National Institute for Public Health
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RIVM report 2014-0009

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This investigation has been performed by order and for the account of the European Commission, Directorate General for Health and Consumer Protection (DG-Sanco), within the framework of RIVM project number V/330604/13 European Reference Laboratory for *Salmonella*

This is a publication of:

National Institute for Public Health and the Environment
P.O. Box 1│3720 BA Bilthoven
The Netherlands
www.rivm.nl/en

Publiekssamenvatting

Achttiende EURL-*Salmonella* ringonderzoek (2013) voor de typering van *Salmonella* spp.

De Nationale Referentie Laboratoria (NRL's) van de 28 Europese lidstaten scoorden in 2013 goed bij de kwaliteitscontrole op *Salmonella*-typering. Twee laboratoria hadden hiervoor een herkansing nodig. Uit de analyse van alle NRL's als groep bleek dat de laboratoria aan 98 procent van de geteste stammen de juiste naam konden geven.

Sinds 1992 zijn de NRL's van de Europese lidstaten verplicht om deel te nemen aan jaarlijkse kwaliteitstoetsen, die bestaan uit zogeheten ringonderzoeken voor *Salmonella*. Elke lidstaat wijst een laboratorium aan, het Nationale Referentie Laboratorium (NRL), dat namens dat land verantwoordelijk is voor het aantonen en typeren van *Salmonella* uit monsters van levensmiddelen of dieren. Om te controleren of de laboratoria hun werk goed uitvoeren moeten zij onder andere 20 *Salmonella*-stammen op juiste wijze identificeren. Soms doen ook landen van buiten de Europese Unie vrijwillig mee. In 2013 waren dat de kandidaat-lidstaten Macedonië en Turkije, en de EFTA-landen IJsland, Noorwegen en Zwitserland, waarbij EFTA staat voor European Free Trade Association.

Van de NRL's zijn er zeven laboratoria die, behalve de standaardtoets (serotypering) op *Salmonella*, preciezere typeringen 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 83 procent van de *S*. Typhimurium-stammen en 93 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 Public Health England in Londen, Engeland.

Trefwoorden: EURL-*Salmonella*, *Salmonella*, serotypering, faagtypering, moleculaire (PFGE) typering, vergelijkend laboratoriumonderzoek

Abstract

Eighteenth EURL-*Salmonella* interlaboratory comparison study (2013) on typing of *Salmonella* spp.

The National Reference Laboratories (NRLs) of all 28 European Union (EU) Member States performed well on the 2013 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 98 per cent of the strains tested.

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*. Each 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. NRLs from countries outside the European Union occasionally participate in these tests on a voluntary basis. EU-candidate-countries Former Yugoslav Republic of Macedonia and Turkey, and EFTA countries Iceland, Norway and Switzerland took part in the 2013 test.

Seven 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 93 per cent of the *S*. Enteritidis strains correctly; for *S*. Typhimurium, 83 per cent of the strains were correctly phage typed.

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

Keywords: EURL-*Salmonella*, *Salmonella*, serotyping, phage typing, molecular (PFGE) typing, interlaboratory comparison study

Contents

Summary

In November 2013, the 18th 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 Public Health England (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 carried out uniformly, and whether comparable results were obtained.

A total of 29 NRLs-*Salmonella* of the 28 Member States of the European Union participated, supplemented by the NRLs of the EU-candidate-countries Former Yugoslav Republic of Macedonia, and Turkey, and the EFTA countries Iceland, Norway and Switzerland.

All 34 laboratories performed serotyping. A total of 20 obligatory *Salmonella* strains plus 1 additional optional *Salmonella* strain 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-Kauffmann-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, nearly 100 per cent of the strains were typed correctly for the O-antigens, 98 per cent of the strains were typed correctly for the H-antigens and 97 per cent of the strains were correctly named by the participants.
At the EURL-*Salmonella* workshop in 2007, the EURL-*Salmonella* proposed a definition for good performance of the NRLs regarding the serotyping. Using this definition, 32 participants achieved good performance. The two 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 NRLs of EU Member States, and the two EU-NRLs concerned obtained good scores in this follow-up study.

Seven of the participating NRLs-*Salmonella* also performed phage typing of both *S*. Enteritidis and *S*. Typhimurium. Public Health England selected the 20 strains for phage typing. Ten strains were of the *Salmonella* serovar Enteritidis and ten concerned serovar Typhimurium. The overall results were good. The seven NRLs phage typed 93 per cent of the *S*. Enteritidis strains correctly and 83 per cent of the *S*. Typhimurium strains.

Twenty laboratories participated in the optional first pilot study on PFGE typing. PFGE results were evaluated on the quality of the images according to the PulseNet International Guidelines. The quality of the PFGE results was promising, though there was quite some variation in results between the large number of participants. Application of relatively simple adjustments will help to improve the results.

1 Introduction

This report describes the 18th 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 2013.

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

A total of 34 laboratories participated in this study. These included 29 NRLs-*Salmonella* situated in the 28 EU Member States, 2 NRLs of an EU-candidate country and 3 NRLs of EFTA countries. 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 20 obligatory strains and all but two of the participants serotyped the optional 21st strain. 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 10 additional strains were serotyped.

Seven of the NRLs-*Salmonella* performed phage typing on 10 *Salmonella* Enteritidis strains and on 10 *Salmonella* Typhimurium strains. The selection of the strains and interpretation of the results of the phage typing were performed in close cooperation with Public Health England, London, UK.

As a pilot, this year the typing study also included PFGE typing. Twenty NRLs participated in this part of the study by PFGE typing ten designated *Salmonella* strains and returning the images for evaluation.

2 Participants

Country	City	Institute
Austria	Graz	AGES / Institute for Medical Microbiology and Hygiene
Belgium	Brussels	CODA-CERVA
Bulgaria	Sofia	National Reference Centre of Food Safety
Croatia	Zagreb	Croatian Veterinary Institute
Cyprus	Nicosia	Laboratory for the Control of Foods of Animal Origin, Cyprus Veterinary Services
Czech Republic	Prague	State Veterinary Institute Prague
Denmark	Søborg	National Food Institute
Estonia	Tartu	Veterinary and Food Laboratory
Finland	Kuopio	Finnish Food Safety Authority Evira
France	Maisons-Alfort	ANSES Laboratoire de Sécurité des Aliments
Germany	Berlin	Federal Institute of Risk Assessment (BFR)
Greece	Chalkida	Veterinary Laboratory of Chalkis
Hungary	Budapest	National Food Chain Safety Office, Food and Feed Safety Directorate
Iceland	Reykjavik	Landspitali University Hospital, Dept. of Clinical Microbiology
Ireland	Celbridge	DAFM Central Veterinary Research Laboratories
Italy	Legnaro	Istituto Zooprofilattico Sperimentale delle Venezie
Latvia	Riga	Institute of Food Safety, Animal Health and Environment BIOR
Lithuania	Vilnius	National Food and Veterinary Risk Assessment Institute
Luxembourg	Dudelange	Laboratoire National de Santé
Macedonia, FYR of	Skopje	UKIM Faculty of Veterinary Medicine - Food Institute
Malta	Valletta	Malta Public Health Laboratory
Netherlands	Bilthoven	National Institute for Public Health and the Environment (RIVM), Center for Infectious Diseases Research, Diagnostics and Screening (IDS)
Norway	Oslo	Norwegian Veterinary Institute
Poland	Pulawy	National Veterinary Research Institute, Department of Microbiology
Portugal	Lisboa	INIAV-Instituto Nacional de Investigação Agrária e Veterinária
Romania	Bucharest	Institute for Diagnosis and Animal Health, Bacteriology Department

Country	City	Institute
Slovak Republic	Bratislava	State Veterinary and Food Institute
Slovenia	Ljubljana	UL, Veterinary faculty, National Veterinary Institute
Spain	Algete-Madrid	Laboratorio Central de Veterinaria
Sweden	Uppsala	National Veterinary Institute SVA
Switzerland	Bern	Institute of Veterinary Bacteriology, ZOBA
Turkey	Etlik-Ankara	Veterinary control Central Research Institute, Bacteriological diagnosis Laboratory
United Kingdom	Addlestone	Animal Health and Veterinary Laboratories Agency
United Kingdom	Belfast	Agri Food & Biosciences Institute

3 Materials and methods

3.1 *Salmonella* strains for serotyping

A total number of 20 *Salmonella* strains (coded S1 - S20) had to be serotyped by the participants. As agreed at the 18th EURL-*Salmonella* Workshop in St. Malo (Mooijman, 2013), one additional strain from an uncommon source and subspecies (S21) was included in the study; 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 distribution. The complete antigenic formulas, according to the most recent White-Kauffmann-Le Minor scheme (Grimont&Weill, 2007), of the 21 serovars are shown in Table 1. However, participants were asked to report only those results, on which the identification of serovar names was based.

Table 1. Antigenic formulas of the 21 Salmonella *strains according to the White-Kauffmann-LeMinor scheme used in the 18th EURL-* Salmonella *typing study*

Strain code	O-antigens	H-antigens **(phase 1)**	H-antigens **(phase 2)**	Serovar
S1	13,23	d	e,n,z_{15}	Telelkebir
S2	6,7	r	1,7	Colindale
S3	1,4,[5],12	e,h	e,n,z_{15}	Sandiego
S4	9,46	d	z_6	Plymouth
S5	6,7,14	r	1,2	Virchow
S6	1,4,[5],12,[27]	g,s,t	[1,2]	Kingston
S7	1,13,23	f,g,[s]	-	Havana
S8	6,8	z_{10}	e,n,x	Hadar
S9	6,7,14	k	1,5	Thompson
S10	1,13,23	z	l,w	Worthington
S11	3,{10}{15}{15,34}	e,h	1,6	Anatum
S12	1,9,12	l,v	1,5	Panama
S13	1,9,12	l,z_{13}	e,n,x	Napoli
S14	8,20	i	z_6	Kentucky
S15	6,7,14	z_{10}	e,n,z_{15}	Mbandaka
S16[a)]	1,4,[5],12	b	1,2	Paratyphi B var. Java
S17	6,7,14	r	1,5	Infantis
S18	1,9,12	g,m	-	Enteritidis
S19[b)]	1,4,[5],12	i	-	1,4,[5],12:i:-
S20	1,4,[5],12	i	1,2	Typhimurium
S21[c)]	42	g,t	-	42:g,t:-

[a)] L(+) tartrate (= d-tartrate) positive variant as determined by PCR, often referred to as var. Java.

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

[c)] *Salmonella enterica* subspecies *salamae*.

3.2 Laboratory codes

The NRLs-*Salmonella* were assigned a laboratory code 1-34, which differed from the previous typing studies.

3.3 Protocol and test report

Three weeks before the start of the study, the NRLs received the protocol by email. As in 2012, the study used web based test reports, a general one for serotyping/phage typing and a separate one for PFGE typing. Instructions for the use of these test reports and the way to enter data were sent to the NRLs in week 46, 2013.
The protocol and test reports can be found on the EURL-*Salmonella* website: http://www.eurlsalmonella.eu/Proficiency_testing/Typing_studies

3.4 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, phage typing, and PFGE typing were sent by the EURL-*Salmonella* in week 47, 2013.

3.5 Guidelines for evaluation

The evaluation of the various serotyping results mentioned in this report is described in Table 2.

Table 2. Evaluation of serotyping results

Results	Evaluation
Auto-agglutination or Incomplete set of antisera (outside range of antisera)	Not typable
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 regarding 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 human health related *Salmonella* serovars (as indicated in EU legislation) and all other strains:

- 4 penalty points: Incorrect typing of *S*. Enteritidis, *S*. Typhimurium (including the monophasic variant), *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.

The total number of penalty points is determined for each NRL-*Salmonella*. The NRL meets the criterion of 'good performance' if it has less 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.

3.6 Follow-up study serotyping

The follow-up study for serotyping consisted of typing an additional set of 10 *Salmonella* strains. The strains for the follow-up study are shown in Table 3. All EU-NRLs with four penalty points or more had to participate in this follow-up study.

Table 3. Antigenic formulas of the ten Salmonella *strains according to the White-Kauffmann-Le Minor scheme used in the follow-up part of the 18th EURL-*Salmonella *typing study*

Strain	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
SF-1	1,4,[5],12	i	1,2	Typhimurium
SF-2	1,9,12	g,m	-	Enteritidis
SF-3	1,4,[5],12	r	1,2	Heidelberg
SF-4	6,8,20	r, [i]	1,5	Bovismorbificans
SF-5	6,7,14	r	1,5	Infantis
SF-6	6,7,14	r	1,2	Virchow
SF-7	6,8	z10	e,n,x	Hadar
SF-8	6,7	r	1,7	Colindale
SF-9	3,{10}{15}{15,34}	e,h	1,6	Anatum
SF-10	1,13,23	f,g,[s]	-	Havana

3.7 *Salmonella* strains for phage typing

The *Salmonella* strains for phage typing were obtained from the culture collection of the *Salmonella* Reference Service, Gastrointestinal Bacteria Reference Unit, Public Health England, London, UK. Ten strains of *Salmonella* Enteritidis and ten strains of *Salmonella* Typhimurium were selected.

