National Institute for Public Health
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Synopsis

20th EURL-*Salmonella* interlaboratory comparison study (2015) on typing of *Salmonella* spp.

The National Reference Laboratories (NRLs) of all 28 European Union (EU) Member States performed well in the 2015 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 97% of the strains tested.

In addition to the standard method for typing *Salmonella* (serotyping), sixteen laboratories performed typing at DNA level, using Pulsed Field Gel Electrophoresis (PFGE). This more detailed typing method is sometimes needed to trace the source of a contamination. For quality control, the participants received another ten strains of *Salmonella* to be tested by this method. Fourteen of the sixteen participating laboratories were suitably equipped to use the PFGE method.

Since 1992, the NRLs of the EU Member States are obliged 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 referred to as the National Reference Laboratories (NRLs). The performance of these NRLs in *Salmonella* typing is assessed annually by testing their ability to identify twenty *Salmonella* strains. NRLs from countries outside the European Union occasionally participate in these tests on a voluntary basis. The EU-candidate-countries Former Yugoslav Republic of Macedonia and Turkey, and EFTA countries Iceland, Norway and Switzerland took part in the 2015 assessment.

The annual interlaboratory comparison study on *Salmonella* typing is organised by the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*). The EURL-*Salmonella* is located at the National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands.

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

Publiekssamenvatting

Twintigste EURL-*Salmonella* ringonderzoek (2015) voor de typering van *Salmonella* spp.

De Nationale Referentie Laboratoria (NRL's) van de 28 Europese lidstaten scoorden in 2015 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 97 procent van de geteste stammen de juiste naam konden geven.

Zestien laboratoria hebben, behalve de standaardtoets (serotypering) op *Salmonella*, extra typeringen op DNA-niveau uitgevoerd met behulp van de zogeheten PFGE-typering (Pulsed Field Gel Electroforese). Deze preciezere typering kan soms nodig zijn om de bron van een besmetting op te sporen. Om de kwaliteit ervan te toetsen moeten de laboratoria tien extra stammen met deze methode typeren. Veertien van de zestien deelnemende laboratoria waren daartoe in staat.

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 twintig *Salmonella*-stammen op juiste wijze identificeren. Soms doen ook landen van buiten de Europese Unie vrijwillig mee. In 2015 waren dat de kandidaat-lidstaten Macedonië en Turkije, en de EFTA-landen IJsland, Noorwegen en Zwitserland. EFTA staat voor European Free Trade Association.

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.

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

Contents

Summary

In November 2015, the 20th interlaboratory comparison study on typing of *Salmonella* was organised by the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*, Bilthoven, the Netherlands). The study's main objective was to evaluate whether the typing of *Salmonella* strains by the National Reference Laboratories (NRLs-*Salmonella*) in 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 (potential) EU-candidate-countries Former Yugoslav Republic of Macedonia and Turkey, and the EFTA countries Iceland, Norway and Switzerland.

All 34 laboratories performed serotyping. A total of twenty obligatory *Salmonella* strains plus one 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, 99% of the strains were typed correctly for the O-antigens, 97% of the strains were typed correctly for the H-antigens and 97% of the strains were correctly named by the participants.
At the EURL-*Salmonella* Workshop in 2007, criteria for 'good performance' during an interlaboratory comparison study on serotyping were defined. Using these criteria, 33 participants achieved good results. The laboratory that did not achieve the level of good performance participated in a follow-up study including ten additional strains for serotyping. This EU-NRL obtained good scores in this obligatory follow-up study.

Sixteen participating laboratories also performed additional typing at DNA level, using Pulsed Field Gel Electrophoresis (PFGE).
The participants received another ten strains of *Salmonella* to be tested by this method. Fourteen of the sixteen participating laboratories were able to produce a PFGE gel of sufficient quality to enable a profile determination suitable for use in inter-laboratory database comparisons. In addition, twelve participants also processed their gel in the dedicated software Bionumerics, and all of them were able to analyse their PFGE profiles in this computer program.
Some more adjustments to follow the guidelines more closely, both on PFGE gel image preparation and on gel analysis in Bionumerics, should still be able to improve the results of PFGE typing in general.

1 Introduction

This report describes the 20th 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 2015.

According to EC Regulation 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*) in 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 34 laboratories participated in this study. These included 29 NRLs-*Salmonella* in the 28 EU Member States, 2 NRLs in (potential) EU-candidate countries and 3 NRLs in EFTA countries. The main objective of this study was to check the performance of the NRLs in serotyping of *Salmonella* spp. and to compare the results of serotyping of *Salmonella* spp. among the NRLs-*Salmonella*. All NRLs performed serotyping of the 20 obligatory strains and all but two of the participants serotyped the optional 21st strain. 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.

For the third time, the typing study also included PFGE typing. Sixteen NRLs participated in this part of the study by PFGE typing ten designated *Salmonella* strains and submitting images for evaluation. Twelve of these participants also used a pre-configured database, provided by the EURL-*Salmonella,* to analyse the profiles on their gel using the computer program BioNumerics.

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	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
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 20th EURL-*Salmonella* Workshop in Berlin (Mooijman, 2015), 1 additional strain (S21), being a less common strain, 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 20th EURL-*Salmonella *typing study*

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
S1	4,12	i	1,6	Agama
S2	6,7	l,v	1,5	Irumu
S3	1,3,19	d	1,5	Ahmadi
S4	8,20	z_4,z_{23}	[z_6]	Corvallis
S5	1,9,12	e,h	1,5	Eastbourne
S6	6,8	z_{10}	e,n,x	Hadar
S7	6,7,14	r	1,2	Virchow
S8	8,20	g,m,s	-	Emek
S9	1,13,23	m,t	-	Kintambo
S10	6,7	y	1,2	Richmond
S11	1,13,23	z_{29}	-	Cubana
S12	6,8	b	1,7	Eboko
S13	1,4,12,27	y	1,7	Teddington
S14[a)]	1,4,[5],12	i	-	1,4,[5],12:i:-
S15	3,{10}{15}{15,34}	e,h	l,w	Meleagridis
S16	1,4,[5],12	i	1,2	Typhimurium
S17	6,7,14	r	1,5	Infantis
S18	6,7,14	z_{10}	l,w	Jerusalem
S19	1,9,12	g,m	-	Enteritidis
S20	6,7,14	g,m,[p],s	[1,2,7]	Montevideo
S21[b)]	1,9,12	a	1,5	Miami

[a)] Typhimurium, monophasic variant as determined by PCR.

