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

Nineteenth EURL-*Salmonella* interlaboratory comparison study (2014) on typing of *Salmonella* spp.

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Colophon

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Publiekssamenvatting

Negentiende EURL-*Salmonella* ringonderzoek (2014) voor de typering van *Salmonella* spp.

De Nationale Referentie Laboratoria (NRL's) van de 28 Europese lidstaten scoorden in 2014 goed bij de kwaliteitscontrole op *Salmonella*-typering. Eén laboratorium had hiervoor een herkansing nodig. Uit de analyse van alle NRL's als groep bleek dat de laboratoria aan 96% 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 om *Salmonella* in monsters van levensmiddelen of dieren aan te tonen en te typeren. Om te controleren of de laboratoria hun werk goed uitvoeren moeten zij onder andere 20 *Salmonella*-stammen op juiste wijze identificeren. Soms doen ook landen van buiten de Europese Unie vrijwillig mee. In 2014 waren dat de kandidaat-lidstaten Macedonië, Servië en Turkije, en de EFTA-landen IJsland, Noorwegen en Zwitserland. 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% van de *S.* Typhimurium-stammen en eveneens 83% van de *S.* Enteritidis-stammen op de juiste wijze.

De organisatie van het ringonderzoek is in handen van het Europese Unie Referentie Laboratorium (EURL) voor *Salmonella* (EURL-*Salmonella*), dat is ondergebracht bij het RIVM in Nederland. De organisatie van het faagtyperingsringonderzoek is uitgevoerd in samenwerking met Public Health England in Londen.

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

Synopsis

Nineteenth EURL-*Salmonella* interlaboratory comparison study (2014) on typing of *Salmonella* spp.

The National Reference Laboratories (NRLs) of all 28 European Union (EU) Member States performed well in the 2014 quality control test on *Salmonella* typing. One laboratory was found to require a follow-up study after the initial test. Overall, the EU-NRLs were able to assign the correct name to 96% 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 in animals and/or food products. These laboratories are then referred to as the National Reference Laboratories. The performance of these NRLs in *Salmonella* typing is assessed annually by testing their ability to 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, Serbia and Turkey, and EFTA countries Iceland, Norway and Switzerland took part in the 2014 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 10 strains of *Salmonella* Enteritidis and 10 strains of *Salmonella* Typhimurium. These NRLs correctly typed 83% of the *S*. Enteritidis strains and also 83% of the *S*. Typhimurium strains.

The European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organises this annual interlaboratory comparison study on the 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 2014, the 19th interlaboratory comparison study on the 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 the typing of *Salmonella* strains by the National Reference Laboratories (NRLs-*Salmonella*) within the European Union was carried out uniformly, and whether comparable results were being 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, Serbia and Turkey, and the EFTA countries Iceland, Norway and Switzerland.

All 35 laboratories performed serotyping. A total of 20 obligatory *Salmonella* strains plus 1 optional *Salmonella* strain were selected by the EURL-*Salmonella* for serotyping. The strains had to be typed according to 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, 97% of the strains were typed correctly for the O-antigens, 94% of the strains were typed correctly for the H-antigens and 94% of the strains were correctly named by the participants.
At the EURL-*Salmonella* Workshop in 2007, the EURL-*Salmonella* proposed a definition of good performance by NRLs with regard to serotyping. Using this definition, 33 participants achieved good performance. The two laboratories that did not achieve the level of good performance were offered a follow-up study including 10 additional strains for serotyping. This follow-up study is obligatory for NRLs of EU Member States, and the EU-NRL concerned obtained good scores in the follow-up study.

Seven of the participating NRLs-*Salmonella* also performed phage typing of both *S*. Enteritidis and *S*. Typhimurium. Public Health England selected 20 strains for phage typing: 10 of the *Salmonella* serovar Enteritidis and 10 of the serovar Typhimurium. The overall results were satisfactory. The seven NRLs phage correctly typed 83% of both serovars.

Eighteen laboratories participated in the optional second study on PFGE typing. PFGE results were evaluated on the quality of the images in accordance with the PulseNet International Guidelines. The quality of the PFGE results was promising, though there was quite some variation in results between the participants. Some simple adjustments should improve the results.

1 Introduction

This report describes the 19th 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 2014.

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 the typing of *Salmonella* strains are that the typing should be carried out uniformly in all Member States, and that comparable results should be obtained. The implementation of typing studies started in 1995.

A total of 35 laboratories participated in this study. These included 29 NRLs-*Salmonella* in the 28 EU Member States, 3 NRLs of EU-candidate countries and 3 NRLs of EFTA countries. The main objective of this study was to check the performance of the NRLs in the typing of *Salmonella* spp. and to compare the results of the typing of *Salmonella* spp. among the NRLs-*Salmonella*. All NRLs performed serotyping of the 20 obligatory strains and all but three of the participants serotyped the optional 21st strain. Any NRLs of EU Member States that 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 to be serotyped.

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

For the second time, the typing study also included PFGE typing. Eighteen NRLs participated in this part of the study by PFGE typing 10 designated *Salmonella* strains and submitting images for evaluation.

2 Participants

Country	City	Institute
Austria	Graz	IMED Graz/AGES
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	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	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	Lisbon	INIAV-Instituto Nacional de Investigação Agrária e Veterinária
Romania	Bucharest	Institute for Diagnosis and Animal Health, Bacteriology Department

Country	City	Institute
Serbia	Belgrade	Laboratory for Bacteriology
Slovak Republic	Bratislava	State Veterinary and Food Institute
Slovenia	Ljubljana	UL, Veterinary Faculty
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
United Kingdom	Addlestone	Animal and Plant Health Agency (APHA)
United Kingdom	Belfast	Agri Food & Biosciences Institute

3 Materials and methods

3.1 *Salmonella* strains for serotyping

A total of 20 *Salmonella* strains (coded S1–S20) had to be serotyped by the participants. As agreed at the 19th EURL-*Salmonella* Workshop in Zaandam (Mooijman, 2014), 1 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 verified by the Centre before distribution. The complete antigenic formulas of the 21 serovars, in accordance with the most recent White-Kauffmann-Le Minor scheme (Grimont & Weill, 2007), 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-Le Minor scheme used in the 19th EURL-*Salmonella *typing study*

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
S1	1,3,19	i	z_6	Taksony
S2	1,4,[5],12	r	l,w	Bochum
S3	13,23	r	1,6	Adjame
S4	18	m,t	-	Langenhorn
S5	8,20	z_4,z_{24}	-	Albany
S6	3,{10},{15}	b	e,n,z_{15}	Yaba
S7	13,23	b	1,6	Bracknell
S8	4,[5],12	a	1,7	Arechavaleta
S9	6,8	z_{10}	e,n,x	Hadar
S10	6,7,14	r	1,5	Infantis
S11	6,7,14	r	1,2	Virchow
S12	1,9,12[Vi]	g,p	-	Dublin
S13	6,8	d	e,n,z_{15}	Herston
S14	6,7,14	l,v	e,n,z_{15}	Potsdam
S15	1,3,19	g,[s],t	-	Senftenberg
S16	1,4,[5],12	i	1,2	Typhimurium
S17[a)]	1,4,[5],12	i	-	1,4,[5],12:i:-
S18	1,9,12	g,m	-	Enteritidis
S19	1,4,[5],12	f,g,s	[1,2]	Agona
S20	3,{10}{15}{15,34}	e,h	1,5	Muenster
S21[b)]	41	z_4,z_{23}	-	41:z_4,z_{23}:-

[a)] Typhimurium, monophasic variant as determined by PCR.
[b)] *Salmonella enterica* subspecies *arizonae*.

3.2 Laboratory codes

Each NRL-*Salmonella* was assigned a laboratory code between 1 and 35, which differed from its codes in previous typing studies.

3.3 Protocol and test report

Two weeks before the start of the study, the NRLs received the protocol by email. As in 2013, the study used web-based test report forms: a combined form for serotyping/phage typing and a separate form for PFGE typing. Instructions for the completion of these test report forms and the entering of data were sent to the NRLs in week 45, 2014.
The protocol and test report forms can be found on the EURL-*Salmonella* website:
http://www.eurlsalmonella.eu/Proficiency_testing/Typing_studies

3.4 Transport

The parcels containing the strains for serotyping, phage typing and PFGE typing were sent by the EURL-*Salmonella* in week 45, 2014. All samples were packed and transported as Biological Substance Category B (UN 3373) and transported by door-to-door courier service.

3.5 Guidelines for evaluation

The evaluation of the various serotyping errors 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
Incomplete set of antisera or Part of the formula (for the name of the serovar) or No serovar name	Partly correct
Wrong serovar or Mixed sera formula	Incorrect

At the EURL-*Salmonella* Workshop in Bilthoven in May 2007 (Mooijman, 2007), the EURL-*Salmonella* made a proposal for the level of performance that the NRLs need to achieve during an interlaboratory comparison study on serotyping – i.e. a definition of good performance – based on a system of penalty points. Penalty points are given for the incorrect typing of strains, but 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 calculated for each NRL-*Salmonella*, which meets the criterion of good performance if it has fewer than four penalty points.
A follow-up study is organised for NRLs with four penalty points or more. All NRLs of EU Member States not meeting the criterion of good performance must 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 selected 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 10 Salmonella *strains according to the White-Kauffmann-Le Minor scheme used in the follow-up part of the 19th EURL-*Salmonella *typing study*

Strain	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
SF-1	6,8	z_{10}	e,n,x	Hadar
SF-2	6,7,14	r	1,5	Infantis
SF-3	6,7,14	r	1,2	Virchow
SF-4	1,4,[5],12	i	1,2	Typhimurium
SF-5	1,9,12	g,m	-	Enteritidis
SF-6	6,8	b	1,6	Stourbridge
SF-7	1,4,[5],12,27	z_4,z_{23}	[1,2]	Stanleyville
SF-8	6,8,20	r, [i]	1,5	Bovismorbificans
SF-9	3,{10}{15}{15,34}	y	1,5	Orion
SF-10	4,12	i	1,6	Agama

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
RDNC	Reacts Does Not Conform
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 19th 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	1b	< CL	SCL	CL	< OL	CL	SCL	CL	< OL	< OL	SCL	CL	CL	CL	< CL	CL	CL	SCL
E2	14b	-	-	-	-	-	SCL	-	-	-	-	-	-	-	-	-	-	SCL
E3	4	-	CL	CL	< OL	CL	SCL	CL	OL	< OL	< OL	CL	CL	CL	-	-	-	SCL
E4	3	OL	-	-	-	-	+	-	OL	-	OL	-	-	-	< CL	-	-	-
E5	33	-	-	-	-	-	-	-	-	-	-	-	-	-	< CL	-	-	-
E6	59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	SCL
E7	35	-	SCL	-	SCL	-	-	-	-	< OL	-	-	-	-	-	-	-	< OL
E8	13a	-	-	-	OL	-	SCL	-	OL	< OL	OL	-	-	-	-	-	-	< OL
E9	8	-	-	CL	OL	CL	SCL	CL	OL	< OL	OL	CL	CL	-	-	-	-	< OL
E10	56	-	-	CL	< OL	SCL	-	SCL	-	< OL	-	+	+	-	-	-	-	< OL

Table 5. Phage reactions of the Salmonella *Typhimurium strains used in the 19th 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	36	CL	CL	CL	OL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL
T2	104	-	-	-	-	-	-	-	-	-	-	SCL	SCL	-	-	-	-	+++	-
T3	U311	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T4	8	-	-	-	-	-	-	-	SCL	CL	SCL	-	-	-	-	+++	-	-	-
T5	41	< OL	OL	OL	OL	OL	CL	OL	-	OL	OL	-	-	OL	OL	OL	OL	< CL	OL
T6	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T7	1	CL	CL	CL	OL	CL	CL	CL	-	CL	OL	OL	OL	CL	OL	CL	OL	CL	CL
T8	10	-	-	-	-	-	-	-	-	CL	CL	CL	CL	-	-	SCL	-	-	-
T9	132	-	CL	CL	OL	CL	++	-	-	SCL	< CL	CL	CL	CL	CL	CL	CL	-	CL
T10	136	-	-	-	OL	CL	CL	-	-	-	CL	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	36	< CL	CL	CL	CL	CL	CL	CL	CL	OL	CL	CL	OL	+++	+++	+++	OL	OL	OL	CL
T2	104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	OL	OL	-
T3	U311	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	-
T4	8	SCL	-	< OL	< CL	-	+	+	-	-	CL	CL	-	+	+	+	< CL	CL	SCL	-
T5	41	+++	OL	OL	OL	OL	OL	OL	OL	-	OL	OL	OL	+++	++	+++	OL	OL	OL	+++
T6	193	-	-	-	-	-	-	-	-	-	-	-	-	+++	+++	+++	-	-	-	-
T7	1	< CL	OL	OL	CL	CL	CL	CL	CL	-	CL	CL	OL	-	-	-	OL	OL	OL	OL
T8	10	< CL	-	OL	CL	-	SCL	SCL	-	-	CL	CL	-	+++	+++	+++	OL	OL	OL	-
T9	132	+	< CL	OL	SCL	< CL	CL	CL	CL	-	CL	CL	OL	+++	+++	SCL	-	-	+	OL
T10	136	+	-	-	-	-	CL	-	-	-	-	-	-	++	+	+++	OL	OL	OL	-

3.8 *Salmonella* strains for PFGE typing

A total of 10 *Salmonella* strains (coded P1–P10) were included in the pilot study on PFGE typing.
After consultation with the Statens Serum Institut (SSI), Copenhagen, Denmark, the same strains were used as in the External Quality Assessment EQA-5 on PFGE typing, organised by the SSI for the Food- and Water-borne Diseases and Zoonoses Laboratories Network (FWD laboratories network) (ECDC, 2014). Background information on the strains is given in Table 6.

Table 6. Background information on the Salmonella *strains used for PFGE typing in 2014*

Strain code in pilot 2014 (EURL-*Salmonella*)	Strain code in EQA-5 (ECDC, 2014)	*Salmonella* serovar
P1	Salm 10	Strathcona
P2	Salm 7	1,4,5,12:i:-
P3	Salm 2	Infantis
P4	Salm 6	Montevideo
P5	Salm 9	Stanley
P6	Salm 5	Rough strain
P7	Salm 3	Enteritidis
P8	Salm 4	Kentucky
P9	Salm 1	Enteritidis
P10	Salm 8	Poona

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 details of the method in the electronic test report. The PFGE gel images were to be emailed as Tagged Image File Format (TIFF) files to the EURL-*Salmonella*, and had to include the laboratory code in the filename.
Evaluation of the PFGE results was based on the quality of the PFGE images only, and not (yet) on gel analysis using BioNumerics. Quality was assessed on seven parameters in accordance with the PulseNet guidelines (www.pulsenetinternational.org), as given in Annex 1. Each parameter is given a score of up to 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 the test reports of the interlaboratory comparison study (for serotyping and phage typing, and for 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. One participant reported a missing cap on one of the tubes. No explanation was found, but it did not have an impact on the final results.