The explanation of the various notations in Table 4 and Table 5 (and in Annex 5 and Annex 6) are as follows:

CL	Confluent (complete) lysis
OL	Opaque lysis (confluent lysis with a heavy central opacity due to secondary (lysogenised) growth
<CL	Intermediate degrees of confluent lysis
<OL	Intermediate degrees of opaque lysis
SCL	Semi-confluent lysis
<SCL	Intermediate degrees of semi-confluent lysis
+++	Over 100 plaques
+++	81 – 100 plaques
++	61 – 80 plaques
++	41 – 60 plaques
+	21 – 40 plaques
+	6 – 20 plaques
1 – 5	1 – 5 plaques
-	No plaques
0	No data entry

Table 4. Phage reactions of the Salmonella *Enteritidis strains used in the 18th EURL-* Salmonella *typing study*

Phage reactions at Routine Test Dilution (*S.* Enteritidis)																	
Strain number	Phage type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
E1	4	-	SCL	CL	SCL	CL	SCL	CL	OL	< OL	OL	CL	CL	CL	-	-	-	<SCL
E2	60	OL	-	CL	-	CL	SCL	-	OL	-	OL	-	CL	CL	< CL	-	-	-
E3	5a	-	SCL	+	OL	CL	SCL	-	-	< OL	-	-	CL	-	-	-	-	< OL
E4	4b	-	OL	CL	SCL	CL	SCL	CL	OL	< OL	OL	CL	CL	CL	-	±	CL	SCL
E5	2	OL	-	CL	SCL	CL	SCL	< CL	OL	< OL	OL	< CL	CL	-	< CL	-	-	< OL
E6	21	OL	< OL	-	OL	-	SCL	-	OL	< OL	OL	-	-	-	< CL	-	-	< OL
E7	5	-	SCL	+	SCL	< CL	SCL	SCL	OL	< OL	+++	<SCL	OL	<SCL	-	±	OL	< OL
E8	6a	-	OL	-	SCL	-	SCL	-	-	OL	-	-	-	-	-	-	-	OL
E9	6	-	SCL	-	SCL	-	SCL	-	OL	< OL	OL	-	-	-	-	-	-	< OL
E10	1b	< CL	SCL	CL	< OL	CL	SCL	CL	OL	< OL	SCL	CL	CL	CL	< CL	CL	CL	SCL

Table 5. Phage reactions of the Salmonella *Typhimurium strains used in the 18th EURL*- Salmonella *typing study*

		Phage reactions at Routine Test Dilution (*S.* Typhimurium)																	
Strain number	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
T1	195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T2	104	-	-	-	-	-	-	-	-	-	-	SCL	SCL	-	-	-	-	+++	-
T3	8	-	-	-	-	-	-	-	SCL	SCL	< CL	-	-	-	-	+++	-	-	-
T4	7a	-	-	-	-	-	-	< CL	±	-	-	-	-	-	-	-	-	CL	-
T5	120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< CL	-
T6	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T7	40	CL	OL	CL	OL	CL	CL	CL	-	CL	CL	-	OL	CL	CL	CL	CL	CL	CL
T8	36	CL	OL	CL	OL	CL	CL	CL	CL	< CL	CL	CL	CL	CL	CL	CL	CL	CL	CL
T9	15a	-	-	-	-	-	-	-	-	-	OL	OL	OL	-	OL	-	OL	-	OL
T10	135	-	++	+++	SCL	SCL	< CL	-	-	SCL	< OL	CL	CL	+	< CL	< CL	CL	-	+++

		Phage reactions at Routine Test Dilution (*S*. Typhimurium)												**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	195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	SCL	-	-	-	-
T2	104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	OL	OL	-
T3	8	SCL	-	SCL	SCL	-	±	±	-	-	CL	CL	-	++	+	++	OL	OL	OL	-
T4	7a	<SCL	-	-	-	-	-	-	-	-	SCL	SCL	-	+++	+++	+++	OL	OL	OL	-
T5	120	-	-	-	-	-	-	-	-	-	-	-	-	++	+	++	OL	OL	OL	-
T6	193	-	-	-	-	-	-	-	-	-	-	-	-	SCL	SCL	SCL	-	-	-	-
T7	40	< CL	OL	OL	< CL	CL	CL	CL	CL	-	CL	CL	OL	++	++	++	OL	OL	OL	OL
T8	36	< CL	OL	OL	< CL	CL	< CL	< CL	CL	CL	CL	CL	OL	+++	+++	+++	OL	OL	OL	OL
T9	15a	OL	-	-	-	-	-	-	±	-	-	OL	±	+++	+++	+++	OL	OL	OL	-
T10	135	+	+	+	SCL	+	+	+	SCL	-	CL	< CL	OL	++	++	+++	< OL	++	++	CL

3.8 *Salmonella* strains for PFGE typing

A total number of 10 *Salmonella* strains (coded P1 – P10) were included in the pilot study on PFGE typing. For the participants' convenience, the general reference strain *Salmonella* Braenderup H9812 was added to the parcel as well. In consultation with the Statens Serum Institut (SSI), Copenhagen, Denmark, the same strains were used as in the External Quality Assessment EQA-4 on PFGE as organised by SSI for the Food- and Waterborne Diseases and Zoonoses Laboratories Network (FWD laboratories network)(ECDC, 2013). Background information on the strains is given in Table 6.

Table 6. Background information on the Salmonella *strains as used in the pilot on PFGE typing 2013*

Strain Code in Pilot 2013 (EURL-*Salmonella*)	Strain Code in EQA-4 (SSI, 2013)	*Salmonella* serovar
P1	Salm01	Mbandaka
P2	Salm10	Typhimurium
P3	Salm09	Enteritidis
P4	Salm06	Infantis
P5	Salm03	Aberdeen
P6	Salm02	Strathcona
P7	Salm04	Dublin
P8	Salm05	Poona
P9	Salm08	Saintpaul
P10	Salm07	Infantis

3.9 Evaluation of PFGE typing results

Participants were asked to test the strains using their own routine PFGE method (*Xba*I digestion) and to give some details on the method in the electronic test report. The PFGE gel images were to be emailed as a Tagged Image File Format (TIFF) file to the EURL-*Salmonella*, and had to include the laboratory code in the name of these .tif files.
Evaluation of the PFGE results was on the quality of the PFGE images only, and not (yet) on gel analysis in Bionumerics. Quality grading was done according to the PulseNet guidelines (www.pulsenetinternational.org) as given in Annex 1. These guidelines describe an assessment of seven parameters. Each parameter is given a score of a maximum of 4 points, where a poor result equals 1 point and an excellent result equals 4 points.

4 Questionnaire

4.1 General

A questionnaire was incorporated in both test reports of the interlaboratory comparison study (serotyping plus phage typing; PFGE typing). The questions and a summary of the answers are listed below.

4.2 General questions

Question 1: Contact details of the participating laboratory?
See Chapter 2.

Question 2: Was your parcel damaged at arrival?
All packages were received in good condition.

Question 3: What was the date of receipt of the parcel at the laboratory?
All participants received their package in the same week it was sent (week 47 of 2013).

Question 4: What kind of medium was used for sub-culturing the strains?
The participants 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 5: What was the frequency of serotyping of *Salmonella* at your laboratory in 2012?

Question 6: How many *Salmonella* strains (approximately) did your laboratory serotype in 2012?
Replies to questions 5 and 6 are summarised in Table 7.

Table 7. Frequency and number of strains serotyped in 2012 (for all 34 NRLs)

Lab code	Typing frequency	Number of strains serotyped in 2012
31	Once a week	60
1	Twice a week	80
14	Weekly	100
28	Twice to 3x a week	126
25	Weekly	150
20	3x a week	167
21	Weekly	~ 200
5	Daily	200
6	Daily	230
23	3x a week	248
32	Daily	251
15	Daily	276
33	3x a week	278
11	Daily	400
19	Once a week	510
18	Daily	748
10	Daily	800
13	Daily	800
4	Daily	1000
24	Daily	1100
7	Daily	1200
2	Daily	1500
34	Daily	1535
27	Daily	2000
29	Daily	4000
12	Daily	4100
3	Daily	4500
17	Once a week	5000

Question 7: What kind of sera do you use (commercially available or prepared in own laboratory)?
The replies to question 7 are summarised in Table 8 and Table 9.

Table 8. Number of laboratories using sera from one or more manufacturers and/or in-house prepared sera

Number of manufacturers from which sera are obtained	Number of NRLs (n=32)
From 1 manufacturer	6
From 2 manufacturers	12
From 3 manufacturers	6
From 4 manufacturers	5
From 5 manufacturers	3

Table 9. Number of laboratories using sera from various manufacturers

Manufacturer	Number of NRLs (n=32)
BD-Difco	1
Biomed	1
Biorad	16
Diachel	1
Immunolab	1
Mast	2
Microgen	1
Own preparation	4
Oxoid	1
Pro-Lab	5
Reagensia	3
Remel	2
Siemens	1
Sifin	21
Statens Serum Institute (SSI)	27

Question 8: Were the strains in this study typed in your own laboratory?
Two NRLs-Salmonella (laboratory codes 18 and 21) sent the additional strain S21 to another laboratory for further serotyping or confirmation. All other laboratories tested all strains in their own laboratory.

4.4 Questions regarding the use of PCR/biochemical tests

Question 9: Did you use PCR for confirmation of any of the serotyped strains S1-S21?

Question 10: For which strains did you use this PCR?
A total of 17 laboratories reported using PCR for confirmation of strains. Three laboratories used PCR to confirm all strains. Eleven laboratories used PCR to confirm strain S19, the monophasic variant of *S*. Typhimurium 1,4,[5],12:i:-, and four of these also used PCR to confirm strain S20, *S*. Typhimurium. Strains S6 (2x), S7, S16 (5x), and S21 were also mentioned to be confirmed by PCR.

Question 11: Is the PCR used commercially available, details and manufacturer?
Only one laboratory used a commercially available PCR: Check & Trace *Salmonella* by Check points.

Question 12: Details of the PCR and literature references?
PCR testing is mainly done to confirm monophasic (Typhimurium) strains. Seven and four laboratories respectively mentioned the following references:
- EFSA Journal, 2010; 8(10):1826;
- Tennant et al., 2010;

Other references mentioned, sometimes in combination with the previous two, were:
- Aabo et al, 1993;
- Lee et al., 2009;
- Barco et al., 2011;

- Bugarel et al., 2012;
- Prendergast et al., 2013.

References regarding molecular serotyping were:
- Herrera-León et al., 2007/Herrera-León et al., 2004/Echeita et al., 2002;
- Fitzgerald et al., 2007/McQuiston et al., 2011.

Three participants referred to Malorny et al., (2003) as a PCR method on d-tartrate fermentation.

Question 13: Do you use this PCR routinely?
Fourteen of the laboratories use PCR routinely.

Question 14: How many samples did you test for *Salmonella* using this PCR in 2012?
The replies to question 14 are summarised in Table 10.

Table 10. Number of strains routinely tested by PCR in 2012

Laboratory code	Number of strains tested by PCR in 2012
15	10
21	15
30	16
33	20
23	21
13	25
3	50
4	50
10	50
19	125
27	~ 400
2	500
17	677
12	Will start to use the test routinely in 2013

Question 15: Did you use any biochemical test, like dulcitol, malonate, tartrate, etc., to distinguish between subspecies?
Unintentionally, only the participants using PCR methods were asked this question. Fifteen of the 17 answers given confirmed the use of biochemical tests. Details are given in Table 11.

Table 11. Details on the biochemical tests used on various strains

Lab code	Beta-glucuronidase	Dulcitol	Galacturonate	Lactose	Lysinecarboxylase	Malonate	Mannitol	Mucate	ONPG	Sacarose	Salicine	Sorbitol	Sorbofosfate	Tartrate	TSI	Urea
2		21	21			21		21	21		21	21				
4																
7		21	21			21		21			21	21		16		
10					all	all	all	all	all	all			all		all	all
12						6/21								16		
15	21	21		21		21			21		21	21		16		
21		all	all			all			all			all		all		
22						16/21										
23		21				21			21		21	21		16	21	
27		x		x		x					x	x				
30						6/21										
34														16		

X: test used, but not stated for which strains

4.5 Questions regarding phage typing

Questions 17/18: Does your laboratory perform phage typing of *S.* Enteritidis, *S.* Typhimurium and/or other strains?

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

Questions 19/20: Which typing system is used for *S.* Enteritidis and *S.* Typhimurium?

All phage typing laboratories use the PHE (HPA)/Colindale system.

Question 21: How many strains did your laboratory phage type in 2012?

Replies to question 21 are summarised in Table 12.

Table 12. Number of strains phage typed in 2012

Laboratory code	Number of strains phage typed in 2012
26	200
27	650
34	789
9	850
12	1000
18	1112
3	2200

4.6 Questions regarding PFGE typing

Question P1: What method do you use for *Salmonella* PFGE?
Nine participants reported using the Standard PulseNet Protocol *Salmonella* PFGE (PulseNet International, 2013), ten participants use this Standard protocol with modifications. One participant uses the 2009 version of this Standard protocol.

Question P2: How many *Salmonella* strains did you approximately PFGE type in 2013
Replies to question P2 are summarised in Table13.

Table 13. Number of strains PFGE typed during 2013

Laboratory code	Number of strains PFGE typed in 2013
24	0
25	0
21	10
19	15
26	25
28	40
7	50
9	50
13	50 or 60
34	100
5	110
17	150
18	150
2	200
12	200
33	220
22	250
1	400
3	400
27	450

Question P3: Which strain did you use as a reference strain?
The replies to question P3 are summarised in Table 14.

Table 14. Reference strains as used by the participants

Reference strain S. Braederup H9812	Number of NRLs
As provided by the EURL-*Salmonella*	10
Own strain	6
Own strain + provided by EURL-*Salmonella*	3
Own strain + provided by SSI	1

Question P4: Manufacturer of the enzyme XbaI?
The replies to question P4 are summarised in Table 15.

Table 15. Number of participants using the enzyme XbaI from different manufacturers

Manufacturer	Number of NRLs
Fermentas	2
New England BioLabs	4
Promega	1
Roche Diagnostics	7
Sigma Life Science	3
Thermo Scientific	3

Question P5: Name/model of the Electrophoresis system?
The replies to question P5 are summarised in Table 16.

Table 16. Name/model of the electrophoresis systems used by the participants

Electrophoresis system	Number of NRLs
Bio-Rad CHEF Mapper	6
Bio-Rad CHEF-DR II System	4
Bio-Rad CHEF-DR III System	10

Question P6: Name/model of the gel documentation system?
The replies to question P6 are summarised in Table 17.

Table 17. Name/model of the gel documentation systems used by the participants

Gel documentation system	Number of NRLs
AlphaDigi Doc	1
Bio-Rad Image lab	1
Bio-Rad Molecular Imager CHEMIDOC XRS+	1
Bio-Rad Quantity ONE	1
Bio-Rad GelDoc	1
Bio-Rad GelDoc 1000	1
Bio-Rad GelDoc 2000	1
Bio-Rad GelDoc XR(+)	2
ChemiDoc	2
ChemiImager 5500	2
EC3 Chemi R 410 Imaging Systems, BioImaging Systems	1
GelDoc-it TS	1
GelLogic200	1
GeneGenius (Syngene)	1
IMAGEMASTER VDS	1
Kodak digital 1D	1
TDI GELPRINTER	1

Note: Different names for the same instruments may have been used.

5 Results

5.1 Serotyping results

5.1.1 General comments on this year's evaluation

As decided at the 18th EURL-Salmonella Workshop (Mooijman, 2013), Strain S21 was added to the study as an additional strain. 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 2, 3 and 4, and the percentages of correct results in Figure 1.

The O-antigens were typed correctly by 32 of the 34 participants (94%). This corresponds to nearly 100% of the total number of strains. The H-antigens were typed correctly by 24 of the 34 participants (71%), corresponding to 98% of the total number of strains. A total of 23 participants (68%) gave the correct serovar names, corresponding to 97% of all strains evaluated.

