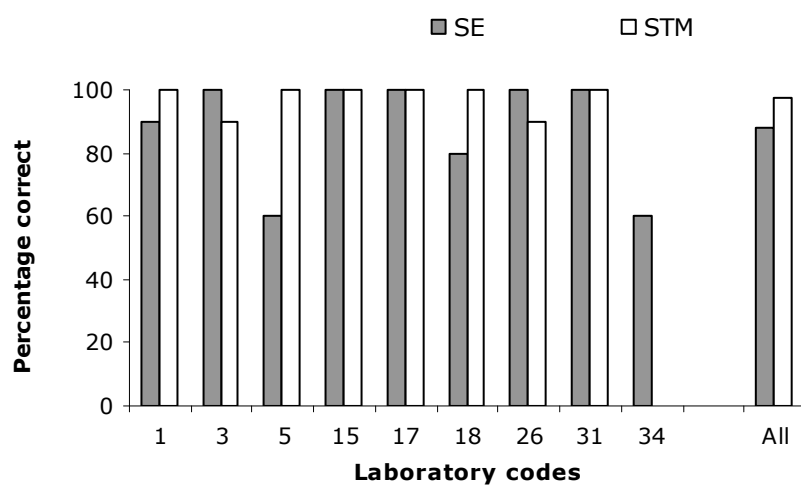


Figure 6 Percentage of strains correctly phage typed by each laboratory



## 6 Discussion

### Serotyping

A total of 36 laboratories participated in this study. These included 28 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) in the 27 EU Member States, 3 NRLs of EU-candidate countries, 2 NRLs of EFTA countries and 3 additional participants.

A total of 20 *Salmonella* strains were sent to the participants in November 2011 for serotyping by all participants.

Overall, 98% of the strains were typed correctly for the O-antigens, 96% of the strains were typed correctly for the H-antigens and 96% of the strains were correctly named by the participants.

At the CRL-*Salmonella* workshop in 2007, the CRL-*Salmonella* proposed a definition for good performance of the NRLs regarding the serotyping. Using this definition, 32 laboratories achieved good performance. The four NRLs that did not achieve the defined level of good performance were offered a follow-up study including ten additional strains for serotyping. This follow-up study is obligatory for EU-NRLs and the two EU-NRLs concerned achieved good performance in this follow-up study. Therefore, in the end all 28 EU-NRLs achieved good performance in the 2011 typing studies.

One of the two EU-candidate countries voluntarily performed the follow-up study but did not achieve good performance.

When evaluating the results of the participants, mistakes in typing five designated *Salmonella* serovars (Enteritidis, Typhimurium, Hadar, Infantis and Virchow) are more severely judged than the other *Salmonella* serovars. This '*Salmonella* top 5' is indicated in European legislation and it is most important that the laboratories are able to type these serovars correctly. In the 2011 study, none of the NRLs had problems with correctly serotyping *S. Enteritidis*, *S. Hadar* or *S. Typhimurium*. One mistake was made with typing *S. Virchow*; and two mistakes were made when serotyping *S. Infantis*.

Table 10 and Table 11 show an overview of the details obtained for the typing studies starting from 2007, when the system of penalty points was used for the first time. Table 10 shows results for EU-NRLs only and Table 11 shows results for all participants per study. The relatively large number of 56 penalty points in 2009 (Table 11) was mainly due to the results of one non-EU NRL, participating for the first time.

The percentages of correctly typed strains remain quite stable over the years, with usually a slightly better performance for the O-antigens than for the H-antigens. The percentage of laboratories that achieved completely correct results for O-antigens, H-antigens or serovar names clearly shows an increase from 2010 onwards, especially for the EU-NRLs.

*Table 10 Details of the serotyping studies for EU-NRLs only*

<b>Study/Year</b>	<b>XII 2007</b>	<b>XIII 2008</b>	<b>XIV 2009</b>	<b>XV 2010</b>	<b>XVI 2011</b>
N participants	25	27	28	30	28
N strains evaluated	20	20	20	19	19*
O-antigens correct/strains	98%	98%	98%	98%	527/532 99%
H-antigens correct/strains	95%	98%	95%	95%	518/532 97%
Names correct/strains	95%	97%	95%	95%	463/476 97%
O-antigens correct/labs	68%	70%	75%	93%	26/28 93%
H-antigens correct/labs	56%	67%	43%	73%	20/28 71%
Names correct/labs	52%	52%	46%	67%	21/28 75%
Total no. penalty points	35	30	36	16	22
Total no. labs with non-good performance	<b>6</b>	<b>3</b>	<b>4</b>	<b>2</b>	<b>2</b>
Total no. labs with non-good performance after follow-up	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>

\*2 of the 19 strains were evaluated only on their O-antigens and H-antigens results.

*Table 11 Details of the serotyping studies for all participants*

<b>Study/Year</b>	<b>XII 2007</b>	<b>XIII 2008</b>	<b>XIV 2009</b>	<b>XV 2010</b>	<b>XVI 2011</b>
N participants	26	29	31	33	36
N strains evaluated	20	20	20	19	19*
O-antigens correct/strains	98%	98%	97%	98%	670/684 98%
H-antigens correct/strains	96%	98%	94%	95%	657/684 96%
Names correct/strains	95%	97%	93%	95%	586/612 96%
O-antigens correct/labs	69%	76%	74%	88%	31/36 86%
H-antigens correct/labs	58%	72%	45%	67%	25/36 69%
Names correct/labs	54%	59%	48%	61%	25/36 69%
Total no. penalty points	36	34	56	37	41
Total no. labs with non-good performance	<b>6</b>	<b>4</b>	<b>5</b>	<b>4</b>	<b>4</b>
Total no. labs with non-good performance after follow-up	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b> (n=3)	<b>1</b> (n=3)

\*2 of the 19 strains were evaluated only on their O-antigens and H-antigens results.

### Phage typing

Ten strains of *S. Enteritidis* and ten strains of *S. Typhimurium* were selected by the *Salmonella* Reference Unit of the Health Protection Agency in London. All ten of the *S. Enteritidis* strains were correctly typed by five of the nine NRLs. One NRL incorrectly typed one of the *S. Enteritidis* strains and one NRL incorrectly typed two of these strains. Two NRLs incorrectly typed four of the ten *S. Enteritidis* strains.

Three of the NRLs incorrectly phage typed strain E2 (PT 35). One NRL did not give a phage type for this strain and commented that they would refer the strain to the reference laboratory. This laboratory obtained reactions with two phages (10 and 14) that should not react with this strain and therefore was unable to give a phage type for this strain. This suggests the titres of these two phages were too high or the inoculum of the culture used for the phage typing was incorrect. The second NRL typed this strain as PT 7. They obtained the correct phage reactions but misinterpreted their results. The third NRL gave the phage type for this strain as RDNC/21. They obtained readings with three phages (1, 8 and 14) that should not react with this strain and also had variable reactions with two phages (6 and 10). As this laboratory correctly phage typed all the other strains this suggests the phages were at the correct titre and the inoculum of the culture used for the phage typing was incorrect.

Two NRLs incorrectly phage typed strain E3 (PT 21). One NRL did not give a phage type for this strain and commented that they would refer the strain to a reference laboratory. This laboratory obtained reactions with three phages (11, 15 and 16) that should not react with this strain and therefore was unable to give a phage type for this strain. This suggests the titres of these three phages were too high or the inoculum of the culture used for the phage typing was incorrect. One NRL typed this strain as PT 21b and this was because they had a low reaction with phage 14, suggesting the titre of this phage was too low. Strain E5 (PT 9b) was incorrectly phage typed as PT 11b by two of the NRLs. Both of these laboratories failed to get a reaction with phage 14, suggesting the titre of this phage was too low.

Strain E8 (PT 4) was incorrectly typed as PT 53 by one laboratory. This incorrect result was due to no reaction being obtained with two phages – phage 8 and phage 10.

One NRL incorrectly phage typed strain E9 (PT 2) as PT 43. This was due to obtaining a reaction with a phage that does not react with this strain and obtaining low or no reactions with several other phages.

Two laboratories incorrectly typed strain E10 (PT 1b). One laboratory typed this strain as PT 1d, as they obtained low reactions with several phages and no reaction with one phage that reacts with this strain. The second laboratory typed this strain as PT 1, as they did not obtain any reaction with phages 15 and 16.

Six of the NRLs correctly phage typed all ten of the *S. Typhimurium* strains and two of the NRLs correctly phage typed nine of the ten strains.

One laboratory incorrectly typed strain T4 (DT 104) as DT 104b because they failed to obtain any reaction with phages 12 and 13. As this laboratory correctly phage typed all the other strains it suggests the phages were at the correct titre and the inoculum of the culture used for phage typing was incorrect.

Strain T9 (DT 8) was incorrectly phage typed by one laboratory as DT 9 because they obtained phage reactions with phages 12, 13 and 21 and DT 8 does not react with these phages. As this laboratory correctly phage typed all the other strains it suggests the phages were at the correct titre and the inoculum of the culture used for phage typing was incorrect.

Strain T6 (DT 66a) was typed as PT U283 by one laboratory. This was the provisional phage type allocated to this phage type and it has since been designated as definitive type (DT) 66a.

Overall, the results for *S. Enteritidis* in this study were average, with 88% of the strains correctly phage typed. This is not as good as the results of the 2010 study when 98% of the *S. Enteritidis* strains were correctly phage typed. Overall, the results for *S. Typhimurium* in this study were very good, with 98% of the strains correctly phage typed. This is equal to the results of the 2010 study.

## 7 Conclusions

### **Serotyping**

- 98% of the strains were typed correctly for the O-antigens.
- 96% of the strains were typed correctly for the H-antigens.
- 96% of the strains were correctly named.
- Serotyping of *S. Krefeld* caused the most problems in this study.
- Four NRLs did not achieve the defined level of good performance.
- In the follow-up study, the two EU-NRLs did achieve the level of good performance. One EU-candidate NRL did not achieve the level of good performance. The other EU-candidate NRL was not able to participate in the follow-up study.

### **Phage typing**

- The performance of the laboratories participating in this study was average for *S. Enteritidis*, with 88% of the strains typed correctly, and very good for *S. Typhimurium*, with 98% of the strains typed correctly.
- Four of the *S. Enteritidis* strains and eight of the *S. Typhimurium* strains were correctly typed by all of the participating laboratories.
- Six of the *S. Enteritidis* strains caused a problem. Strain E2 (PT 35) was incorrectly typed by three laboratories, Strains E3 (PT 21), E5 (PT 9b) and E10 (PT 1b) were incorrectly typed by two laboratories and strains E8 (PT 4) and E9 (PT 2) were incorrectly phage typed by one laboratory.
- Two *S. Typhimurium* strains caused a problem, T4 (DT7) and T9 (DT 8). Each of these strains was incorrectly typed by one laboratory.





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## Annex 1 Protocol

### PROTOCOL OF THE 16<sup>TH</sup> EURL-SALMONELLA INTERLABORATORY COMPARISON STUDY (NOVEMBER 2011) ON SEROTYPING AND PHAGE TYPING OF *SALMONELLA* STRAINS, FOR THE NRL-SALMONELLA LABORATORIES

#### Introduction

The European Union Reference Laboratory (EURL) - *Salmonella* organises the sixteenth interlaboratory comparison study on the typing of *Salmonella* strains amongst the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*).

The main objective of this typing study is to test the performance of the participating laboratories for serotyping and phage typing of *Salmonella* spp.

The study will take place in week 45 (starting on 7 November 2011). The timetable can be found on the last page of this protocol.

All data have to be reported in the test report, and in the relevant Excell sheets and sent by e-mail to the EURL-*Salmonella*. The data on phage typing will be forwarded by the EURL-*Salmonella* to the Health Protection Agency (HPA, London, United Kingdom) for further analyses.

#### Transportation of the *Salmonella* strains to the laboratories

The strains for the serotyping part and the phage typing part of the study will be transported in a separate parcel. The strains will be sent as Biological Substance Category B (UN 3373) with a door-to-door courier to your laboratory.