Question 3: Date of receipt at your laboratory:
All but three participants received their package in the same week it was sent (week 45 of 2014). The remaining parcels were delivered in week 46.

Question 4: Medium 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 the most commonly used medium.

4.3 Questions serotyping

Question 5: What was the frequency of serotyping of Salmonella *at your laboratory in 2013?*

Question 6: How many Salmonella strains did your laboratory (approximately) serotype in 2013?
The replies to questions 5 and 6 are summarised in Table 7.

Table 7. Frequency and number of strains serotyped, and number of strains phage typed and/or PFGE typed (for all 35 NRLs)

Lab code	Serotyping frequency in 2013	No. of strains serotyped in 2013	No. of strains phage typed in 2013	No. of strains PFGE typed in 2014
9	Once a week	60		
4	Daily	100		50
28	Twice a week	130		
11	Thrice a week	148		
34	Once a week	155		
19	Daily	180		50
27	Daily	191		
16	Weekly	200		10
23	Twice a week	200		17
22	Weekly	212		
33	Daily	221		
7	Thrice a week	251		
30	Thrice a week	285		
2	Thrice a week	300		
8	Thrice a week	325		50
17	Daily	476	766	
3	Daily	500		
24	Daily	500		
31	Daily	500		
18	Daily	514		
35	Twice a week	650		36
32	Daily	700		
20	Daily	1000		
25	Daily	1325		6
10	Daily	1400	800	100
15	Daily	1400		10
14	Daily	1500		100
21	Daily	1700	1100	100
13	Once a week	3000		120
6	Daily	3500	350	125
5	Daily	3600		300
29	Daily	3988	220	30
1	Daily	4500	1600	330
26	Daily	5200		450
12	Daily	5466	977	50
n=35		44377	5813	1934

Question 7: What kind of sera do you use [commercially available and/or prepared in own laboratory]?

The replies to question 7 are summarised in Tables 8 and 9.

Table 8. Number of sources of sera (including in-house prepared sera) used by laboratories

Number of manufacturers from which sera are obtained (including in-house preparations)	Number of NRLs (n=35)
1	9
2	9
3	9
4	6
5	2

Table 9. Number of laboratories using each source of sera

Source of sera	Number of NRLs (n=35)
BD-Difco	2
Biomed	1
Biorad	15
Immunolab	1
Mast	1
Microgen	1
Own preparation	4
Pro-Lab	6
Reagensia	3
Remel	2
Siemens	1
Sifin	19
Statens Serum Institute (SSI)	31

Question 8: The strains in this study were serotyped by: own laboratory/Other laboratory

Two NRLs-*Salmonella* (lab codes 16 and 17) sent the additional strain S21 to another laboratory for further serotyping or confirmation. All the other laboratories tested all the strains in their own laboratory.

4.4 Questions on the use of PCR / biochemical tests

Question 9: Did you use any biochemical test, like dulcitol, malonate, tartrate, etc., to distinguish between subspecies?

Twenty-five participants confirmed the use of biochemical tests. Details are given in Table 10.

Table 10. Strains (as numbered 1 – 21) on which biochemical tests were used

Lab-code	Biochemical test														
	API20E	beta-glucuronidase	Dulcitol	Enterotest 24	Galacturonate	Gelatinase	Lactose	Maldi Tof	Malonate	Mucate	ONPG	PGUA	Salicine	Sorbitol	Tartrate
1[a)]															
3			21								21			21	
5			21						21		21		21		
6			21						21		21	21		21	
8		21	21				21	all	21		21		21		
12			X						X		X		X	X	
13			21						21		21		21	21	
14			4/21		4/21				4/21	4/21			4/21	4/21	
15			4/21		4/21				4/21	4/21			4/21	4/21	
16			21		21				21	21	21			21	21
17	21	21	21						21				21		
18	3/4/21								3/4/21	3/4/21					
19					4/21	4/21			4/21		4/21			4/21	4/21
20				21											
21			4/21				4/21		4/21		4/21		4/21	4/21	
22	all														
23									4/21						
24									4/15/21						4/15/21
25			4/21						4/21		4/21		4/21	4/21	4/21
26[b)]															
27		21	21				21		21		21		21	21	21
29						4			4/21		all				4
30			21				21		21		21		21		
31			21						4/21		21				
32									21						
33			4/21						4/21						

a) All strains tested, but type of test not stated

b) Strains 4/6/7/13/15/21 tested, but type of test not stated

X Strains 4/6/7/8/21 tested

Question 10: Did you use PCR for confirmation of any of the serotyped strains S1–S21?
A total of 14 laboratories reported using PCR for the confirmation of strains.

Question 11: For which strains did you use this PCR?
Three laboratories used PCR to confirm all the strains. Eleven laboratories used PCR to confirm strain S17, the monophasic variant of *S*. Typhimurium 1,4,[5],12:i:-, and three of these also used PCR to confirm strain S16, *S*. Typhimurium. Strains S4 (x1), S5 (x1) and S21 (x1) were also reported to have been confirmed using PCR.

Question 12: Is the PCR used commercially available, details and manufacturer?
Only one laboratory used a commercially available PCR: Check & Trace *Salmonella* by Check points.

Question 13: Reference literature
PCR testing is mainly done to confirm monophasic (Typhimurium) strains.

Seven laboratories mentioned the following reference:
- EFSA Journal, 2010.

Other references mentioned, sometimes in combination with others, were:
- Barco et al., 2011;
- Bugarel et al., 2012;
- Lee et al., 2009;
- Prendergast et al., 2013;
- Tennant et al., 2010.

References regarding molecular serotyping were:
- Herrera-León et al., 2007/Herrera-León et al., 2004/Echeita et al., 2002;
- Hong et al., 2008;
- Fitzgerald et al., 2007/McQuiston et al., 2011.

Question 14: Do you use this PCR routinely?
Twelve of the laboratories use this PCR routinely.

Question 15: How many samples did you test for Salmonella *using this PCR in 2013?*
The replies to question 15 are summarised in Table 11.

Table 11. Number of strains routinely tested by PCR in 2013

Laboratory code	Number of strains tested by PCR in 2013
27	7
8	ca 20
32	20
3	23
30	32
16	38
20	50
14	120
35	170
13	260
21	780
6	3000

4.5 Questions phage typing

Question 18: Does your laboratory perform phage typing?

Question 19: Which Salmonella strains do you phage type?
Seven NRLs perform phage typing of *S*. Typhimurium and *S*. Enteritidis strains. One NRL also phage typed other strains for routine purposes, including *S*. Hadar, *S*. Virchow, *S*. Paratyphi B and *S*. Typhi.

Question 20: Which phage typing system is used for Salmonella *Typhimurium?*

Question 21: Which phage typing system is used for Salmonella Enteritidis?
All phage typing laboratories use the PHE (HPA)/Colindale system.

Question 22: How many strains did your laboratory (approximately) phage type in 2013?
The replies to question 22 are summarised in Table 7 (above).

4.6 Questions regarding PFGE typing

What method do you use for Salmonella PFGE?
Ten participants reported using the Standard PulseNet Protocol *Salmonella* PFGE (PulseNet International, 2013). Eight participants use this Standard protocol with modifications.

How many strains did you approximately PFGE type in 2014?
Replies to this question are summarised in Table 7 (above).

Manufacturer of the XbaI Enzyme
The replies to this question are summarised in Table 12.

Table 12. Number of participants using the enzyme XbaI per manufacturer

Manufacturer	Number of NRLs
New England BioLabs	2
Promega	1
Roche Diagnostics	9
Sigma Life Science	2
Thermo Scientific	4

Name/Model of the Electrophoresis System (e.g. CHEF Mapper II)?
The replies to this question are summarised in Table 13.

Table 13. Number of participants using electrophoresis systems per name/model

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

Name/Model of the Gel Documentation System (e.g. GelDoc 2000)?
The replies to this question are summarised in Table 14.

Table 14. Number of participants using gel documentation systems per name/model

Gel documentation system	Number of NRLs
BIO-RAD VersaDoc with Quantity One software	1
G:Box (Syngene)	1
GelDoc 1000	1
GelDoc 2000	4
GelDoc EQ	1
GelDoc XR+	4
GeneGenious (Syngene)	1
GenSnap v6.00.22	1
INGENIUS Syngene Bio Imaging	1
Kodak Digital Science 1D	1
TDI GELPRINTER	1
UVP BioImaging Systems/EC3 Chemi HR	1

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

5 Results

5.1 Serotyping results

5.1.1 General comments on this year's evaluation

As decided at the 19th EURL-*Salmonella* Workshop (Mooijman, 2014), strain S21 was added to the study for optional testing and results were not included in the evaluation.

5.1.2 Serotyping results per laboratory

The percentages of correct results per laboratory are shown in Figure 1 and the evaluation of the type of errors for O- and H-antigens and identification of the strains are shown in Figures 2, 3 and 4.
The O-antigens were correctly typed by 29 of the 35 participants (83%). This corresponds to 97% of the total number of strains. The H-antigens were correctly typed by 22 of the 35 participants (63%), corresponding to 94% of the total number of strains. A total of 20 participants (57%) gave the correct serovar names, corresponding to 94% of all strains evaluated.