[b)] Miami, as determined after biochemical testing for malonate and minimal medium (Simmon's citrate).

3.2 Laboratory codes

Each NRL-*Salmonella* was randomly assigned a laboratory code between 1 and 34.

3.3 Protocol and test report

Two weeks before the start of the study, the NRLs received the protocol by email. As before, the study used web-based test report forms: a form for serotyping 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 44, 2015.
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 and PFGE typing were sent by the EURL-*Salmonella* in week 44, 2015. 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 presented 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

In 2007, criteria for 'good performance' during an interlaboratory comparison study on serotyping were defined (Mooijman, 2007). 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*. The criterion for good performance is set at less than four penalty points.
All EU Member State NRLs not meeting the criterion of good performance (four penalty points or more) have to participate in a 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 10 Salmonella *strains according to the White-Kauffmann-Le Minor scheme used in the follow-up part of the 20th EURL-*Salmonella *typing study*

Strain	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
SF-1	9,46	a	e,n,x	Baildon
SF-2	9,46	d	z_6	Plymouth
SF-3	1,9,12	l,z_{13}	e,n,x	Napoli
SF-4	1,4,[5],12	r	l,w	Bochum
SF-5	6,8	z10	e,n,x	Hadar
SF-6	6,7,14	l,v	e,n,z_{15}	Potsdam
SF-7	8,20	z_4,z_{23}	[z_6]	Corvallis
SF-8	6,8	z_{10}	e,n,x	Hadar
SF-9	6,7,14	r	1,5	Infantis
SF-10	6,7,14	z_{10}	l,w	Jerusalem

3.7 *Salmonella* strains for PFGE typing

A total of 10 *Salmonella* strains (coded P1–P10) were included in the 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-6 on PFGE typing, organised by the SSI for the Food- and Water-borne Diseases and Zoonoses Laboratories Network (FWD laboratories network) (ECDC, 2015). Background information on the strains is given in Table 4.

Table 4. Background information on the Salmonella *strains used for PFGE typing in 2015*

Strain code in study 2015 (EURL-*Salmonella*)	Strain code in EQA-6 (ECDC, 2015)	*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

3.8 Evaluation of the PFGE gel image

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. However, the EURL-*Salmonella*-recommended

method can be found in EFSA supporting publication 2014:EN-703 (Jacobs et al., 2014). The PFGE gel images were to be emailed as uncompressed 8-bit gray scale 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. Quality was assessed on seven parameters in accordance with the PulseNet guidelines (www.pulsenetinternational.org), as given in Annex 1. To comply with these guidelines the reference strain *S*. Braenderup H9812 must be run in every 6 lanes as a minimum. 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.
In general, an acceptable quality should be obtained for each parameter as a low quality score in just one category can still have a large impact on the suitability to further analyse the image and compare it to other profiles.

3.9 Evaluation of the analysis of the PFGE gel in BioNumerics

This year we introduced the evaluation of the (optional) analysis of the PFGE gel in the dedicated program BioNumerics.
In short, this included the following actions by the participants:

- start a new database in BioNumerics,
- import the pre-configured database set-up as sent by email on 30 November 2015,
- import the TIFF image and analyse the gel (also see the protocol EURL-*Salmonella* typing study XX-2015 for further reference, which is available on the website at https://www.eurlsalmonella.eu/proficiency-testing/typing-studies),
- export the analysed data in either XML plus TIFF files (BN 6.0 and below) or in one .ZIP file (BN 7),
- email the correctly named files in a zipped format to the EURL-*Salmonella*.

Evaluation of the analysis of the gel in BioNumerics was done according to the guidelines as used in the EQAs for the FWD laboratories (Annex 2).These guidelines use 5 parameters, which are scored with 1 (poor), 2 (fair/good) or 3 (excellent) points.

4 Questionnaire

4.1 General

A questionnaire was incorporated in both test reports of the interlaboratory comparison study (for serotyping 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.

Question 3: Date of receipt at your laboratory:
All participants received their package in the same week it was sent (week 44 of 2015).

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 2015?*

Question 6: How many Salmonella strains did your laboratory (approximately) serotype in 2015?
The replies to questions 5 and 6 are summarised in Table 5.

Table 5. Frequency and (approximate) number of strains serotyped, and (approximate) number of strains PFGE typed (for all 34 participants)

Lab code	Serotyping frequency in 2015	No. of strains serotyped in 2015	No. of strains PFGE typed in 2015
12	Daily	128	
1	Daily	150	250
11	Daily	200	
6	Daily	242	
13	Daily	300	
25	Daily	350	260
5	Daily	400	
4	Daily	400	
2	Daily	500	
16	Daily	600	20
32	Daily	800	
9	Daily	800	
3	Daily	1000	300
23	Daily	1200	
14	Daily	1200	10
30	Daily	1500	350
28	Daily	1600	
17	Daily	2500	50-100
18	Daily	3000	200
22	Daily	3500	20
15	Daily	4500	75
8	Daily	5500	150
24	Daily	6400	50
7	Thrice a week	139	
20	Thrice a week	530	40
34	Twice a week	109	
26	Twice a week	120	
19	Twice a week	124	
33	Twice a week	150	
31	Twice a week	243	
10	Twice a week	300	
21	Once a week	40	*
29	Once a week	750	70
27	Once a week	2500	150
n=34		41775	1945

*no answer available

Question 7: Is your laboratory accredited for Salmonella serotyping?
Out of the 34 participants, 31 mentioned to be accredited for serotyping of all *Salmonella* serovars, mainly according to ISO 17025, or more specifically mentioning ISO/TR 6579-3. The other three laboratories indicated to be planning to go for accreditation of *Salmonella* serotyping.

Question 8: What kind of sera do you use?
The replies to question 8 are summarised in Tables 6 and 7.

Table 6. Number of laboratories using sera from one or more manufacturers and/or in-house prepared sera

Number of manufacturers from which sera are obtained (including in-house preparations)	Number of NRLs (n=34)
1	10
2	8
3	11
4	5

Table 7. Number of laboratories using sera from various manufacturers

Manufacturer	Number of NRLs (n=34)
Biorad	15
Own preparation	4
Pro-Lab	6
Reagensia	2
Remel	2
Sifin	20
Statens Serum Institute (SSI)	30
Other	2

Question 9: The strains in this study were serotyped by: own laboratory/Other laboratory

One NRL-*Salmonella* (lab code 13) sent strain S4 to another laboratory for further serotyping or confirmation. All other laboratories tested all strains in their own laboratory.