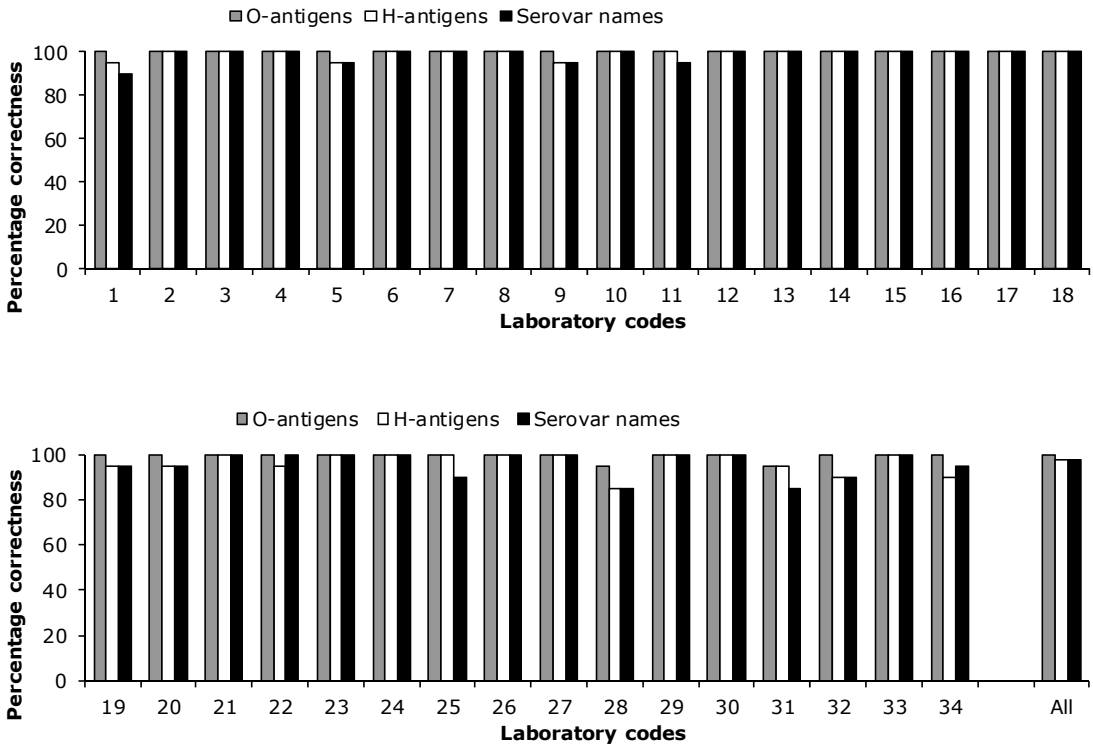

Figure 1. Percentages of correct serotyping results

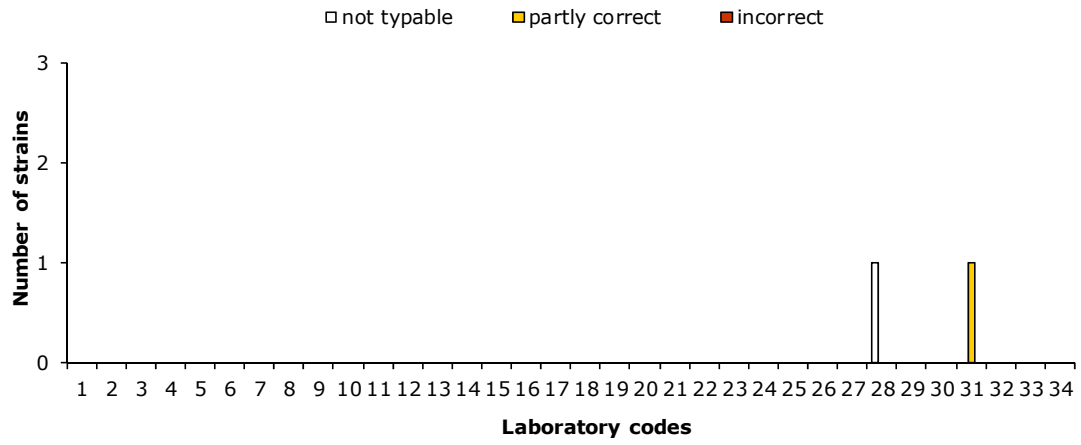

Figure 2. Evaluation of serotyping of O-antigens per NRL

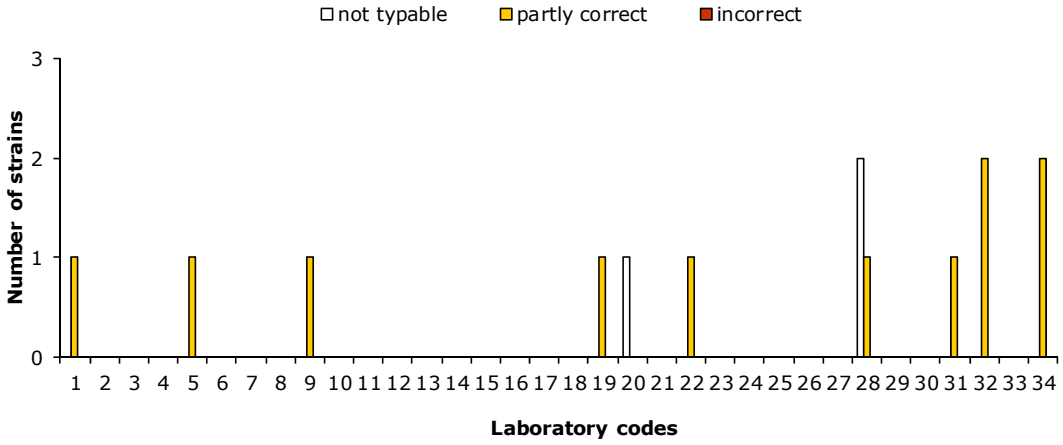

Figure 3. Evaluation of serotyping of H-antigens per NRL

Figure 4. Evaluation of assigning the correct serovar names per NRL

For each NRL, the number of penalty points was determined using the guidelines in section 3.5. Table 18 shows the number of penalty points for each NRL, including the information on whether the level of good performance was achieved (yes or no). Two 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.

Table 18. Evaluation of serotyping results per NRL

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

5.1.3 *Serotyping results per strain*

Results found per strain and per laboratory are given in Annex 2, except for the more complicated strains S19 and S21 which are separately reported in Annex 3.
A completely correct identification by all participants was obtained for twelve strains: *S*. Telelkebir (S1), *S*. Sandiego (S3), *S*. Plymouth (S4), *S*. Kingston (S6), *S*. Havana (S7), *S*. Hadar (S8), *S*. Worthington (S10), *S*. Kentucky (S14). *S*. Infantis (S17), *S*. Enteritidis (S18), 1,4,[5],12:i:-(S19), and *S*. Typhimurium (S20).
Most problems occurred with the serovar *S*. Thompson (S9). Eight laboratories had difficulties correctly assigning the correct serovar name to this strain, though in two cases this was caused by the (partly) non-typable nature of the strain. The characterisations of strains that caused problems in serotyping are shown in Annex 4.
The reported serovar name for strain S19 again showed a large variation of "Typhimurium-like" names, despite the example given in both the protocol and the electronic test report on how to preferably report this serovar name. Therefore the reported serovar names are summarised separately in Annex 3. Details on the additional and optional strain S21 are also given in Annex 3.

All but two participants actually did serotype this additional strain S21, being a *Salmonella enterica* subspecies *salamae* 42:g,t:-. Thirty laboratories correctly serotyped the O-antigens and the H-antigens for this strain.

5.1.4 *Follow-up*

Two NRLs did not achieve the level of good performance (Table 18; Lab codes 32 and 34) and were offered a follow-up study. This follow-up study is obligatory for laboratories from EU Member States, and the two laboratories received ten additional strains for serotyping in week 14, 2014.
For both participants, the number of penalty points was determined using the guidelines in section 3.5. Table 19 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 a level of good performance in this follow-up study.

Table 19. Evaluation of serotyping results per NRL in the follow-up study

Lab code	Penalty points	Good performance
32	0	Yes
34	0	Yes

5.2 Phage typing results

Seven NRLs participated in the phage typing study of both *S*. Enteritidis and *S*. Typhimurium. The phage typing results for *S*. Enteritidis and *S*. Typhimurium are shown in Table 20. The percentages of strains correctly phage typed for each laboratory for both *S*. Enteritidis and *S*. Typhimurium are shown in Figure 5.
Separate notations per phage type and per laboratory are given in Annex 5 (*S*. Enteritidis) and Annex 6 (*S*. Typhimurium).

Table 20. Results of Salmonella *Enteritidis and* Salmonella *Typhimurium phage typing*

S. Enteritidis strain numbers

Lab code	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	Y
PHE	**4**	**60**	**5a**	**4b**	**2**	**21**	**5**	**6a**	**6**	**1b**	
3	4	60	5a	4b	2	21	5	6a	6	1b	0
9	4	60	5a	4b	2	21	5	6a	6	1b	0
12	4	60	5a	4b	2	21	5	6a	6	1b	0
18	4	60	5A	4B	2	21	5	6A	6	1B	0
26	PT 4	PT 60	PT 5a	PT 4b	PT 2	PT 21	PT 61	PT 6a	PT 6	PT 1b	1
27	PT4	PT60	PT5A	PT4B	PT2	PT21	PT5	PT6A	PT6	PT1B	0
34	4	*20(60?)	36	4b	2	21c	4a	6a	6	1b	4
X	0	1	1	0	0	1	2	0	0	0	5

S. Typhimurium strain numbers

Lab code	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	Y
PHE	**195**	**104**	**8**	**7a**	**120**	**193**	**40**	**36**	**15a**	**135**	
3	195	U302	8	7a	120	193	40	36	15a	135	1
9	195	104	8	7	120	193	40	36	46	2	3
12	195	104b	8	7	120	193	40	36	15a	2	3
18	195	104B	8	7A	120	193	40	36	15A	135	1
26	DT 195	DT 104	DT 8	DT 7a	DT 104B	DT 193	DT 40	DT 36	DT 15a	DT 2	2
27	DT195	DT104L	DT8	DT7a	DT120	DT193	DT40	DT36	DT15a	DT2	1
34	195	104L	8	7a	120	193	40	36	15	135	1
X	0	3	0	2	1	0	0	0	2	4	12

PHE = reference results X = number of deviating laboratories per strain Y = number of deviating strains per laboratory

incorrect result

incorrect result with remark
Strain E2, lab 34 — the phage reactions were not correct for PT 20 or PT 60

correct result with remark
Strain T2, labs 27, 34 — the phage type has been given as DT 104L, this is a low variant of DT 104

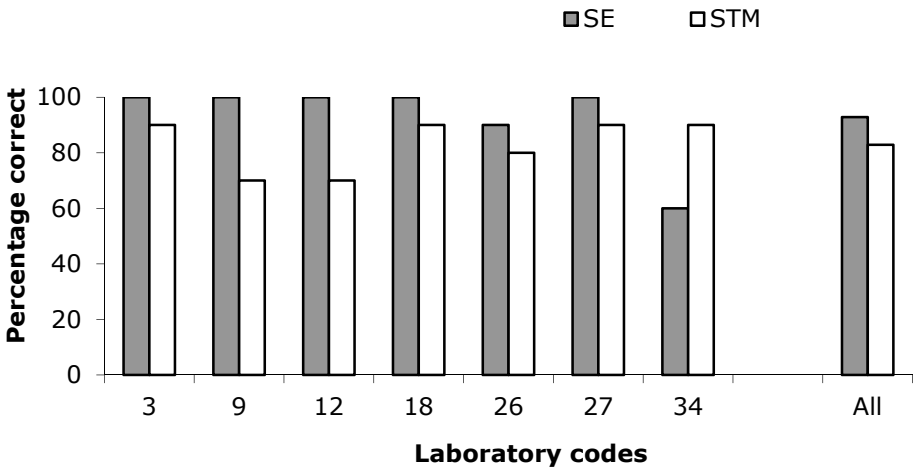

Figure 5. Percentage of strains correctly phage typed for each participating laboratory

Five laboratories correctly phage typed all ten strains of *S*. Enteritidis. The laboratory with labcode 26 assigned the incorrect phage type to one of the strains (E7). Laboratory 34 incorrectly phage typed four of the strains, E2, E3, E6 and E7.
None of the laboratories correctly phage typed all ten strains of *S*. Typhimurium. Four laboratories (3, 18, 27 and 34) assigned the correct phage type to nine of the ten strains. Laboratory 26 incorrectly phage typed two of the strains, T5 and T10. Two laboratories (9 and 12) incorrectly phage typed three of the *S*. Typhimurium strains.
Overall, 93% of the *S*. Enteritidis strains and 83% of the *S*. Typhimurium strains were correctly phage typed.

5.3 PFGE typing results

A promising number of 20 NRLs participated in the first pilot study on PFGE typing. For this initial study, the results were evaluated on the quality of the PFGE images only (submitted as TIFF files).
The quality of the gels was quite variable, as shown in two examples in Annex 7. An example of the individual laboratory evaluation report in this pilot study is given in Annex 8. In addition to the scores given in accordance to the PulseNet Guidelines, the EURL-*Salmonella* also included some general comments per individual report, for example the resolution of the TIFF file was too low (< 300 KB), or too dark to see any details. The protocol request to include at least the lab code in the name of the .tif file, as returned to the EURL-*Salmonella,* was also commented on; only 50% of the participants followed this request, which was intended to avoid any confusion in the evaluation of the results.

The scores per NRL, as obtained for the seven parameters (Annex 1) are given in Table 21. The scores per parameter are visualised in Figure 6.
The parameter "Image Acquisition/Running Conditions" yielded rather low scores, with Poor or Fair only. The parameter "Bands" yielded scores ranging from Poor (5x) to Excellent (10x). The other five parameters all yielded a majority of Excellent scores.

Table 21. Evaluation of PFGE results per participants and per parameter

Lab code/ Parameter	2	27	1	25	28	17	21	13	19	33	22	26	7	9	12	3	5	18	24	34	Total score per parameter	Average per parameter
Image Acquisition and Running Conditions	1	2	1	2	2	1	1	1	2	1	2	1	1	1	2	2	2	2	2	2	31	1,6
Cell Suspension	4	4	4	1	4	4	3	4	4	3	4	4	4	4	4	4	4	4	4	4	75	3,8
Bands	1	2	2	1	1	1	1	2	4	3	4	3	4	4	4	4	4	4	4	4	57	2,9
Lanes	4	4	4	4	3	2	4	4	4	4	3	4	4	4	3	4	4	4	4	4	75	3,8
Restriction	1	4	1	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	73	3,7
Gel Background	4	1	3	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4	4	4	75	3,8
DNA Degradation (smearing in the lanes)	3	1	4	3	3	4	4	4	1	4	4	4	4	4	4	4	4	4	4	4	71	3,6
Total score per participant	18	18	19	19	20	20	21	23	23	23	24	24	25	25	25	26	26	26	26	26		
Average per participant	2,6	2,6	2,7	2,7	2,9	2,9	3,0	3,3	3,3	3,3	3,4	3,4	3,6	3,6	3,6	3,7	3,7	3,7	3,7	3,7	65,3	3,3

Each of the seven parameters was given a score of a maximum of 4 points; poor quality equals 1 point and excellent quality equals 4 points.