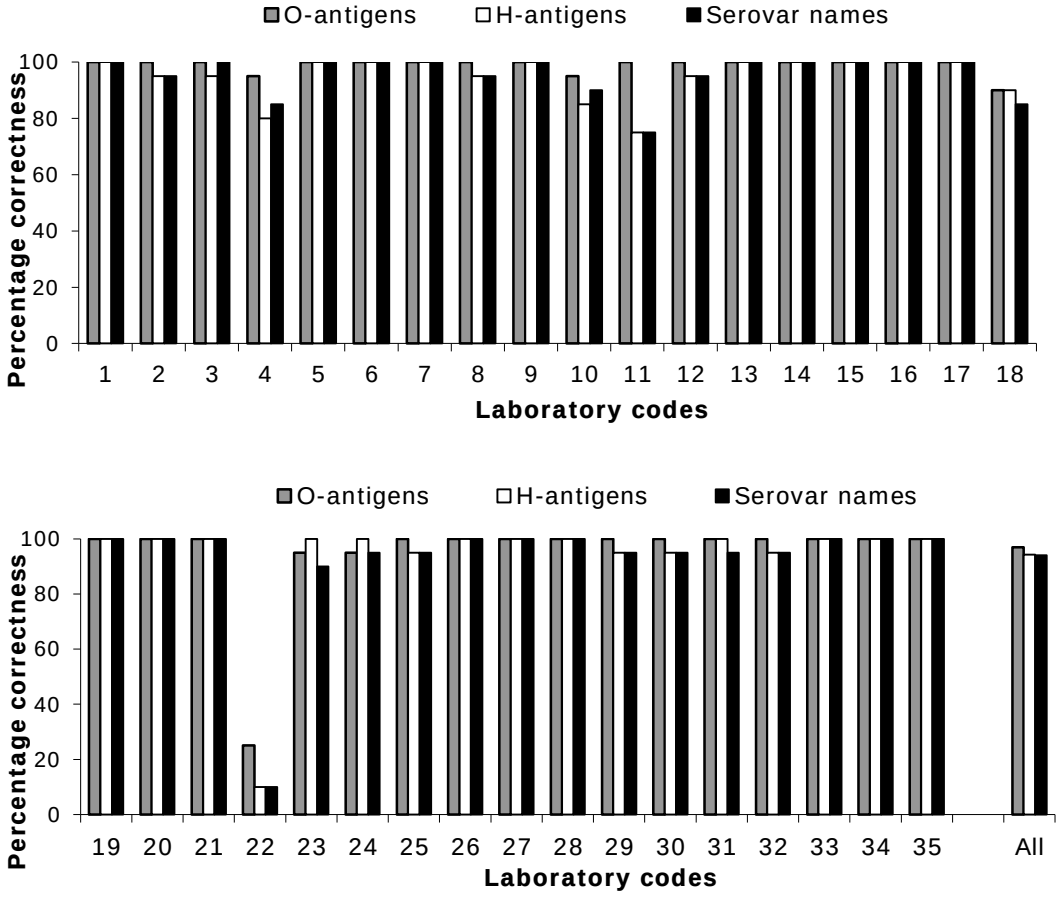


Figure 1. Percentages of correct serotyping results

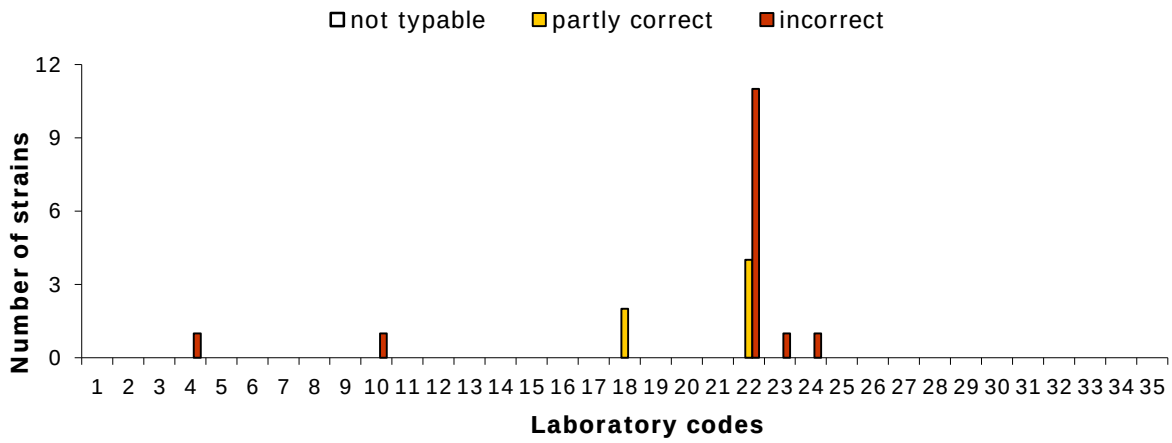


Figure 2. Evaluation of type of errors for O-antigens per NRL

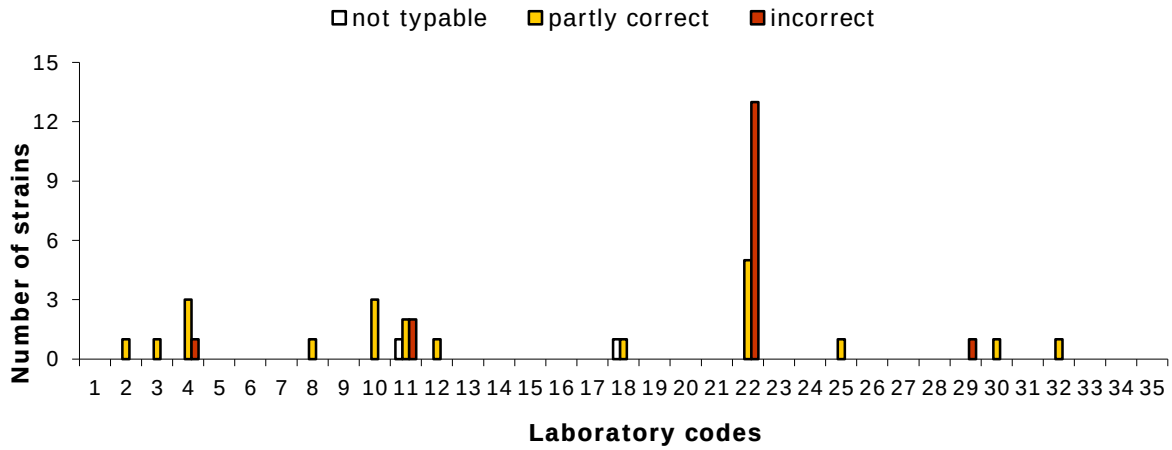


Figure 3. Evaluation of type of errors for H-antigens per NRL

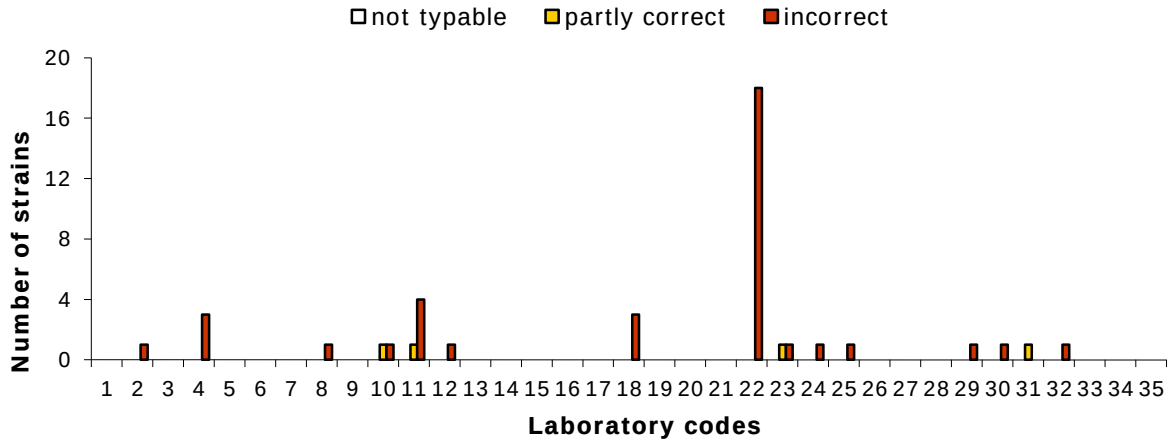


Figure 4. Evaluation of type of errors in the identification of serovar names

For each NRL, the number of penalty points was determined using the guidelines described in Section 3.5. Table 15 shows the number of penalty points for each NRL and indicates 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 15. Evaluation of serotyping results per NRL

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

5.1.3 *Serotyping results per strain*

The results found per strain and per laboratory are given in Annex 2, except for the more complicated strains S17 and S21, which are separately reported in Annex 3.
With the exception of participant 22, which encountered many problems during the serotyping study, a completely correct identification by all participants was obtained for eight strains: *S.* Arechavaleta (S8), *S.* Hadar (S9), *S.* Infantis (S10), *S.* Virchow (S11), *S.* Dublin (S12), *S.* Herston (S13), *S.* Typhimurium (S16) and *S.* Enteritidis (S18). The most problems occurred with the serovar *S.* Bochum (S2). Eight laboratories failed to assign the correct serovar name to this strain. Details of the problems encountered in serotyping are shown in Annex 4. The reported serovar names for strain S17 (Annex 3) still show some variation of 'Typhimurium-like' names, but the example given in both the protocol and the electronic test report on how to preferably report this serovar name (4,5,12:i:-) seems to be of help.
Details of the results for the additional and optional strain S21 are also given in Annex 3. All but three participants actually did serotype this strain, which was a *Salmonella enterica* subspecies *arizona*e 41:z_4,z_{23}:-. Twenty-eight 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 15; Lab codes 11 and 22) and were offered a follow-up study. A follow-up study is obligatory for laboratories from EU Member States that do not achieve the level of good performance, and laboratory 11 received 10 additional strains for serotyping in week 14, 2015. Non-EU Laboratory 22 was not able to participate due to lack of resources.
Again, the number of penalty points was determined using the guidelines described in Section 3.5. Table 16 shows the results of the follow-up study for participant 11, which achieved the level of good performance.

Table 16. Evaluation of serotyping results per NRL in the follow-up study

Lab code	Penalty points	Good performance
11	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 17. The percentages of strains correctly phage typed for each laboratory for both *S*. Enteritidis and *S*. Typhimurium are shown in Figure 5. Separate figures per phage type and per laboratory are given in Annex 5 (*S*. Enteritidis) and Annex 6 (*S*. Typhimurium).

Table 17. 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	**1b**	**14b**	**4**	**3**	**33**	**59**	**35**	**13a**	**8**	**56**	
1	1b	14b	4	22	33	59	35	13a	8	2	2
6	1b	14b	4	3	33	rdnc or 23	35	13a	8	56	1
10	1b	14b	4	21	33	59	35	13a	8	2	2
12	1b	14b	4	3	33	23	35	13a	8	56	1
17	1B	14B	4	3	33	14B	21	13	8	2	4
21	PT1b	PT14b	PT4	PT21	PT33	PT59	PT35	PT13a	PT8	PT56	1
29	1b	14b	4	3	33	23	35	13a	8	56	1
X	0	0	0	3	0	4	1	1	0	3	12

S. Typhimurium strain numbers

Lab code	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	Y
PHE	**36**	**104**	**U311**	**8**	**41**	**193**	**1**	**10**	**132**	**136**	
1	36	104L	U311	8	41	193	1	10	2	136	1
6	36	104	U311	8	41	193	1	10	rdnc	136	1
10	36	104L	U311	8	41	193	1	67	2	136	2
12	36	104	U311	8	41	193	1	10	2	136	1
17	36	12	U311	8	41	195	1	10	2	136	3
21	DT36	DT104	U311	DT8	DT41b	DT195	DT1	DT10	DT135	DT136	3
29	36	104	U311	8	41	193	1	10	47	136	1
X	0	1	0	0	1	2	0	1	7	0	12

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

incorrect result

incorrect result with remark

correct result with remark

Laboratories 1 and 10 reported strain T2 as DT 104L. This is a low variant of DT 104.

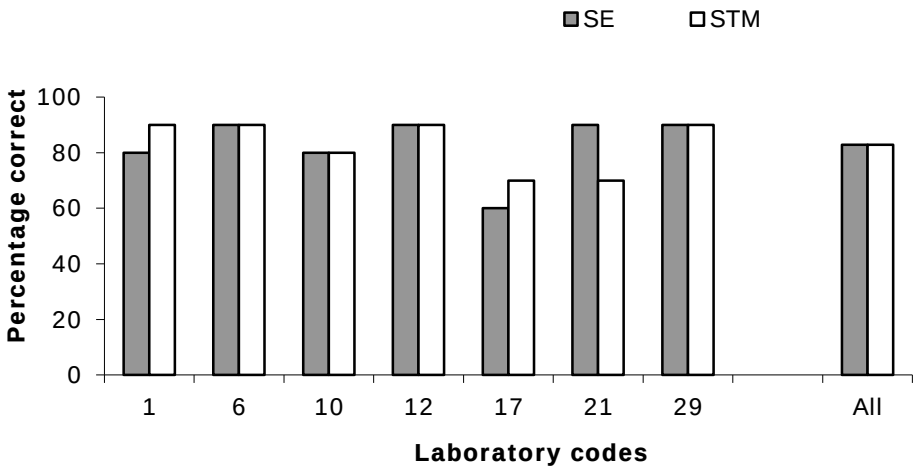


Figure 5. Percentage of strains correctly phage typed for each participating laboratory

None of the laboratories correctly phage typed all 10 strains of *S*. Enteritidis. Four laboratories (6, 12, 21 and 29) assigned the correct phage type to 9 of the 10 strains. Two laboratories (1 and 10) correctly phage typed 8 of the *S*. Enteritidis strains. Laboratory 17 incorrectly phage typed 4 of the strains: E6, E7, E8 and E10.
None of the laboratories correctly phage typed all 10 strains of *S*. Typhimurium. Four laboratories (1, 6, 12 and 29) assigned the correct phage type to 9 of the 10 strains. Laboratory 10 incorrectly phage typed 2 of the strains, T8 and T9. Two laboratories (17 and 21) incorrectly phage typed 3 of the *S*. Typhimurium strains.
Overall, 83% of the *S*. Enteritidis strains and 83% of the *S*. Typhimurium strains were correctly phage typed.

5.3 PFGE typing results

A total of 18 NRLs participated in the second study on PFGE typing, including one that was participating for the first time. Regrettably, one participant encountered severe problems with its documentation system after running the gel and was not able to submit an adequate image.
As in the previous typing study, the results were evaluated only on the quality of the PFGE images (submitted as TIFF files). As in 2013, the quality of the gels was variable, as shown by the two examples in Annex 7.
An example of an individual laboratory evaluation report is given in Annex 8. In addition to the scores given in accordance with the PulseNet Guidelines, the EURL-*Salmonella* included some general comments in individual reports, such as 'the resolution of the TIFF file was too low (< 300 KB)' or 'the use of the *S*. Braenderup H9812 standard was deviating'. The report showed a display of the 'Distortion bar' option in BioNumerics of the participants gel, in addition to the display of the comparison of the participants profiles to the reference profiles.
The protocol request to include at least the lab code in the filenames of the images submitted was also commented on; 7 of the 18 participants

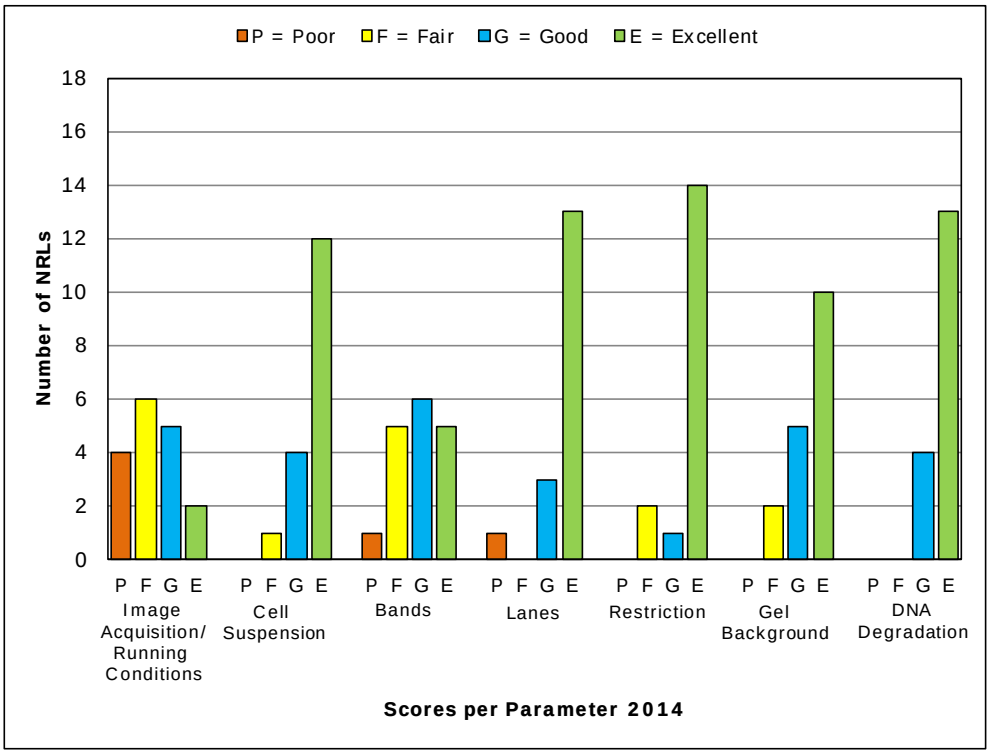
did not adhere to this request, which was intended to prevent confusion in the evaluation of the results.

The scores per NRL, broken down across the seven parameters (see Annex 1), are given in Table 18. The scores per parameter are shown in Figure 6, which includes for comparison both the 2013 and 2014 results. Scores on the parameter ‘Image Acquisition/Running Conditions’ improved from only Poor or Fair in 2013 to Poor (x4), Fair (x6), Good (x5) and Excellent (x2). Scores on the parameter ‘Bands’ also improved; where in 2013 they were polarised between Poor (x5) and Excellent (x10), in 2014 there was only 1 Poor score, the remainder being spread across Fair, Good and Excellent. The other five parameters all yielded a majority of Excellent scores, as in 2013.
Four of the 17 images resulted in a Poor score on at least one of the seven parameters (all four in ‘Image Acquisition/Running Conditions’), which may indicate that these four images are not suitable for use in interlaboratory comparisons.