#### Serotyping

A total number of 20 *Salmonella* strains (coded S1 - S20) have to be serotyped. The method routinely performed in your laboratory can be used in this study. Each laboratory is allowed to send strains for serotyping to another reference laboratory in their country, if this is part of the normal routine procedure. An additional strain from a reptile source (S-21) is included in the package and serotyping of this strain is optional.

The results for each strain have to be reported with the formula for the O-antigens and H-antigens **and** the serovar names according to the White-Kauffman-le Minor scheme of 2007 (<http://www.pasteur.fr/ip/portal/action/WebdriveActionEvent/oid/01s-000036-089>). Laboratories have to report only those results, on which the identification of serovar names is based. 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 by the EURL-*Salmonella* according to Table 1.

Table 1 Evaluation of serotyping results

Results	Evaluation	Abbreviation
Auto-agglutination 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	-

**Phage typing**

A total number of 20 *Salmonella* strains have to be phage typed:

- 10 strains of *S. Enteritidis* coded E1 - E10
- 10 strains of *S. Typhimurium* coded T1 - T10

The evaluation of the phage typing results will be done in collaboration with the *Salmonella* Reference Unit of the HPA, London, UK.

Please record all test results in the separately provided Test Report and Excel sheet file and return this file by e-mail to [Irene.Pol@rivm.nl](mailto:Irene.Pol@rivm.nl) on 9 December 2011 at the latest.

A check-up by the participants of the submitted results is no longer needed when the results are sent by email in the provided file format. This will save time, *but participants need to be sure to fill in the right results at once.*

If you have questions or remarks about this study, please contact:

Irene Pol-Hofstad  
P.O. Box 1  
3720 BA Bilthoven  
tel. number: +31-30-2747057  
fax. number: +31-30-2744434  
e-mail: [Irene.Pol@rivm.nl](mailto:Irene.Pol@rivm.nl)

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

Elizabeth de Pinna  
Public Health Laboratory Service, Laboratory of Enteric Pathogens  
61 Colindale Avenue, London NW9 5HT  
tel. number: + 44-20-8327 6136  
fax number: + 44-20-8905 9929  
e-mail: [Elizabeth.DePinna@HPA.org.uk](mailto:Elizabeth.DePinna@HPA.org.uk)

**Timetable of the 16<sup>th</sup> EURL-*Salmonella* interlaboratory comparison study  
(2011) on serotyping and phage typing of *Salmonella* spp.  
for NRLs-*Salmonella***

Week	Date	Topic
43	31 October- 3 November	Mailing of the protocol and test report 2011 to the NRLs.
45	7-11 November	Mailing of the parcels to the participants as diagnostic specimens by door-to-door courier service. After arrival at the laboratory the strains need to be sub-cultured and stored until the performance of the typing. If you did not receive the parcel <b>by 11 November</b> , do contact the EURL- <i>Salmonella</i> immediately.
46	14-18 November	Starting with the identification of the strains.
49	9 December	Send the electronically completed test report by email to the EURL- <i>Salmonella</i> . <b>Deadline: 9 December 2011</b>
50	12-16 December	Data input at the EURL- <i>Salmonella</i> . A check-up of the submitted results by the NRLs is no longer needed when the results are sent by email in the provided excel file format. This will save time, but NRLs need to be sure to fill out the right results at once.
	January 2012	Reporting of individual laboratory results.
	March 2012	Interim Summary Report.
	Summer 2012	Final report.

## Annex 2 Test report



Test report *Salmonella* typing study EURL-*Salmonella* XVI-2011

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**TEST REPORT**

**THE 16<sup>TH</sup> EURL-*SALMONELLA* INTERLABORATORY COMPARISON STUDY (2011) ON SEROTYPING AND  
PHAGE TYPING OF *SALMONELLA* STRAINS FOR THE NRL-*SALMONELLA* LABORATORIES**

Laboratory code	
Name contact person	
Email address contact person	
Name of laboratory or institute	
Country	

**Please enter your remarks and comments on page 12 of the test report.**

EURL-*Salmonella*, Bilthoven, the Netherlands

**GENERAL QUESTIONS****Shipment of the strains**

Was your parcel damaged at arrival?

Date of receipt at your laboratory:

**Sub-culturing**

Medium used for sub-culturing the strains

Name:

Manufacturer:

**REMARKS CONCERNING THE ADDITIONAL TABLES FOR SEROTYPING**

Two **optional** tables are included this test report, to give the EURL-*Salmonella* more information about the antisera used. The tables on pages 4 and 5 concern reactions obtained with O-antisera and the tables on pages 6 and 7 with H-antisera. At the bottom of the table space is left to fill in other antisera than mentioned in the table.

Please mention the manufacturer of the antisera used in the column next to the antisera. Indicate for each combination of strain and antiserum if there was agglutination (+) or not (-). If the cell remains empty this indicates that the agglutination was not determined for the specific combination of antiserum and strain.

**Please note that in case of deviating results you will be asked to fill in these tables retrospectively!**

**QUESTIONS SEROTYPING**

What was the frequency of serotyping of <i>Salmonella</i> at your laboratory in 2010?	<input type="checkbox"/> Daily <input type="checkbox"/> Once a week <input type="checkbox"/> Twice a week <input type="checkbox"/> Thrice a week <input type="checkbox"/> Weekly <input type="checkbox"/> Monthly <input type="checkbox"/> Other:
How many <i>Salmonella</i> strains did your laboratory (approximately) serotype in 2010?	Number of strains:
What kind of sera do you use?	<input type="checkbox"/> Prepared in own laboratory <input type="checkbox"/> Commercial sera Manufacturer(s):
The strains in this study were serotyped by:	<input type="checkbox"/> Own laboratory, <input type="checkbox"/> Other laboratory, namely:  Strains:

EURL-*Salmonella*, Bilthoven, the Netherlands

Test report *Salmonella* typing study EURL-*Salmonella* XVI-2011

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O-antisera	Manufacturer	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21
<b>Group B</b>																						
1, 4, 12, 27																						
1, 4, 5, 12																						
4, 5, 12																						
4, 5, 27																						
4, 5																						
4																						
5																						
<b>Group C</b>																						
7, 8																						
6, 7, 8																						
6, 7																						
6 <sub>1</sub> , 6 <sub>2</sub> , 7																						
6, 8																						
8, 20																						
6 <sub>1</sub>																						
6																						
7																						
8																						
14																						
20																						
<b>Group D</b>																						
9																						
9, 12																						
1, 9, 12																						
12																						

EURL-*Salmonella*, Bilthoven, the Netherlands

O-antisera	Manufacturer	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21
9, 46																						
46																						
Group E																						
1, 3, 10, 15, 19, 34																						
3, 10, 15, 19, 34																						
(3), (15), 34																						
3, 10, 15																						
3, 10																						
3, 15																						
10																						
15																						
1, 3, 19																						
19																						
Group G																						
13																						
13, 22, 23																						
22																						
23																						
Other O-antisera																						
O11																						
O16																						
O17																						

		Strains																				
H-antiser	Manufacturer	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21
a																						
b																						
c																						
d																						
E (complex)																						
e, h																						
e, n																						
e, n, x																						
e, n, z <sub>15</sub>																						
h																						
x																						
x (z <sub>16</sub> )																						
z <sub>15</sub>																						
G (complex)																						
g, p																						
g, m																						
f																						
m																						
p																						
q																						
s																						
t																						
u																						
q, s, t, p, u																						

EURL-*Salmonella*, Bilthoven, the Netherlands

		Strains																				
H-antiser	Manufacturer	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21
i																						
k																						
L (complex)																						
l, v																						
l, w																						
v																						
w																						
r																						
y																						
z																						
z <sub>6</sub>																						
z <sub>10</sub>																						
1 (complex)																						
2																						
5																						
6																						
7																						
Other H-antiser																						

EURL-*Salmonella*, Bilthoven, the Netherlands

Test report *Salmonella* typing study EURL-*Salmonella* XVI-2011

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EURL-*Salmonella*, Bilthoven, the Netherlands



TEST RESULTS SEROTYPING

Labcode:		Starting date of serotyping:		Finishing date of serotyping:	
----------	--	------------------------------	--	-------------------------------	--

Strain no.	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar name
S-1				
S-2				
S-3				
S-4				
S-5				
S-6				
S-7				
S-8				
S-9				
S-10				
S-11				
S-12				
S-13				
S-14				
S-15				
S-16				
S-17				
S-18				
S-19				
S-20				
S-21				

EURL-*Salmonella*, Bilthoven, the Netherlands

QUESTIONS PHAGE TYPING

Does your laboratory perform phage typing?	<input type="checkbox"/> Yes <input type="checkbox"/> No
If yes, which <i>Salmonella</i> strains do you phage type?	<input type="checkbox"/> <i>Salmonella</i> Typhimurium <input type="checkbox"/> <i>Salmonella</i> Enteritidis <input type="checkbox"/> Other(s):
Which typing system is used for:	<i>Salmonella</i> Typhimurium:  <i>Salmonella</i> Enteritidis:
How many strains did your laboratory (approximately) phage type in 2010?	Number of strains:

TEST RESULTS PHAGETYPING

Labcode:		Starting date of phage typing:		Finishing date of phage typing:	
----------	--	--------------------------------	--	---------------------------------	--

Notations:    - :    no reaction                      O\*:    O pooled  
                  + :    5-20 plaques                    (<) CL: Clear Lysis  
                  + :    21-40 plaques                   (<) OL: Opaque Lysis  
                  ++ :    41-80 plaques                   SCL:    Semi Confluent Lysis  
                  +++ :    81-100 plaques                << :    Merging plaques towards semi-confluent lysis

		Phage reactions at Routine Test Dilution ( <i>S. Enteritidis</i> )																
Strain number	Phage type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
E1																		
E2																		
E3																		
E4																		
E5																		
E6																		
E7																		
E8																		
E9																		
E10																		

TEST RESULTS PHAGE TYPING

Labcode:		Starting date of phage typing:		Finishing date of phage typing:	
----------	--	--------------------------------	--	---------------------------------	--

		Phage reactions at Routine Test Dilution ( <i>S. Typhimurium</i> )																	
Strain number	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
T1																			
T2																			
T3																			
T4																			
T5																			
T6																			
T7																			
T8																			
T9																			
T10																			

		Phage reactions at Routine Test Dilution ( <i>S. Typhimurium</i> )												Additional phages						
Strain number	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10 var 2	10 var 3	18
T1																				
T2																				
T3																				
T4																				
T5																				
T6																				
T7																				
T8																				
T9																				
T10																				

DETECTION BY PCR (I)	
<b>General questions</b>	
Is the PCR used commercially available ?	<input type="checkbox"/> Yes <input type="checkbox"/> No
If yes, give name of PCR, manufacturer and batch used in the study:	<input type="checkbox"/> Real time PCR <input type="checkbox"/> Other PCR ..... Manufacturer : ..... Batch : .....
Is the PCR validated ?	<input type="checkbox"/> Yes <input type="checkbox"/> No
If yes, for which matrix/matrices and by which organisation?	Matrices:..... Validated by:..... Ref. number:.....
If no, is the PCR published in the open literature ?	Reference literature :
Do you use the PCR routinely ?	<input type="checkbox"/> Yes <input type="checkbox"/> No
How many samples did you test for <i>Salmonella</i> using this PCR in 2010 ?	..... number/year
Volume of DNA-sample added to PCR-mixture	..... .....

Composition of PCR-mixture		
Compound	Volume per sample	Manufacturer and batch of specific compound
Name of thermocycler		
Number of cycles		
What kind of detection system is used ?		

Results of detection using PCR		
Strain no.	Serovar name	Remarks
S1		
S2		
S3		
S4		
S5		
S6		
S7		
S8		
S9		
S10		
S11		
S12		
S13		
S14		
S15		
S16		
S17		
S18		
S19		
S20		
S21		

EURL-*Salmonella*, Bilthoven, the Netherlands

REMARKS AND COMMENTS
<div></div>

Name of person(s) carrying out the typing:	
Date:	

Name of person in charge:	
Date:	





## Annex 3 Protocol for Follow-up study

### **PROTOCOL OF THE FOLLOW-UP OF THE SIXTEENTH INTERLABORATORY COMPARISON STUDY (XVI, 2011) ON SEROTYPING OF *SALMONELLA* STRAINS, FOR THE NRL-*SALMONELLA* LABORATORIES**

#### **Introduction**

In November 2011 the European Reference Laboratory (EURL)-*Salmonella* has organised the sixteenth interlaboratory comparison study on the typing of *Salmonella* strains amongst the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*).