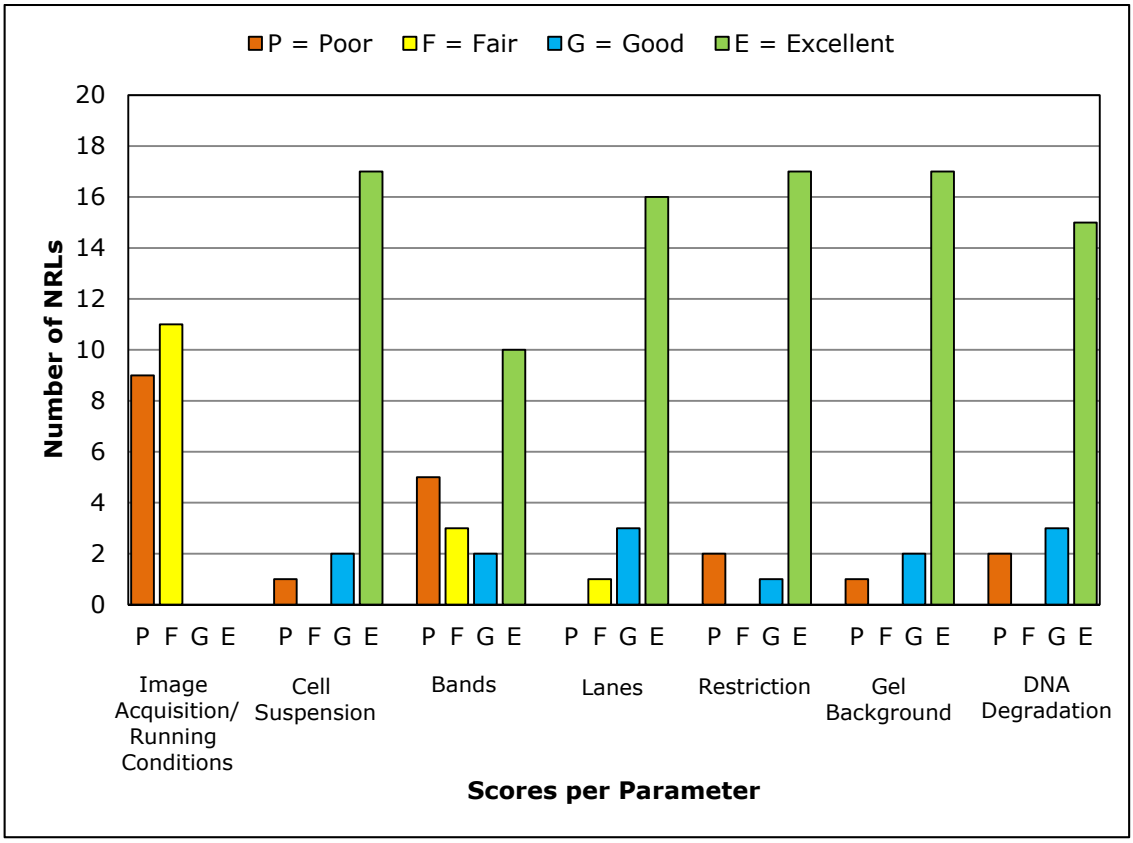

Figure 6. Evaluation of the quality of the PFGE images in scores per parameter

6 Discussion

6.1 Serotyping

A total of 34 laboratories participated in this study. These included 29 National Reference Laboratories for Salmonella (NRLs-Salmonella) situated in the 28 EU Member States, 2 NRLs of EU-candidate countries, and 3 NRLs of EFTA countries.
A total of 21 Salmonella strains were sent to the participants in November 2013 for serotyping by all participants, however the 21st strain was optional and not included in the evaluation.
Overall, nearly 100% of the strains were typed correctly for the O-antigens, 98% of the strains were typed correctly for the H-antigens and 97% of the strains were correctly named by the participants.

At the EURL-*Salmonella* workshop in 2007, the EURL-*Salmonella* proposed a definition for good performance of the NRLs regarding the serotyping. Using this definition, 32 laboratories achieved good performance. The two 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. Therefore, in the end all 34 participants achieved good performance in the 2013 serotyping study.

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 when judging 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 current study, none of the NRLs had problems with correctly serotyping all these serovars, except for one mistake that was made in typing *S*. Virchow. Table 22 and Table 23 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 22 shows results for EU-NRLs only and Table 23 shows results for all participants per study. The relatively large number of 56 penalty points in 2009 (Table 23) 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 with a better performance for the O-antigens than for the H-antigens.

Compared to the 2011/2012 studies, the number of penalty points was lower and the NRLs that had to participate in the follow-up studies differed each year. The mistakes which led to the 2 NRLs in 2013 to participate in the follow-up study were, in both cases, due to one mistype in the "top five" *Salmonella* strains.

Table 13. Historical overview of the EURL-Salmonella interlaboratory comparison studies on serotyping of Salmonella, for EU-NRLs only

Study/Year	XII 2007	XIII 2008	XIV 2009	XV 2010	XVI 2011	XVII 2012	XVIII 2013
# participants	25	27	28	30	28	28	29
# strains evaluated	20	20	20	19	19	20	20
O-antigens correct/strains	98%	98%	98%	98%	99%	99%	100%
H-antigens correct/strains	95%	98%	95%	95%	97%	98%	98%
Names correct/strains	95%	97%	95%	95%	97%	96%	98%
O-antigens correct/labs	68%	70%	75%	93%	93%	82%	97%
H-antigens correct/labs	56%	67%	43%	73%	71%	64%	72%
Names correct/labs	52%	52%	46%	67%	75%	57%	69%
# Penalty Points	35	30	36	16	22	20	17
# labs with non-Good Performance	6	3	4	2	2	2	2
# labs with non-Good Performance after follow-up	0	0	0	0	0	0	0

*Table 14. Historical overview of the EURL-*Salmonella *interlaboratory comparison studies on serotyping of* Salmonella*, for all participants*

Study/Year	XII 2007	XIII 2008	XIV 2009	XV 2010	XVI 2011	XVII 2012	XVIII 2013
# participants	26	29	31	33	36	31	34
# strains evaluated	20	20	20	19	19	20	20
O-antigens correct/strains	98%	98%	97%	98%	98%	99%	100%
H-antigens correct/strains	96%	98%	94%	95%	96%	98%	98%
Names correct/strains	95%	97%	93%	95%	96%	96%	97%
O-antigens correct/labs	69%	76%	74%	88%	86%	77%	94%
H-antigens correct/labs	58%	72%	45%	67%	69%	61%	71%
Names correct/labs	54%	59%	48%	61%	69%	55%	68%
# Penalty Points	36	34	56	37	41	20	20
# labs with non-Good Performance	6	4	5	4	4	2	2
# labs with non-Good Performance after follow-up	0	0	0	0 (n=3)	1 (n=3)	0	0

6.2 Phage typing

Ten strains of *S*. Enteritidis and ten strains of *S*. Typhimurium were selected by the *Salmonella* Reference Service of Public Health England, London, UK.
All ten *S*. Enteritidis strains were correctly phage typed by five of the seven NRLs. One NRL incorrectly phage typed one of the *S*. Enteritidis strains and one NRL incorrectly phage typed four of the ten strains.
One laboratory incorrectly phage typed strain E2 (PT 60) as PT 20/PT 60?. The reactions obtained were not correct for either of these phage types: they obtained reactions with phages 15 and 16, and as PT 60 does not react with these two phages, this suggests that the titre of these phages was incorrect.
One laboratory phage typed strain E3 (PT 5a) incorrectly as PT 36. This strain was typed as PT 36 because they obtained a high reaction with phage 3 and reactions with phages 8 and 10. As PT 5a does not react with these two phages, these incorrect reactions could either be due to the titre of the phages being too

high, or the inoculum of the broth culture used for the phage typing was incorrect. Strain E6 (PT 21) was also incorrectly phage typed by one laboratory. This laboratory typed it as PT 21c because it obtained reactions with phages 15 and 16; however PT 21 does not react with these two phages. This laboratory also had a problem with these two phages for strain E2, which suggests the titres were too high.
Two laboratories phage typed strain E7 (PT5) incorrectly. This strain was typed as PT 61 by one laboratory because they did not get a reaction with phage 2 and the reaction they obtained with phage 3 was too high. The second laboratory typed this strain as PT 4a as the reaction they obtained with phage 3 was too high and they had no reaction with phage 16.

None of the seven NRLs correctly phage typed all ten strains of *S*. Typhimurium. Four of the NRLs correctly phage typed nine of the *S*. Typhimurium strains. One NRL correctly typed eight of the S. Typhimurium strains, and two of the NLRs correctly typed seven of the ten strains.
Three laboratories incorrectly phage typed strain T2 (DT 104). One laboratory typed it as PT U302 due to reactions being obtained with three phages (12, 13 and 18). Two laboratories typed it as DT 104b because no reactions were obtained with phages 12 and 13. As none of these three laboratories had problems with these phages on the other strains in this study, the reason for the incorrect typing was probably due to the inoculum of the culture used for the phage typing being incorrect.

Strain T4 (DT 7a) was incorrectly typed as DT 7 by two laboratories. One laboratory did not get a phage reaction with phage 29 and the other laboratory only had a low reaction with phage 29. This suggests the titre of this phage was too low.

T5 (DT 120) was typed as DT 104b by one laboratory. This incorrect result was due to no reaction being obtained with additional phages 1, 2 and 3. DT 120 has an intermediate reaction with these three phages, so the incorrect typing was probably due to the titre of these three phages being slightly low.

Two laboratories incorrectly phage typed T9 (DT 15a). One typed it as DT 46 because they obtained phage reactions with several phages that do not react with DT 15a. This may have been due to the inoculum of the culture used for the phage typing being incorrect. One laboratory incorrectly typed this strain as DT 15 as a reaction was obtained with phage 18 and DT 15a does not react with this phage. This may have been due to the titre of this phage being too high.

Four laboratories incorrectly typed strain T10 (DT 135) as DT2. DT2 and DT 135 react with the same phages but DT 2 gives high reactions with all of these phages, whereas DT 135 gives variable reactions. The distinguishing feature for DT 135 is that the reactions with phages 2 to 6 show an increase from ++ for phage 2 up to CL for phage 6.

6.3 PFGE typing

A large number (20) of NRLs participated in this first pilot study on PFGE typing. Evaluation was based on the quality of the generated images only, and did not include the gel analysis in Bionumerics.
The quality of the PFGE results was promising, though there was some variation in results between the participants. The evaluation of the PFGE images was

based on the assessment of seven parameters, using a scoring from 1 (Poor), 2 (Fair), 3 (Good), to 4 (Excellent) points per parameter.
Although variation within parameter scores per participant was noticed, a laboratory could still score an "acceptable" average (e.g. lab code 13, Table 21, score=3,3). However, it should be kept in mind that, in general, an acceptable quality should be obtained for each parameter as a low quality score in just one category can have a high impact on the ability to further analyse the image and compare it to other profiles.
The parameter "Image Acquisition/Running Conditions" yielded rather low scores, with Poor or Fair scores only. Application of adjustments related to this parameter will help improve the results. These adjustments may be relatively easy to implement, for example:

- the gel fills whole TIFF
- the wells are included on the TIFF
- the bottom band of the standard is 1 – 1,5 cm from the bottom of the gel
- use the standard strain correctly (placing in first and last lane, and at least in every 6 lanes for narrow plugs, or in at least every 5 lanes for wide plugs
- to check on the resolution of the image (preferably > 300 KB file size).

7 Conclusions

7.1 Serotyping

- Nearly 100% of the strains were typed correctly for the O-antigens.
- 98% of the strains were typed correctly for the H-antigens.
- 97% of the strains were correctly named.
- Serotyping of *S*. Thomson caused most problems in this study (eight participants).
- All participants correctly serotyped the 'top 5' strains *S*. Enteritidis, *S*. Hadar, *S*. Infantis, and *S*. Typhimurium (including the monophasic variant). Only one mistake was made in typing *S*. Virchow.
- Two NRLs had to participate in the follow-up study, typing an additional set of ten strains.
- In the end, all 34 participants achieved the defined level of good performance.

7.2 Phage typing

- The performance of the 7 laboratories participating in this study showed an improvement for *S*. Enteritidis when compared to the 2012 study. In 2012, 90% of the *S*. Enteritidis strains were correctly phage typed and in the 2013 study, 93% of the strains were correctly typed.
- The performance in the phage typing of the *S*. Typhimurium strains in the current study was not as good as in the previous study. In 2012, 92% of the *S*. Typhimurium strains were correctly phage typed and in the 2013 study, 83% of the strains were correctly typed.
- Six of the *S*. Enteritidis strains and five of the *S*. Typhimurium strains were correctly phage typed by all participating laboratories.

7.3 PFGE typing

- Twenty participants also performed PFGE typing in this first pilot study.
- Evaluation of the PFGE results was on the quality of the generated images only, assessing seven parameters with a score of either Poor, Fair, Good or Excellent.
- The quality of the PFGE results was promising, although there was some variation in results between the participants.
- The parameter "Image Acquisition/Running Conditions" yielded low scores, with Poor or Fair only.
- Application of adjustments related to this parameter will help to improve the results.

List of abbreviations

#	Total number
CRL-*Salmonella*	Community Reference Laboratory for *Salmonella* (nowadays EURL-*Salmonella*)
DT	Definitive type
ECDC	European Centre for Disease prevention and Control
EFTA	European Free Trade Association
EQA	External Quality Assessment
EU	European Union
EURL-*Salmonella*	European Union Reference Laboratory for *Salmonella*
FWD	Food- and Waterborne Diseases and Zoonoses Programme
HPA	Health Protection Agency (nowadays Public Health England)
NRLs-*Salmonella*	National Reference Laboratories for *Salmonella*
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PHE	Public Health England (formerly Health Protection Agency)
PT	Phage Type
REF	Reference
RIVM	National Institute for Public Health and the Environment (Bilthoven, The Netherlands)
SE	*Salmonella* Enteritidis
SSI	Statens Serum Institut (Copenhagen, Denmark)
STM	*Salmonella* Typhimurium
TIFF	Tagged Image File Format
UK	United Kingdom

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Acknowledgements

The authors would like to thank Sjoerd Kuiling and Kim van der Zwaluw (Centre for Infectious Diseases, Diagnostics and Screening, RIVM, Bilthoven, The Netherlands) for their expert contribution in the evaluation of the PFGE typing results.

Annex 1 PulseNet Guidelines for PFGE image quality assessment (PNQ01)

As copied from www.pulsenetinternation.org :

STANDARD OPERATING PROCEDURE FOR TIFF QUALITY GRADING	CODE: PNQ01 Effective Date: 5 09 2005

1. **PURPOSE:** To describe guidelines for the quality of TIFF images submitted to the PulseNet national databases.

2. **SCOPE:** This applies to all TIFF images submitted to PulseNet, thereby allowing comparison of results with other PulseNet laboratories.