Table 18. Evaluation of PFGE results per participants and per parameter

Lab code / Parameter	16	21	10	13	15	25	14	26	5	6	19	35	4	12	29	8	1	Total score per parameter	Average per parameter
Image Acquisition and Running Conditions	1	2	1	2	3	2	3	1	1	3	2	2	3	2	3	4	4	39	2,3
Cell Suspension	3	2	4	4	3	4	3	4	4	4	3	4	4	4	4	4	4	62	3,6
Bands	1	2	2	3	2	3	2	2	3	3	4	3	4	4	3	4	4	49	2,9
Lanes	1	3	4	3	4	4	4	4	4	4	4	4	4	4	4	3	4	62	3,6
Restriction	3	4	2	4	4	2	4	4	4	4	4	4	4	4	4	4	4	63	3,7
Gel Background	4	2	3	3	2	3	4	4	4	3	4	4	3	4	4	4	4	59	3,5
DNA Degradation (smearing in lanes)	4	3	3	3	4	4	3	4	4	4	4	4	4	4	4	4	4	64	3,8
Total score per participant	17	18	19	22	22	22	23	23	24	25	25	25	26	26	26	27	28		
Average per participant	2,4	2,6	2,7	3,1	3,1	3,1	3,3	3,3	3,4	3,6	3,6	3,6	3,7	3,7	3,7	3,9	4		

1=Poor; 2=Fair; 3=Good; 4=Excellent



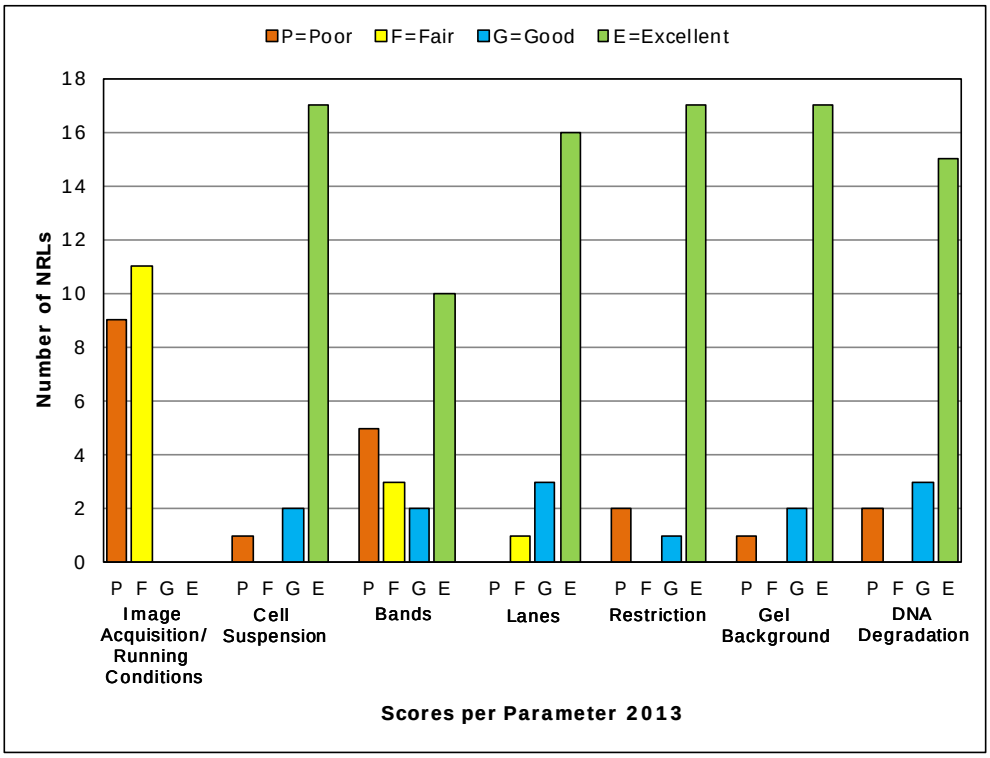


Figure 6. Evaluation of the quality of the PFGE images in scores per parameter in the 2014 study and the 2013 study

6 Discussion

6.1 Serotyping

A total of 35 laboratories participated in this study. These included 29 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) in the 28 EU Member States, 3 NRLs of EU-candidate countries, and 3 NRLs of EFTA countries.
A total of 21 *Salmonella* strains were sent to the participants in November 2014 for serotyping by all participants; however, testing of the 21st strain was optional and the results were not included in the evaluation.
Overall, 97% of the strains were correctly typed for the O-antigens, 94% of the strains were correctly typed for the H-antigens and 94% of the strains were correctly named by the participants.

At the EURL-Salmonella Workshop in 2007, the EURL-*Salmonella* proposed a definition of good performance in serotyping for NRLs. Using this definition, 33 laboratories achieved good performance. The two participants that did not achieve the defined level of good performance were offered a follow-up study including 10 additional strains for serotyping. A follow-up study is obligatory for EU-NRLs and the EU-MS-NRL concerned achieved good performance.

In the evaluation of the results obtained by the participants, mistakes in typing the five designated *Salmonella* serovars (Enteritidis, Typhimurium, Hadar, Infantis and Virchow) are more severely judged than errors in typing 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 serotyping the 'top 5' serovars, except for participant 22, which encountered many problems during this serotyping study.
Tables 19 and 20 give an overview of the results of the typing studies from 2007, when the system of penalty points was introduced. Table 19 shows results for EU-NRLs only; Table 20 shows results for all participants per study. The relatively large number of 56 penalty points in 2009 (Table 20) was mainly due to the results of one non-EU NRL, participating for the first time. Similarly, the large number of penalty points in the current 2014 study (57) was mainly due to the results of another non-EU-MS NRL, which encountered many problems during this serotyping study.
The percentages of correctly typed strains remain quite stable over the years, usually with a better performance for the O-antigens than for the H-antigens.
The most problems in 2014 occurred with the serovar *S*. Bochum (S2). Eight laboratories assigned the serovar name *S*. Africa to this strain, on account of a positive reaction for both r and i in the first H-phase. When this strain was tested by the EURL using a monoclonal serum as well as a molecular technique (Luminex), there was no i reaction. Although it is not known which sera the participants used to type this strain, all 8 laboratories reported using mainly non-monoclonal sera. Therefore, a cross-reaction is the most likely explanation for this finding.

*Table 19. Historical overview of the EURL-*Salmonella *interlaboratory comparison studies on the serotyping of* Salmonella*, for EU-NRLs only*

Study / Year	XII 2007	XIII 2008	XIV 2009	XV 2010	XVI 2011	XVII 2012	XVIII 2013	XIX 2014
No. of participants	25	27	28	30	28	28	29	29
No. of strains evaluated	20	20	20	19	19	20	20	20
O-antigens correct/strains	98%	98%	98%	98%	99%	99%	100%	99%
H-antigens correct/strains	95%	98%	95%	95%	97%	98%	98%	97%
Names correct/strains	95%	97%	95%	95%	97%	96%	98%	96%
O-antigens correct/labs	68%	70%	75%	93%	93%	82%	97%	86%
H-antigens correct/labs	56%	67%	43%	73%	71%	64%	72%	66%
Names correct/labs	52%	52%	46%	67%	75%	57%	69%	55%
No. of penalty points	35	30	36	16	22	20	17	18
No. of labs not achieving good performance	6	3	4	2	2	2	2	1
No. of labs not achieving good performance after follow-up	0	0	0	0	0	0	0	0

*Table 20. 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	XIX 2014
No. of participants	26	29	31	33	36	31	34	35
No. of strains evaluated	20	20	20	19	19	20	20	20
O-antigens correct/strains	98%	98%	97%	98%	98%	99%	100%	97%
H-antigens correct/strains	96%	98%	94%	95%	96%	98%	98%	94%
Names correct/strains	95%	97%	93%	95%	96%	96%	97%	94%
O-antigens correct/labs	69%	76%	74%	88%	86%	77%	94%	83%
H-antigens correct/labs	58%	72%	45%	67%	69%	61%	71%	63%
Names correct/labs	54%	59%	48%	61%	69%	55%	68%	57%
No. of penalty points	36	34	56	37	41	20	20	57
No. of labs not achieving good performance	6	4	5	4	4	2	2	2
No. of labs not achieving good performance after follow-up	0	0	0	0 (n=3)	1 (n=3)	0	0	0 (n=1)

6.2 Phage typing

Ten strains of *S*. Enteritidis and 10 strains of *S*. Typhimurium were selected by the *Salmonella* Reference Service of Public Health England, London, UK.
None of the seven NRLs correctly phage typed all 10 of the *S*. Enteritidis strains. Four of the NRLs incorrectly phage typed 1 of the *S*. Enteritidis strains. Two of the NRLs incorrectly phage typed 2 of the 10 strains and one NRL incorrectly phage typed 4 of the *S*. Enteritidis strains.

Two laboratories incorrectly phage typed strain E4 (PT 3) as PT 21 and one laboratory incorrectly phage typed this strain as PT 22. These three laboratories all obtained reactions with phages that do not react with PT 3. The two laboratories that typed this strain as PT 21 had reactions

with phages 2, 4, 9 and 17 and the laboratory that typed it as PT 22 had reactions with phages 4, 9 and 17; PT 3 does not react with these phages. These incorrect reactions could be due to the titres of the phages being too high or the inoculum of the broth used for the phage typing being incorrect.

Two laboratories phage typed strain E6 (PT 59) incorrectly as PT 23 and one laboratory typed it as RDNC or PT 23. A fourth laboratory phage typed this strain as PT 14b. PT 59 reacts with only one phage, 17. The three laboratories that phage typed this strain as PT 23 obtained reactions with phages 4 and 9. Occasionally, PT 59 will give a few plaques with phage 9, as phage 17 was adapted from phage 9. PT 23 is a rough phage type, so it is possible that this strain had become rough in the laboratories that typed it as PT 23. The laboratory that typed this strain as PT 14b obtained a reaction with phage 6, which suggests that the titre of this phage was too high.

Strain E7 (PT 35) was also incorrectly phage typed by one laboratory. This laboratory typed it as PT 21 because it obtained phage reactions with phages 1, 6, 8, 10 and 14. PT 35 does not react with these phages, which suggests that the titres of these phages were too high.

One laboratory phage typed strain E8 (PT 13a) incorrectly. This strain was typed as PT 13 because they did not get anythere were no reactions with phages 8 and 10. This may have been due to the titres of these two phages being incorrect.

Strain E10 (PT 56) was incorrectly phage typed by three laboratories as PT 2. These three laboratories all obtained reactions with phages 1, 6, 8, 10 and 14; PT 56 does not react with these phages, which suggests that the titres of these phages were too high.

None of the seven NRLs correctly phage typed all 10 strains of *S*. Typhimurium. Four of the NRLs correctly phage typed 9 of the *S*. Typhimurium strains. One NRL correctly typed 8 of the S. Typhimurium strains and two of the NRLs correctly typed 7 of the 10 strains.

One laboratory incorrectly phage typed strain T2 (DT 104) as DT 12. This incorrect result was due to no reaction being obtained with phage 18, which suggests that the titre of this phage was too low.
Strain T5 (DT 41) was incorrectly typed as DT 41b by one laboratory because they got low reactions with several phages, which suggests that the titres of these phages were too low.

T6 (DT 193) was typed as DT 195 by two laboratories. Neither of these laboratories got any phage reactions with additional phages 1 and 2, which suggests that the titres of these two phages were too low.

One laboratory incorrectly phage typed T8 (DT 10) as DT 67 because they did not get any reaction with phage 11, which suggests that the titre of this phage was too low.

All seven of the NRLS incorrectly phage typed strain T9 (DT 132). Four laboratories typed this strain as DT 2, one laboratory typed it as DT 135, one as DT 47 and one laboratory was unable to allocate a phage type and called it RDNC. The four laboratories that typed this strain as DT 2 incorrectly interpreted the phage reactions they obtained. DT 2 and DT 132 react with the same phages and DT 2 gives a high reaction (CL) with all the phages it reacts with, whereas DT 132 gives a low reaction with some of the phages. The laboratory that typed this strain as DT 135 also interpreted the phage reactions incorrectly. This strain was phage typed as DT 47 by one laboratory because it failed to get any phage reactions with two phages, 27 and 35. The laboratory that phage typed this strain as RDNC did not get any reactions with phages 6, 20 and 23; DT 132 should react with these phages.

6.3 PFGE typing

A large number (18) of NRLs participated in this second 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 as in 2013 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 system of 1 (Poor), 2 (Fair), 3 (Good) or 4 (Excellent) points per parameter.
Scores on the parameter ‘Image Acquisition/Running Conditions’ improved from only Poor or Fair in 2013 to Poor (x4), Fair (x6), Good (x5) and Excellent (x2). Scores on the parameter ‘Bands’ also improved; where in 2013 they were polarised between Poor (x5) and Excellent (x10), in 2014 there was only 1 Poor score, the remainder being spread across Fair, Good and Excellent. The other five parameters all yielded a majority of Excellent scores, as in 2013.
Four of the 17 images submitted obtained a Poor score on at least one of the seven parameters (all four on ‘Image Acquisition/Running Conditions’), which may indicate that these four images are not suitable for use in interlaboratory comparisons.
The following simple guidelines related to this, the most complicated parameter should improve the results:

- Ensure that the gel fills the whole image.
- Ensure that the wells are included in the image.
- The bottom band of the standard should be 1–1,5 cm from the bottom of the gel.
- Use the standard strain, S. Braenderup H9812, placing at least in every six lanes.
- Check the resolution of the image (preferably > 300 KB file size).

7 Conclusions

7.1 Serotyping

- 97% of the strains were typed correctly for the O-antigens.
- 94% of the strains were typed correctly for the H-antigens.
- 94% of the strains were correctly named.
- Serotyping of S. Bochum caused the most problems in this study (eight participants).
- All participants except one correctly serotyped the 'top 5' strains S. Enteritidis, S. Hadar, S. Infantis, S. Typhimurium and S. Virchow.
- Two NRLs initially did not achieve the defined level of good performance and were offered a follow-up study, typing an additional set of 10 strains.
- In the end, 34 participants, including all the EU-NRLs, achieved the defined level of good performance.

7.2 Phage typing

- The performance of the laboratories participating in this study was not as good for *S*. Enteritidis as that of the laboratories participating in the 2013 study. In 2013, 93% of the *S*. Enteritidis strains were correctly phage typed; in this 2014 study, only 83% of the strains were correctly typed.
- The participants' performance in the phage typing of the *S*. Typhimurium strains in this study was the same as that in the 2013 study. In both the 2013 and 2014 studies, 83% of the *S*. Typhimurium strains were correctly phage typed.
- Five of the *S*. Enteritidis strains and five of the *S*. Typhimurium strains were correctly phage typed by all of the participating laboratories.