Four (candidate) NRLs did not achieve the level of Good Performance for serotyping in this study, therefore this follow-up is planned in which these NRLs have to serotype an additional set of 10 strains.

The parcel containing the strains will be sent in week 12 (19 March 2012). The timetable can be found on the last page of this protocol.

All data have to be reported in the test report, to be sent by e-mail to the EURL-*Salmonella* and will be used for analysis.

#### **Transportation of the *Salmonella* strains to the NRLs-*Salmonella*.**

The strains will be sent as Biological Substance Category B (UN 3373) with a door-to-door courier to your laboratory.

#### **Serotyping**

A total number of 10 *Salmonella* strains (indicated S1 till S10) have to be serotyped. The method routinely performed in your laboratory can be used in this study. Each laboratory is allowed to send strains for serotyping to another reference laboratory in their country, if this is part of the normal routine procedure.

**IN THE TEST REPORT OF THIS STUDY 2 EXTRA TABLES ARE ADDED. PLEASE INDICATE THE REACTIONS FOR EVERY STRAIN-ANTISERUM COMBINATION USED. THIS SUPPLIES THE EURL-*SALMONELLA* WITH MORE INFORMATION IN CASE OF ANY DEVIATING RESULTS.**

The results for each strain have to be reported with the formula for the O-antigens and H-antigens and the serovar names according to the White-Kauffman-le Minor scheme of 2007 (<http://www.pasteur.fr/ip/portal/action/WebdriveActionEvent/oid/01s-000036-089>). Laboratories have to report only those results, on which the identification of the serovar names is based. 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 by the EURL-*Salmonella* according to Table 1.

Table 1 Evaluation of serotyping results

Results	Evaluation	Abbreviation
Auto-agglutination 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	-

Please record all test results in the separately provided Test Report file and return this file by e-mail to [Irene.Pol@rivm.nl](mailto:Irene.Pol@rivm.nl) on **Friday 13 April 2012 at the latest**.

A check-up by the participants of the submitted results is no longer needed when the results are sent by email in the provided file format. This will save time, *but participants need to be sure to fill in the right results at once*.

If you have questions or remarks about the interlaboratory comparison study, please contact:

Irene Pol  
P.O. Box 1  
3720 BA Bilthoven  
tel. number: +31-30-2747057  
fax number: +31-30-2744434  
e-mail: [Irene.Pol@rivm.nl](mailto:Irene.Pol@rivm.nl)

**Timetable of the Follow-up of 16<sup>th</sup> interlaboratory comparison study  
(2011) on serotyping of *Salmonella* spp.**

Week	Date	Topic
11	12 March 2012	Mailing of the protocol and test report Follow-up study 2011.
12	19 March 2012	Mailing of the parcels to the participants as Biological Substance Category B (UN 3373) by door-to-door courier service. After arrival at the laboratory the strains need to be sub-cultured and stored until the performance of the typing. <b>If you did not receive the parcel by 23 March, do contact the EU-RL immediately.</b>
13	26 March – 30 March	Starting with the identification of the strains.
15	9 - 13 April 2012	Send the completed test report preferably by e-mail to EU-RL- <i>Salmonella</i> . <b>Deadline: 13 April 2012</b>
16	16 – 20 April 2012	Data input at the EU-RL- <i>Salmonella</i> . A check-up of the submitted results by the NRLs is no longer needed when the results are sent by email in the provided file format. This will save time, <i>but NRLs need to be sure to fill out the right results at once.</i>
17	23 – 27 April 2012	Reporting of the individual laboratory results.



## Annex 4 Test report, Follow-up study

Test report Follow-up Interlaboratory Comparison Study on typing of *Salmonella* XVI (2011) page 1 of 7

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**TEST REPORT**

**FOLLOW-UP  
OF THE SIXTEENTH INTERLABORATORY COMPARISON STUDY (XVI,  
2011) ON SEROTYPING OF *SALMONELLA* STRAINS, FOR THE NRL-  
*SALMONELLA* LABORATORIES**

Laboratory code	
Name contact person	
Email address contact person	
Name of laboratory or institute	
Country	

**Please write your remarks and comments on page 7 of the test report.**

**GENERAL QUESTIONS****Shipment of the strains**

Was your parcel damaged at arrival?	
Date of receipt at your laboratory:	

**Sub-culturing**

Medium used for sub-culturing the strains	Name: Manufacturer:
---	------------------------

**QUESTIONS SEROTYPING**

What kind of sera do you use?	<input type="checkbox"/> Prepared in own laboratory <input type="checkbox"/> Commercial sera Manufacturer(s):
The strains in this collaborative study were serotyped by:	<input type="checkbox"/> Own laboratory, <input type="checkbox"/> Other laboratory, namely  Strains:

**REMARKS CONCERNING THE TABLES FOR SEROTYPING**

Two tables are added to this test report, to give the EURL-*Salmonella* more information about the antisera used. The table on page 4 concern reactions obtained with O-antisera and the table on page 5 with H-antisera. At the bottom of the table space is left to fill in other antisera than already mentioned in the table.

Please mention the manufacturer of the antisera used in the column next to the antisera.

Indicate for each combination of strain and antisera tested if there was agglutination (+) or not (-). If the cell remains empty this indicates that the agglutination was not determined for the specific combination of antisera and strain.



O-antisera	Manufacturer	Strains									
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
<b>Group B</b>											
1, 4, 12, 27											
1, 4, 5, 12											
4, 5, 12											
4, 5, 27											
4, 5											
4											
5											
<b>Group C</b>											
7, 8											
6, 7, 8											
6, 7											
6 <sub>1</sub> , 6 <sub>2</sub> , 7											
6, 8											
8, 20											
6 <sub>1</sub>											
6											
7											
8											
14											
20											
<b>Group D</b>											
9											
9, 12											
1, 9, 12											
12											
9, 46											
46											
<b>Group E</b>											
1, 3, 10, 15, 19, 34											
3, 10, 15, 19, 34											
(3), (15), 34											
3, 10, 15											
3, 10											
3, 15											
10											
15											
1, 3, 19											
19											
<b>Group G</b>											
13											
13, 22, 23											
22											
23											
<b>Other O-antisera</b>											

H-antiserum	Manufacturer	Strains									
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
b											
d											
E (complex)											
e, h											
e, n											
e, n, x											
e, n, z <sub>15</sub>											
h											
x											
x (z <sub>16</sub> )											
z <sub>15</sub>											
G (complex)											
g, p											
g, m											
f											
m											
p											
q											
s											
t											
u											
q, s, t, p, u											
i											
k											
L (complex)											
l, v											
l, w											
v											
w											
r											
y											
z											
z <sub>6</sub>											
z <sub>10</sub>											
z <sub>29</sub>											
1 (complex)											
2											
5											
6											
7											
Other H-antisera											

**TEST RESULTS SEROTYPING**

Labcode	
Starting date of serotyping	
Finishing date of serotyping	

Strain no.	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar name
S 1				
S 2				
S 3				
S 4				
S 5				
S 6				
S 7				
S 8				
S 9				
S 10				

## REMARKS AND COMMENTS

Name of person(s) carrying out the typing:	
Date:	

Name of person in charge:	
Date:	

## Annex 5 Serotyping results per strain and laboratory

X= number of deviating laboratories per strain,

Grey = deviating results,

Yellow = typographical errors

\*Green = Colonial form variation may occur with the expression of the O:6<sub>1</sub> antigen by some serogroup C<sub>2</sub> serovars (Hendriksen et al., 2009).

Lab REF	S1 Putten	S3 Infantis	S5 Stourbridge	S7 Hadar	S8 Virchow	S9 Enteritidis	S10 Krefeld	S11 Saintpaul
1	Putten	Infantis	Stourbridge	Hadar	Virchow	Enteritidis	Krefeld	Saintpaul
2	Putten	Infantis	Stourbridge	Hadar	Virchow	Enteritidis	Krefeld	Saintpaul
3	Putten	Infantis	Stourbridge	Hadar	Virchow	Enteritidis	Krefeld	Saintpaul
4	S. Putten	S. Infantis	S. Stourbridge	S. Hadar	S. Virchow	S. Enteritidis	S. Krefeld	S. Saintpaul
5	Putten	Infantis	Stourbridge	Hadar	Virchow	Enteritidis	Krefeld	Saintpaul
6	S. Putten	S. Infantis	S. Stourbridge	S. Hadar	S. Virchow	S. Enteritidis	S. Krefeld	S. Saintpaul
7	Putten	Infantis	Stourbridge	Hadar	Virchow	Enteritidis	1,3,19:y:-	Saintpaul
8	Putten	Infantis	Stourbridge	Hadar	Virchow	Enteritidis	Krefeld	Saintpaul
9	S.Putten	S.Infantis	S.Stourbridge	S.Hadar	S.Virchow	S.Enteritidis	S.Krefeld	S.Saintpaul
10	Putten	Infantis	Stourbridge	Hadar	Virchow	Enteritidis	Krefeld	Saintpaul
11	S. Putten	S. Infantis	S. Stourbridge	S. Hadar	S. Virchow	S. Enteritidis	S. Krefeld	S. Saintpaul
12	S.Putten	S.Bareilly	S. Staurbridge	S.Hadar	S.Virchow	S.Enteritidis	S.Krefeld	S.Chester
13	S. Putten	S. Infantis	S. Stourbridge	S. Hadar	S. Virchow	S. Enteritidis	S. Krefeld	S. Saintpaul
14	S. Putten	S. Infantis	S. Stourbridge	S. Hadar	S. Virchow	S. Enteritidis	S. Krefeld	S. Saintpaul
15	S. Putten	S. Infantis	S. Stourbridge	S. Istanbul*	S. Virchow	S. Enteritidis	S. Krefeld	S. Saintpaul
16	Putten	Infantis	Wagenia	Hadar	Virchow	Enteritidis	Langensalza	Saintpaul
17	S. Putten	S. Infantis	S. Stourbridge	S. Hadar	S. Virchow	S. Enteritidis	S. Krefeld	S. Saintpaul
18	Putten	Infantis	Stourbridge	Hadar	Virchow	Enteritidis	Krefeld	Saintpaul
19	Putten	Infantis	Stourbridge	Hadar	Virchow	Enteritidis	Svedvi	Chester
20	S. Putten	S. Infantis	S. Stourbridge	S. Hadar	S. Virchow	S. Enteritidis	S. Krefeld	S. Saintpaul
21	S.Putten	S.Infantis	S.Stourbridge	S.Hadar	S.Virchow	S.Enteritidis	S.Krefeld	S.Saintpaul
22	Putten	Infantis	Stourbridge	Hadar	Virchow	Enteritidis	Krefeld	Saintpaul
23	Putten	Infantis	Stourbridge	Hadar	Virchow	Enteritidis	Krefeld	Saintpaul
24	S. Putten	S. Infantis	S. Stourbridge	S. Hadar	S. Virchow	S. Enteritidis	S. Slade	S. Saintpaul
25	Putten	Infantis	Stourbridge	Hadar	Stourbridge	Enteritidis	Krefeld	Saintpaul
26	S. Putten	S. Infantis	S. Stourbridge	S. Hadar	S. Virchow	S. Enteritidis	S. Krefeld	S. Saintpaul
27	S. Kedougou	S. Bareilly	S. Stourbridge	S. Hadar	S. Virchow	S. Enteritidis	S. Krefeld	S. Saintpaul
28	S. Putten	S. Infantis	S. Stourbridge	S. Hadar	S. Virchow	S. Enteritidis	S. Krefeld	S. Saintpaul
29	Putten	Infantis	Stourbridge	Hadar	Virchow	Enteritidis	Krefeld	Saintpaul
30	Putten	Infantis	Stourbridge	Hadar	Virchow	Enteritidis	Cannonhill	Saintpaul
31	Putten	Infantis	Stourbridge	Hadar	Virchow	Enteritidis	Krefeld	Saintpaul
32	Putten	Infantis	Stourbridge	Hadar	Virchow	Enteritidis	Krefeld	Saintpaul
33	S.Putten	S.Infantis	S.Stourbridge	S.Hadar	S.Virchow	S.Enteritidis	S.Krefeld	S.Saintpaul
34	Putten	Infantis	Stourbridge	Hadar	Virchow	Enteritidis	Krefeld	Saintpaul
35	Putten	Infantis	Stourbridge	Hadar	Virchow	Enteritidis	Krefeld	Saintpaul
36	Putten	Infantis	Stourbridge	Hadar	Virchow	Enteritidis	Krefeld	Saintpaul
X	13	25	1	0	4	0	5	4