4.4 Questions on the use of PCR / biochemical tests

Question 10: Did you use any biochemical test, like dulcitol, malonate, tartrate, minimal medium, etc., to distinguish between subspecies?

Twenty-seven participants confirmed the use of biochemical tests. Details are given in Table 8.

Table 8. Strains (as numbered 1 – 21, or grouped) on which biochemical tests were used

Lab code	API20E	Arabinose	beta-glucuronidase	Dulcitol	Galacturonate	Gelatinase	Glucose	H2S	Lactose	Malonate	Minimal medium	Mucate	ONPG	Rhamnose	Salicine	Simmon's citrate	Sorbitol	Tartrate	Xylose
1	21				21					21							21	21	
2																21			
3		21		x	x		x	x	x	x		x	x		x	21	x	21	
4a)																			
5b)										n									
6				y						y									
8c)																			
9				z						z						21		z	
12		21	p	p				21	p	p	21		p		p	21	p	p	21
13				p	p					p	p	p	p				p	p	
14				p	p					p		p			p		p		
15d)																			
16	21			21						21					21	21			
17										y									
18				21						p									
19						21												21	
20e)																			
22						q				q	21		q						
23				p						p			p		p		p	p	
24				9/21						p			9/21		9/21		9/21		
25b)																			
27a)																			
28					11					11									
30		21		2			21	21	2	s			s	21			2		21
31				21					21	21			21		21				
32										z									
33										r									

a) Strains S9, S11, S21 tested, but tests not stated
b) Malonate tested, but strains not stated
c) Strains S9, S11, S12, S21 tested, but tests not stated
d) All strains S1-S21 tested, but tests not stated
e) Strain S-21 tested, but tests not stated

n: All strains S1-S21
p: Strains S9, S11, S21
q: Strains S9, S11, S14, S20, S21
r: Strains S2, S9, S11, S12, S21
s: Strains S2, S9, S20
x: Strains S2, S9, S11, S20, S21
y: Strains S2, S9, S11, S21
z: Strains S2, S9, S20, S21

Question 11: Did you use PCR for confirmation of any of the serotyped strains S1–S21?

A total of 19 laboratories reported using PCR for the confirmation of strains.

Question 12: For which strains did you use this PCR?

Five laboratories used PCR to confirm all or almost all strains. Sixteen laboratories used PCR to confirm strain S14, the monophasic variant of

S. Typhimurium 1,4,[5],12:i:-, and three of these laboratories also used PCR to confirm strain S16, *S*. Typhimurium. Strains S1 (1x), S19 (1x) and S21 (2x) were also reported to have been confirmed using PCR.

Question 13: Is the PCR used commercially available, details and manufacturer?
One laboratory used a commercially available PCR: Check & Trace *Salmonella* by Check points.

Question 14: Reference literature
PCR testing is mainly done to confirm monophasic (Typhimurium) strains.

Ten 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:
- Fitzgerald et al., 2007/McQuiston et al., 2011.

Question 15: Do you use this PCR routinely?
Fifteen of the laboratories use this PCR routinely.

Question 16: How many samples did you test for Salmonella *using this PCR in 2015?*
The replies to question 16 are summarised in Table 9.

Table 9. Number of strains routinely tested by PCR in 2015

Laboratory code	Number of strains tested by PCR in 2015
12	4
25	5
5	5
4	12
20	approx. 20
1	25
13	30
29	40
31	42
32	40-50
3	150
27	543
30	approx. 600
17	2000

4.5 Questions regarding PFGE typing

Note that answers from one participant (Labcode 21) were missing, so generally only 15 answers per question are shown.

What method do you use for Salmonella PFGE?
Nine participants reported using the Standard PulseNet Protocol *Salmonella* PFGE (PulseNet International, 2013). Six participants use this Standard protocol with modifications.
How many strains did you approximately PFGE type in 2015?
Replies to this question are summarised in Table 5 (above).

Manufacturer of the XbaI Enzyme?
The replies to this question are summarised in Table 10

Table 10. Manufacturers of the enzyme XbaI used by the participants

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

Name/Model of the Electrophoresis System (e.g. CHEF Mapper II)?
The replies to this question are summarised in Table 11.

Table 11. Electrophoresis system used by the participants

Electrophoresis system	Number of NRLs
Bio-Rad CHEF Mapper (XA)	1
Bio-Rad CHEF-DR III System	11
Bio-Rad CHEF-DR II System	3

Name/Model of the Gel Documentation System (e.g. GelDoc 2000)?
The replies to this question are summarised in Table 12.

Table 12. Gel documentation system used by the participants

Gel documentation system	Number of NRLs
BIO-RAD VersaDoc 4000MP	1
EC3 Chemi HR 410 Imaging System	1
G:Box (Syngene)	1
GelDoc 2000	1
GelDoc XR	2
GelDoc XR+	6
GeneGenious (Syngene)	1
Image J	1
UVP Gel Doc It	1

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

Solution to stain the gel, and approximate time of staining and de-staining?
For straining the gel, two participants used GelRed and one particpant used SYBR SAfe; all other participants were using Ethidium Bromide. The duration of the staining varied between 10 minutes (1x) and 2

hours (1x), but most participants used 30 minutes (9x). De-staining (not applicable for GelRed) was even more diverse; varying between 30 minutes and 24 hours, but a majority of participants used up to 60 minutes.

Did you use a wide or narrow comb?
Nine participants used a comb with wide teeth, and six participants used a comb with narrow teeth.

5 Results

5.1 Serotyping results

5.1.1 *General comments on this year's evaluation*

As decided at the 20th EURL-*Salmonella* Workshop (Mooijman, 2015), 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 31 of the 34 participants (91%). This corresponds to 99% of the total number of strains. The H-antigens were correctly typed by 21 of the 34 participants (62%), corresponding to 97% of the total number of strains. A total of 19 participants (56%) gave the correct serovar names, corresponding to 97% of all strains evaluated.