7.3 PFGE typing

- Eighteen participants also performed PFGE typing in this second study.
- Evaluation of the PFGE results was based on the quality of the generated images only and was expressed in terms of scores on seven parameters: Poor, Fair, Good or Excellent.
- The quality of the PFGE results was promising, although as in 2013 there was some variation in results between the participants.
- Four of the 17 images resulted a Poor score on at least one of the seven parameters, which may indicate that these four images are not suitable for use in interlaboratory comparisons.
- Adherence to simple guidelines, especially in relation to the parameter 'Image Acquisition/Running Conditions', should improve the results.

List of abbreviations

CRL-*Salmonella*	Community Reference Laboratory for *Salmonella* (now 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 Water-borne Diseases and Zoonoses Programme
HPA	Health Protection Agency (now Public Health England)
NRL-*Salmonella*	National Reference Laboratory 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

References

Barco, L., A.A. Lettini, E. Ramon, A. Longo, C. Saccardin, M.C. Pozza and A. Ricci (2011). Rapid and sensitive method to identify and differentiate *Salmonella enterica* serotype Typhimurium and *Salmonella enterica* serotype 4,[5],12:i:- by combining traditional serotyping and multiplex polymerase chain reaction. Foodborne Pathog. Dis. 8(6): 741–743.

Bugarel M., M.L. Vignaud, F. Moury, P. Fach and A. Brisabois (2012). Molecular identification in monophasic and nonmotile variants of *Salmonella enterica* serovar Typhimurium. Microbiology Open. doi:10.1002/mbo3.39.

EC (2004). European Regulation EC No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. Official Journal of the European Union L 165: 30 April 2004. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:165:0001:0141:EN:PDF (accessed 9/2/2015).

ECDC (2014). Fifth external quality assessment scheme for *Salmonella* typing. Stockholm, Sweden. doi 10.2900/35432 http://ecdc.europa.eu/en/publications/Publications/fifth-EQA-salmonella-typing-November-2014.pdf (accessed 16/11/2015).

Echeita, M.A., S. Herrera, J. Garaizar and M.A. Usera (2002) Multiplex PCR-based detection and identification of the most common Salmonella second-phase flagellar antigens. Res. Microbiol. 153(2): 107–113.

EFSA Panel on Biological Hazards (BIOHAZ) (2010) Scientific Opinion on monitoring and assessment of the public health risk of 'Salmonella Typhimurium-like' strains. EFSA Journal 8(10): 1826. http://www.efsa.europa.eu/en/efsajournal/pub/1826.htm (accessed 9/2/2015).

Fitzgerald, C., M. Collins, S. van Duyne, M. Mikoleit, T. Brown and P. Fields (2007) Multiplex, bead-based suspension array for molecular determination of common Salmonella serogroups. J. Clin. Microbiol. 45(10): 3323–3334.

Grimont, P.A.D. and F.-X. Weill (2007) Antigenic formulae of the Salmonella serovars, 9th ed. WHO Collaborating Centre for Reference and Research on Salmonella. Institute Pasteur, Paris, France. https://www.pasteur.fr/sites/www.pasteur.fr/files/wklm_en.pdf (accessed 15/2/2016).

Herrera-León, S., J.R. McQuiston, M.A. Usera, P.I. Fields, J. Garaizar and M.A. Echeita (2004) Multiplex PCR for distinguishing the most common phase-1 flagellar antigens of Salmonella spp. J. Clin. Microbiol. 42(6): 2581–2586.

Herrera-León, S., R. Ramiro, M. Arroyo, R. Díez, M.A. Usera and M.A. Echeita (2007) Blind comparison of traditional serotyping with three multiplex PCRs for the identification of Salmonella serotypes. Res. Microbiol. 158(2): 122–127.

Hong, Y., T. Liu, M.D. Lee, C.L. Hofacre, M. Maier, D.G. White, S. Ayers, L. Wang, R. Berghaus, and J.J. Maurer (2008). Rapid screening of Salmonella enterica serovars Enteritidis, Hadar, Heidelberg and

Typhimurium using a serologically-correlative allelotyping PCR targeting the O and H antigen alleles. BMC Microbiol. 8: 178.

Lee, K., T. Iwata, M. Shimizu, T. Taniguchi, A. Nakadai, Y. Hirota and H. Hayashidani (2009) A novel multiplex PCR assay for Salmonella subspecies identification. J. Appl. Microbiol. 107(3): 805–811.

McQuiston, J.R., R.J. Waters, B.A. Dinsmore, M.L. Mikoleit and P.I. Fields (2011) Molecular determination of H antigens of Salmonella by use of a microsphere-based liquid array. J Clin Microbiol, 49(2): 565–573.

Mooijman, K.A. (2007) The twelfth CRL-*Salmonella* Workshop; 7 and 8 May 2007, Bilthoven, the Netherlands. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 330604006. http://www.eurlsalmonella.eu/Publications/Workshop_Reports (accessed 9/2/2015).

Mooijman, K.A. (2014) The 19th EURL-*Salmonella* Workshop; 26 and 27 May 2014, Zaandam, The Netherlands. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2014-0147. http://www.eurlsalmonella.eu/Publications/Workshop_Reports (accessed 9/2/2015).

Prendergast, D.M., D. Hand, E. Ní Ghallchóir, E. McCabe, S. Fanning, M. Griffin, J. Egan and M. Gutierrez (2013) A multiplex real-time PCR assay for the identification and differentiation of *Salmonella enterica* serovar Typhimurium and monophasic serovar 4,[5],12:i:-. Int. J. Food Microbiol. 16;166(1): 48–53.

PulseNet international (2013) Standard Operating Procedure for PulseNet PFGE of *Escherichia coli* O157:H7, *Escherichia coli* non-O157 (STEC), *Salmonella* serotypes, *Shigella sonnei* and *Shigella flexneri*. PNL05, effective date 03-04-2013. Available at: http://www.pulsenetinternational.org/assets/PulseNet/uploads/pfge/PNL05_Ec-Sal-ShigPFGEprotocol.pdf (accessed 9/2/2015).

Tennant, S.M., S. Diallo, H. Levy, S. Livio, S.O. Sow, M. Tapia, P.I. Fields, M. Mikoleit, B. Tamboura, K.L. Kotloff, J.P. Nataro, J.E. Galen and M.M. Levine (2010) Identification by PCR of non-typhoidal Salmonella enterica serovars associated with invasive infections among febrile patients in Mali. PLoS. Negl. Trop. Dis 4(3): 621.

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

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 Taksony	S2 Bochum	S3 Adjame	S4 Langenhorn	S5 Albany	S6 Yaba	S7 Bracknell	S8 Arechavaleta	S9 Hadar	S10 Infantis
1	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
2	Taksony	Bochum	Adjame	Pontypridd	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
3	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
4	Taksony	Bohum	Adjame	Langenhorn	Albany	Java	Bracknell	Arechavaleta	Hadar	Infantis
5	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
6	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
7	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
8	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
9	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
10	Taksony	Africana	Adjame	Langenhorn	O8,20 : Z4 : -	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
11	Chichester	Africana	Adjame	Langenhorn	Bovismorbificans	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
12	Taksony	Africana	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arachevaleta	Hadar	Infantis
13	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
14	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
15	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
16	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
17	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
18	Taksony	Africana	13,22:r:-	Langenhorn	Albany	Yaba	Oudwijk	Arechavaleta	Hadar	Infantis
19	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
20	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
21	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
22	Fulda	Gloucester	II	Wien	Enteritidis	Koessen	Rottnest	Paratypfi A	Hadar	Typfimurium
23	Taksony	Bochum	Adjame	Langenhorn	Kalamu	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
24	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
25	Taksony	Africana	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
26	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
27	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
28	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
29	Taksony	Bochum	Adjame	Langenhorn	Albany	Lamberhurst	Bracknell	Arechavaleta	Hadar	Infantis
30	Taksony	Africana	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
31	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
32	Taksony	Africana	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
33	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
34	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
35	Taksony	Bochum	Adjame	Langenhorn	Albany	Yaba	Bracknell	Arechavaleta	Hadar	Infantis
X	2	8	2	2	3	3	2	1	0	1

S11 Virchow	S12 Dublin	S13 Herston	S14 Potsdam	S15 Senftenberg	S16 Typhimurium	S18 Enteritidis	S19 Agona	S20 Muenster	Lab REF
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	1
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	2
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	3
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Derby	Muenster	4
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	5
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	6
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	7
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Nyborg	8
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	9
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Reading	10
Virchow	Dublin	Herston	6,7,14:l,v:-	Senftenberg	Typhimurium	Enteritidis	Agona	Anatum	11
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	12
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	13
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	14
Virchow	Dublín	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	15
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	16
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	17
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	18
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	19
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	20
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	21
Krefeld	Infantis	Colindale	Legon	Aberden	Oritamerin	Gallinarum	Nitra	III b	22
Virchow	Dublin	Herston	Potsdam	1,19:g,s,t:-	Typhimurium	Enteritidis	Agona	Muenster	23
Virchow	Dublin	Herston	Potsdam	Menston	Typhimurium	Enteritidis	Agona	Muenster	24
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	25
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	26
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	27
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	28
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	29
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	30
Virchow	Dublin	Herston	Potsdam	Senftenberg (or Dessau)	Typhimurium	Enteritidis	Agona	Muenster	31
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	32
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	33
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	34
Virchow	Dublin	Herston	Potsdam	Senftenberg	Typhimurium	Enteritidis	Agona	Muenster	35
1	1	1	1	2	1	1	2	4	**X**

remark
partly correct, in the naming: no penalty points
incorrect, in the naming: 1 penalty point
incorrect, in the naming: 4 penalty points

X = number of deviating laboratories per strain

Results for Strains S17 and S21 are given in Annex 3

Annex 3 Details of serotyping results for strains S17 and S21

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	Lab code
S-17	**1,4,[5],12**	**i**	**-**	**1,4,[5],12:i:-**	**REF**
S-17	4,5	i	-	4,5,12:i:-	1
S-17	4,5,12	i	-	4,5,12:i:-	2
S-17	4,12	i	-	Typhimurium - monophasic	3
S-17	4, 5	i	-	Typhimurium	4
S-17	4,5,12	i	-	4,5,12:i:-	5
S-17	4,5,12	i	-	4,5,12:i.-	6
S-17	4	i	-	4,i,-	7
S-17	1,4,[5],12	i	-	4,[5],12:i:-	8
S-17	4, 5, 12	i	-	4, 5, 12; i; -	9
S-17	1,4,5,12	i	-	1,4,5,12:i:-	10
S-17	4,5,12	i	-	4,5,12:i:-	11
S-17	4,5,12	i	-	4,5,12:i:-	12
S-17	4,5,12	i	-	4,5,12:i:-	13
S-17	4, 5	i	-	Monophasic S. Typhimurium	14
S-17	4,5,12	i	-	4,5,12: i : -	15
S-17	4,5	i	-	Typhimurium monophasic variant	16
S-17	4,5	i	-	4,5:i:-	17
S-17	4,5,12	i	-	4,5,12:i:-	18
S-17	4,5,12	i	-	4,5,12:i:-	19
S-17	1,4,[5],12	i	-	S. enterica ssp. enterica (1,4,[5],12:i:-)	20
S-17	4,5	i	-	S. 4,5:i:-	21
S-17	1,4,(5),12	i	-	-	22
S-17	4,5,12	i	-	4,5,12:i:-	23
S-17	4,5,12	i	-	4,5,12:i:-	24
S-17	4,5,12	i	-	4,5,12:i:-	25
S-17	4,5,12	i	-	4,5,12:i:-	26
S-17	4,5,12	i	-	4,5,12:i:-	27
S-17	4,5,12	i	-	4,5,12:i:-	28
S-17	4,5,12	i	-	4,5,12:i:-	29
S-17	4,5,12	i	-	4,5,12:i :-	30
S-17	4,5,12	i	-	4,5,12:i:-	31
S-17	4, 5	i	-	Monophasic S. Typhimurium	32
S-17	4,5,12	i	-	4,5,12:i:-	33
S-17	1,4,[5],12	i	-	1,4,[5],12: i :-	34
S-17	4,5,12	i	-	4,5,12:i:-	35

remark
partly correct, in the naming: no penalty points
incorrect, in the naming: 1 penalty point
incorrect, in the naming: 4 penalty points

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	Lab code
S-21	**41**	**z4,z23**	**-**	**41:z4,z23:-**	**REF**
S-21	41	z4,z23	-	S IIIa 41:z4,z23:-	1
S-21	41	z4,z23	-	41:z4,z23:-	2
S-21	41	z4,z23	-	S. enterica subsp. houtenae ser. 41 : z4,z23 : -	3
S-21					4
S-21	41	z4,z23	-	41:z4,z23	5
S-21	41	z4,z23	-	IIIa 41:z4,z23:-	6
S-21	41	z4	-	41,z4	7
S-21	41	z4,z23	-	IIIa 41:z4,z23	8
S-21					9
S-21	39/40/41/42/43/44/45			NT (OMD)	10
S-21	41	z4,z23	-	41:z4,z23:-	11
S-21	41	z4,z23	-	SG IIIa 41: z4, z23:-	12
S-21	41	z4,z23	-	41:z4,z23:-	13
S-21	41	z4, z23	-	S. enterica subsp. arizonae 41:z4z23:-	14
S-21	41	z4,z23	-	41:z4,z23:- (IIIa)	15
S-21	41	z4,z23	-	enterica subsp. arizonae 41 : z4,z23 :-	16
S-21	41	z4,z23	-	41:z4,z23:-	17
S-21	41	z4,z23	-	41:z4,z23:- (arizonae)	18
S-21	41	z4,z23	-	41:z4,z23:-	19
S-21	41	z4,z23	-	S. enterica ssp. arizonae	20
S-21	41	z4,z23	-	S. enterica subsp. arizonae	21
S-21	-	-	-	-	22
S-21	41	z4,z23	-	IIIa	23
S-21	41	z4,z23	-	41:z4,z23:-	24
S-21	41	z4,z23	-	41:z4,z23:-	25
S-21	41	z4,z23	-	S.IIIa 41:z4,z23:-	26
S-21	41	z4,z23	-	41:z4,z23:-	27
S-21	OMD (SSI)	?	?	Salmonella sp.	28
S-21	41	z4,z23	-	S. enterica subsp.arizonae (III.a) 41:z4,z23:-	29
S-21	41	z4,z23	-	41:z4,z23 :-	30
S-21	41	z4,z23	-	S. enterica subsp. arizonae 41:z4,z23:-	31
S-21	41	z4, z23	-	S. enterica subsp arizonae= 41:z4, z23:-	32
S-21	41	z4,z23	-	Salmonella enterica subsp. arizonae (IIIa) 41:z4,z23:-	33
S-21	41	z4,z23	-	IIIa or IV	34
S-21	41	k,Z4,Z23	-	41:k,Z4,Z23;-	35