3. **DEFINITIONS/TERMS:**

   3.1 TIFF: Tagged Image File Format
   3.2 TIFF Quality: The grading of the appearance and ease of analysis of a TIFF, according to the TIFF Quality Grading Guidelines within this SOP. This is a main component of the evaluation of a TIFF submitted for certification or proficiency testing.
   3.3 SOP: Standard Operating Procedure

4. **RESPONSIBILITIES/PROCEDURE:**

Parameter	TIFF Quality Grading Guidelines			
	Excellent	**Good**	**Fair**	**Poor**
Image Acquisition and Running Conditions	By protocol, for example: - Gel fills whole TIFF - Wells included on TIFF - Bottom band of standard 1-1.5 cm from bottom of gel	- Gel doesn't fill whole TIFF but band finding is not affected	Not protocol; for example, one of the following: - Gel doesn't fill whole TIFF and band finding is affected - Wells not included on TIFF - Bottom band of standard not 1-1.5 cm from bottom of gel - Band spacing of standards doesn't match global standard	Not protocol; for example, >1 of the following: - Gel doesn't fill whole TIFF and this affects band finding - Wells not included on TIFF - Bottom band of standard not 1-1.5 cm from bottom of gel - Band spacing of standards doesn't match global standard
Cell Suspensions	The cell concentration is approximately the same in each lane	1-2 lanes contain darker or lighter bands than the other lanes	- >2 lanes contain darker or lighter bands than the other lanes, or - At least 1 lane is much darker or lighter than the other lanes, making the gel difficult to analyze	The cell concentrations are uneven from lane to lane, making the gel impossible to analyze
Bands	Clear and distinct all the way to the bottom of the gel	- Slight band distortion in 1 lane but doesn't interfere with analysis - Bands are slightly fuzzy and/or slanted - A few bands (e.g., ≤3) difficult to see clearly (e.g., DNA overload), especially at bottom of gel	- Some band distortion (e.g., nicks) in 2-3 lanes but still analyzable - Fuzzy bands - Some bands (e.g., 4-5) are too thick - Bands at the bottom of the gel are light, but analyzable	- Band distortion that makes analysis difficult - Very fuzzy bands. - Many bands too thick to distinguish - Bands at the bottom of the gel too light to distinguish
Lanes	Straight	- Slight smiling (higher bands in the outside lanes vs. the inside) - Lanes gradually run longer toward the right or left - Still analyzable	- Significant smiling - Slight curves on the outside lanes - Still analyzable	- Smiling or curving that interferes with analysis

VERSION:	REPLACED BY:	AUTHORIZED BY:	Page 1 of 2

Restriction	Complete restriction in all lanes	- One to two faint shadow bands on gel	- One lane with many shadow bands - A few shadow bands spread out over several lanes	- Greater than 1 lane with several shadow bands - Lots of shadow bands over the whole gel
Gel Background	Clear	- Mostly clear background - Minor debris present that doesn't affect analysis	- Some debris present that may or may not make analysis difficult (e.g., auto band search finds too many bands) - Background caused by photographing a gel with very light bands (image contrast was "brought up" in photographing gel-makes image look grainy)	- Lots of debris present that may or may not make analysis difficult (i.e., auto band search finds too many bands)
DNA Degradation (smearing in the lanes)	Not present	- Minor background (smearing) in a few lanes but bands are clear	- Significant smearing in 1-2 lanes that may or may not make analysis difficult - Minor background (smearing) in many lanes	- Significant smearing in >2 lanes that may or may not make analysis difficult - Smearing so that a lane is not analyzable (except if untypeable [thiourea required])

5. FLOW CHART:

6. BIBLIOGRAPHY:

7. CONTACTS:

8. AMENDMENTS:

Annex 2 Serotyping results per strain and per laboratory

Lab REF	S1 Telelkebir	S2 Colindale	S3 Sandiego	S4 Plymouth	S5 Virchow	S6 Kingston	S7 Havana	S8 Hadar	S9 Thompson	S10 Worthington
1	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingstone	Havana	Hadar	Nessziona	Worthington
2	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
3	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
4	S. Telelkebir	S.Colindale	S. Sandiego	S. Plymouth	S. Virchow	S. Kingston	S.Havana	S. Hadar	S .Thompson	S. Worthington
5	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
6	S. Telelkebir	S. Colindale	S. Sandiego	S.Plymouth	S.Virchow	S. Kingston	S.Havana	S.Hadar	S.Thompson	S.Worthington
7	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
8	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
9	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Bareilly	Worthington
10	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	6,7:-:1,5*	Worthington
11	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
12	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
13	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
14	Salmonella Telelkebir	Salmonella Colindale	Salmonella Sandiego	Salmonella Plymouth	Salmonella Virchow	Salmonella Kingston	Salmonella Havana	Salmonella Hadar	Salmonella Thompson	Salmonella Worthington
15	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
16	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
17	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
18	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
19	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Alamo	Worthington
20	Telelkerib	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	6,7:-:1,5	Worthington
21	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
22	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
23	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
24	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
25	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
26	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
27	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
28	Telelkebir	Auto-agglutination	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Poitiers	Worthington
29	S.Telekebir	S.Colindale	S.Sandiago	S.Plymouth	S.Virchow	S.Kingston	S.Havana	S.Hadar	S.Thompson	S.Worthington
30	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
31	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
32	Telelkebir	Colindale	Sandiego	Plymouth	Infantis	Kingston	Havana	Hadar	Nessziona	Worthington
33	Telelkebir	Colindale	Sandiego	Plymouth	Virchow	Kingston	Havana	Hadar	Thompson	Worthington
34	S. Telelkebir	S. Colindale	S. Sandiego	S. Plymouth	S. Virchow	S. Kingston	S. Havana	S. Hadar	S. Infantis	S. Worthington
X	0	1	0	0	1	0	0	0	8	0

S11	S12	S13	S14	S15	S16	S17	S18	S20	Lab
Anatum	**Panama**	**Napoli**	**Kentucky**	**Mbandaka**	**Paratyphi B var. Java**	**Infantis**	**Enteritidis**	**Typhimurium**	**REF**
Anatum	Houston	Napoli	Kentacky	Mbandaka	Paratyphi B	Infantis	Enteritidis	Typhimurium	1
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B var. Java	Infantis	Enteritidis	Typhimurium	2
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B var. Java (dT+)	Infantis	Enteritidis	Typhimurium	3
S. Anatum	S.Panama	S. Napoli	S. Kentucky	S. Mbandaka	S. Paratyphi B var. Java	S. Infantis	S. Enteritidis	S. Typhimurium	4
Anatum	Houston*	Napoli	Kentucky	Mbandaka	Paratyphi B var. Java	Infantis	Enteritidis	Typhimurium	5
S.Anatum	S.Panama	S.Napoli	S. Kentucky	S.Mbandaka	S.Paratyphi B	S.Infantis	S.Enteritidis	S.Typhimurium	6
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B , Var.Java	Infantis	Enteritidis	Typhimurium	7
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B, var. Java	Infantis	Enteritidis	Typhimurium	8
Anatum (var 15+)	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B var Java	Infantis	Enteritidis	Typhimurium	9
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B/Java**	Infantis	Enteritidis	Typhimurium	10
Anatum	Panama subgroup	Napoli	Kentucky	Mbandaka	Paratyphi B	Infantis	Enteritidis	Typhimurium	11
Anatum var. 15	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B var. Java	Infantis	Enteritidis	Typhimurium	12
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B var Java	Infantis	Enteritidis	Typhimurium	13
Salmonella Anatum	Salmonella Panama	Salmonella Napoli	Salmonella Kentucky	Salmonella Mbandaka	Salmonella Paratyphi-B	Salmonella Infantis	Salmonella Enteritidis	Salmonella Typhimurium	14
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B var. Java	Infantis	Enteritidis	Typhimurium	15
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B	Infantis	Enteritidis	Typhimurium	16
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B variatie Java	Infantis	Enteritidis	Typhimurium	17
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B var. Java	Infantis	Enteritidis	Typhimurium	18
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B	Infantis	Enteritidis	Typhimurium	19
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B	Infantis	Enteritidis	Typhimurium	20
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B	Infantis	Enteritidis	Typhimurium	21
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B	Infantis	Enteritidis	Typhimurium	22
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B var. Java	Infantis	Enteritidis	Typhimurium	23
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B, var L(+) tartrate Java	Infantis	Enteritidis	Typhimurium	24
Anatum	Salmonella spp.*	Napoli	Kentucky	Mbandaka	Paratyphi	Infantis	Enteritidis	Typhimurium	25
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B var Java	Infantis	Enteritidis	Typhimurium	26
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B	Infantis	Enteritidis	Typhimurium	27
Anatum	9,12:?	Napoli	Kentucky	Mbandaka	Paratyphi B var. Java	Infantis	Enteritidis	Typhimurium	28
S.Anatum	S.Panama	S.Napoli	S.Kentucky	S.Mbandaka	S.Paratyphi B	S.Infantis	S.Enteritidis	S.Typhimurium	29
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B	Infantis	Enteritidis	Typhimurium	30
Hayindogo	Houston	Napoli	Kentucky	Braenderup	Paratyphi B	Infantis	Enteritidis	Typhimurium	31
Anatum	Panama	Napoli	Kentucky	Mbandaka	Paratyphi B	Infantis	Enteritidis	Typhimurium	32
Anatum	Panama	Napoli	Kentacky	Mbandaka	Paratyphi B var. Java	Infantis	Enteritidis	Typhimurium	33
S. Anatum	S. Panama	O9,12:L:x*	S. Kentucky	S. Mbandaka	S. Paratyphi B var. Java	S. Infantis	S. Enteritidis	S. Typhimurium	34
1	5	1	0	1	1	0	0	0	**X**

mistake in writing

not typable

incorrect (1 penalty point)

incorrect (4 penalty points)

X = number of deviating laboratories per strain

Results for Strain S19 and Strain S21 are given in Annex 3

Annex 3 Details on serotyping results of strains S19 and S21

Strain	O-antigens	H-antigens phase 1	H-antigens phase 2	Serovar	Labcode
S19	**1,4,[5],12**	**i**	**-**	**1,4,[5],12:i:-**	**REF**
S19	4, 5, 12	i	-	4, 5, 12:i:-	1
S19	4,5	i	-	Monophasic S.Typhimurium	2
S19	4,5	i	-	1,4,5,12:i:- (monophasic Typhimurium)	3
S19	4,5,12	i	-	S. enterica ssp. enterica I 4,5,12:i:-	4
S19	4,5,12	i	-	4,5,12 : i : -	5
S19	4,5,12	i	-	S. enterica subsp.enterica 4,5,12:i:-	6
S19	4,5,12	i	-	4,5,12: i: -	7
S19	4,5,12	i	-	4,5,12:i:-	8
S19	4,5,12	i	-	4,5,12:i:-	9
S19	4,5	i	-	Monophasic Typhimurium	10
S19	4,5	i	-	Monophasic Salmonella Typhimurium	11
S19	4,5,12	i	-	4,5,12:i:-	12
S19	4,5,12	i	-	Monophasic Salmonella Typhimurium	13
S19	4,[5],12	i	-	?	14
S19	4,5,12	i	-	4,5,12:i:-	15
S19	4,5,12	i	-	Salmonella enterica subsp. enterica seroty	16
S19	4,5,12	i	-	1,4,5,12:i:-	17
S19	4,5	i	-	monophasic Typhimurium	18
S19	4,5,12	i	-	4,5,12:i:-	19
S19	4,5,12	i	-	4,5,12:i:-	20
S19	4,5,12	i	-	Typhimurium monophasic variant	21
S19	1,4,[5],12	i	-	1,4,[5],12:i:-	22
S19	4,5,12	i	-	enterica subsp. enterica	23
S19	4,5,12	i	-	4,5,12:i:-	24
S19	4,5	i	-	4,5:i:-	25
S19	1,4,5,12	i	-	monophasic S. Typhimurium	26
S19	4,12	i	-	4,12:i.-	27
S19	4,5,12	i	-	4,5,12:i:-	28
S19	4,5,12	i	-	S.Typhimurium, monophasic	29
S19	4,5,12	i	-	4,5,12:i:-	30
S19	4, 12	i	-	4, 12: i: -	31
S19	1,4 (5) 12	i	-	4,12 :i: -	32
S19	4,12	i	-	4,12:i:-	33
S19	4,5,12	i	-	O4,5,12:i:-	34

Strain	O-antigens	H-antigens phase 1	H-antigens phase 2	Serovar	Labcode
S21	**42**	**g,t**	**-**	**42:g,t:-**	**REF**
S21	-	-	-	-	1
S21	42	g,t	-	S. enterica subsp. salamae 42:g,t:-	2
S21	42	g,t	-	S. II 42:g,t:-	3
S21	42	g,t	-	S. enterica ssp. salamae II	4
S21	42	g,t	-	42 : g,t : -	5
S21	42	g,t	-	S.enterica subsp.salamae 42:g,t:-	6
S21	42	g,t	-	42:g,t:- (II)	7
S21	42	g,t	-	II 42:g,t:-	8
S21	42	gt	-	II 42:gt:-	9
S21	42	g,t	-	S. salamae=42:g,t:-	10
S21	42	g,t	-	Kampala II	11
S21	42	g,t	-	II 42:gt:-	12
S21	42	gt	-	Salmonella enterica subsp. salamae ser. 4	13
S21	42	g,t	-	Salmonella II	14
S21	42	g,t	-	42:g,t:-	15
S21	42	g,t	-	Salmonella enterica subsp. salamae (II)	16
S21	42	g,t	-	42:g,t:-	17
S21	42	g,t	-	Salmonella ssp. II	18
S21	42	g,t	-	Enterica subsp salamae	19
S21	42	g,t	-	42:g,t:-	20
S21	42	g,t	-	subspecies salamae	21
S21	42	g,t	-	S.II 42:g,t:-	22
S21	42	g,t	-	enterica subsp. salamae	23
S21	42	g,t	-	42:g,t:-	24
S21	42	g,t	-	II	25
S21	42	g,t	-	Salmonella enterica subsp. salamae (II.) 4	26
S21	42	g,t	-	S. enterica subsp. salamae	27
S21	OMD (SSI)	?	?	Salmonella sp.	28
S21	42	g,t	-	S.II 42:g,t:-	29
S21	42	g,t	-	42: g,t subsp salamae	30
S21	not tested	not tested	not tested	no result	31
S21	42	gt	-	II - enterica subsp.salamae	32
S21	42	g,t	-	II:42:g,t:-	33
S21	OMD	not tested	not tested	no result	34

Grey = deviating results of any kind.