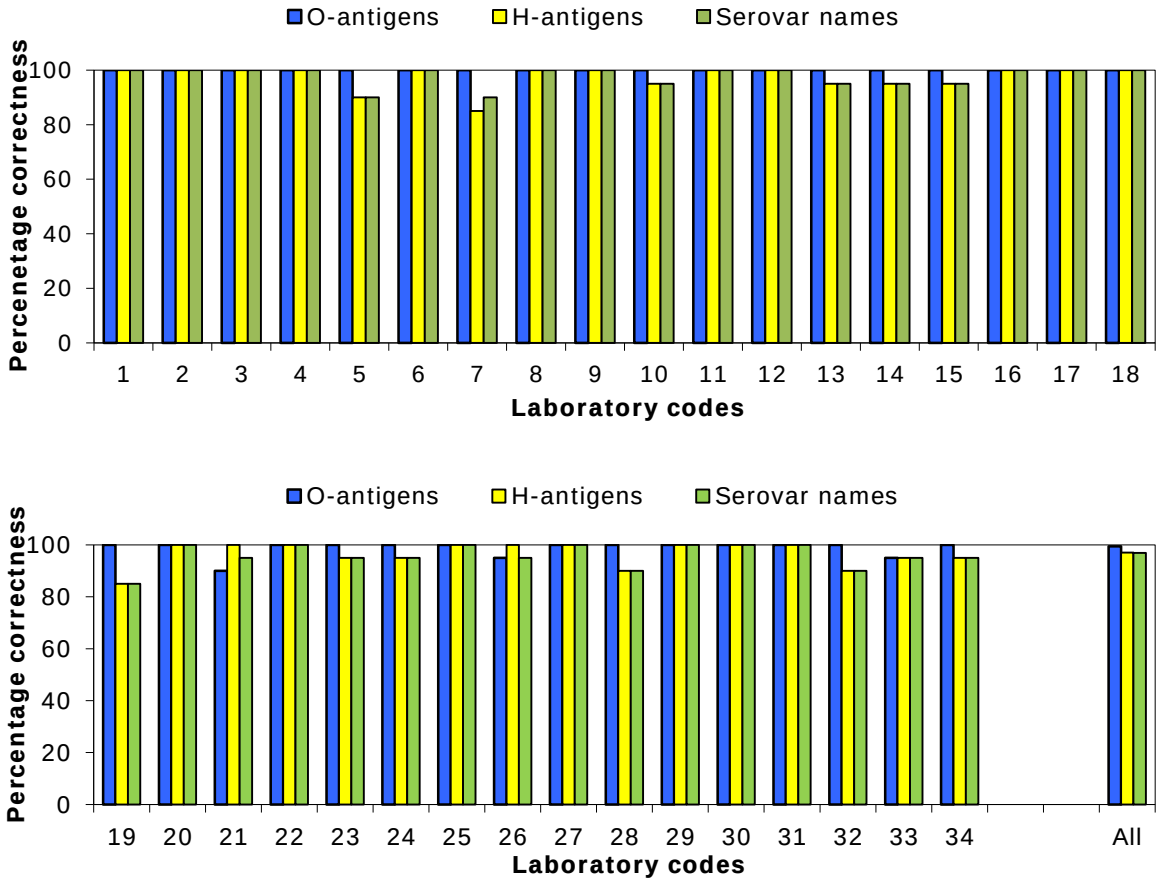

Figure 1. Percentages of correct serotyping results

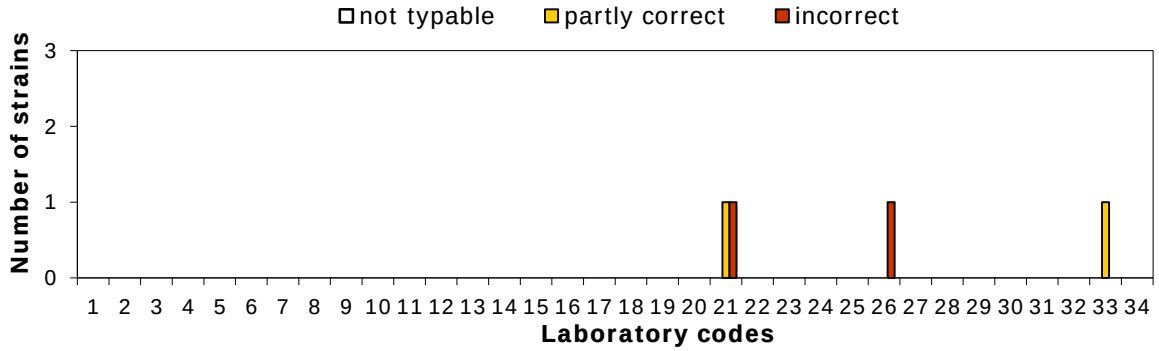

Figure 2. Evaluation of type of errors for O-antigens per NRL

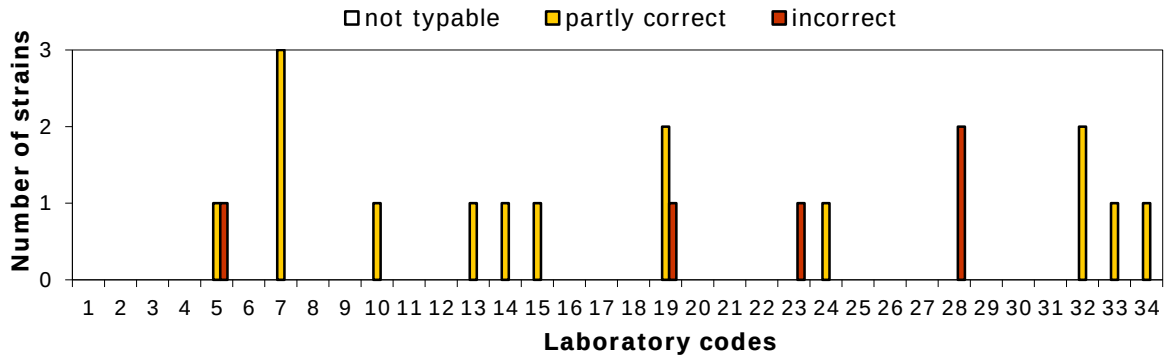

Figure 3. Evaluation of type of errors for H-antigens per NRL

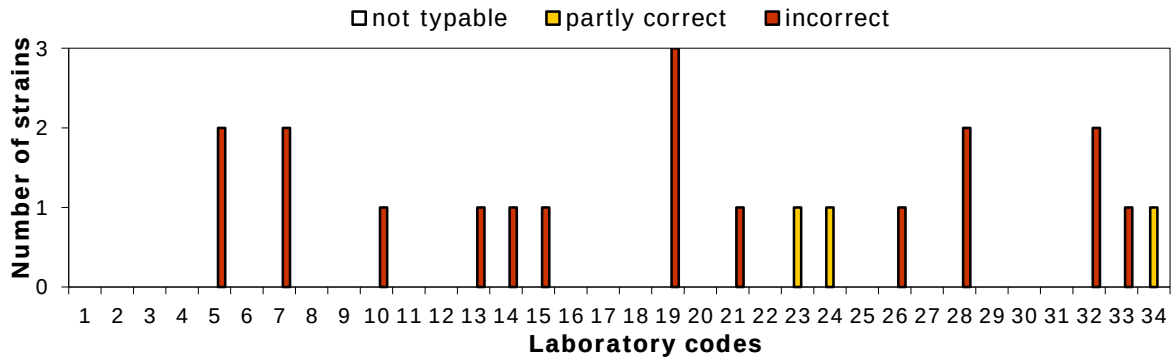

Figure 4. Evaluation of type of errors in the identification of serovar names

The number of penalty points was determined for each NRL using the guidelines described in Section 3.5. Table 13 shows the number of penalty points for each NRL and indicates whether the level of good performance was achieved (yes or no). One EU-NRL did not meet the level of good performance at this stage of the study and for this laboratory, a follow-up study was organised.

Table 13. Evaluation of serotyping results per NRL

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

5.1.3 *Serotyping results per strain*

The results found per strain and per laboratory are given in Annex 3, except for the more complicated strains S14 and S21; these are reported separately in Annex 4.
A completely correct identification was obtained for eleven *Salmonella* serovars: Agama (S1), Eastborne (S5), Virchow (S7), Emek (S8), Teddington (S13), 1,4,[5],12:i:- (S14), Meleagridis (S15), Typhimurium (S16), Infantis (S17), Enteritidis (S19), and Montevideo (S20). Most problems occurred with the serovar Kintambo (S9). Four laboratories had difficulties assigning the correct serovar name to this strain. Details on the strains that caused problems in serotyping are shown in Annex 5. The reported serovar names for strain S14 (Annex 4) continue to show some variation of 'Typhimurium-like' names.
Details on the additional and optional strain S21 are given in Annex 4 as well. All but two participants actually did serotype this strain S21, being a *Salmonella* Miami. All 32 laboratories correctly serotyped the O-antigens and the H-antigens for this strain, but in order to be able to correctly name this strain, some additional biochemistry was required. Six laboratories replied not to have done any biochemical tests on this strain and three participants therefore correctly named this strain 9,12:1:1,5. The other three laboratories named this strain Miami (2x) or II (1x, incorrect), but without the 'evidence' to do so.