remark

Annex 4 Details of strains that caused problems in serotyping

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	Lab code
S-1	**1,3,19**	**i**	**z6**	**Taksony**	**REF**
S-1	1,3,19	i	1,6	Chichester	11
S-1	1,3,19	l,w	1,5	Fulda	22
S-2	**1,4,[5],12**	**r**	**l,w**	**Bochum**	**REF**
S-2	4	r,i	w	Africana	10
S-2	4,12	r,i	l,w	Africana	11
S-2	4,12	r,i	l,w	Africana	12
S-2	4,12	r,i	l,w	Africana	18
S-2	1,4,5,12	i	l,w	Gloucester	22
S-2	4,12	r,i	l,w	Africana	25
S-2	4,12	r,i	l,w	Africana	30
S-2	4	r, i	l, w	Africana	32
S-3	**13,23**	**r**	**1,6**	**Adjame**	**REF**
S-3	13,22	r	-	13,22:r:- Gp V Salmonella bongori	18
S-3	3,10	a	l,w	II	22
S-4	**18**	**m,t**	**-**	**Langenhorn**	**REF**
S-4	18	g,m	-	Pontypridd	2
S-4	1,4,5,12 (27)	b	l,w	Wien	22
S-5	**8,20**	**z4,z24**	**-**	**Albany**	**REF**
S-5	8,20	z4	-	O8,20 : Z4 : -	10
S-5	8,20	r	1,5	Bovismorbificans	11
S-5	1,9,12	gm	-	Enteritidis	22
S-5	1,4,5,12	z4,z24	-	Kalamu	23
S-6	**3,{10},{15}**	**b**	**e,n,z15**	**Yaba**	**REF**
S-6	1, 4, 12	b	1, 2	Java	4
S-6	2,12	l,v	1,5	Koessen	22
S-6	3,10	e,h	e,n,z15	Lamberhurst	29
S-7	**13,23**	**b**	**1,6**	**Bracknell**	**REF**
S-7	13,22	b	1,6	Oudwijk	18
S-7	1,13,22	b	1,7	Rottnest	22
S-8	**4,[5],12**	**a**	**1,7**	**Arechavaleta**	**REF**
S-8	1,2,12	a	-1,5	Paratypfi A	22
S-9	**6,8**	**z10**	**e,n,x**	**Hadar**	**REF**
S-10	**6,7,14**	**r**	**1,5**	**Infantis**	**REF**
S-10	1,4,(5), 12	i	1,2	Typfimurium	22
S-11	**6,7,14**	**r**	**1,2**	**Virchow**	**REF**
S-11	1,3,19	y	l,w	Krefeld	22
S-12	**1,9,12[Vi]**	**g,p**	**-**	**Dublin**	**REF**
S-12	6,7,14	r	1,5	Infantis	22
S-13	**6,8**	**d**	**e,n,z15**	**Herston**	**REF**
S-13	6,7	r	1,7	Colindale	22
S-14	**6,7,14**	**l,v**	**e,n,z15**	**Potsdam**	**REF**
S-14	6,7,14	l,v	-	6,7,14:l,v:-	11
S-14	1,4,12,(27)	c	1,5	Legon	22
S-15	**1,3,19**	**g,[s],t**	**-**	**Senftenberg**	**REF**
S-15	11	i	1,2	Aberden	22
S-15	1,19	g,s,t	-	1,19:g,s,t:-	23
S-15	7	g,s,t	-	Menston	24
S-16	**1,4,[5],12**	**i**	**1,2**	**Typhimurium**	**REF**
S-16	6,7	i	1,5	Oritamerin	22
S-18	**1,9,12**	**g,m**	**-**	**Enteritidis**	**REF**
S-18	1,9,12	-	-	Gallinarum	22
S-19	**1,4,[5],12**	**f,g,s**	**[1,2]**	**Agona**	**REF**
S-19	4, 12	g, f	1, 2	Derby	4
S-19	2,12	gm	-	Nitra	22
S-20	**3,{10}{15}{15,34}**	**e,h**	**1,5**	**Muenster**	**REF**
S-20	3,{10}{15}	e,h	1,7	Nyborg	8
S-20	4	h	1	Reading	10
S-20	3,10	e,h	1,6	Anatum	11
S-20	11	l,v	z	III b	22

remark
partly correct, in the naming: no penalty points
incorrect, in the naming: 1 penalty point
incorrect, in the naming: 4 penalty points

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	**1b**	**< CL**	**SCL**	**CL**	**< OL**	**CL**	**SCL**	**CL**	**< OL**	**< OL**	**SCL**	**CL**	**CL**	**CL**	**< CL**	**CL**	**CL**	**SCL**
1	1b	OL	SCL	CL	< OL	CL	SCL	CL	OL	OL	OL	CL	CL	CL	CL	CL	CL	< OL
6	1b	< CL	CL	CL	OL	CL	< OL	CL	CL	< OL	< OL	SCL	OL	CL	< CL	0	CL	OL
10	1b	+++	SCL	OL	SCL	SCL	+++	SCL	+++	+++	+++	SCL	SCL	SCL	+++	SCL	SCL	SCL
12	1b	<SCL	SCL	CL	OL	CL	SCL	CL	CL	SCL	SCL	CL	CL	<SCL	CL	CL	CL	< OL
17	1B	+++	+++	CL	++	CL	SCL	< CL	+++	+++	++	< CL	CL	CL	CL	CL	CL	OL
21	PT1b	+++	+++	< CL	OL	CL	+++	SCL	OL	< OL	< OL	SCL	OL	SCL	+++	SCL	SCL	< OL
29	1b	OL	CL	CL	OL	CL	SCL	CL	OL	OL	OL	CL	CL	CL	CL	CL	CL	< OL

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	**14b**	**-**	**-**	**-**	**-**	**-**	**SCL**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**SCL**
1	14b	-	-	-	±	-	SCL	-	-	±	-	-	-	-	-	-	-	< OL
6	14b	-	-	-	1 – 5	-	++	-	-	1 – 5	-	-	-	-	-	0	-	< OL
10	14b	0	0	0	+	0	+++	0	0	+	0	0	0	0	0	0	0	+++
12	14b	-	-	-	±	-	SCL	-	-	±	-	-	-	-	-	-	-	OL
17	14B	-	-	-	-	-	SCL	-	-	-	-	-	-	-	-	-	-	SCL
21	PT14b	-	-	-	±	-	+	-	-	±	-	-	-	-	-	-	-	±±±
29	14b	-	-	-	-	-	SCL	-	-	±	-	-	-	-	-	-	-	OL

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	**4**	**-**	**CL**	**CL**	**< OL**	**CL**	**SCL**	**CL**	**OL**	**< OL**	**< OL**	**CL**	**CL**	**CL**	**-**	**-**	**-**	**SCL**
1	4	-	< CL	CL	OL	CL	SCL	CL	OL	OL	SCL	CL	CL	CL	-	-	-	< OL
6	4	-	CL	CL	OL	CL	SCL	CL	OL	OL	< OL	CL	CL	CL	-	0	-	OL
10	4	0	SCL	OL	OL	SCL	+++	SCL	+++	OL	+++	SCL	SCL	SCL	0	0	0	+++
12	4	-	SCL	CL	SCL	CL	SCL	CL	CL	SCL	CL	CL	CL	SCL	-	-	-	OL
17	4	-	+++	CL	+++	CL	< CL	CL	OL	+++	< CL	SCL	CL	CL	-	-	-	SCL
21	PT4	-	SCL	+++	OL	CL	+++	SCL	OL	< OL	< OL	SCL	CL	SCL	-	-	-	+++
29	4	-	SCL	CL	OL	CL	SCL	CL	OL	OL	OL	CL	CL	CL	-	-	-	< 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	**3**	**OL**	**-**	**-**	**-**	**-**	**+**	**-**	**OL**	**-**	**OL**	**-**	**-**	**-**	**< CL**	**-**	**-**	**-**
1	22	OL	-	-	SCL	-	SCL	-	OL	OL	OL	-	-	-	SCL	-	-	SCL
6	3	CL	-	-	-	-	+	-	OL	-	< OL	-	-	-	< CL	0	-	-
10	21	SCL	+++	0	+++	0	+++	0	SCL	+++	SCL	0	0	0	SCL	0	0	+++
12	3	OL	-	-	-	-	+	-	CL	-	CL	-	-	-	CL	-	-	-
17	3	CL	-	-	-	-	+++	-	CL	-	CL	-	-	-	SCL	-	-	-
21	PT21	CL	+++	-	+++	-	++	-	OL	< OL	< OL	-	±	-	± ± ±	-	-	± ± ±
29	3	CL	-	-	-	-	±	-	CL	-	CL	-	-	-	CL	-	-	-

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	**33**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**< CL**	**-**	**-**	**-**
1	33	-	-	-	-	-	-	-	-	-	-	-	-	-	CL	-	-	-
6	33	-	-	-	-	-	-	-	-	-	-	-	-	-	< CL	0	-	-
10	33	0	0	0	0	0	0	0	0	0	0	0	0	0	SCL	0	0	0
12	33	-	-	-	-	-	-	-	-	-	-	-	-	-	CL	-	-	-
17	33	-	-	-	-	-	-	-	-	-	-	-	-	-	SCL	-	-	-
21	PT33	-	-	-	-	-	-	-	-	-	-	-	-	-	< OL	-	-	-
29	33	-	-	-	-	-	-	-	-	-	-	-	-	-	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	**59**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**SCL**
1	59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< OL
6	rdnc or 23	-	-	-	+	-	-	-	-	±	-	-	-	-	-	0	-	OL
10	59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	SCL
12	23	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	SCL
17	14B	-	-	-	-	-	< OL	-	-	++	-	-	-	-	-	-	-	< OL
21	PT59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< OL
29	23	-	-	-	+++	-	-	-	-	++	-	-	-	-	-	-	-	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	**35**	**-**	**SCL**	**-**	**SCL**	**-**	**-**	**-**	**-**	**< OL**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**< OL**
1	35	-	CL	-	OL	-	-	-	-	OL	-	-	-	-	-	-	-	< OL
6	35	-	CL	-	OL	-	-	-	-	OL	-	-	-	-	-	0	-	OL
10	35	0	SCL	0	OL	0	0	0	0	OL	0	0	0	0	0	0	0	<SCL
12	35	-	SCL	-	OL	-	-	-	-	<SCL	-	-	-	-	-	-	-	OL
17	21	+++	+++	-	< OL	-	+++	-	SCL	< OL	< OL	-	-	-	SCL	-	-	< OL
21	PT35	-	+++	-	OL	-	-	-	-	OL	-	-	-	-	-	-	-	OL
29	35	-	SCL	-	SCL	-	-	-	-	OL	-	-	-	-	-	-	-	< OL

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	**13a**	**-**	**-**	**-**	**OL**	**-**	**SCL**	**-**	**OL**	**< OL**	**OL**	**-**	**-**	**-**	**-**	**-**	**-**	**< OL**
1	13a	-	-	-	OL	-	SCL	-	OL	OL	OL	-	-	-	-	-	-	< OL
6	13a	1 – 5	-	-	OL	-	++	-	OL	OL	OL	-	-	-	-	0	-	OL
10	13a	0	0	0	SCL	0	+++	0	SCL	OL	SCL	0	0	0	0	0	0	+++
12	13a	-	-	-	OL	-	<SCL	-	OL	SCL	CL	-	-	-	-	-	-	< OL
17	13	-	-	-	< OL	-	SCL	-	-	< OL	-	-	-	-	-	-	-	< OL
21	PT13a	-	-	-	SCL	-	±	-	OL	< OL	< OL	-	1 – 5	-	-	-	-	< OL
29	13a	-	-	-	SCL	-	++	-	OL	OL	OL	-	-	-	-	-	-	< 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	**8**	**-**	**-**	**CL**	**OL**	**CL**	**SCL**	**CL**	**OL**	**< OL**	**OL**	**CL**	**CL**	**-**	**-**	**-**	**-**	**< OL**
1	8	-	-	OL	OL	CL	SCL	CL	OL	OL	SCL	CL	CL	-	-	-	-	< OL
6	8	-	-	< CL	OL	CL	SCL	< CL	OL	OL	OL	SCL	CL	-	-	0	-	OL
10	8	0	0	OL	OL	OL	+++	+++	OL	OL	SCL	++	SCL	0	0	0	0	+++
12	8	-	-	<SCL	CL	CL	SCL	SCL	OL	SCL	CL	SCL	CL	-	-	-	-	< OL
17	8	-	-	SCL	+++	CL	SCL	< CL	OL	+++	< CL	SCL	CL	-	-	-	-	< OL
21	PT8	-	-	+	SCL	SCL	±	SCL	OL	< OL	< OL	SCL	SCL	-	-	-	-	+++
29	8	-	-	SCL	SCL	CL	+++	SCL	OL	OL	OL	SCL	CL	-	-	-	-	< OL

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	**56**	**-**	**-**	**CL**	**< OL**	**SCL**	**-**	**SCL**	**-**	**< OL**	**-**	**+**	**+**	**-**	**-**	**-**	**-**	**< OL**
1	2	< OL	-	OL	OL	OL	SCL	<SCL	OL	CL	SCL	<SCL	<SCL	-	<SCL	-	-	< OL
6	56	-	-	< OL	OL	±	-	SCL	-	OL	-	< OL	±	-	-	0	-	OL
10	2	+++	0	OL	OL	OL	++	++	+++	SCL	++	+++	OL	0	++	0	0	+++
12	56	-	-	SCL	CL	SCL	-	+++	-	SCL	±	±±±	1 – 5	-	-	-	-	< OL
17	2	+++	-	SCL	+++	SCL	SCL	+++	+++	+++	+++	++	+++	-	SCL	-	-	< OL
21	PT56	-	-	+	OL	±±	-	SCL	-	< OL	-	±	+	-	-	-	-	< OL
29	56	-	-	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	**36**	**CL**	**CL**	**CL**	**OL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**
1	36	SCL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL
6	36	< CL	SCL	CL	OL	CL	CL	SCL	CL	< CL	CL	CL	CL	CL	CL	CL	SCL	CL	CL
10	36	SCL	SCL	SCL	CL	CL	CL	CL	CL	CL	SCL	CL	CL	CL	CL	CL	CL	CL	CL
12	36	SCL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL
17	36	SCL	CL	CL	OL	CL	CL	CL	SCL	CL	CL	SCL	SCL	CL	CL	CL	CL	CL	CL
21	DT36	SCL	+++	CL	CL	CL	SCL	SCL	SCL	SCL	CL	SCL	SCL	SCL	CL	CL	SCL	SCL	CL
29	36	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	< CL	CL	CL	CL	CL	CL	CL	CL