Annex 4 Identifications per strain that caused problems in serotyping

Strain	O-antigens	H-antigens phase 1	H-antigens phase 2	Serovar	Labcode
S2	**6,7**	**r**	**1,7**	**Colindale**	**REF**
S2	Auto-agglutination			Not typable. Auto-agglutination	28
S5	**6,7,14**	**r**	**1,2**	**Virchow**	**REF**
S5	6,7,14	r	1,5	Infantis	32
S6	**1,4,[5],12,[27]**	**g,s,t**	**[1,2]**	**Kingston**	**REF**
S6	1,4,[5],12,[27]	g,s,t	[1,6]	Kingston	22
S9	**6,7,14**	**k**	**1,5**	**Thompson**	**REF**
S9	6, 7	l, z13	1, 5	Nessziona	1
S9	6,7	y	1,5	Bareilly	9
S9	6,7	-	1,5	6,7:-:1,5	10
S9	6,7	g,z51	1,5	Alamo	19
S9	6,7	-	1,5	6,7:-:1,5	20
S9	6,7	z	1,5	Poitiers	28
S9	6,7	l,z13	1,5	Nessziona	32
S9	7	r	5	S. Infantis	34
S11	**3,{10}{15}{15,34}**	**e,h**	**1,6**	**Anatum**	**REF**
S11	1, 3, 19	e, h	1, 6	Hayindogo	32
S12	**1,9,12**	**l,v**	**1,5**	**Panama**	**REF**
S12	9, 12	l, v	1, 5	Houston	1
S12	9,12	l,v	1,5	Houston	5
S12	9,12	l,v	1,5	Salmonella spp.	25
S12	9,12	Poly h:L pos. ?		9,12:?	28
S12	9, 12	l, v	1, 5	Houston	31
S13	**1,9,12**	**l,z13**	**e,n,x**	**Napoli**	**REF**
S13	9,12	L	x	O9,12:L:x	34
S15	**6,7,14**	**z10**	**e,n,z15**	**Mbandaka**	**REF**
S15	6, 7	e, h	e, n, z15	Braenderup	31
S16	**1,4,[5],12**	**b**	**1,2**	**Paratyphi B var. Java**	**REF**
S16	4,5	b	1,2	Paratyphi	25

Grey = deviating results of any kind.

Annex 5 Phage typing results per *S*. Enteritidis strain for all participating laboratories

Strain E1		Phages reactions at Routine Test Dilution (*S.* Enteritidis)																
Lab code	**Phage type**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
PHE	**4**	**-**	**SCL**	**CL**	**SCL**	**CL**	**SCL**	**CL**	**OL**	**< OL**	**OL**	**CL**	**CL**	**CL**	**-**	**-**	**-**	**<SCL**
3	4	-	SCL	CL	OL	CL	SCL	CL	OL	< OL	OL	CL	CL	CL	-	-	-	< OL
9	4	-	SCL	CL	SCL	CL	SCL	CL	OL	OL	OL	CL	CL	CL	-	-	-	OL
12	4	-	< CL	CL	OL	CL	< OL	CL	OL	OL	OL	< CL	CL	CL	-	0	-	OL
18	4	-	+++	CL	+++	CL	SCL	SCL	OL	+++	CL	SCL	CL	CL	-	-	-	+++
26	PT 4	-	SCL	CL	SCL	CL	SCL	CL	OL	OL	OL	CL	CL	CL	-	-	-	OL
27	PT4	-	SCL	SCL	OL	OL	+++	CL	OL	OL	OL	SCL	CL	SCL	-	-	-	< OL
34	4	0	SCL	SCL	SCL	< CL	+++	SCL	< OL	< OL	< OL	SCL	SCL	SCL	0	0	0	SCL

Strain E2		Phages reactions at Routine Test Dilution (*S.* Enteritidis)																
Lab code	**Phage type**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
PHE	**60**	**OL**	**-**	**CL**	**-**	**CL**	**SCL**	**-**	**OL**	**-**	**OL**	**-**	**CL**	**CL**	**< CL**	**-**	**-**	**-**
3	60	OL	-	CL	-	CL	< OL	-	OL	-	OL	-	CL	CL	CL	-	-	-
9	60	OL	-	CL	-	CL	SCL	-	OL	-	OL	-	CL	CL	CL	-	-	-
12	60	OL	-	CL	-	CL	SCL	-	OL	-	OL	-	CL	CL	CL	0	-	-
18	60	SCL	-	CL	-	CL	+++	-	OL	-	SCL	-	SCL	CL	CL	++	±	-
26	PT 60	OL	-	CL	-	CL	<SCL	-	CL	-	CL	-	CL	CL	CL	-	-	-
27	PT60	SCL	-	SCL	-	SCL	+++	-	OL	-	SCL	-	SCL	SCL	SCL	-	-	-
34	*20(60?)	SCL	0	SCL	0	< CL	±±	0	< OL	0	< OL	0	SCL	<SCL	SCL	++	SCL	0

incorrect result

incorrect result with remark
Strain E2, lab 34 the phage reactions were not correct for PT 20 or PT 60

correct result with remark
Strain T2, labs 27, 34 the phage type has been given as DT 104L, this is a low variant of DT 104

Strain E3		Phages reactions at Routine Test Dilution (*S.* Enteritidis)																
Lab code	**Phage type**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
PHE	**5a**	**-**	**SCL**	**+**	**OL**	**CL**	**SCL**	**-**	**-**	**< OL**	**-**	**-**	**CL**	**-**	**-**	**-**	**-**	**< OL**
3	5a	-	SCL	-	OL	CL	<SCL	-	-	OL	-	1 – 5	CL	-	-	-	-	< OL
9	5a	-	SCL	+	OL	CL	SCL	-	-	OL	-	+	CL	-	-	-	-	OL
12	5a	-	< CL	+	OL	CL	SCL	-	-	OL	-	-	CL	-	-	0	-	OL
18	5A	-	<SCL	++	+++	CL	SCL	-	-	+++	-	-	OL	-	-	-	-	<SCL
26	PT 5a	-	SCL	±	OL	CL	SCL	-	-	OL	-	-	SCL	-	-	-	-	OL
27	PT5A	-	+++	-	SCL	SCL	+++	-	-	SCL	-	-	SCL	-	-	-	-	< OL
34	36	0	SCL	SCL	OL	< CL	+++	+	+++	< OL	++	0	SCL	0	0	0	0	< OL

Strain E4		Phages reactions at Routine Test Dilution (*S.* Enteritidis)																
Lab code	**Phage type**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
PHE	**4b**	**-**	**OL**	**CL**	**SCL**	**CL**	**SCL**	**CL**	**OL**	**< OL**	**OL**	**CL**	**CL**	**CL**	**-**	**±**	**CL**	**SCL**
3	4b	-	SCL	CL	OL	CL	SCL	CL	OL	OL	OL	CL	CL	CL	-	1 – 5	CL	< OL
9	4b	-	SCL	CL	SCL	CL	SCL	CL	OL	OL	OL	CL	CL	CL	-	±	SCL	OL
12	4b	-	< CL	CL	OL	CL	SCL	CL	OL	< OL	OL	CL	CL	CL	-	0	CL	OL
18	4B	-	+++	CL	+++	CL	SCL	SCL	OL	+++	OL	SCL	CL	CL	-	-	SCL	SCL
26	PT 4b	-	SCL	CL	OL	CL	SCL	CL	OL	OL	OL	CL	CL	CL	-	±	CL	OL
27	PT4B	-	SCL	SCL	CL	+++	SCL	OL	< OL	< OL	SCL	CL	SCL	SCL	-	CL	CL	+++
34	4b	0	SCL	SCL	OL	< CL	+++	SCL	SCL	SCL	< OL	SCL	SCL	SCL	0	±	<SCL	< OL

Strain E5		Phages reactions at Routine Test Dilution (*S.* Enteritidis)																
Lab code	**Phage type**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
PHE	**2**	**OL**	**-**	**CL**	**SCL**	**CL**	**SCL**	**< CL**	**OL**	**< OL**	**OL**	**< CL**	**CL**	**-**	**< CL**	**-**	**-**	**< OL**
3	2	OL	-	CL	OL	CL	SCL	CL	OL	< OL	OL	CL	CL	-	CL	-	-	< OL
9	2	OL	-	CL	SCL	CL	SCL	CL	OL	OL	OL	OL	CL	-	CL	-	-	OL
12	2	OL	-	CL	OL	CL	SCL	SCL	OL	OL	OL	+	CL	-	CL	0	-	< OL
18	2	OL	-	SCL	SCL	OL	SCL	SCL	OL	SCL	OL	<SCL	OL	-	SCL	-	-	SCL
26	PT 2	OL	-	CL	OL	SCL	SCL	CL	OL	OL	OL	SCL	SCL	-	CL	-	-	OL
27	PT2	SCL	-	SCL	SCL	SCL	SCL	SCL	OL	< OL	OL	+++	SCL	-	SCL	-	-	<SCL
34	2	SCL	0	SCL	+++	SCL	+++	SCL	SCL	SCL	< OL	SCL	SCL	0	SCL	0	0	SCL

Strain E6		Phages reactions at Routine Test Dilution (*S.* Enteritidis)																
Lab code	**Phage type**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
PHE	**21**	**OL**	**< OL**	**-**	**OL**	**-**	**SCL**	**-**	**OL**	**< OL**	**OL**	**-**	**-**	**-**	**< CL**	**-**	**-**	**< OL**
3	21	OL	SCL	-	< OL	-	< OL	-	OL	< OL	OL	-	-	-	CL	-	-	< OL
9	21	CL	SCL	-	OL	-	OL	-	OL	OL	OL	-	-	-	CL	-	-	OL
12	21	OL	< CL	-	OL	-	SCL	-	OL	< OL	OL	-	-	-	CL	0	-	< OL
18	21	SCL	SCL	-	SCL	-	SCL	-	OL	SCL	OL	-	-	-	< CL	-	-	OL
26	PT 21	OL	SCL	-	SCL	-	SCL	-	OL	OL	OL	-	-	-	CL	-	-	OL
27	PT21	SCL	SCL	-	SCL	-	SCL	-	OL	< OL	OL	-	-	-	SCL	-	-	SCL
34	21c	SCL	SCL	0	OL	0	+++	0	SCL	< OL	< OL	0	0	0	SCL	++	SCL	< OL

Strain E7		Phages reactions at Routine Test Dilution (*S.* Enteritidis)																
Lab code	**Phage type**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
PHE	**5**	**-**	**SCL**	**+**	**SCL**	**< CL**	**SCL**	**SCL**	**OL**	**< OL**	**+++**	**<SCL**	**OL**	**<SCL**	**-**	**±**	**OL**	**< OL**
3	5	-	SCL	+	< OL	< CL	SCL	++	OL	< OL	+++	++	< OL	< OL	-	1 – 5	CL	< OL
9	5	-	SCL	+	SCL	CL	SCL	+	+++	OL	+++	±	CL	SCL	-	±	CL	OL
12	5	-	< CL	+	OL	CL	SCL	+	OL	OL	< OL	1 – 5	CL	SCL	-	0	CL	OL
18	5	-	<SCL	+++	+++	SCL	SCL	+++	<SCL	<SCL	+++	++	< CL	<SCL	-	-	+++	SCL
26	PT 61	-	-	CL	OL	CL	SCL	SCL	OL	OL	OL	CL	CL	SCL	-	-	CL	< OL
27	PT5	-	+++	-	OL	SCL	+++	SCL	OL	< OL	< OL	SCL	SCL	SCL	-	+	OL	< OL
34	4a	0	SCL	SCL	OL	< CL	SCL	SCL	±±±	< OL	++	SCL	SCL	SCL	0	0	0	SCL

Strain E8		Phages reactions at Routine Test Dilution (*S.* Enteritidis)																
Lab code	**Phage type**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
PHE	**6a**	**-**	**OL**	**-**	**SCL**	**-**	**SCL**	**-**	**-**	**OL**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**OL**
3	6a	-	SCL	-	OL	-	SCL	-	-	< OL	-	-	-	-	-	-	-	< OL
9	6a	-	SCL	-	SCL	-	SCL	-	-	OL	-	-	-	-	-	-	-	OL
12	6a	-	< CL	-	OL	-	SCL	-	-	OL	-	-	-	-	-	0	-	OL
18	6A	-	+++	-	+++	-	SCL	-	-	<SCL	-	-	-	-	-	-	-	SCL
26	PT 6a	-	SCL	-	SCL	-	SCL	-	-	OL	-	-	-	-	-	-	-	OL
27	PT6A	-	SCL	-	SCL	-	+++	-	-	OL	-	-	-	-	-	-	-	< OL
34	6a	0	SCL	0	OL	0	+++	0	0	< OL	0	0	0	0	0	0	0	< OL

Strain E9		Phages reactions at Routine Test Dilution (*S.* Enteritidis)																
Lab code	**Phage type**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
PHE	**6**	**-**	**SCL**	**-**	**SCL**	**-**	**SCL**	**-**	**OL**	**< OL**	**OL**	**-**	**-**	**-**	**-**	**-**	**-**	**< OL**
3	6	-	SCL	-	< OL	-	SCL	-	OL	< OL	OL	-	-	-	-	-	-	< OL
9	6	-	SCL	-	SCL	-	SCL	-	OL	OL	OL	-	-	-	-	-	-	OL
12	6	-	< CL	-	OL	-	SCL	-	OL	< OL	OL	-	-	-	-	0	-	< OL
18	6	-	+++	-	+++	-	SCL	-	OL	<SCL	SCL	-	-	-	-	-	-	SCL
26	PT 6	-	SCL	-	SCL	-	SCL	-	OL	OL	OL	-	-	-	-	-	-	OL
27	PT6	-	SCL	-	SCL	-	+++	-	OL	< OL	OL	-	-	-	-	-	-	< OL
34	6	0	SCL	0	OL	0	+++	0	SCL	< OL	SCL	0	0	0	0	0	0	SCL

Strain E10		Phages reactions at Routine Test Dilution (*S.* Enteritidis)																
Lab code	**Phage type**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
PHE	**1b**	**< CL**	**SCL**	**CL**	**< OL**	**CL**	**SCL**	**CL**	**OL**	**< OL**	**SCL**	**CL**	**CL**	**CL**	**< CL**	**CL**	**CL**	**SCL**
3	1b	OL	SCL	CL	< OL	CL	SCL	CL	OL	< OL	< OL	CL	CL	CL	CL	CL	CL	< OL
9	1b	OL	SCL	CL	OL	CL	SCL	CL	OL	OL	OL	CL	CL	CL	CL	OL	OL	OL
12	1b	< CL	CL	CL	OL	CL	SCL	CL	OL	OL	< OL	< CL	CL	CL	CL	0	CL	OL
18	1B	<SCL	<SCL	CL	+++	CL	SCL	SCL	SCL	SCL	+++	+++	CL	SCL	SCL	<SCL	+++	<SCL
26	PT 1b	OL	SCL	CL	OL	CL	SCL	CL	OL	OL	OL	CL	CL	CL	CL	SCL	CL	OL
27	PT1B	+++	SCL	CL	SCL	CL	+++	SCL	OL	SCL	< OL	SCL	CL	SCL	SCL	SCL	SCL	< OL
34	1b	SCL	+++	SCL	+++	< CL	+++	<SCL	SCL	< OL	< OL	SCL	SCL	SCL	SCL	SCL	< OL	OL