The majority of the participants named S21 Miami, ruling out the possibility of a *S. enterica* subspecies *salamae* (II) result by testing on e.g. malonate or tartrate (also see Table 8). However, the 'proof' on how to have differentiated between Miami and Sendai was not always given.

The results as applicable from the White-Kauffmann-LeMinor (WKLM) scheme (Grimont and Weill, 2007) are given in Figure 5 and the WKLM

scheme (page 10) writes about differentiation of serovars with formula 1,9,12:a:1,5:
'Serovars Miami and Sendai are both kept in this scheme because they might be different. Biochemical characters formerly used for their differentiation (xylose, arabinose, rhamnose, H_2S) can only be used to define biovars. The differentiation is now based on an essential character: Sendai, which is adapted to man, is auxotrophic, i.e. does not grow on a minimal medium with glucose or on Simmons's citrate agar. On the contrary, Miami, which is ubiquist, is prototrophic, i.e. grows on such minimal media.'
The (primary) distinction between *Salmonella enterica* subspecies *enterica* (I) and subspecies *salamae* (II) could be made as usual, e.g. by testing on malonate or tartrate (Grimont and Weill, 2007; page 7).

Type	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase2
Sendai[1]	1,9,12	a	1,5
Miami[1]	1,9,12	a	1,5
II	9,12	a	1,5

[1] Sendai (adapted to man) is auxotrophic, Miami is prototrophic

Figure 5. Relevant information from the White-Kauffmann-LeMinor scheme on serovar 1,9,12:a:1,5

5.1.4 *Follow-up*

One EU-NRL did not achieve the level of good performance (Table 13; Lab code 5) and participated in a follow-up study, receiving 10 additional strains for serotyping in week 15, 2016.
The number of penalty points was determined using the guidelines described in Section 3.5. Table 14 shows the results of the follow-up study for participant 5, which achieved the level of good performance.

Table 14. Evaluation of serotyping results of the NRL in the follow-up study

Lab code	Penalty points	Good performance
5	0	Yes

5.2 PFGE typing results

5.2.1 *Results on the evaluation of the PFGE gel image*

A total of 16 participants sent in a PFGE gel image for evaluation.
The evaluation was done on the quality of the PFGE images and quality grading was done according to the PulseNet guidelines (Annex 1). These guidelines use seven parameters, which are scored with 1 (poor) to 4 (excellent) points.
The scores per NRL (n=16), broken down across the seven parameters, are given in Annex 6. The overall scores per parameter are shown in Figure 6.