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	**36**	**< CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**OL**	**CL**	**CL**	**OL**	**+++**	**+++**	**+++**	**OL**	**OL**	**OL**	**CL**
1	36	CL	CL	CL	+++	CL	CL	CL	CL	CL	CL	CL	CL	-	-	-	OL	OL	OL	CL
6	36	SCL	SCL	< CL	< CL	CL	OL	< CL	< CL	CL	CL	CL	OL	+++	±	+	OL	OL	< OL	CL
10	36	SCL	+++	SCL	CL	CL	CL	CL	CL	CL	CL	SCL	CL	+++	+++	+++	OL	CL	OL	SCL
12	36	SCL	CL	SCL	CL	CL	CL	SCL	CL	CL	CL	CL	OL	0	0	0	0	0	0	0
17	36	CL	CL	< CL	CL	CL	CL	< CL	< CL	< CL	OL	CL	OL	-	±	+++	< CL	< OL	< OL	OL
21	DT36	+++	+++	+++	+++	+++	SCL	+++	+++	+++	+++	±±	OL	-	±	-	< OL	< OL	< OL	< OL
29	36	SCL	CL	SCL	SCL	CL	CL	CL	CL	CL	CL	CL	CL	-	-	-	OL	OL	< OL	CL

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**	**-**	**-**	**-**	**-**	**+++**	**-**
1	104L	-	-	-	-	-	-	-	-	-	-	OL	OL	-	-	-	-	+++	-
6	104	-	-	-	-	-	-	-	-	-	-	< CL	SCL	-	-	-	-	< CL	-
10	104L	0	0	0	0	0	0	0	0	0	0	SCL	CL	0	0	0	0	+++	0
12	104	-	-	-	-	-	-	-	-	-	-	CL	CL	-	-	-	-	+	-
17	12	-	-	-	-	-	-	-	-	-	-	< OL	OL	-	-	-	-	-	-
21	DT104	-	-	-	-	-	-	-	-	-	-	SCL	SCL	-	-	-	-	+++	-
29	104	-	-	-	-	-	-	-	-	-	-	SCL	CL	-	-	-	-	+++	-

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**	**-**
1	104L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	OL	OL	-
6	104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CL	CL	< OL	-
10	104L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+++	CL	+++	0
12	104	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0
17	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< CL	< OL	< OL	-
21	DT104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< OL	< OL	< OL	-
29	104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	OL	< OL	-

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	**U311**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
1	U311	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	U311	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	U311	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	U311	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	U311	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	U311	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	U311	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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	**U311**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**OL**	**-**
1	U311	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	-
6	U311	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< OL	-
10	U311	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	<SCL	0
12	U311	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL
17	U311	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	-
21	U311	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< OL	-
29	U311	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OL	-

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	**8**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**SCL**	**CL**	**SCL**	**-**	**-**	**-**	**-**	**+++**	**-**	**-**	**-**
1	8	-	-	-	-	-	-	-	<SCL	<SCL	<SCL	-	-	-	-	+++	-	-	-
6	8	-	-	-	-	-	-	-	+++	+++	+++	-	-	-	-	±	-	-	-
10	8	0	0	0	0	0	0	0	SCL	SCL	++	0	0	0	0	0	0	+++	0
12	8	-	-	-	-	-	-	-	SCL	SCL	<SCL	-	-	-	-	<SCL	-	-	-
17	8	-	-	-	-	-	-	-	+++	+++	+++	-	-	-	-	++	-	-	-
21	DT8	-	-	-	-	-	-	-	< OL	OL	OL	-	-	-	-	< OL	-	-	-
29	8	-	-	-	-	-	-	-	SCL	SCL	+	-	-	-	-	++	-	-	-

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	**8**	**SCL**	**-**	**< OL**	**< CL**	**-**	**+**	**+**	**-**	**-**	**CL**	**CL**	**-**	**+**	**+**	**+**	**< CL**	**CL**	**SCL**	**-**
1	8	< OL	-	<SCL	+++	-	-	-	-	-	OL	OL	-	-	-	-	OL	OL	OL	-
6	8	++	-	+	+++	-	1 – 5	-	-	-	SCL	< CL	-	++	-	++	OL	OL	< OL	-
10	8	+++	0	++	SCL	0	++	+	0	0	SCL	++	0	++	++	+++	SCL	+++	OL	0
12	8	+++	-	<SCL	<SCL	-	1 – 5	1 – 5	-	-	<SCL	CL	-	0	0	0	0	0	0	0
17	8	+++	-	<SCL	CL	-	++	+	-	-	CL	CL	-	-	-	+++	< CL	OL	OL	-
21	DT8	± ± ±	-	+	± ± ±	-	+	1 – 5	-	-	+++	± ± ±	-	-	-	-	OL	OL	OL	-
29	8	SCL	-	SCL	SCL	-	-	-	-	-	SCL	+++	-	-	-	-	OL	OL	< OL	-

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	**41**	**< OL**	**OL**	**OL**	**OL**	**OL**	**CL**	**OL**	**-**	**OL**	**OL**	**-**	**-**	**OL**	**OL**	**OL**	**OL**	**< CL**	**OL**
1	41	+++	OL	OL	OL	<SCL	OL	<SCL	-	<SCL	OL	-	-	OL	OL	OL	OL	CL	OL
6	41	SCL	+	CL	OL	< CL	< CL	++	-	SCL	CL	-	-	CL	CL	< CL	SCL	CL	CL
10	41	+++	SCL	SCL	OL	SCL	SCL	SCL	0	SCL	+++	0	0	SCL	CL	CL	SCL	SCL	CL
12	41	SCL	CL	CL	OL	CL	CL	CL	-	SCL	CL	-	-	SCL	CL	CL	CL	CL	CL
17	41	+++	SCL	CL	OL	SCL	CL	+++	-	+++	+++	-	-	SCL	CL	SCL	CL	+++	CL
21	DT41b	SCL	SCL	SCL	SCL	+++	SCL	SCL	-	+++	CL	-	-	+++	CL	SCL	SCL	SCL	SCL
29	41	SCL	CL	CL	CL	CL	SCL	SCL	-	SCL	SCL	-	-	CL	CL	CL	SCL	CL	CL

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	**41**	**+++**	**OL**	**OL**	**OL**	**OL**	**OL**	**OL**	**OL**	**-**	**OL**	**OL**	**OL**	**+++**	**++**	**+++**	**OL**	**OL**	**OL**	**+++**
1	41	<SCL	<SCL	<SCL	±	<SCL	<SCL	<SCL	<SCL	1 – 5	< OL	SCL	OL	+++	+++	+++	-	-	-	OL
6	41	++	SCL	SCL	++	< CL	< CL	SCL	SCL	1 – 5	< CL	SCL	OL	+++	-	++	-	-	-	CL
10	41	+++	+++	++	SCL	SCL	SCL	SCL	SCL	0	SCL	+++	SCL	SCL	+++	SCL	0	0	0	SCL
12	41	SCL	CL	SCL	<SCL	CL	CL	SCL	CL	-	SCL	CL	OL	0	0	0	0	0	0	0
17	41	+++	<SCL	SCL	+++	+++	SCL	+++	+++	-	CL	+++	OL	-	-	+++	-	-	SCL	OL
21	DT41b	±±±	±±	+	+	SCL	±±±	±±	±±	-	++	±	OL	±	-	SCL	-	-	+	OL
29	41	SCL	CL	SCL	SCL	CL	CL	SCL	CL	-	CL	SCL	CL	-	-	-	-	-	-	CL

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**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
1	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	193	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	DT195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**+++**	**+++**	**+++**	**-**	**-**	**-**	**-**
1	193	-	-	-	-	-	-	-	-	-	-	-	-	+++	+++	+++	-	-	-	-
6	193	-	-	-	-	-	-	-	-	-	-	-	-	+++	±	++	1 – 5	-	-	-
10	193	0	0	0	0	0	0	0	0	0	0	0	0	+++	+++	+++	0	0	0	0
12	193	-	-	-	-	-	-	-	-	-	-	-	-	+	+++	+	-	-	-	-
17	195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<SCL	-	-	-	-
21	DT195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+++	+	-	-	-
29	193	-	-	-	-	-	-	-	-	-	-	-	-	++	+	+	-	-	-	-

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	1	CL	CL	CL	OL	CL	CL	CL	-	CL	OL	OL	OL	CL	OL	CL	OL	CL	CL
1	1	CL	CL	CL	CL	CL	CL	CL	-	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL
6	1	SCL	SCL	CL	OL	CL	CL	SCL	-	SCL	CL	CL	SCL	CL	CL	CL	< CL	CL	CL
10	1	SCL	SCL	SCL	SCL	SCL	SCL	SCL	0	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL
12	1	SCL	CL	CL	OL	CL	CL	CL	-	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL
17	1	<SCL	< OL	CL	OL	CL	CL	< CL	-	CL	CL	SCL	CL	OL	OL	OL	CL	SCL	CL
21	DT1	SCL	SCL	CL	CL	CL	SCL	SCL	-	SCL	CL	CL	SCL	SCL	CL	CL	CL	SCL	SCL
29	1	SCL	CL	CL	CL	CL	CL	SCL	-	CL	CL	SCL	CL	CL	CL	CL	CL	CL	CL

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	1	< CL	OL	OL	CL	CL	CL	CL	CL	-	CL	CL	OL	-	-	-	OL	OL	OL	OL
1	1	CL	CL	CL	SCL	CL	CL	SCL	SCL	-	CL	CL	CL	-	-	-	OL	OL	OL	CL
6	1	+	SCL	+	+	CL	CL	SCL	SCL	-	< CL	CL	OL	-	-	-	OL	< OL	< OL	CL
10	1	+++	+++	SCL	SCL	SCL	SCL	SCL	SCL	0	SCL	+++	SCL	0	0	0	SCL	SCL	SCL	SCL
12	1	+++	CL	CL	CL	CL	CL	SCL	CL	-	SCL	SCL	CL	0	0	0	0	0	0	0
17	1	SCL	< OL	< OL	CL	SCL	CL	SCL	CL	-	OL	OL	OL	-	-	-	< CL	OL	OL	OL
21	DT1	+++	+++	±±±	±±±	SCL	SCL	+++	SCL	-	SCL	SCL	OL	-	-	-	OL	OL	OL	OL
29	1	SCL	CL	CL	CL	CL	CL	SCL	CL	-	CL	SCL	CL	-	-	-	OL	OL	< OL	CL

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	10	-	-	-	-	-	-	-	-	CL	CL	CL	CL	-	-	SCL	-	-	-
1	10	-	-	-	-	-	-	-	-	OL	OL	OL	OL	-	-	< OL	-	-	-
6	10	-	-	-	-	-	-	-	1 – 5	CL	OL	CL	CL	-	-	++	-	-	-
10	67	0	0	0	0	0	0	0	0	SCL	0	SCL	SCL	0	0	+++	0	0	0
12	10	-	-	-	-	-	-	-	-	CL	CL	CL	CL	-	-	SCL	-	-	-
17	10	-	-	-	-	-	-	-	-	CL	SCL	OL	OL	-	-	+++	-	-	-
21	DT10	-	-	-	-	-	-	-	-	+++	CL	CL	CL	-	-	+++	-	-	-
29	10	-	-	-	-	-	-	-	-	SCL	CL	SCL	CL	-	-	+++	-	-	-

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	10	< CL	-	OL	CL	-	SCL	SCL	-	-	CL	CL	-	+++	+++	+++	OL	OL	OL	-
1	10	OL	-	OL	<SCL	-	±	±	-	-	CL	CL	-	-	-	-	OL	OL	OL	-
6	10	SCL	1 – 5	SCL	< OL	-	±	1 – 5	-	-	CL	CL	-	±	-	+	OL	OL	< OL	-
10	67	+++	0	+++	SCL	0	0	+++	+++	0	SCL	+++	0	+++	+++	+++	+++	+++	+++	0
12	10	SCL	1 – 5	SCL	SCL	-	1 – 5	±	-	-	<SCL	CL	-	0	0	0	0	0	0	0
17	10	SCL	-	SCL	CL	-	++	-	-	-	CL	CL	-	-	-	+++	< CL	< OL	< OL	-
21	DT10	±±±	+	±±	±±	-	++	-	-	-	SCL	SCL	-	-	-	-	OL	OL	OL	-
29	10	SCL	+	SCL	SCL	-	-	±	-	-	CL	SCL	-	-	-	-	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	**132**	**-**	**CL**	**CL**	**OL**	**CL**	**++**	**-**	**-**	**SCL**	**< CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**-**	**CL**
1	2	-	OL	OL	OL	OL	+++	-	-	++	OL	OL	OL	OL	OL	OL	< OL	-	OL
6	rdnc	-	SCL	CL	OL	< CL	-	-	-	1 – 5	CL	CL	CL	CL	CL	< OL	SCL	-	SCL
10	2	0	SCL	SCL	SCL	< OL	OL	0	0	+++	+++	SCL	SCL	SCL	SCL	SCL	SCL	0	SCL
12	2	-	CL	CL	CL	CL	CL	-	-	<SCL	CL	CL	CL	CL	CL	CL	CL	-	CL
17	2	-	OL	CL	CL	CL	SCL	-	-	+++	CL	< OL	CL	CL	CL	CL	SCL	-	CL
21	DT135	-	SCL	SCL	SCL	SCL	±±	-	-	±±	CL	CL	CL	CL	CL	SCL	SCL	-	SCL
29	47	-	CL	SCL	SCL	SCL	+	-	-	++	SCL	CL	CL	SCL	CL	SCL	CL	-	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	**132**	**+**	**< CL**	**OL**	**SCL**	**< CL**	**CL**	**CL**	**CL**	**-**	**CL**	**CL**	**OL**	**+++**	**+++**	**SCL**	**-**	**-**	**+**	**OL**
1	2	+++	+++	<SCL	+	<SCL	<SCL	<SCL	<SCL	-	< OL	< OL	< OL	-	-	-	OL	OL	OL	+++
6	rdnc	-	< OL	+	-	SCL	< OL	< OL	+	-	SCL	< CL	< OL	++	1 – 5	++	OL	OL	< OL	1 – 5
10	2	++	+++	+++	+++	SCL	SCL	SCL	SCL	0	SCL	+++	SCL	+++	+++	+++	SCL	SCL	SCL	0
12	2	SCL	SCL	<SCL	±	CL	SCL	SCL	CL	-	SCL	CL	CL	0	0	0	0	0	0	0
17	2	++	< OL	SCL	+++	+++	CL	SCL	SCL	-	CL	CL	OL	-	-	+++	< CL	< OL	< OL	SCL
21	DT135	+	SCL	+	1 – 5	OL	SCL	+++	+++	-	SCL	±±	SCL	-	-	-	OL	OL	OL	-
29	47	+	CL	CL	SCL	SCL	SCL	SCL	-	-	CL	SCL	-	+	+	+	OL	OL	< OL	-