<b>S12</b>	<b>S13</b>	<b>S14</b>	<b>S15</b>	<b>S17</b>	<b>S18</b>	<b>S19</b>	<b>S20</b>	<b>Lab</b>
<b>Haifa</b>	<b>Durban</b>	<b>Havana</b>	<b>Bovismorbificans</b>	<b>Abaetetuba</b>	<b>Amsterdam</b>	<b>Typhimurium</b>	<b>Kentucky</b>	<b>REF</b>
Haifa	Durban	Havana	Bovismorbificans	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>1</b>
Haifa	Durban	Havana	Bovismorbificans	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>2</b>
Haifa	Durban	Havana	Bovismorbificans	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>3</b>
S. Haifa	S. Durban	S. Havana	S. Bovismorbificans	S. Abaetetuba	S. Amsterdam	S. Typhimurium	S. Kentucky	<b>4</b>
Haifa	Durban	Havana	Bovismorbificans	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>5</b>
S. Haifa	S. Durban	S. Havana	<b>S. Bovismorbificans</b>	S. Abaetetuba	S. Amsterdam	S. Typhimurium	S. Kentucky	<b>6</b>
Haifa	Durban	Havana	Bovismorbificans	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>7</b>
Haifa	Durban	Havana	Bovismorbificans	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>8</b>
S. Haifa	S. Durban	S. Havana	S. Bovismorbificans	S. Abaetetuba	S. Amsterdam	S. Typhimurium	S. Kentucky	<b>9</b>
Haifa	Durban	Havana	Bovismorbificans	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>10</b>
S. Haifa	S. Durban	S. Havana	S. Bovismorbificans	S. Abaetetuba	S. Amsterdam	S. Typhimurium	S. Kentucky	<b>11</b>
<b>S. Agona</b>	S. Durban	S. Havana	S. Bovismorbificans	S. Abaetetuba	S. Amsterdam	S. Typhimurium	S. Kentucky	<b>12</b>
S. Haifa	S. Durban	S. Havana	S. Bovismorbificans	S. Abaetetuba	S. Amsterdam	S. Typhimurium	S. Kentucky	<b>13</b>
S. Haifa	S. Durban	S. Havana	S. Bovismorbificans	S. Abaetetuba	S. Amsterdam	S. Typhimurium	<b>S. Altona</b>	<b>14</b>
S. Haifa	S. Durban	S. Havana	S. Bovismorbificans	S. Abaetetuba	S. Amsterdam	S. Typhimurium	<b>S. Kentucky</b>	<b>15</b>
Haifa	<b>Babylor</b>	<b>Maiduguri</b>	<b>Hindmarsh*</b>	Abaetetuba	<b>Suberu</b>	Typhimurium	Kentucky	<b>16</b>
S. Haifa	S. Durban	S. Havana	S. Bovismorbificans	S. Abaetetuba	S. Amsterdam	S. Typhimurium	S. Kentucky	<b>17</b>
Haifa	Durban	Havana	Bovismorbificans	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>18</b>
Haifa	<b>0</b>	Havana	Bovismorbificans	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>19</b>
S. Haifa	S. Durban	S. Havana	S. Bovismorbificans	S. Abaetetuba	S. Amsterdam	S. Typhimurium	S. Kentucky	<b>20</b>
S. Haifa	S. Durban	S. Havana	S. Bovismorbificans	S. Abaetetuba	S. Amsterdam	S. Typhimurium	S. Kentucky	<b>21</b>
Haifa	Durban	Havana	<b>Bovismorbificans</b>	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>22</b>
Haifa	Durban	Havana	Bovismorbificans	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>23</b>
S. Haifa	S. Durban	S. Havana	S. Bovismorbificans	S. Abaetetuba	S. Amsterdam	S. Typhimurium	S. Kentucky	<b>24</b>
Haifa	Durban	Havana	Bovismorbificans	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>25</b>
S. Haifa	S. Durban	S. Havana	S. Bovismorbificans	S. Abaetetuba	S. Amsterdam	S. Typhimurium	S. Kentucky	<b>26</b>
S. Haifa	S. Durban	S. Havana	<b>S. Infantis</b>	S. Abaetetuba	S. Amsterdam	S. Typhimurium	S. Kentucky	<b>27</b>
S. Haifa	S. Durban	S. Havana	S. Bovismorbificans	S. Abaetetuba	S. Amsterdam	S. Typhimurium	S. Kentucky	<b>28</b>
Haifa	Durban	Havana	Bovismorbificans	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>29</b>
Haifa	Durban	Havana	Bovismorbificans	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>30</b>
Haifa	Durban	Havana	Bovismorbificans	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>31</b>
<b>Stanleyville</b>	Durban	Havana	Bovismorbificans	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>32</b>
S. Haifa	S. Durban	S. Havana	S. Bovismorbificans	S. Abaetetuba	S. Amsterdam	S. Typhimurium	S. Kentucky	<b>33</b>
Haifa	Durban	Havana	Bovismorbificans	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>34</b>
Haifa	Durban	Havana	Bovismorbificans	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>35</b>
Haifa	Durban	Havana	Bovismorbificans	Abaetetuba	Amsterdam	Typhimurium	Kentucky	<b>36</b>
2	3	6	1	2	1	12	1	<b>X</b>

## Annex 6 Identifications per strain that caused problems in serotyping

Strain	O-antigens	H-antigens, phase 1	H-antigens, phase 2	Serovar	Labcode
<b>S-1</b>	<b>13,23</b>	<b>d</b>	<b>l,w</b>	<b>Putten</b>	<b>REF</b>
S-1	1,13,23	i	l,w	S. Kedougou	27
<b>S-2</b>	<b>6,7</b>	<b>c</b>	<b>1,5</b>	<b>Choleraesuis</b>	<b>REF</b>
S-2	6,7	c	5	Choleraesuis	1
S-2	6,7	c	1,5	Choleraesuis	2
S-2	6,7	c	1,5	Choleraesuis	3
S-2	6,7	c	1,5	S. Choleraesuis var. Kunzendorf	4
S-2	6,7	c	-	I 06, 7 : c : -	5
S-2	7	c	5	S. Choleraesuis	6
S-2	6,7	c	1,5	Choleraesuis	7
S-2	6,7	c	1,5	Choleraesuis/Typhisuis*	8
S-2	6,7	c	5	S.Choleraesuis	9
S-2	7	c	1,5	Choleraesuis	10
S-2	6,7	c	-	S. Typhisuis	11
S-2	6,7	c	1,5	S.Choleraesuis	12
S-2	6,7	c	1,5	S. Choleraesuis	13
S-2	6,7	c	1,5	S. Choleraesuis	14
S-2	6,7	c	1,5	S. Choleraesuis*	15
S-2	1, 9, 12 Vi	g, p	-	Dublin	16
S-2	7	c	5	S. Choleraesuis	17
S-2	6,7	c	1,5	Choleraesuis	18
S-2	6,7	g,m,t	1,5	II	19
S-2	6,7	c	1,5	S. Choleraesuis	20
S-2	6,7	c	1,5	S.Choleraesuis	21
S-2	6,7	c	1,5	Choleraesuis	22
S-2	6,7	c	1,5	Choleraesuis	23
S-2	6,7	c	1,5	S. Paratyphi C subgroup	24
S-2	6,7	c	1,5	Choleraesuis	25
S-2	6,7	c	1,5	S. Choleraesuis	26
S-2	6,7	c	1,5	S. Typhisuis	27
S-2	7	c	1,5	S. Choleraesuis	28
S-2	6,7	c	1,5	Choleraesuis	29
S-2	6,7	c	1,5	See comment page 17	30
S-2	7	c	1,5	Choleraesuis	31
S-2	6,7	c	-	Species	32
S-2	6,7	c	1,5	S.Choleraesuis (H2S-,Dulcitol-,d-tartrate+)	33
S-2	6,7	c	1,5	Choleraesuis	34
S-2	6,7	c	1,5	*Typhisuis *(dulcitol -ve, H2S -ve,tartrate -ve)	35
S-2	6,7	c	1,5	Choleraesuis	36
<b>S-3</b>	<b>6,7,14</b>	<b>r</b>	<b>1,5</b>	<b>Infantis</b>	<b>REF</b>
S-3	6,7,14	y	1,5	S.Bareilly	12
S-3	6,7,14	y	1,5	S. Bareilly	27
<b>S-4</b>	<b>1,4,[5],12</b>	<b>i</b>	<b>-</b>	<b>1,4,[5],12:i:-</b>	<b>REF</b>
S-4	4,5	i	-	4,5:i:-	1
S-4	4,5	i	-	MonophasicstrainofS.Typhimurium	2
S-4	4,5,12	i	-	Typhimurium monophasic	3
S-4	4	i	-	4: i : -	4
S-4	4, 5, 12	i	-	I 04, 5, 12 : i : -	5
S-4	4,5,12	i	-	Monophasic S. Typhimurium	6
S-4	4,5,12	i	-	4,5,12:i:-	7
S-4	1,4,[5],12	i	-	4,[5],12:i:- (monophasic ST) *	8
S-4	4,5	i	-	O4,5:i:-	9
S-4	4,5	i	-	4,5,12 : i : -	10
S-4	4,5,12	i	-	Salmonella spp.	11
S-4	4,5,12	i	-	S.enterica subsp.enterica, 4,5,12 : i : -	12
S-4	4,5,12	i	-	S. enterica subsp. enterica 4,5,12: i : -	13
S-4	1,4,5,12	i	-	S. Typhimurium-like ( S. 1,4,5,12 : i : -)	14
S-4	1,4,[5],12	i	-	S. 4,5,12:i:-	15
S-4	1,4,12,27	i	l, w	Gloucester	16
S-4	4,5	i	-	Monophasic strain Group B -monophasic S. Typhimurium	17
S-4	1,4,[5],12	i	-	1,4,[5],12:i:- (monophasic S. Typhimurium )	18
S-4	4,12	i	0	Typhimurium (monophasic)	19
S-4	4,5,12	i	-	S. enterica ssp. enterica Gr. O:4 mon.var.	20
S-4	1, 4, 5, 12	i	-	S.enterica subsp. enterica	21
S-4	1,4,[5],12	i	-	Monophasic Typhimurium	22
S-4	4,5,12	i	-	enterica subsp. enterica Serotype 4, 5, 12: i: -	23
S-4	4,5,12	i	-	S. Enterica subspecies enterica	24
S-4	4,5,12	i	1,2	Typhimurium	25
S-4	4,5	i	-	Monophasic variant of S. Typhimurium	26
S-4	1,4,[5],12	i	-	monophasic S. Typhimurium	27
S-4	4,5	i	-	S.I=4,5:i:-	28
S-4	1,4, , [5], 12	i	-	Typhimurium monophasic	29
S-4	4,5,12	i	-	Typhimurium monophasic	30
S-4	4,5	i	1,2	Typhimurium	31
S-4	4,5,12	i	-	Species	32
S-4	4,5	i	-	S.enterica ssp. enterica I 4,[5],12:i:-	33
S-4	4,5,12	i	-	Monophasic Typhimurium (by PCR)	34
S-4	4,5,12	i	-	Salm. enterica subsp. enterica serovar 4,5,12:i:-	35
S-4	4,5,12	i	-	Unnamed ( 4,5,12: i : - )	36