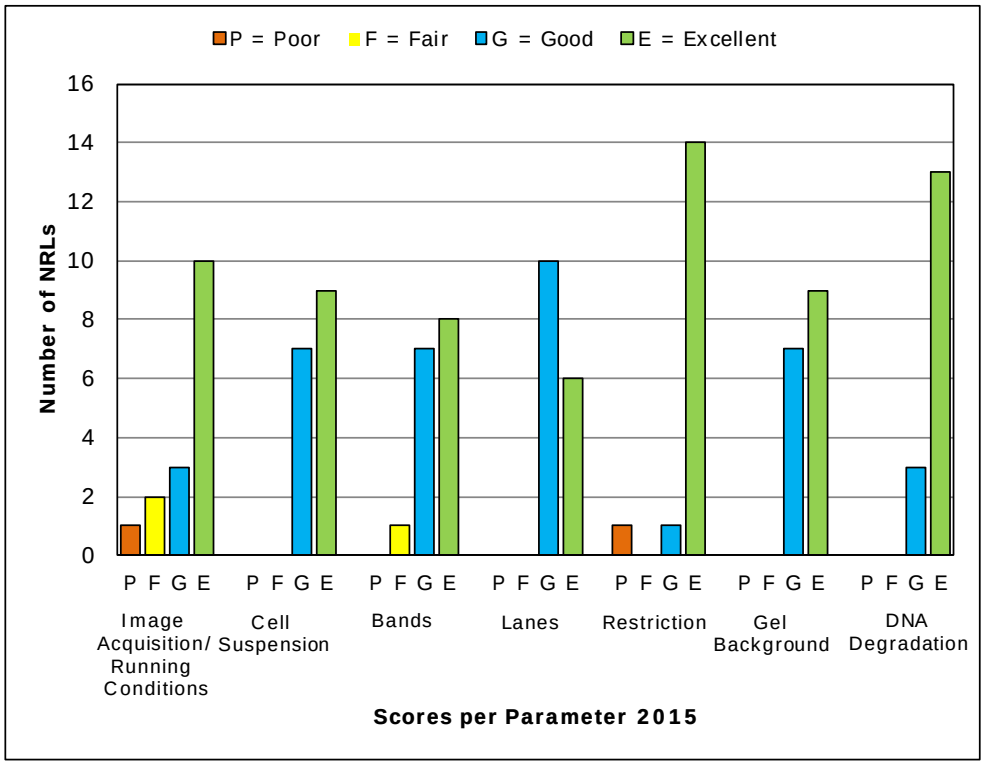

Figure 6. Evaluation of the quality of the PFGE images in scores per parameter in the 2015 study

As in the previous two studies, the quality of the gels was variable, as shown by the two examples in Annex 8.
Two of the 16 images resulted in a Poor score on at least one of the seven parameters, which may indicate that these two images are not suitable for use in interlaboratory comparisons. The Poor score for Lab code 25 mainly concerned the 'Image Acquisition', which could relatively easy be improved in the future by including the wells and the bottom of the gel on the TIFF image.
An example of an individual laboratory evaluation report is given in Annex 9.
Figure 7 shows the results of the evaluation of the TIFF images from the studies 2013 – 2015. Improvements in time are clearly seen, particularly in the reduction of red (Poor) result.

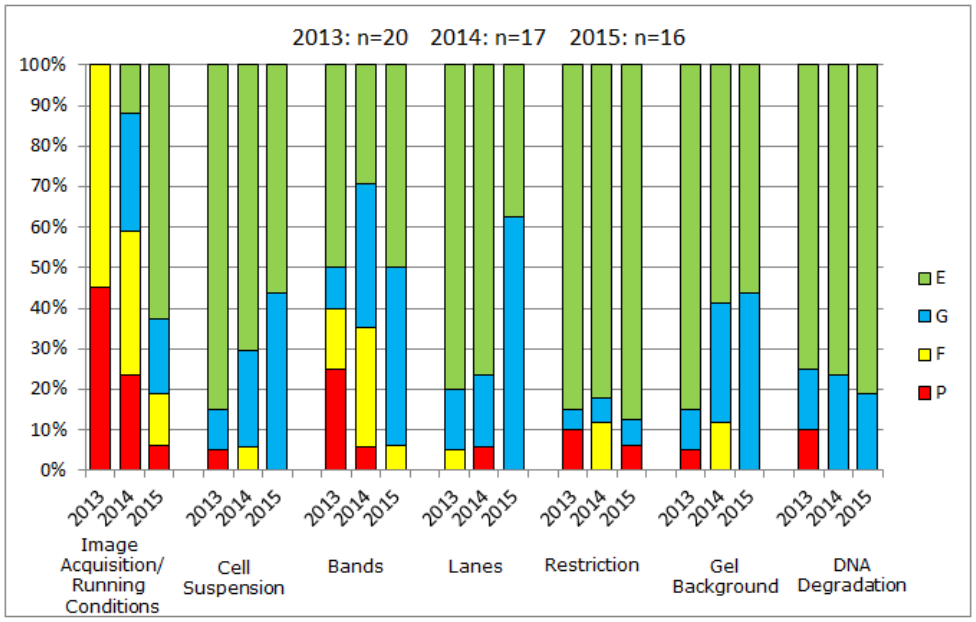

Figure 7. Evaluation of the quality of the PFGE images in scores per parameter, 2013 – 2015 studies

5.2.2 *Evaluation of the analysis of the gel in BioNumerics*

This year we introduced the evaluation of the (optional) analysis of the produced PFGE gel in BioNumerics as well.
A total of 12 participants sent in their analysed gel data for evaluation. The participants were all using the pre-configured database as provided by the EURL-*Salmonella*, and therefore were using identical initial experimental settings in BioNumerics.
Evaluation of the analysis of the gel in BioNumerics was done according to the guidelines as used in the EQAs for the FWD laboratories (Annex 2).
These guidelines use 5 parameters, which are scored with 1 (poor), 2 (fair/good) or 3 (excellent) points.
The scores per NRL (n=12), broken down across the five parameters, are given in Annex 7. The overall scores per parameter are shown in Figure 8.
Overall, 67% of the scores were Excellent. Only one participant scored a Poor for one of the parameters. This concerned “position of gel frame”, and was due to wrongly included well when placing the frame. This will be easy to correct in future analysis.
Six participants were seen to assign bands for the test strains under 33 kb, which should not be done according to the protocol, but could be easily avoided in the future as well.
Most problems were seen in the parameters ‘curves’ and ‘band assignment’. The former mostly due to participants defining the curves by encompassing almost the whole lane, whereas the average thickness is recommended to be reduced to about 1/3 of the lane. The latter due to the assignment of double bands as single bands or single bands a double bands, which is a well know difficulty of PFGE interpretations.