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	**136**	**-**	**-**	**-**	**OL**	**CL**	**CL**	**-**	**-**	**-**	**CL**	**CL**	**CL**	**-**	**CL**	**CL**	**-**	**-**	**CL**
1	136	-	-	-	OL	OL	OL	-	-	-	OL	OL	OL	-	OL	OL	-	-	OL
6	136	-	-	-	OL	CL	CL	-	-	-	CL	CL	CL	-	CL	CL	-	-	CL
10	136	0	0	0	SCL	SCL	SCL	0	0	0	++	SCL	SCL	0	SCL	SCL	0	0	SCL
12	136	-	-	-	OL	OL	OL	-	-	-	CL	CL	CL	-	OL	OL	-	-	CL
17	136	-	-	-	OL	CL	CL	-	-	-	CL	OL	OL	-	CL	CL	-	-	CL
21	DT136	-	-	-	OL	< OL	SCL	-	-	-	CL	OL	OL	-	CL	CL	-	-	CL
29	136	-	-	-	CL	CL	SCL	-	-	-	SCL	CL	CL	-	CL	CL	-	-	CL

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	**136**	**+**	**-**	**-**	**-**	**-**	**CL**	**-**	**-**	**-**	**-**	**-**	**-**	**++**	**+**	**+++**	**OL**	**OL**	**OL**	**-**
1	136	+++	-	-	-	-	OL	-	-	-	-	-	-	-	-	-	OL	OL	OL	-
6	136	±	-	-	-	-	CL	-	-	-	-	-	-	-	-	-	OL	OL	< OL	-
10	136	++	0	0	0	0	SCL	0	0	0	0	0	0	0	0	0	+++	SCL	+++	0
12	136	±	-	-	-	-	CL	-	-	-	-	-	-	0	0	0	0	0	0	0
17	136	-	-	-	-	-	CL	-	-	-	-	-	-	-	-	-	OL	OL	OL	-
21	DT136	+	-	-	-	-	SCL	-	-	-	-	-	-	-	-	-	OL	OL	OL	-
29	136	+	-	-	-	-	CL	-	-	-	-	-	-	-	-	-	OL	OL	< OL	±

Annex 7 Examples of PFGE images obtained by the participants

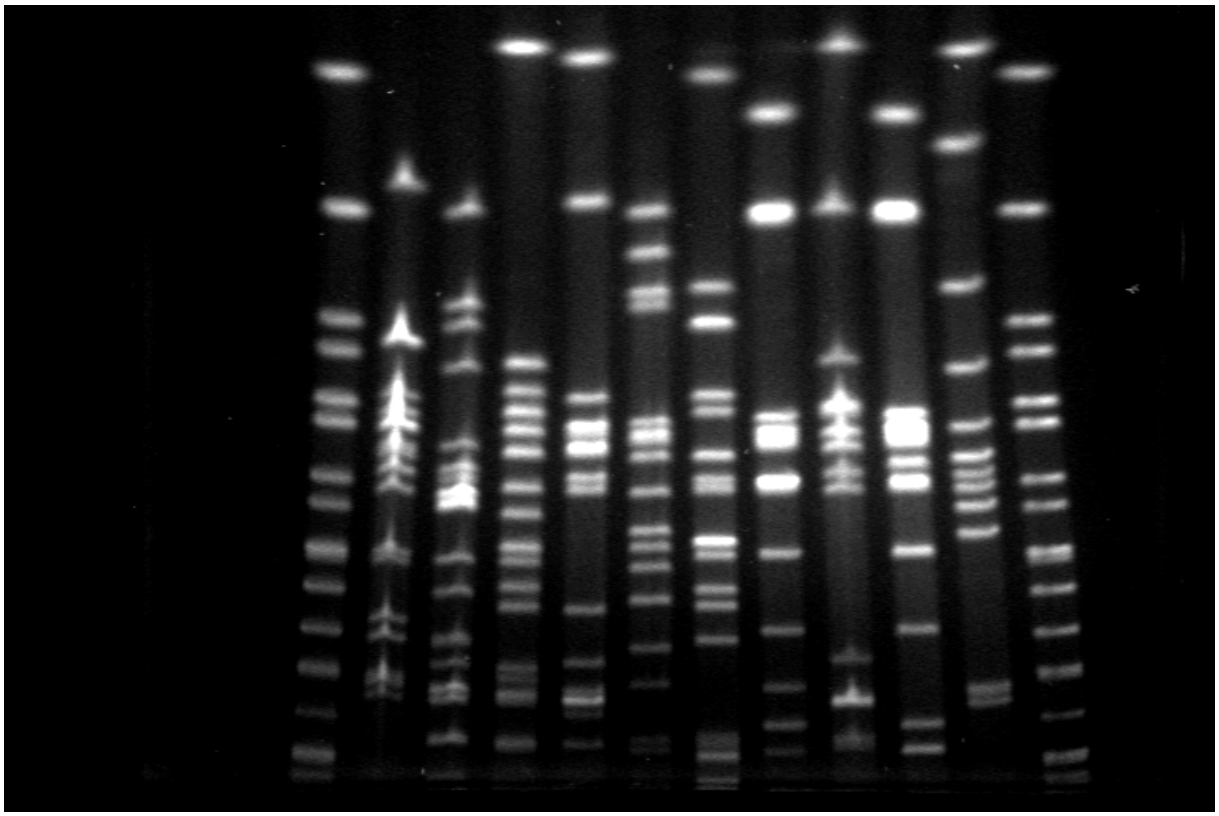


Figure A7.1. Example of a gel (lab code 16) with a generally low score

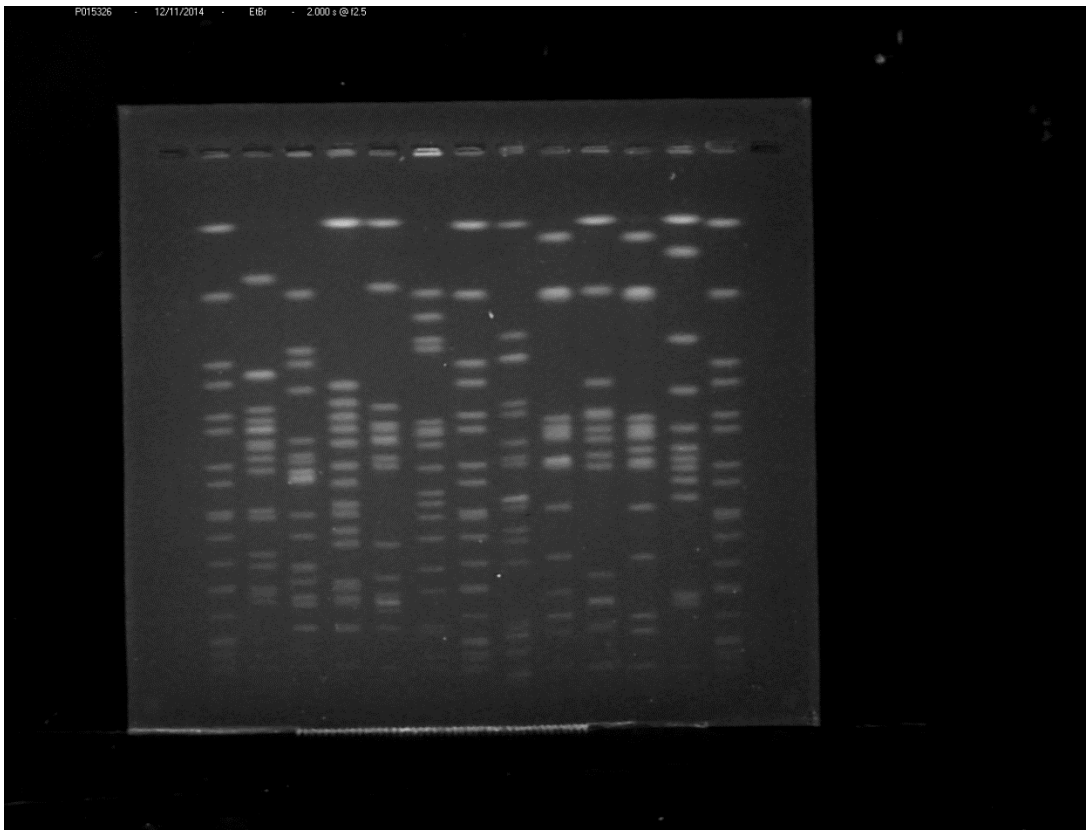


Figure A7.2. Example of a gel (lab code 29) with a generally high score

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

Individual Laboratory Results Interlaboratory Comparison Study *Salmonella* PFGE typing (November 2014)

NRL Laboratory code: **16**

Also at this second time that a PFGE typing study was included in the EURL-*Salmonella* interlaboratory comparison study on typing of *Salmonella*, participants were asked to test the 10 P-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 TIFF file only.
Like the first time, 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 17 participants in the 2014-PFGE study will be discussed at the Workshop in May 2015, and will be reported in the final report on the *Salmonella* typing study XIX (2014).
In Figure 1, your own laboratory PFGE profiles are compared to the reference profiles (in this study obtained from the NRL Austria). Figure 2 shows the display of the "Distortion bar" option in Bionumerics of your gel. Darker colours indicate critical normalisation.

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;
This reference strain has to be placed every 6 lanes at least.

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

Parameter	Evaluation	Comments	Points*
Image Acquisition and Running Conditions	Poor	Deviation in the use of standards. Wells not included on TIFF. Bottom of gel not visible. Bottom band of standard may not be 1-1,5 cm from bottom of gel.	1
Cell Suspension	Good	1-2 lanes contain lighter or darker bands than the other lanes.	3
Bands	Poor	Many bands are too thick, fuzzy or slanted, wich makes analysis too difficult.	1
Lanes	Poor	Smiling or curving that interferes with analysis.	1
Restriction	Good	One to two faint shadow bands on gel.	3
Gel Background	Excellent	Clear.	4
DNA Degradation	Excellent	No DNA degradation visible (no smearing).	4
Total score:			17

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

Individual Laboratory Results Interlaboratory Comparison Study *Salmonella* PFGE typing (November 2014)

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

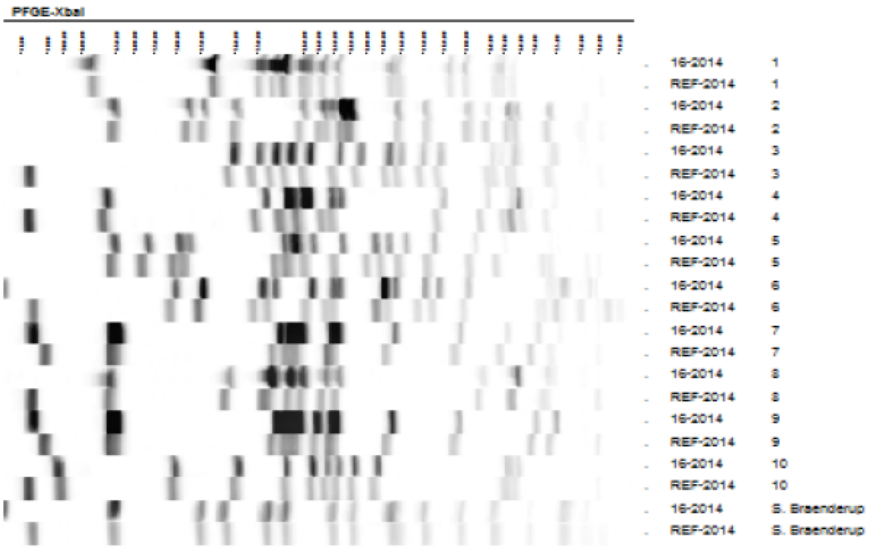
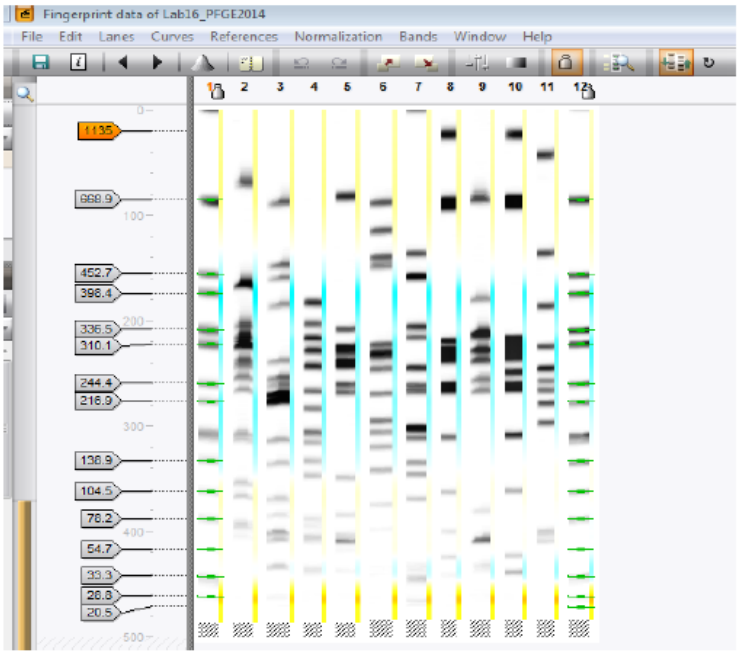


Figure 2. Display of the "Distortion bar" option in Bionumerics of your gel. Darker colours indicate critical normalisation.



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