<b>S-5</b>	<b>6,8</b>	<b>b</b>	<b>1,6</b>	<b>Stourbridge</b>	<b>REF</b>
S-5	1, 4, 12, 27	b	e, n, z15	Wagenia	16
<b>S-6</b>	<b>1,13,23</b>	<b>g,t</b>	-	<b>1,13,23:g,t:-</b>	<b>REF</b>
S-6	13,23	g,t	-	Okatie	1
S-6	1,13,23	g,t	-	S.entericasubsp.salamae1,13,23:g,t:-	2
S-6	13,23	g,t	-	Okatie	3
S-6	13,23	g,[s],t	-	S. Okatie	4
S-6	13, 23	g, t	-	Okatie	5
S-6	13,23	g,t	-	S. Okatie	6
S-6	13,23	g,[s],[t]	-	Okatie	7
S-6	13,23	g,[s],t	-	Okatie	8
S-6	13,23	g,t	0	O13,23:g,t	9
S-6	13,23	g,t	-	subsp. enterica or salamae; 13,23 : g,t : - , see remarks	10
S-6	1,13,23	t,g	-	Salmonella spp.	11
S-6	13,23	g,t	-	S.Okatie	12
S-6	13,23	g,t	-	S. Okatie	13
S-6	1,13, 23	g	-	S. subsp. enterica (I)	14
S-6	13,23	g,[s],t	-	S. Okatie *	15
S-6	1,3,19	g, t	-	Senftenberg	16
S-6	1,13,23	g,t	-	S. II (Salmonella enterica subsp. salamae)	17
S-6	13,23	g,t	-	Okatie	18
S-6	13,23	g,t	0	Okatie	19
S-6	1,13,23	g,t	-	S. enterica ssp. salamae Gr. O:13 mon. var.	20
S-6	13,23	g,[s],t	-	S.Okatie	21
S-6	1,13,23	g,t	-	1,13,23 :g,t :- (II)	22
S-6	13,23	g,t	-	Okatie	23
S-6	13	g,t	-	S. Okatie	24
S-6	13,23	g,t	-	Okatie	25
S-6	13,23	g,t	-	S. Okatie	26
S-6	16	g,[s],[m],t	[1,5]	S. enterica subsp.salamae (II16:g,[s],[m],t:[1,5])	27
S-6	23	g,t	-	S. Okatie	28
S-6	13,23	g,[s],t	-	Okatie	29
S-6	1,3,19	g,t	-	Senftenberg	30
S-6	13,23	g,s,t	-	Okatie	31
S-6	13,23	g,t	-	Okatie	32
S-6	13,23	g,t	-	S.Okatie	33
S-6	13,23	g,t	-	Okatie	34
S-6	13,23	gt	-	Okatie	35
S-6	1,13,23	g,t	z42	Unnamed (sub-species II)	36
<b>S-8</b>	<b>6,7,14</b>	<b>r</b>	<b>1,2</b>	<b>Virchow</b>	<b>REF</b>
S-8	6,8	b	1,6	Stourbridge	25
<b>S-10</b>	<b>1,3,19</b>	<b>y</b>	<b>l,w</b>	<b>Krefeld</b>	<b>REF</b>
S-10	1,3,19	y	-	1,3,19:y:-	7
S-10	3, 10	y	l, w	Langensalza	16
S-10	1,3,19	l,v	e,n,z15	Svedvi	19
S-10	1,3,19	y	e,n,z15	S. Slade	24
S-10	1,3,15,19	y	-	Cannonhill*	30
<b>S-11</b>	<b>1,4,[5],12</b>	<b>e,h</b>	<b>1,2</b>	<b>Saintpaul</b>	<b>REF</b>
S-11	4,5,12	e,h	e,n,x	S.Chester	12
S-11	4,12	e,h	e,n,x	Chester	19
<b>S-12</b>	<b>1,4,[5],12</b>	<b>z10</b>	<b>1,2</b>	<b>Haifa</b>	<b>REF</b>
S-12	4,5,12	f,g,t	-	S.Agona	12
S-12	4,5,12	z4z23	1,2	Stanleyville	32
<b>S-13</b>	<b>1,9,12</b>	<b>a</b>	<b>e,n,z15</b>	<b>Durban</b>	<b>REF</b>
S-13	9, 46	z	e,n,z15	Babylor	16
S-13	12	0	0	0	19
<b>S-14</b>	<b>1,13,23</b>	<b>f,g,[s]</b>	-	<b>Havana</b>	<b>REF</b>
S-14	1, 3, 19	f, g, t	e, n, z15	Maiduguri	16
<b>S-15</b>	<b>6,8,20</b>	<b>r,[i]</b>	<b>1,5</b>	<b>Bovismorbificans</b>	<b>REF</b>
S-15	8, 20	R	1, 5	Hindmarsh	16
S-15	6,7,14	r	1,5	S. Infantis	27

S-16	6,7,14	e,h	e,n,z15	Braenderup	REF
S-16	6,7	e,h	e,n,z15	Braenderup	1
S-16	6,7	e,h	e,n,z15	Braenderup	2
S-16	6,7	e,h	e,n,z15	Braenderup	3
S-16	6,7,14	e,h	e,n,z15	S. Braenderup	4
S-16	6, 7	e,h	e,n,z15	Braenderup	5
S-16	7,14	e,h	e,n,z15	S. Braenderup	6
S-16	6,7,14	e,h	e,n,z15	Braenderup	7
S-16	-	-	-	Untypable*	8
S-16	4,5	h	z15	S.Sandiego	9
S-16	7	E,h	e,z15	Braenderup	10
S-16	1,4,5	h	z15	S. Sandiego	11
S-16	4,12	d	e,n,z15	S.Duisburg	12
S-16	6,7	e,h	e,n,z15	S. Braenderup	13
S-16	6,7,14	e,h	e,n,z15	S. Braenderup	14
S-16	see remark	e,h	e,n,z15	see p15 test report	15
S-16	6,7 Vi	c	1, 5	Paratyphi C	16
S-16	7	h	z15	S. Braenderup	17
S-16	6,7,14	e,h	e,n,z15	Braenderup	18
S-16	4,12	i	e,n,z15	Tsevie	19
S-16	6,7	e,h	e,n,z15	S. Braenderup	20
S-16	6, 7, 14	e, h	e,n,z15	S.Braenderup	21
S-16	6,7,14	e,h	e,n,z15	Braenderup	22
S-16	6,7,14	e,h	e,n,z15	Braenderup	23
S-16	7	e,h	e,n,z15	S. Braenderup	24
S-16	6,7,14	e,h	e,n,z15	Braenderup	25
S-16	6,7,14	e,h	e,n,z15	S. Braenderup	26
S-16	6,7,14+	e,h	e,n,z15	S. Braenderup	27
S-16	7	h	z15	S. Braenderup	28
S-16	6,7,14	e,h	e,n,z15	Braenderup	29
S-16	4,12	e,h	e,n,z15	Sandiego	30
S-16	45	e,h	e,n,z15	45:e,h:e,n,z15?	31
S-16	0	0	0	Species	32
S-16	6,7	e,h	e,n,z15	S.Braenderup	33
S-16	6,7	e,h	e,n,z15	Braenderup	34
S-16	6,7	eh	e,n,z15	Braenderup	35
S-16	6,7	e,h	e,n,z15	Braenderup	36
S-18	3	g,m,s	-	Amsterdam	REF
S-18	3,1	g, m	-	Suberu	16
S-20	8,20	i	z6	Kentucky	REF
S-20	8,2	r,i	z6	S. Altona	14
S-21	38	r	z	38:r:z	REF
S-21	38	r	z	38:r:z	1
S-21	38	r	z	S.entericasubsp.diarizonae38:r:z	2
S-21	38	r	z	IIIb 38:r:z	3
S-21	38	r	z	S. enterica subsp. diarizonae /IIIb/	4
S-21	38	r	z	III O38: r : z	5
S-21	38	r	z	S. enterica subsp diarizonae	6
S-21	38	r	z	IIIb 38:r:z	7
S-21	38	r	z	IIIb 38:r:z (subsp. diarizonae)	8
S-21	OMC	r	0	OMC	9
S-21	-	r	z	subsp. diarizonae 38 : r : z	10
S-21	38	r	z	III b	11
S-21	38	r	1,5	S.Lindi	12
S-21	38	r	z	S. enterica subsp. diarizonae 38:r:z	13
S-21	38	r	z	S. IIIb 38: r : z	14
S-21	38	r	z54	S. IIIb 38:r:z54 *	15
S-21	38	r	z	S. IIIb (Salmonella enterica subsp. diarizonae)	16
S-21	38	r	z	enterica subsp. diarizonae (III.b) 38:r:z	17
S-21	38	r	z	S. enterica ssp. diarizonae	18
S-21	38	r	z	S.enterica subsp. diarizonae	19
S-21	38	r	z	38: r : z (IIIb)	20
S-21	38	r	z	enterica subsp. diarizonae (IIIb)	21
S-21	38	r	z	S. Emmastad IIIb	22
S-21	4,5,12	e,h	1,2	Saintpaul	23
S-21	38	r	z	S. enterica subsp.diarizonae - Gr. P 38:r:z	24
S-21	38	r	z [z57]	S. enterica subsp.diarizonae (IIIb:38:r:z [z57])	25
S-21	38	r	z	S.IIIb=38:r:z	26
S-21	38	r	z	IIIb	27
S-21	38	r	z	Enterica subsp. diarizonae	28
S-21	38	r	-	IIIb38:r:-	29
S-21	38	r	z	Diarizonae	30
S-21	38	r	z	S.enterica ssp. arizonae IIIb 38:r:z:[z57]	31
S-21	38	r	z	38:r:z (subspecies diarizonae)	32
S-21	38	r	z	Salm.enterica subsp.diarizonae serovar 38:r:z	33
S-21	38	r	-	Arizonae (sub-species IIIb)	34
S-21	38	r	-		35
S-21	38	r	-		36

Deviating results only are shown for strains that caused problems, but as background information complete results for all labs are shown for strains S2, S4, S6, S16, and S21.

## Annex 7     Phage typing results per strain and laboratory

-	= no reaction
+	= 5-20 plaques
+	= 21-40 plaques
++	= 41-80 plaques
+++	= 81-100 plaques
SCL	= semi-confluent lysis
CL	= confluent clear lysis
OL	= confluent opaque lysis
<<	= merging plaques towards semi-confluent lysis

Strain E1		Phage 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	17
HPA	21c	CL	SCL	-	OL	-	SCL	-	OL	OL	SCL	-	-	-	CL	CL	CL	+++
1	21c	+++	+++	-	+++	-	SCL	-	<OL	+++	++	-	-	-	<SCL	<SCL	SCL	+++
3	21c	SCL	SCL	-	SCL	±	SCL	-	OL	<OL	+++	-	++	-	<CL	<CL	<CL	SCL
5	21c	+++	+++	-	+	-	<OL	-	SCL	<OL	+++	+++	-	-	CL	CL	CL	OL
15	21c	OL	CL	-	OL	-	SCL	-	<OL	<OL	SCL	-	-	-	OL		<OL	OL
17	21c	OL	+++	-	SCL	-	+++	-	<CL	<OL	<CL	-	-	-	CL	+++	<OL	OL
18	21c	OL	SCL	-	SCL	-	SCL	-	OL	OL	<OL	-	-	-	SCL	<CL	SCL	<OL
26	21c	OL	SCL	+	OL	-	SCL	-	OL	OL	<OL	-	CL	-	SCL	SCL	SCL	<OL
31	21c	+++	SCL	-	SCL	-	++	-	+++	SCL	+++	-	-	-	SCL	SCL	SCL	+++
34	21c	OL	SCL	-	<OL	-	SCL	-	OL	OL	<OL	-	-	-	SCL	SCL	CL	SCL