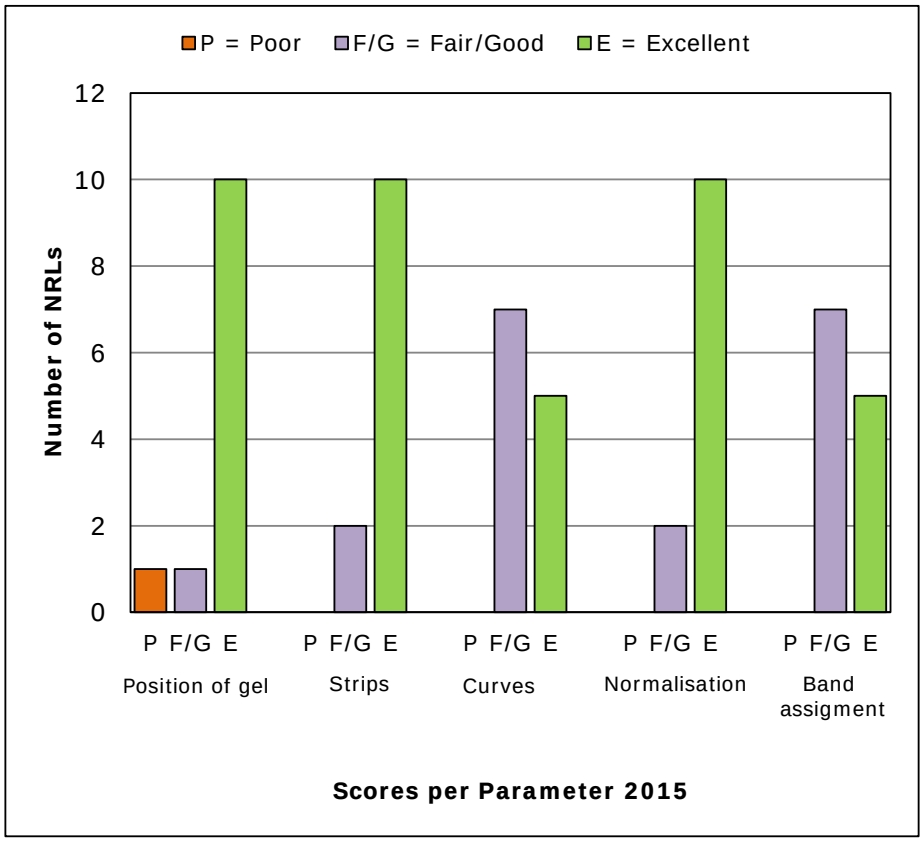

Figure 8. Evaluation of the analysis of the gel in BioNumerics in scores per parameter, 2015 study

6 Discussion

A total of 34 laboratories participated in this serotyping study. These included 29 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) in the 28 EU Member States, 2 NRLs of EU-candidate countries, and 3 NRLs of EFTA countries.
A total of 21 *Salmonella* strains were sent to the participants in November 2015 for serotyping by all participants; however, testing of the 21st strain was optional and the results were not included in the evaluation.
Overall, 99% of the strains were correctly typed for the O-antigens, 97% of the strains were correctly typed for the H-antigens and 97% of the strains were correctly named by the participants.

At the EURL-*Salmonella* Workshop in 2007, criteria for 'good performance' during an interlaboratory comparison study on serotyping were defined. According to these criteria, 33 participants achieved good results. The laboratory that did not achieve the level of good performance participated in a follow-up study including 10 additional strains for serotyping. This EU-NRL obtained good scores in this obligatory follow-up study.

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, one NRL mistyped the *S*. Hadar strain, but no other problems were noticed in serotyping the 'top 5' serovars.

Tables 15 and 16 give an overview of the results of the typing studies from 2007, when the system of penalty points was introduced. Table 15 shows results for EU-NRLs only; Table 16 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 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.

*Table 15. 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	XX 2015
No. of participants	25	27	28	30	28	28	29	29	29
No. of strains evaluated	20	20	20	19	19	20	20	20	20
O-antigens correct/strains	98%	98%	98%	98%	99%	99%	100%	99%	99%
H-antigens correct/strains	95%	98%	95%	95%	97%	98%	98%	97%	97%
Names correct/strains	95%	97%	95%	95%	97%	96%	98%	96%	97%
O-antigens correct/labs	68%	70%	75%	93%	93%	82%	97%	86%	93%
H-antigens correct/labs	56%	67%	43%	73%	71%	64%	72%	66%	62%
Names correct/labs	52%	52%	46%	67%	75%	57%	69%	55%	59%
No. of penalty points	35	30	36	16	22	20	17	18	16
No. of labs not achieving good performance	6	3	4	2	2	2	2	1	1
No. of labs not achieving good performance after follow-up	0	0	0	0	0	0	0	0	0

*Table 16. 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	XX 2015
No. of participants	26	29	31	33	36	31	34	35	34
No. of strains evaluated	20	20	20	19	19	20	20	20	20
O-antigens correct/strains	98%	98%	97%	98%	98%	99%	100%	97%	99%
H-antigens correct/strains	96%	98%	94%	95%	96%	98%	98%	94%	97%
Names correct/strains	95%	97%	93%	95%	96%	96%	97%	94%	97%
O-antigens correct/labs	69%	76%	74%	88%	86%	77%	94%	83%	91%
H-antigens correct/labs	58%	72%	45%	67%	69%	61%	71%	63%	62%
Names correct/labs	54%	59%	48%	61%	69%	55%	68%	57%	56%
No. of penalty points	36	34	56	37	41	20	20	57	21
No. of labs not achieving good performance	6	4	5	4	4	2	2	2	1
No. of labs not achieving good performance after follow-up	0	0	0	0 (n=3)	1 (n=3)	0	0	0 (n=1)	0

7 Conclusions

7.1 Serotyping

- 99% of the strains were typed correctly for the O-antigens.
- 97% of the strains were typed correctly for the H-antigens.
- 97% of the strains were correctly named.
- Apart from one mistake in serotyping a *S*. Hadar strain by one participant, all 'top 5' strains *S*. Enteritidis, *S*. Hadar, *S*. Infantis, *S*. Typhimurium and *S*. Virchow were correctly serotyped.
- One NRL initially did not achieve the defined level of good performance and was 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 PFGE typing

- For the third time, the typing study also included PFGE typing.
- Evaluation of the PFGE results was initially based on the quality of the generated images and was expressed in terms of scores on seven parameters: Poor, Fair, Good or Excellent.
- Two of the 16 images resulted in a Poor score on at least one of the seven parameters, which may indicate that these two images are not suitable for use in interlaboratory comparisons.
- For the first time also the analysis of the PFGE images in the dedicated software BioNumerics was evaluated, which was expressed in terms of scores on five parameters.
- Only one of the twelve participants scored a Poor for one of the parameters.
- Overall, 67% of the scores were Excellent; most problems were seen in the parameters 'curves' and 'band assignment'. The latter due to the assignment of double bands as single bands or single bands a double bands, which is a well know difficulty of PFGE interpretations.
- Adherence to the guidelines, both on PFGE gel image preparation and on gel analysis in BioNumerics, should be helpful to improve the results.