Annex 6 Phage typing results per *S*. Typhimurium strain for all participating laboratories

Strain T1		Phages 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**
PHE	**195**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
3	195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	DT 195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	DT195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	195	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Strain T1		Phages 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**	**10 var 2**	**10 var 3**	**18**
PHE	**195**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**SCL**	**-**	**-**	**-**	**-**
3	195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+++	1 – 5	+	-	-
9	195	-	-	-	-	-	-	-	-	-	-	-	-	-	±	SCL	-	-	-	-
12	195	-	-	-	-	-	-	-	-	-	-	-	-	1 – 5	-	< CL	±	1 – 5	-	-
18	195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	SCL	-	+	-	-
26	DT 195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-
27	DT195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	±±	±	1 – 5	-
34	195	0	0	0	0	0	0	0	0	0	0	0	0	0	0	SCL	±	±	±	0

Strain T2		Phages 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**
PHE	**104**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**SCL**	**SCL**	**-**	**-**	**-**	**-**	**+++**	**-**
3	U302	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	104	-	-	-	-	-	-	-	-	-	-	++	++	-	-	-	-	+	-
12	104b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-
18	104B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<SCL	-
26	DT 104	-	-	-	-	-	-	-	-	-	-	++	< CL	-	-	-	-	+++	-
27	DT104L	-	-	-	-	-	-	-	-	-	-	SCL	SCL	-	-	-	-	+++	-
34	104L	0	0	0	0	0	0	0	0	0	0	++	++	0	0	0	0	SCL	0

Strain T2		Phages 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**	**10 var 2**	**10 var 3**	**18**
PHE	**104**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**OL**	**OL**	**OL**	**-**
3	U302	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< OL	OL	< OL	-
9	104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	OL	SCL
12	104b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	OL	< OL	-
18	104B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	OL	OL	-
26	DT 104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	OL	< OL	-
27	DT104L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+++	OL	OL	-
34	104L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	OL	OL	OL	0

Strain T3		Phages 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**
PHE	**8**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**SCL**	**SCL**	**< CL**	**-**	**-**	**-**	**-**	**+++**	**-**	**-**	**-**
3	8	-	-	-	-	-	-	-	CL	SCL	<SCL	-	-	-	-	<SCL	-	-	-
9	8	-	-	-	-	-	-	-	CL	SCL	++	-	-	-	-	+++	-	-	-
12	8	-	-	-	-	-	-	-	+++	+++	+++	-	-	-	-	1 – 5	-	-	-
18	8	-	-	-	-	-	-	-	++	+++	<SCL	-	-	-	-	<SCL	-	-	-
26	DT 8	-	-	-	-	-	-	-	SCL	SCL	+++	-	-	-	-	+++	-	-	-
27	DT8	-	-	-	-	-	-	-	SCL	SCL	SCL	-	-	-	-	SCL	-	-	-
34	8	0	0	0	0	0	0	0	< CL	+++	±±±	0	0	0	0	±±±	0	0	0

Strain T3		Phages 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**	**10 var 2**	**10 var 3**	**18**
PHE	**8**	**SCL**	**-**	**SCL**	**SCL**	**-**	**±**	**±**	**-**	**-**	**CL**	**CL**	**-**	**++**	**+**	**++**	**OL**	**OL**	**OL**	**-**
3	8	SCL	-	SCL	SCL	-	+	-	-	-	CL	CL	-	1 – 5	+	-	< OL	OL	< OL	-
9	8	SCL	-	SCL	SCL	-	±	±	-	-	CL	CL	-	-	-	-	-	OL	OL	SCL
12	8	+	-	++	++	-	±	±	-	-	< CL	SCL	-	±	-	+	OL	OL	< OL	-
18	8	+++	-	+++	SCL	-	-	-	-	-	CL	CL	-	-	±	++	OL	OL	OL	-
26	DT 8	SCL	-	SCL	SCL	-	-	±	-	-	CL	SCL	-	-	-	-	OL	OL	< OL	-
27	DT8	+++	-	±±	±±	-	-	-	-	-	SCL	±±±	-	0	0	0	0	0	0	0
34	8	SCL	0	<SCL	<SCL	0	±	±	0	0	< CL	< CL	0	+	±	+++	OL	OL	OL	0

Strain T4		Phages 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**
PHE	**7a**	**-**	**-**	**-**	**-**	**-**	**-**	**< CL**	**±**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**CL**	**-**
3	7a	-	-	-	-	-	-	<SCL	-	-	-	-	-	-	-	-	-	CL	-
9	7	-	-	-	-	-	-	CL	±	-	-	-	-	-	-	-	-	CL	-
12	7	-	-	-	-	-	-	++	1 – 5	-	-	-	-	-	-	-	-	CL	-
18	7A	-	-	-	-	-	-	<SCL	-	-	-	-	-	-	-	-	-	SCL	-
26	DT 7a	-	-	-	-	-	-	+++	++	-	-	-	-	-	-	-	-	CL	-
27	DT7a	-	-	-	-	-	-	+++	1 – 5	-	-	-	±	-	-	-	-	CL	-
34	7a	0	0	0	0	0	0	< CL	0	0	0	0	0	0	0	0	0	< CL	0

Strain T4		Phages 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**	**10 var 2**	**10 var 3**	**18**
PHE	**7a**	**<SCL**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**SCL**	**SCL**	**-**	**+++**	**+++**	**+++**	**OL**	**OL**	**OL**	**-**
3	7a	SCL	-	-	-	-	-	-	-	-	<SCL	CL	-	+	++	±	< OL	OL	< OL	-
9	7	SCL	-	-	-	-	-	-	-	-	-	SCL	-	±	±	±	-	OL	OL	SCL
12	7	+	-	-	-	-	-	-	-	-	++	< CL	-	++	-	+++	OL	OL	< OL	-
18	7A	±±±	-	-	-	-	-	-	-	-	<SCL	CL	-	-	+++	+++	OL	OL	OL	-
26	DT 7a	SCL	-	-	-	-	-	-	-	-	SCL	SCL	-	-	-	-	OL	OL	< OL	-
27	DT7a	+++	-	-	-	-	-	-	-	-	+++	<SCL	-	0	0	0	0	0	0	0
34	7a	SCL	0	0	0	0	0	0	0	0	SCL	<SCL	0	++	++	+++	OL	OL	OL	0

Strain T5		Phages 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**
PHE	**120**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**< CL**	**-**
3	120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<SCL	-
9	120	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	SCL	-
12	120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CL	-
18	120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< CL	-
26	DT 104B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	SCL	-
27	DT120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	SCL	-
34	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	< CL	0

Strain T5		Phages 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**	**10 var 2**	**10 var 3**	**18**
PHE	**120**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**++**	**+**	**++**	**OL**	**OL**	**OL**	**-**
3	120	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	< OL	OL	< OL	-
9	120	-	-	-	-	-	-	-	-	-	-	-	-	±	±	±	-	OL	OL	OL
12	120	-	-	-	-	-	-	-	-	-	-	-	-	+	1 – 5	++	OL	OL	< OL	-
18	120	-	-	-	-	-	-	-	-	-	-	-	-	-	++	+++	OL	OL	OL	-
26	DT 104B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	OL	< OL	-
27	DT120	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0
34	120	0	0	0	0	0	0	0	0	0	0	0	0	+	++	+++	OL	OL	OL	0

Strain T6		Phages 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**
PHE	**193**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
3	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	DT 193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	DT193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	193	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Strain T6		Phages 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**	**10 var 2**	**10 var 3**	**18**
PHE	**193**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**SCL**	**SCL**	**SCL**	**-**	**-**	**-**	**-**
3	193	-	-	-	-	-	-	-	-	-	-	-	-	SCL	SCL	SCL	-	-	-	-
9	193	-	-	-	-	-	-	-	-	-	-	-	-	++	++	++	-	-	-	-
12	193	-	-	-	-	-	-	-	-	-	-	-	-	+++	±	< CL	< OL	++	1 – 5	-
18	193	-	-	-	-	-	-	-	-	-	-	-	-	+++	+++	SCL	+++	+++	+++	-
26	DT 193	-	-	-	-	-	-	-	-	-	-	-	-	+	±	+	OL	OL	OL	-
27	DT193	-	-	-	-	-	-	-	-	-	-	-	-	+	+	OL	OL	-	-	-
34	193	0	0	0	0	0	0	0	0	0	0	0	0	SCL	SCL	SCL	OL	OL	OL	0

Strain T7		Phages 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**
PHE	**40**	**CL**	**OL**	**CL**	**OL**	**CL**	**CL**	**CL**	**-**	**CL**	**CL**	**-**	**OL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**
3	40	SCL	CL	CL	CL	CL	CL	CL	-	CL	CL	-	CL	CL	CL	CL	CL	< CL	CL
9	40	SCL	CL	CL	CL	CL	CL	CL	-	CL	CL	-	CL	CL	CL	CL	CL	CL	CL
12	40	CL	CL	CL	OL	CL	CL	CL	-	CL	CL	-	CL	CL	CL	CL	CL	CL	CL
18	40	SCL	OL	CL	OL	SCL	SCL	SCL	-	<SCL	SCL	-	CL	CL	CL	CL	CL	CL	CL
26	DT 40	CL	CL	CL	CL	SCL	CL	CL	-	CL	CL	-	CL	CL	CL	CL	CL	CL	CL
27	DT40	OL	SCL	CL	CL	SCL	OL	OL	1 – 5	SCL	CL	-	OL	CL	CL	CL	SCL	CL	SCL
34	40	SCL	SCL	SCL	SCL	SCL	SCL	SCL	±	SCL	SCL	0	SCL	SCL	SCL	SCL	SCL	< CL	SCL

Strain T7		Phages 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**	**10 var 2**	**10 var 3**	**18**
PHE	**40**	**< CL**	**OL**	**OL**	**< CL**	**CL**	**CL**	**CL**	**CL**	**-**	**CL**	**CL**	**OL**	**++**	**++**	**++**	**OL**	**OL**	**OL**	**OL**
3	40	CL	CL	CL	CL	CL	CL	CL	CL	-	CL	CL	OL	1 – 5	+	-	OL	OL	< OL	CL
9	40	CL	CL	CL	CL	CL	CL	CL	CL	-	CL	CL	CL	+	±	±	CL	OL	OL	OL
12	40	SCL	CL	< CL	< CL	CL	CL	CL	CL	-	CL	< CL	OL	+	-	+	OL	OL	< OL	CL
18	40	<SCL	SCL	+++	SCL	SCL	+++	<SCL	SCL	-	CL	SCL	±±±	-	±	±±±	OL	OL	OL	CL
26	DT 40	CL	CL	SCL	SCL	CL	SCL	CL	CL	-	CL	SCL	CL	-	-	-	OL	OL	< OL	CL
27	DT40	SCL	+++	+++	+++	SCL	SCL	+++	SCL	-	SCL	+++	OL	0	0	0	0	0	0	0
34	40	SCL	+++	SCL	SCL	SCL	SCL	SCL	SCL	±	< CL	SCL	SCL	++	+++	SCL	OL	OL	OL	OL

Strain T8		Phages 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**
PHE	**36**	**CL**	**OL**	**CL**	**OL**	**CL**	**CL**	**CL**	**CL**	**< CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**
3	36	SCL	SCL	CL	CL	CL	CL	CL	CL	CL	CL	SCL	SCL	CL	CL	CL	CL	CL	CL
9	36	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL
12	36	< CL	< CL	CL	OL	CL	CL	< CL	CL	CL	CL	CL	< CL	CL	CL	< CL	< CL	CL	CL
18	36	SCL	CL	CL	OL	SCL	SCL	SCL	+++	+++	SCL	SCL	SCL	CL	CL	CL	CL	CL	SCL
26	DT 36	CL	< CL	CL	CL	SCL	CL	CL	CL	SCL	CL	SCL	CL	CL	CL	CL	CL	CL	CL
27	DT36	SCL	SCL	CL	CL	SCL	SCL	SCL	SCL	SCL	CL	SCL	SCL	CL	CL	CL	SCL	< CL	SCL
34	36	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	+++	++	SCL	SCL	SCL	SCL	< CL	SCL

Strain T8		Phages 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**	**10 var 2**	**10 var 3**	**18**
PHE	**36**	**< CL**	**OL**	**OL**	**< CL**	**CL**	**< CL**	**< CL**	**CL**	**CL**	**CL**	**CL**	**OL**	**+++**	**+++**	**+++**	**OL**	**OL**	**OL**	**OL**
3	36	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	OL	±	++	±	< OL	OL	< OL	CL
9	36	CL	CL	CL	CL	CL	CL	SCL	CL	CL	OL	CL	OL	±	±	±	OL	OL	OL	OL
12	36	SCL	CL	< OL	< CL	CL	CL	< OL	< CL	OL	CL	CL	OL	+++	-	+++	OL	OL	< OL	CL
18	36	+++	SCL	±±±	SCL	SCL	++	++	SCL	+++	CL	CL	-	++	+++	<SCL	OL	OL	OL	CL
26	DT 36	CL	CL	CL	CL	CL	< CL	CL	CL	CL	CL	CL	CL	-	-	-	OL	OL	< OL	CL
27	DT36	SCL	SCL	+++	SCL	SCL	SCL	SCL	SCL	SCL	SCL	<SCL	OL	0	0	0	0	0	0	0
34	36	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	< OL	SCL	< OL	++	+++	SCL	OL	OL	OL	OL

Strain T9		Phages 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**
PHE	**15a**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**OL**	**OL**	**OL**	**-**	**OL**	**-**	**OL**	**-**	**OL**
3	15a	-	-	-	-	-	-	-	-	-	OL	< OL	< OL	-	OL	-	< OL	-	< OL
9	46	-	CL	CL	CL	CL	CL	-	-	CL	CL	CL	CL	CL	CL	CL	CL	-	CL
12	15a	-	-	-	-	-	-	-	-	-	OL	CL	SCL	-	CL	-	< CL	-	SCL
18	15A	-	-	-	-	-	-	-	-	-	SCL	SCL	SCL	-	+++	-	SCL	-	+++
26	DT 15a	-	-	-	-	-	-	-	-	-	SCL	CL	SCL	-	SCL	-	SCL	-	CL
27	DT15a	-	-	-	-	-	-	-	-	-	CL	CL	CL	-	SCL	-	CL	-	+++
34	15	0	0	0	0	0	0	0	0	0	<SCL	SCL	<SCL	0	< OL	0	SCL	< OL	SCL