Strain E2		Phage 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	17
HPA	35	-	SCL	-	OL	-	-	-	-	OL	-	-	-	-	-	-	-	SCL
1	RDNC/21	+++	+++	-	+++	-	-/++	-	+++	+++	-/++	-	-	-	+++	-	-	+++
3	35	-	SCL	-	<OL	-	-	-	-	<OL	-	-	-	-	-	-	-	<SCL
5	See comment	-	+++	-	+	-	-	-	-	SCL	+++	-	-	-	+++	-	-	OL
15	35	-	++	-	SCL	-	-	-	-	++	-	-	-	-	-	-	-	SCL
17	35	-	SCL	-	SCL	-	-	-	-	<OL	-	-	-	-	-	-	-	OL
18	35	-	SCL	-	SCL	-	-	-	-	OL	-	-	-	-	-	-	-	<OL
26	35	-	SCL	-	SCL	-	-	-	-	OL	-	-	-	-	-	-	-	<OL
31	35	-	SCL	-	SCL	-	-	-	-	SCL	-	-	-	-	-	-	-	SCL
34	7	-	SCL	-	OL	-	-	-	-	OL	-	-	-	-	-	-	-	OL

Strain E3		Phage 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	17
HPA	21	OL	SCL	-	OL	-	SCL	-	OL	OL	OL	-	-	-	CL	-	-	+++
1	21/21c	OL	+++	-	+++	-	SCL	-	OL	+++	+++	-	-	-	SCL	-/++	-/+++	+++
3	21	OL	<CL	-	OL	-	<OL	-	OL	OL	<OL	-	-	-	SCL	-	-	<OL
5	See comment	CL	+++	-	+	-	SCL	-	OL	OL	+++	SCL	-	-	CL	SCL	SCL	OL
15	21	OL	<CL	-	OL	-	SCL	-	OL	<OL	OL	-	-	-	OL		-	OL
17	21	OL	SCL	-	<OL	-	<SCL	-	OL	OL	OL	-	-	-	CL	-	±	OL
18	21	OL	SCL	-	SCL	-	SCL	-	OL	OL	<OL	-	-	-	SCL	-	-	<OL
26	21	OL	SCL	-	OL	-	SCL	-	OL	<OL	<OL	-	-	-	CL	-	-	<OL
31	21	+++	SCL	-	SCL	-	++	-	-	SCL	+++	-	-	-	SCL	-	++	SCL
34	21b	OL	+++	-	SCL	-	+++	-	OL	OL	SCL	-	-	-	++	-	-	SCL

Strain E4		Phage 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	17
HPA	14b	-	-	-	±	-	SCL	-	-	±	-	-	-	-	-	-	-	+++
1	14b	-	-	-	-	-	SCL	-	-	-	-	-	-	-	-	-	-	+++
3	14b	-	-	-	2	-	SCL	-	-	-	-	-	-	-	-	-	-	<OL
5	14b	-	-	-	+	-	SCL	-	-	+	-	-	-	-	-	-	-	OL
15	14b	-	-	-	±	-	SCL	-	-	-	-	-	-	-	-		-	SCL
17	14b	-	-	-	-	-	+++	-	-	1	-	-	-	-	-	-	-	OL
18	14b	-	-	-	-	-	SCL	-	-	-	-	-	-	-	-	-	-	<OL
26	14b	-	-	-	-	-	SCL	-	-	±	-	-	-	-	-	-	-	<OL
31	14b	-	-	-	+	-	++	-	-	+	-	-	-	-	-	-	-	+++
34	14b	-	-	-	-	-	SCL	-	-	-	-	-	-	-	-	-	-	SCL

Strain E5		Phage 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	17
HPA	9b	-	-	CL	-	CL	-	-	-	-	-	-	CL	-	+++	-	-	-
1	9b	-	-	CL	-	CL	-	-	-	-	-	-	CL	-	+++	-	-	-
3	9b	-	-	CL	-	SCL	-	-	-	-	-	-	CL	-	<SCL	-	-	-
5	9b	<<	-	CL	-	CL	-	-	-	-	-	-	CL	-	SCL	-	-	-
15	9b	-	-	CL	-	CL	-	-	-	-	-	-	CL	-	SCL	-	-	-
17	9b	-	-	CL	-	CL	-	-	-	-	-	-	CL	-	<CL	-	-	-
18	11b	-	-	CL	-	CL	-	-	-	-	-	-	CL	-	-	-	-	-
26	9b	-	-	CL	-	CL	-	-	-	-	-	-	CL	-	SCL	-	-	-
31	9b	-	-	SCL	-	SCL	-	-	-	-	-	-	SCL	-	SCL	-	-	-
34	11b	-	-	SCL	-	CL	-	-	-	-	-	-	CL	-	-	-	-	-

Strain E6		Phage 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	17
HPA	8	-	-	SCL	OL	CL	SCL	CL	OL	OL	OL	CL	CL	-	-	-	-	+++
1	8	-	-	SCL	+++	CL	SCL	+++	OL	+++	+++	++	CL	-	-	-	-	+++
3	8	-	-	SCL	<CL	CL	<SCL	<CL	<OL	<OL	<OL	SCL	CL	-	-	-	-	<OL
5	8	-	-	++	+	CL	SCL	<<	CL	+++	+++	OL	OL	-	-	-	-	OL
15	8	-	-	<CL	SCL	CL	SCL	+	<OL	+	OL	++	CL	-	-	-	-	++
17	8	-	-	SCL	SCL	CL	<SCL	CL	OL	OL	OL	SCL	CL	-	-	-	-	OL
18	8	-	-	SCL	SCL	CL	SCL	+++	OL	OL	OL	+++	CL	-	-	-	-	<OL
26	8	-	-	SCL	SCL	SCL	SCL	SCL	<OL	<OL	<OL	SCL	CL	-	-	-	-	<OL
31	8	+	-	SCL	SCL	SCL	++	SCL	SCL	SCL	SCL	SCL	SCL	-	-	-	-	SCL
34	8	-	-	SCL	SCL	CL	SCL	CL	OL	SCL	SCL	CL	CL	-	-	-	-	<OL

Strain E7		Phage 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	17
HPA	4b	-	SCL	CL	OL	CL	SCL	CL	OL	OL	OL	CL	CL	CL	-	±	CL	+++
1	4b	-	+++	CL	+++	CL	+++	SCL	OL	+++	+++	++	CL	CL	-	-	SCL	+++
3	4b	-	<CL	CL	<CL	CL	SCL	CL	OL	OL	<OL	<CL	CL	CL	-	-	SCL	<OL
5	4b	-	+++	SCL	+	OL	SCL	SCL	OL	OL	SCL	CL	CL	+++	-	+++	CL	OL
15	4b	-	SCL	CL	OL	CL	SCL	SCL	OL	SCL	OL	SCL	CL	CL	-		SCL	<OL
17	4b	-	SCL	CL	SCL	CL	<SCL	CL	OL	OL	OL	CL	CL	CL	-	1	SCL	OL
18	4b	-	SCL	CL	SCL	CL	SCL	CL	OL	OL	OL	CL	CL	CL	-	-	+++	<OL
26	4b	-	SCL	CL	OL	CL	SCL	SCL	<OL	<OL	<OL	CL	CL	<SCL	-	+	SCL	<OL
31	4b	-	SCL	SCL	SCL	SCL	++	SCL	+++	SCL	SCL	SCL	SCL	SCL	-	+	SCL	SCL
34	4b	-	SCL	CL	SCL	CL	SCL	CL	OL	<OL	SCL	CL	CL	CL	-	±	<SCL	<OL

Strain E8		Phage 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	17
HPA	4	-	SCL	CL	OL	CL	SCL	CL	OL	OL	SCL	CL	CL	CL	-	-	-	+++
1	4	-	+++	SCL	+++	CL	+++	+++	+++	+++	+++	+++	CL	CL	-	-	++	+++
3	4	-	<CL	CL	<CL	CL	SCL	CL	OL	<OL	OL	CL	CL	CL	-	-	-	<OL
5	4	-	+++	SCL	+	CL	SCL	+++	CL	OL	+++	OL	OL	SCL	-	-	-	OL
15	4		<CL	CL	OL	CL	<OL	<CL	OL	SCL	<OL	SCL	CL	CL	-		-	OL
17	4	-	SCL	CL	SCL	CL	<SCL	CL	OL	OL	OL	CL	CL	CL	-	-	-	OL
18	4	-	SCL	CL	SCL	CL	SCL	CL	OL	OL	OL	CL	CL	CL	-	-	-	<OL
26	4	-	SCL	CL	OL	CL	SCL	SCL	OL	<OL	<OL	CL	CL	CL	-	-	-	<OL
31	4	-	SCL	SCL	+++	SCL	++	SCL	SCL	SCL	SCL	SCL	SCL	SCL	-	-	+	+++
34	53	-	<CL	CL	SCL	CL	++	CL	-	OL	-	CL	SCL	-	-	-	-	OL

Strain E9		Phage 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	17
HPA	2	OL	-	CL	OL	CL	SCL	CL	OL	OL	OL	CL	CL	-	SCL	-	-	+++
1	2	+++	-	SCL	+++	<CL	+++	+++	+++	+++	+++	+++	<CL	-	+++	-	-	+++
3	2	OL	-	CL	<<SCL	CL	<<SCL	<CL	OL	<<OL	OL	<SCL	CL	-	SCL	-	-	++
5	43	OL	SCL	+++	+	SCL	+++	-	SCL	OL	SCL	CL	CL	-	CL	-	-	OL
15	2	OL	-	CL	OL	CL	SCL	±	OL	SCL	<OL	+	CL	-	<CL		-	<OL
17	2	OL	-	CL	SCL	CL	<SCL	<CL	OL	<OL	OL	<CL	CL	-	CL	-	-	OL
18	2	OL	-	SCL	SCL	CL	<SCL	SCL	OL	OL	OL	SCL	CL	-	SCL	-	-	<OL
26	2	OL	-	SCL	SCL	SCL	SCL	+++	CL	SCL	SCL	+++	CL	-	SCL	-	-	<OL
31	2	SCL	-	SCL	SCL	SCL	++	SCL	SCL	SCL	SCL	SCL	SCL	-	SCL	-	-	SCL
34	2	OL	-	CL	SCL	SCL	SCL	SCL	OL	SCL	OL	CL	CL	±	SCL	-	-	<OL

Strain E10		Phage 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	17
HPA	1b	OL	SCL	CL	OL	CL	SCL	CL	OL	OL	SCL	CL	CL	CL	CL	++	CL	+++
1	1b	OL	SCL	CL	+++	CL	SCL	+++	OL	+++	++	+++	CL	+++	SCL	+++	+++	+++
3	1b	OL	<CL	CL	CL	CL	SCL	CL	OL	OL	OL	SCL	CL	SCL	<CL	+	++	<OL
5	1d	OL	+++	CL	+	SCL	SCL	SCL	CL	OL	+++	SCL	OL	-	OL	++	++	OL
15	1b	OL	<CL	CL	OL	CL	<OL	SCL	OL	SCL	OL	SCL	CL	SCL	OL		+	<OL
17	1b	OL	SCL	CL	<OL	CL	<SCL	<CL	OL	OL	OL	<CL	CL	SCL	OL	<SCL	<OL	OL
18	1	OL	SCL	CL	OL	CL	SCL	CL	OL	OL	OL	CL	CL	<SCL	SCL	-	-	<OL
26	1b	OL	SCL	CL	CL	CL	SCL	SCL	OL	OL	OL	SCL	CL	+++	SCL	+++	+++	<OL
31	1b	SCL	SCL	SCL	SCL	SCL	++	SCL	SCL	+++	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL
34	1b	OL	SCL	CL	<OL	CL	SCL	CL	OL	OL	SCL	CL	CL	CL	CL	SCL	OL	<OL



Strain T1		Phage reactions at routine test dilution (S.Typhimurium)																	
Lab code	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
HPA	36	CL	OL	CL	OL	OL	OL	OL	CL	CL	OL	CL	CL	CL	OL	OL	OL	CL	OL
1	36	SCL	SCL	SCL	SCL	+++	+++	SCL	+++	<CL	CL	<SCL	<SCL	CL	CL	CL	CL	CL	CL
3	36	SCL	SCL	<SCL	OL	<CL	<CL	<CL	<CL	<CL	CL	<CL	<CL	CL	CL	CL	CL	CL	<CL
5	36	+++	+	OL	OL	CL	CL	<CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL
15	36	<CL	CL	CL	OL	CL	CL	SCL	<CL	SCL	CL	SCL	SCL	CL	CL	CL	<CL	CL	CL
17	36	CL	OL	CL	CL	CL	CL	CL	CL	CL	CL	<SCL	<SCL	CL	CL	CL	CL	CL	CL
18	36	CL	CL	CL	CL	SCL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	SCL	CL
26	36	<SCL	CL	CL	CL	CL	CL	<CL	SCL	<CL	CL	SCL	CL	CL	CL	CL	CL	CL	CL
31	36	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	+++	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL

Strain T1		Phage reactions at routine test dilution (S.Typhimurium)												Additional phages							
Lab code	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10VAR2	10VAR3	18	
HPA	36	OL	OL	OL	CL	OL	OL	CL	OL	OL	OL	CL	OL	+++	+++	+++	SCL	OL	OL	OL	
1	36	+++	CL	SCL	<CL	CL	<SCL	SCL	CL	OL	++	CL	SCL	+	++	<SCL	OL	OL	OL	OL	
3	36	<CL	CL	CL	<CL	<CL	CL	<CL	CL	OL	CL	CL	OL								
5	36	SCL	OL	SCL	SCL	CL	OL	SCL	OL	CL	CL	CL	SCL	++	++	++	SCL	SCL	SCL	SCL	
15	36	SCL	CL	<CL	<CL	<CL	CL	CL	<CL	OL	SCL	<CL	OL	±	±	±	OL	OL	SCL	CL	
17	36	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	OL	++	+++	++	<OL	SCL	SCL	OL	
18	36	CL	CL	CL	CL	SCL	SCL	SCL	SCL	CL	CL	SCL	CL	+	+	+	OL	OL	<OL	OL	
26	36	<CL	CL	CL	CL	CL	CL	SCL	CL	OL	CL	CL	OL								
31	36	+++	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	+	+	+++	SCL	SCL	SCL	SCL	

Strain T2		Phage reactions at routine test dilution (S.Typhimurium)																	
Lab code	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
HPA	136	-	-	-	OL	OL	OL	-	-	-	OL	OL	OL	-	OL	OL	-	-	OL
1	136	-	-	-	SCL	+++	+++	-	-	-	CL	SCL	SCL	-	CL	CL	-	-	SCL
3	136	-	-	-	OL	<CL	CL	-	-	-	CL	<CL	<CL	1	CL	CL	-	-	<CL
5	136	-	-	-	SCL	SCL	OL	-	-	-	CL	OL	CL	-	CL	CL	-	-	CL
15	136	-	-	-	OL	OL	OL	-	-	-	OL	SCL	<CL	-	OL	OL	-	-	OL
17	136	-	-	-	OL	CL	CL	-	-	-	OL	SCL	SCL	-	OL	OL	-	-	<OL
18	136	-	-	-	CL	+++	SCL	-	-	-	+++	CL	CL	-	CL	CL	-	-	SCL
26	136	-	-	-	OL	OL	CL	-	-	-	CL	CL	CL	-	CL	CL	-	-	SCL
31	136	-	-	-	SCL	SCL	SCL	-	-	-	SCL	SCL	SCL	-	SCL	SCL	-	-	SCL

Strain T2		Phage reactions at routine test dilution (S.Typhimurium)												Additional phages						
Lab code	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10VAR2	10VAR3	18
HPA	136	±	-	-	-	-	CL	-	-	-	-	-	-	+	±	±	SCL	SCL	SCL	-
1	136	+	-	-	-	-	+++	-	-	-	-	-	-	-	-	-	OL	OL	OL	-
3	136	4	-	-	-	-	CL	-	-	-	-	-	-							
5	136	-	-	-	-	-	CL	-	-	-	-	-	-	-	-	-	-	SCL	SCL	SCL
15	136	1	-	-	-	-	<OL	-	-	-	-	-	-	1	±	3	OL	OL	SCL	
17	136	+	-	-	-	-	<SCL	-	-	-	-	-	-	2	±	1	OL	<OL	<OL	-
18	136	+++	-	-	-	-	SCL	-	-	-	-	-	-	-	-	-	OL	OL	<OL	-
26	136	+	-	-	-	-	<CL	-	-	-	-	-	-							
31	136	+	-	-	-	-	SCL	-	-	-	-	-	-	-	-	++	+++	SCL	+++	-

Strain T3		Phage reactions at routine test dilution (S.Typhimurium)																	
Lab code	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
HPA	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Strain T3		Phage reactions at routine test dilution (S.Typhimurium)												Additional phages						
Lab code	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10VAR2	10VAR3	18
HPA	193	-	-	-	-	-	-	-	-	-	-	-	-	+++	+++	+++	-	-	-	-
1	193	-	-	-	-	-	-	-	-	-	-	-	-	+++	++	SCL	<u>OL</u>	+++	+++	-
3	193	-	-	-	-	-	-	-	-	-	-	-	-	SCL	SCL	SCL	<u>±</u>	+	+	-
5	193	-	-	-	-	-	-	-	-	-	-	-	-	SCL	SCL	SCL	-	-	-	-
15	193	-	-	-	-	-	-	-	-	-	-	-	-	++	++	++	+	-	-	-
17	193	-	-	-	-	-	-	-	-	-	-	-	-	SCL	SCL	SCL	-	-	-	-
18	193	-	-	-	-	-	-	-	-	-	-	-	-	++	++	++	-	-	-	-
26	193	-	-	-	-	-	-	-	-	-	-	-	-	+++	+++	+++	-	-	-	-
31	193	-	-	-	-	-	-	-	-	-	-	-	-	+++	++	SCL	-	-	-	-

Strain T4		Phage reactions at routine test dilution (S.Typhimurium)																	
Lab code	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
HPA	104	-	-	-	-	-	-	-	-	-	-	SCL	SCL	-	-	-	-	++	-
1	104	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	++	-
3	104b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
5	104	-	-	-	-	-	-	-	-	-	-	+++	SCL	-	-	-	-	±	-
15	104	-	-	-	-	-	-	-	-	-	-	±	±	-	-	-	-	SCL	-
17	104	-	-	-	-	-	-	-	-	-	-	+	++	-	-	-	-	+	-
18	104	-	-	-	-	-	-	-	-	-	-	+++	SCL	-	-	-	-	++	-
26	104	-	-	-	-	-	-	-	-	-	-	+	++	-	-	-	-	+++	-
31	104	-	-	-	-	-	-	-	-	-	-	+	+++	-	-	-	-	+++	-

Strain T4		Phage reactions at routine test dilution (S.Typhimurium)												Additional phages						
Lab code	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10VAR2	10VAR3	18
HPA	104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	OL	OL	-
1	104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	OL	OL	-
3	104b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	SCL	SCL	SCL
15	104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	OL	SCL	-
17	104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<OL	<SCL	<SCL	-
18	104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	OL	<OL	-
26	104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	SCL	SCL	SCL	-

Strain T5		Phage reactions at routine test dilution (S.Typhimurium)																		
Lab code	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19	
HPA	99	-	-	-	-	-	-	-	-	-	<CL	-	-	-	-	-	-	-	-	
1	99	-	-	-	-	-	-	-	-	-	CL	-	-	-	-	-	-	-	-	
3	99	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-	
5	99	-	-	-	-	-	-	-	-	-	SCL	-	-	-	-	-	-	-	-	
15	99	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-	
17	99	-	-	-	-	-	-	-	-	-	+++	-	-	-	-	-	-	-	-	
18	99	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-	
26	99	-	-	-	-	-	-	-	-	-	+++	-	-	-	-	-	-	-	-	
31	99	-	-	-	-	-	-	-	-	-	+++	-	-	-	-	-	-	-	-	

Strain T5		Phage reactions at routine test dilution (S.Typhimurium)												Additional phages						
Lab code	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10VAR2	10VAR3	18
HPA	99	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	SCL	OL	OL	-
1	99	-	-	-	-	-	-	-	-	-	-	-	-	-	+	++	OL	OL	OL	-
3	99	-	-	-	-	-	-	-	-	-	-	-	-							
5	99	-	-	-	-	-	-	-	-	-	-	-	-	-	++	++	-	SCL	SCL	SCL
15	99	-	-	-	-	-	-	-	-	-	-	-	-	±	+	±	OL	OL	SCL	
17	99	-	-	-	-	-	-	-	-	-	-	-	-	++	+++	++	<OL	<SCL	<SCL	-
18	99	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	OL	OL	<OL	-
26	99	-	-	-	-	-	-	-	-	-	-	-	-							
31	99	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+++	OL	OL	OL	-

Strain T6		Phage reactions at routine test dilution (S.Typhimurium)																		
Lab code	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19	
HPA	66a	-	-	-	-	-	-	-	-	OL	CL	-	-	-	-	++	-	-	-	
1	66a	-	-	-	-	-	-	-	-	CL	OL	-	-	-	-	++	-	-	-	
3	66a	-	-	-	-	-	-	-	-	SCL	CL	-	-	-	-	+	-	-	-	
5	U283(66a)	-	-	-	-	-	-	-	-	SCL	SCL	-	-	-	-	SCL	-	-	-	
15		66a	-	-	-	-	-	-	-	SCL	SCL	-	-	-	-	±	-	-	-	
17		66a	-	-	-	-	-	-	-	SCL	<OL	-	-	-	-	<SCL	-	-	-	
18		66a	-	-	-	-	-	-	-	SCL	+++	-	-	-	-	+++	-	-	-	
26		66a	-	-	-	-	-	-	-	SCL	SCL	-	-	-	-	SCL	-	-	-	
31	66a	-	-	-	-	-	-	-	-	SCL	SCL	-	-	-	-	+++	-	-	-	

Strain T6		Phage reactions at routine test dilution (S.Typhimurium)												Additional phages						
Lab code	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10VAR2	10VAR3	18
HPA	66a	SCL	-	SCL	OL	-	<u>±</u>	<u>±</u>	-	-	CL	-	-	-	-	-	-	-	-	-
1	66a	+++	-	++	SCL	-	-	-	-	-	++	-	-	-	-	-	-	-	-	-
3	66a	<CL	-	<SCL	<CL	-	+	1	-	-	CL	-	-	-	-	-	-	-	-	-
5	U283(66a)	SCL	-	<u>±</u>	SCL	-	-	-	-	-	CL	-	-	-	-	-	-	-	-	-
15		66a	++	-	+	SCL	-	<u>±</u>	+	-	-	SCL	-	-	-	-	-	-	-	-
17	66a	SCL	-	<SCL	OL	-	-	++	-	-	CL	-	-	-	-	-	-	-	-	-
18	66a	CL	-	+++	SCL	-	+	+	-	-	CL	-	-	-	-	-	-	-	-	-
26	66a	SCL	-	SCL	SCL	-	<u>++</u>	<u>++</u>	-	-	CL	-	-	-	-	-	-	-	-	-
31	66a	+++	-	SCL	SCL	-	+	++	-	-	SCL	-	-	-	-	-	-	-	-	-

Strain T7		Phage reactions at routine test dilution (S.Typhimurium)																	
Lab code	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
HPA	66	-	-	-	-	-	-	-	-	OL	OL	++	++	-	-	SCL	-	-	-
1	66	-	-	-	-	-	-	-	-	CL	OL	+	+	-	-	SCL	-	-	-
3	66	-	-	-	-	-	-	-	-	<CL	<CL	±	±	-	-	SCL	-	-	-
5	66	-	-	-	-	-	-	-	-	SCL	SCL	-	+	-	-	SCL	-	-	-
15	66	-	-	-	-	-	-	-	-	<CL	<OL	+	+	-	-	+	-	-	-
17	66	-	-	-	-	-	-	-	-	OL	<SCL	-	-	-	-	+++	-	-	-
18	66	-	-	-	-	-	-	-	-	SCL	SCL	+++	+++	-	-	+++	-	-	-
26	66	-	-	-	-	-	-	-	-	SCL	SCL	-	-	-	-	SCL	-	-	-
31	66	-	-	-	-	-	-	-	-	SCL	SCL	+	+++	-	-	SCL	-	-	-