Strain T9		Phages 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**	**10 var 2**	**10 var 3**	**18**
PHE	**15a**	**OL**	**-**	**-**	**-**	**-**	**-**	**-**	**±**	**-**	**-**	**OL**	**±**	**+++**	**+++**	**+++**	**OL**	**OL**	**OL**	**-**
3	15a	< OL	-	-	-	-	-	-	1 – 5	1 – 5	-	OL	++	++	+++	++	OL	OL	< OL	-
9	46	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	±	±	±	CL	OL	OL	OL
12	15a	SCL	-	-	-	-	-	-	-	-	-	OL	-	+++	+	+++	OL	< OL	< OL	-
18	15A	±±±	-	-	-	-	-	-	-	-	-	SCL	-	++	+++	<SCL	OL	OL	OL	-
26	DT 15a	SCL	-	-	-	-	-	-	-	-	-	SCL	+	-	-	-	OL	OL	< OL	-
27	DT15a	+++	-	-	-	-	-	-	-	-	-	SCL	-	0	0	0	0	0	0	0
34	15	SCL	0	0	0	0	0	0	+	±	0	SCL	0	+	++	+++	OL	OL	OL	0

Strain T10		Phages 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**
PHE	**135**	**-**	**++**	**+++**	**SCL**	**SCL**	**< CL**	**-**	**-**	**SCL**	**< OL**	**CL**	**CL**	**+**	**< CL**	**< CL**	**CL**	**-**	**+++**
3	135	-	+	++	<SCL	<SCL	< CL	-	-	SCL	< OL	CL	CL	< OL	< OL	< CL	< CL	-	++
9	2	-	CL	CL	CL	CL	CL	-	-	CL	CL	CL	CL	CL	CL	CL	CL	-	CL
12	2	-	SCL	CL	OL	< CL	< CL	-	-	< CL	< CL	OL	OL	< CL	CL	< CL	< CL	-	< CL
18	135	-	<SCL	< CL	SCL	++	SCL	-	-	++	SCL	CL	CL	SCL	SCL	SCL	CL	-	+++
26	DT 2	-	< CL	SCL	CL	< CL	CL	-	-	SCL	SCL	CL	CL	CL	CL	CL	CL	-	CL
27	DT2	-	SCL	SCL	SCL	SCL	SCL	-	-	SCL	SCL	CL	CL	SCL	SCL	SCL	SCL	-	SCL
34	135	0	+++	<SCL	SCL	+++	SCL	0	0	SCL	SCL	SCL	SCL	+++	SCL	SCL	SCL	0	<SCL

Strain T10		Phages 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**	**10 var 2**	**10 var 3**	**18**
PHE	**135**	**+**	**+**	**+**	**SCL**	**+**	**+**	**+**	**SCL**	**-**	**CL**	**< CL**	**OL**	**++**	**++**	**+++**	**< OL**	**++**	**++**	**CL**
3	135	+++	+++	++	SCL	+	<SCL	±	<SCL	1 – 5	CL	<SCL	<SCL	-	-	-	-	-	-	++
9	2	CL	CL	CL	CL	CL	CL	CL	CL	-	CL	CL	CL	-	-	-	SCL	±	-	-
12	2	+	SCL	+	SCL	<SCL	< CL	++	SCL	-	CL	< CL	OL	++	1 – 5	+++	OL	< OL	+	< CL
18	135	±±±	++	-	SCL	+++	-	-	++	-	CL	SCL	±	-	++	+++	OL	<SCL	<SCL	SCL
26	DT 2	SCL	SCL	< CL	SCL	SCL	< CL	SCL	SCL	-	CL	SCL	CL	-	-	-	OL	OL	< OL	CL
27	DT2	SCL	SCL	±	SCL	SCL	+++	+++	+++	-	SCL	<SCL	OL	0	0	0	0	0	0	0
34	135	SCL	+	+	SCL	+++	+++	±±±	SCL	±	SCL	++	SCL	+	++	+++	OL	OL	OL	SCL

Annex 7 Examples of PFGE images obtained by the participants

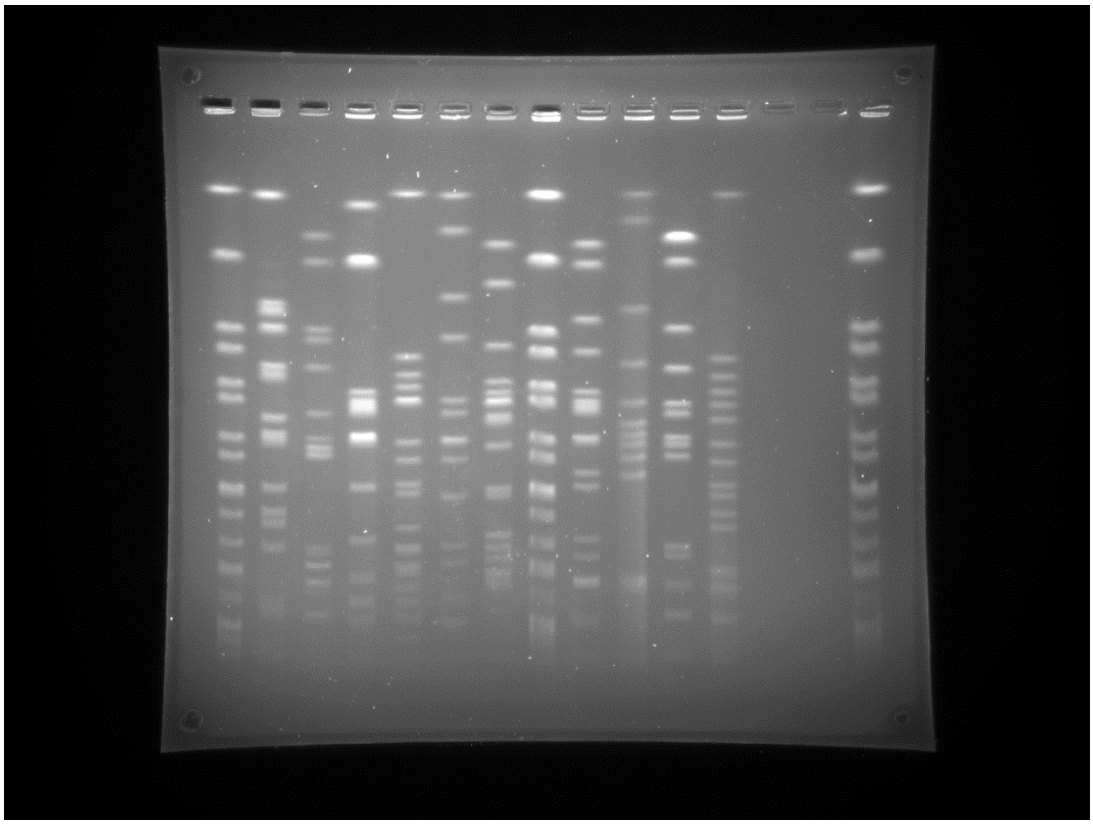


Figure A7.1. Example of a gel (Labcode 2) with a generally lower score

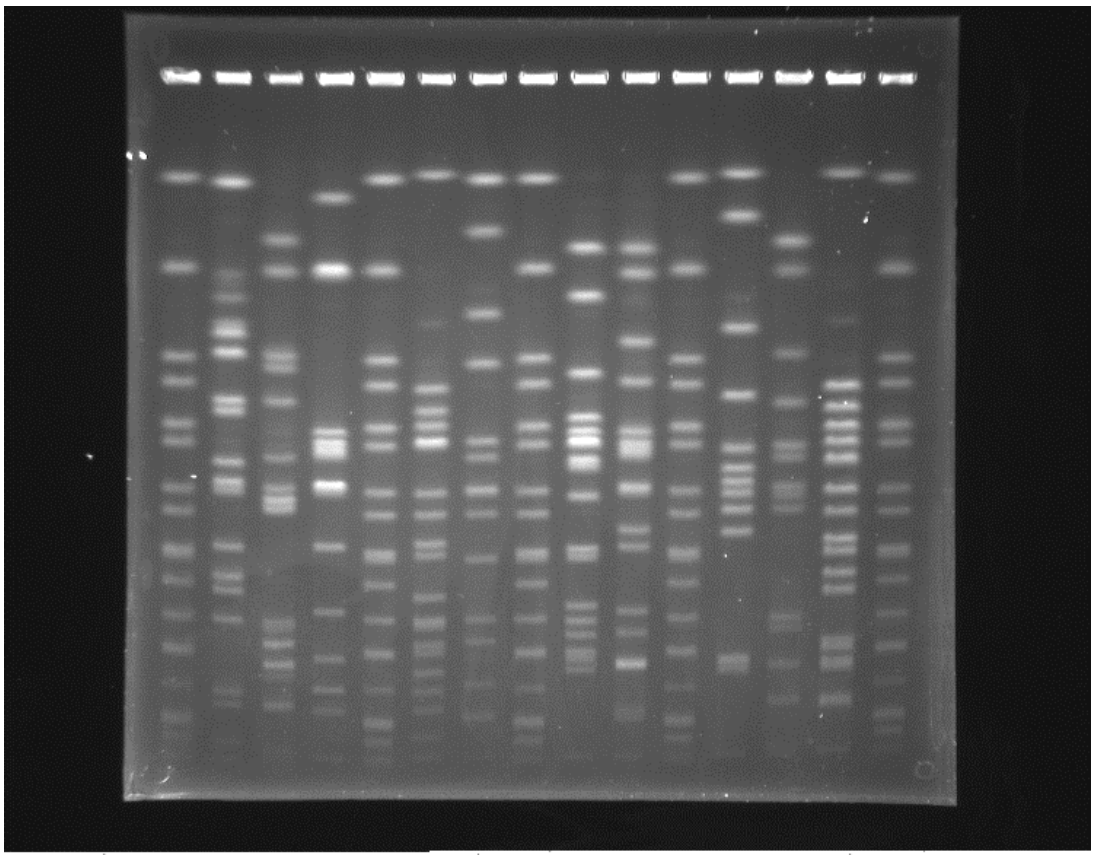


Figure A7.2. Example of a gel (Labcode 34) with a generally higher score

Annex 8 Example of an individual laboratory evaluation report on PFGE typing results

Individual Laboratory Results Interlaboratory Comparison Study *Salmonella* PFGE typing (pilot November 2013)

NRL Laboratory code: **2**

As this was the first time a PFGE typing study was included as a pilot in the EURL-*Salmonella* interlaboratory comparison study on typing of *Salmonella*, participants were asked to test these 10 strains using their own routine PFGE method.
Participants were requested to email their PFGE images as a TIFF file to the EURL-*Salmonella* and to be sure to include at least their laboratory code in the name of these .tif files.

The evaluation of the PFGE typing results was done on the quality of the PFGE images only. This quality grading was done according to the PulseNet guidelines (www.pulsenetinternational.org and attached as pdf: PNQ01 PulseNet US protocol PFGE image quality assessment.pdf).
These guidelines use 7 parameters, which are scored with 1 (poor) to 4 (excellent) points. In general, an acceptable quality should be obtained for each parameter since a low quality score in just one category can have a high impact on the ability to further analyse the image and compare to other profiles.

Your individual laboratory results are given in Table 1.
Overall results for all 20 participants in this pilot PFGE study will be discussed at the Workshop in May 2014, and will be reported in the final report on the *Salmonella* typing study XVIII (2013).
In Figure 1, your own laboratory PFGE profiles are compared to the reference profiles (in this study obtained from the NRL Austria).

General comments:

Your .tif file ~~did~~/did not include your laboratory code in its name.

The use of the *S*. Braenderup H9812 standard was deviating;
Ususally, this reference strain has to be placed every 6 lanes at least.
It is also advised to place the last reference strain directly next to the last test strain (instead of having two empty lanes in-between).

Table 1. Individual results evaluation tif file according to PulseNet guidelines

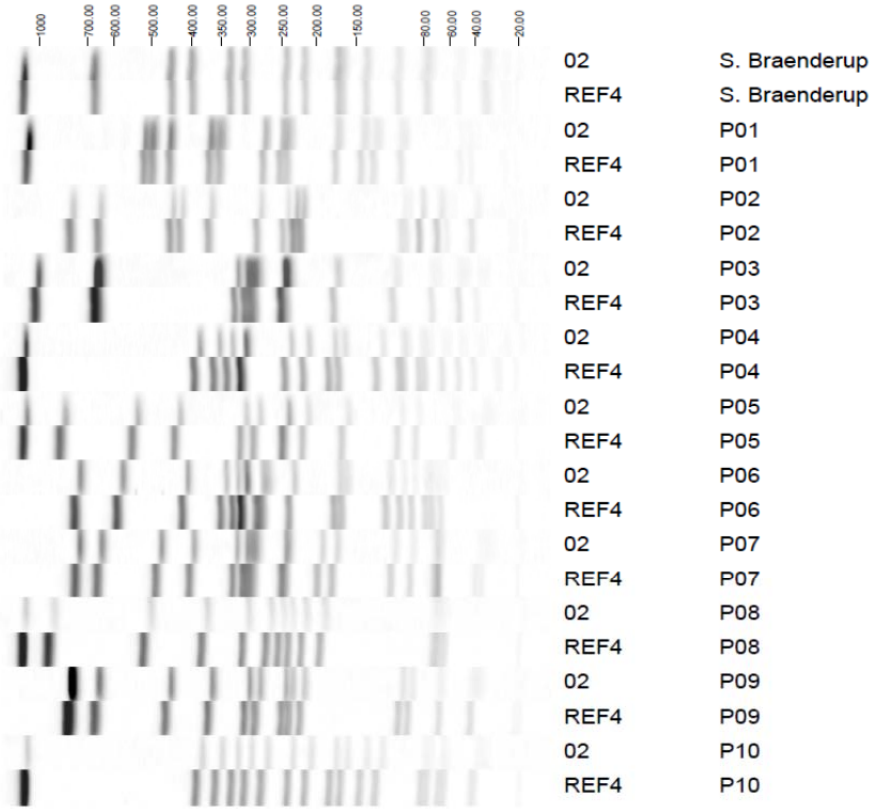
Parameter	Evaluation	Comments	Points
Image Acquisition and Running Conditions	Poor	Gel does not fill whole TIFF, Deviation in the use of standards	1
Cell Suspension	Excellent	The cell concentration is approximately the same in each lane	4
Bands	Poor	Many fuzzy bands, especially at the bottom of the gel	1
Lanes	Excellent	Straight	4
Restriction	Poor	More than 1 lane with shadow bands	1
Gel Background	Excellent	Clear	4
DNA Degradation (smearing in the lanes)	Good	Minor background (smearing) in 1 lane	3
Total score:			18

* 1=Poor, 2=Fair, 3= Good, 4= Excellent
At maximum 4 points per parameter

EURL-*Salmonella*, Bilthoven, The Netherlands Page 1 of 2

Individual Laboratory Results Interlaboratory Comparison Study *Salmonella* PFGE typing (pilot November 2013)

Figure 1. Comparison of your PFGE profiles with the reference profiles



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