Strain T7		Phage reactions at routine test dilution (S.Typhimurium)												Additional phages							
Lab code	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10VAR2	10VAR3	18	
HPA	66	SCL	-	OL	OL	-	±	±	-	-	CL	OL	-	++	++	++	OL	OL	OL	-	
1	66	SCL	-	<SCL	<CL	-	-	7	-	-	SCL	CL	-	-	-	+++	OL	OL	OL	-	
3	66	SCL	-	<CL	<CL	-	<SCL	2	-	-	CL	CL	-								
5	66	SCL	-	SCL	OL	-	-	-	-	-	CL	CL	-	-	-	+	-	SCL	SCL	SCL	
15	66	++		SCL	SCL		+	+			SCL	<CL		2	2	2	OL	OL	SCL		
17	66	<SCL	-	<OL	OL	-	-	++	-	-	CL	SCL	-	-	++	±	<OL	<SCL	<SCL	-	
18	66	CL	-	+++	SCL	-	+	+	-	-	CL	+++	-	-	-	-	OL	OL	<OL	-	
26	66	SCL	-	SCL	SCL	-	<SCL	+	-	-	CL	<CL	-								
31	66	+++	-	SCL	SCL	-	+	++	-	-	SCL	SCL	-	-	+	+++	SCL	SCL	SCL	-	

Strain T8		Phage reactions at routine test dilution (S.Typhimurium)																	
Lab code	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
HPA	10	-	-	-	-	-	-	-	-	CL	OL	OL	OL	-	-	CL	-	-	-
1	10	-	-	-	-	-	-	-	-	CL	OL	SCL	SCL	-	-	SCL	-	-	-
3	10	-	-	-	-	-	-	-	-	SCL	<CL	CL	CL	-	-	SCL	-	-	-
5	10	-	-	-	-	-	-	-	-	SCL	OL	CL	CL	-	-	OL	-	-	-
15	10	-	-	-	-	-	-	-	-	SCL	SCL	SCL	<CL	-	-	+	-	-	-
17	10	-	-	-	-	-	-	-	-	<CL	<CL	<CL	<CL	-	-	SCL	-	-	-
18	10	-	-	-	-	-	-	-	-	SCL	CL	CL	CL	-	-	+++	-	-	-
26	10	-	-	-	-	-	-	-	-	SCL	SCL	SCL	CL	-	-	SCL	-	-	-
31	10	-	-	-	-	-	-	-	-	SCL	SCL	SCL	SCL	-	-	SCL	-	-	-

Strain T8		Phage reactions at routine test dilution (S.Typhimurium)												Additional phages							
Lab code	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10VAR2	10VAR3	18	
HPA	10	SCL	-	SCL	SCL	-	±	±	-	-	CL	CL	-	++	+	++	SCL	OL	OL	-	
1	10	<SCL	-	++	<SCL	-	-	-	-	-	+++	CL	-	-	-	+++	OL	OL	OL	-	
3	10	SCL	-	<CL	<CL	-	++	1	-	-	CL	CL	-								
5	10	SCL	-	-	SCL	-	-	-	-	-	CL	CL	-	-	-	-	-	OL	OL	OL	
15	10	+	-	+	SCL	-	2	±	-	-	SCL	SCL	-	-	-	-	OL	OL	SCL	-	
17	10	<CL	-	<CL	CL	-	2	+	-	-	CL	<CL	-	-	±	-	OL	SCL	SCL	-	
18	10	CL	-	SCL	SCL	-	+	+	-	-	CL	SCL	-	-	-	-	OL	OL	<OL	-	
26	10	SCL	-	SCL	SCL	-	±	±	-	-	CL	CL	-								
31	10	SCL	-	SCL	SCL	-	+	++	-	-	SCL	SCL	-	-	+	+++	SCL	SCL	SCL	-	



Strain T9		Phage reactions at routine test dilution (S.Typhimurium)																	
Lab code	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
HPA	8	-	-	-	-	-	-	-	SCL	SCL	CL	-	-	-	-	+++	-	-	-
1	8	-	-	-	-	-	-	-	+++	CL	CL	+	+	-	-	+++	-	-	-
3	8	-	-	-	-	-	-	-	<<SCL	++	<<SCL	-	-	-	-	++	-	-	-
5	8	-	-	-	-	-	-	-	SCL	SCL	SCL	-	-	-	-	SCL	-	-	-
15	8	-	-	-	-	-	-	-	+++	+++	++	-	-	-	-	+	-	-	-
17	8	-	-	-	-	-	-	-	SCL	SCL	<SCL	-	-	-	-	SCL	-	-	-
18	8	-	-	-	-	-	-	-	SCL	SCL	++	-	-	-	-	+++	-	-	-
26	9	-	-	-	-	-	-	-	+++	+++	+++	+	+++	-	-	+++	-	-	-
31	8	-	-	-	-	-	-	-	SCL	+++	++	-	-	-	-	+++	-	-	-

Strain T9		Phage reactions at routine test dilution (S.Typhimurium)												Additional phages							
Lab code	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10VAR2	10VAR3	18	
HPA	8	++	-	SCL	SCL	-	±	±	-	-	CL	CL	-	+++	+++	+++	SCL	OL	OL	-	
1	8	+++	+	++	<SCL	-	-	-	-	-	+++	CL	-	+	+	+++	OL	OL	OL	-	
3	8	+++	-	<<SCL	<<SCL	-	++	-	-	-	<CL	<CL	-								
5	8	SCL	-	++	SCL	-	-	-	-	-	OL	OL	-	-	-	-	-	SCL	SCL	SCL	
15	8	±	-	++	+++	-	+	±	-	-	++	++		-	-	-	OL	OL	SCL	-	
17	8	<SCL	-	<OL	OL	-	-	±	-	-	OL	SCL	-	±	++	++	OL	SCL	SCL	-	
18	8	SCL	-	+++	SCL	-	+	+	-	-	CL	+++	-	-	-	-	OL	OL	<OL	-	
26	9	+++	±	SCL	SCL	-	<SCL	+	-	-	CL	SCL	-								
31	8	+++	-	SCL	SCL	-	+	++	-	-	SCL	SCL	-	+	+	+++	SCL	SCL	SCL	-	

Strain T10		Phage reactions at routine test dilution (S.Typhimurium)																	
Lab code	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
HPA	1	OL	OL	CL	OL	CL	CL	SCL	-	CL	OL	CL	CL	OL	OL	OL	OL	CL	CL
1	1	<SCL	CL	SCL	SCL	<SCL	+++	SCL	-	CL	CL	SCL	SCL	CL	CL	CL	CL	CL	CL
3	1	<CL	<CL	<CL	OL	<CL	CL	CL	-	<CL	CL	<CL	CL	CL	CL	CL	CL	CL	<CL
5	1	OL	OL	OL	OL	OL	OL	OL	-	OL	OL	OL	OL	OL	OL	OL	OL	OL	OL
15	1	CL	CL	CL	OL	CL	<CL	SCL	-	<CL	CL	SCL	<CL	CL	CL	CL	SCL	CL	CL
17	1	CL	CL	CL	CL	CL	CL	CL	-	CL	CL	<CL	<CL	CL	CL	CL	CL	CL	CL
18	1	<CL	CL	CL	CL	SCL	CL	SCL	-	SCL	SCL	CL	CL	CL	CL	CL	CL	SCL	SCL
26	1	<CL	<CL	CL	CL	CL	CL	SCL	-	SCL	CL	CL	CL	CL	CL	CL	CL	CL	CL
31	1	SCL	SCL	SCL	SCL	SCL	SCL	SCL	-	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL

Strain T10		Phage reactions at routine test dilution (S.Typhimurium)												Additional phages							
Lab code	Phage type	20	21	22	23	24	25	26	27	28	29	32	35	1	2	3	10	10VAR2	10VAR3	18	
HPA	1	OL	OL	OL	OL	CL	CL	CL	CL	-	CL	CL	OL	+++	++	++	SCL	OL	OL	CL	
1	1	<SCL	CL	SCL	SCL	SCL	<SCL	SCL	SCL	++	++	CL	SCL	+	+	++	OL	OL	OL	OL	
3	1	<CL	CL	CL	<CL	CL	CL	<CL	CL	1	CL	CL	OL								
5	1	SCL	OL	-	SCL	CL	CL	SCL	OL	-	CL	CL	CL	++	++	++	SCL	SCL	SCL	SCL	
15	1	+	CL	SCL	SCL	<CL	CL	CL	SCL	-	SCL	<CL	<OL	±	±	±	OL	OL	SCL	CL	
17	1	CL	OL	CL	CL	CL	CL	CL	CL	-	CL	CL	CL	+	++	++	OL	SCL	SCL	CL	
18	1	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	-	CL	SCL	CL	+	+	+	OL	OL	<OL	OL	
26	1	SCL	CL	SCL	SCL	SCL	CL	CL	CL	±	CL	OL	OL								
31	1	+++	SCL	SCL	SCL	SCL	SCL	SCL	SCL	-	SCL	SCL	SCL	+	+	+++	SCL	SCL	SCL	SCL	

## Annex 8 Serotyping results as obtained by two laboratories using PCR

*Table 8.1 Serotyping results by laboratory 15, as obtained by using the CDC, Atlanta, system for molecular serotyping*

Strain no.	O-gruppe	H1 gruppe	H2 gruppe	H-3 gruppe	Serovar name	Remarks
S1	grp G	L-Complex				
S2	grp C1	c	1-complex	5	S.Cholerasuis	biochemical test need to be performed
S3	grp C1	r	1-complex	5	S.Infantis	
S4	grp B	i				(4,5,12:i:-/4,12:1:-)
S5	grp C2	b	1-complex	6	S.Stourbridge	
S6		G-complex				
S7	grp C2	z10	EN-complex	x		Istanbul(8) / Hadar(6,8)
S8	grp C1	r	1-complex	2	S.Virchow	
S9	grp D	G-complex	m(gm)			Enteritidis (9,12) / Hillingdon (9,46)
S10	grp E	y	L-complex			Langensalza (3,10) /Krefeld (1,3,19)
S11	grp B	eh	1-complex	2	S.Saintpaul	
S12	grp B	z10	1-complex	2	S.Haifa	
S13	grp D	a	EN-complex	z15		Durban (9,12) /Doba (9,46)
S14	grp G	G-complex	f		S.Havana	
S15	grp C2	r	1-complex	5		(Hindmarch(8,20) / Bovismorbificans(6,8,20))
S16	grp C1	eh	EN-complex	z15	S.Braenderup	
S17		k	1-complex	5		
S18	grp E	G-complex	m(gm)	s	S.Amsterdam	
S19	grp B	i	1-complex	2	S.Typhimurium	
S20	grp C2	i	z6		S.Kentucky	
S21		r				

Table 8.2 Serotyping results by laboratory 22, using PCR

Strain	Serovar name	Remarks
S1	- : d : -	*
S2	- : - : 1,5	*
S3	- : r : 1,5	*
S4	Monophasic Typhimurium	
S5	Stourbridge	
S6	- : G : -	*
S7	O8: z10 : e,n,x Hadar or Istambul	
S8	- : r : 1,2	*
S9	Enteritidis	
S10	O3,10 or O1,3,19 : - : l,w	*
S11	Saintpaul	
S12	Haifa	
S13	9,12 : - : e,n,z15	*
S14	- : G : -	*
S15	Bovismorbificans	
S16	- : e,h : e,n,z15	*
S17	- : - : 1,5	*
S18	O3,10 or O1,3,19 : G : -	*
S19	Typhimurium	
S20	O8 : i : -	*
S21	- : r : -	*

\*These results are only referred to antigenic factors covered by the PCR used.

#### References:

- \* Somatic antigens multiplex PCR: Herrera-León et al., Research Microbiology 2007;158(2):122-127
- \* Phase-1 flagellar antigens multiplex PCR: Herrera-León et al., J Clin Microbiol 2004:2581-2586
- \* Phase-2 flagellar antigens multiplex PCR: Echeita and Usera, Research Microbiology 2002;153:107-113