List of abbreviations

BN	BioNumerics
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
NRL-*Salmonella*	National Reference Laboratory for *Salmonella*
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
RIVM	National Institute for Public Health and the Environment (Bilthoven, The Netherlands)
SSI	Statens Serum Institut (Copenhagen, Denmark)
TIFF	Tagged Image File Format

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

Copied from www.pulsenetinternational.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

VERSION:	REPLACED BY:	AUTHORIZED BY:	

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., :S3) 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
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])

1. FLOW CHART:

2. BIBLIOGRAPHY:

3. CONTACTS:

4. AMENDMENTS:

VERSION:	REPLACED BY:	AUTHORIZED BY:	

Annex 2 Evaluation of gel analysis of PFGE images in BioNumerics

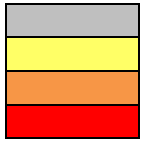
Evaluation of gel analysis of PFGE images in BioNumerics according to the EQAs for the FWD laboratories (European Centre for Disease Prevention and Control. Fifth external quality assessment scheme for *Salmonella* typing. Stockholm: ECDC; 2014. Available at: http://ecdc.europa.eu/en/publications/Publications/fifth-EQA-salmonella-typing-November-2014.pdf).

Parameters/ scores	Excellent	Fair	Poor
Position of gel	Excellent placement of frame and gel inverted.	The image frame is positioned too low. Too much space framed at the bottom of the gel. Too much space framed on the sides of the gel.	Wells wrongly included when placing the frame. Gel is not inverted
Strips	All lanes correctly defined.	Lanes are defined to narrow (or wide). Lanes are defined outside profile. A single lane is not correctly defined.	Lanes not defined correctly.
Curves	1/3 or more of the lane is used for averaging curve thickness.	Curve extraction defined either to narrow or including almost the whole lane.	Curve set so that artefacts will cause wrong band assignment.
Normalisation	All bands assigned correctly in all reference lanes.	Bottom bands <33kb were not assigned in some or all of the reference lanes	Many bands not assigned in the reference lanes. The references were not included when submitting the XML-file.
Band assignment	Excellent band assignment with regard to the quality of the gel.	Few double bands assigned as single bands or single bands assigned as double bands. Few shadow bands are assigned.	Band assignment not done correctly, making it impossible to make an inter-laboratory comparison.

Annex 3 Serotyping results per strain and per laboratory

Lab REF	S1 Agama	S2 Irumu	S3 Ahmadi	S4 Corvallis	S5 Eastbourne	S6 Hadar	S7 Virchow	S8 Emek	S9 Kintambo	S10 Richmond
1	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
2	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
3	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
4	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
5	Agama	Irumu	Ahmadi	Dabou	Eastbourne	Glostrup	Virchow	Emek	Kintambo	Richmond
6	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
7	Agama	Irumu	Wanatah	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
8	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kitambo	Richmond
9	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
10	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Agbeni	Richmond
11	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
12	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
13	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Okatie	Richmond
14	Agama	Nessziona	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
15	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Okatie	Richmond
16	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
17	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
18	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
19	Agama	Irumu	Wanatah	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
20	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
21	Agama	Azteca	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
22	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
23	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
24	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	SG I: 13, 23: t : -	Richmond
25	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
26	Agama	Azteca	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
27	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
28	Agama	Irumu	Ahmadi	Dabou	Eastbourne	Hadar	Virchow	Emek	Kintambo	Bareilly
29	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
30	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
31	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
32	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Okatie	Richmond
33	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Bareilly
34	Agama	Irumu	Ahmadi	Corvallis	Eastbourne	Hadar	Virchow	Emek	Kintambo	Richmond
X	0	3	2	2	0	1	0	0	4	2

S11 Cubana	S12 Eboko	S13 Teddington	S15 Meleagridis	S16 Typhimurium	S17 Infantis	S18 Jerusalem	S19 Enteritidis	S20 Montevideo	Lab REF
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	1
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	2
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	3
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	4
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	5
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	6
Cubana	Stourbridge	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	7
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	8
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	9
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	10
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	11
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	12
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	13
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	14
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	15
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	16
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	17
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	18
Cubana	Stourbridge	Teddington	Meleagridis	Typhimurium	Infantis	Eschweiler	Enteritidis	Montevideo	19
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	20
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	21
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	22
1,13,23:z29:e,n,x	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	23
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	24
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	25
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	26
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	27
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	28
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	29
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	30
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	31
Cubana	Skansen	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	32
Cubana	Eboko	Teddington	Meleagridis	Typhimurium	Infantis	Jerusalem	Enteritidis	Montevideo	33
1,13,23:z29:	Eboko	Teddington	Meleagridis	Typhimurium	İnfantis	Jerusalem	Enteritidis	Montevideo	34
1	3	0	0	0	0	1	0	0	**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 S14 and S21 are given in Annex 4

Annex 4 Details of serotyping results for strains S14 and S21

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

remark

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	Lab code
S-21	**1,9,12**	**a**	**1,5**	**Miami**	**REF**
S-21	9,12	a	1,5	Miami/Sendai	1
S-21	9,12	a	1,5	Miami	2
S-21	9	a	1, 5	Miami	3
S-21	9,12	a	5	Miami	4
S-21	9,12	a	1,5	Miami	5
S-21	9,12	a	1,5	Miami	6
S-21	9,12	a	1,5	9,12:a:1,5	7
S-21	9,12	a	1,5	Miami	8
S-21	9	a	1,5	Miami	9
S-21	9,12	a	1,5	9,12:a:1,5	10
S-21					11
S-21	9,12	a	5	Miami	12
S-21	9	a	1,5	Miami	13
S-21	9,12	a	1,5	Miami	14
S-21	9	a	1,5	Miami / Sendai	15
S-21	9	a	5	Miami	16
S-21	9,12	a	5	Miami (or Sendai)	17
S-21	9,12	a	1,5	Miami	18
S-21	9,12	a	1,5	Sendai/Miami	19
S-21	1,9,12	a	1,5	Miami	20
S-21					21
S-21	9,12	a	1,5	Miami	22
S-21	1,9,12	a	1,5	S. Miami	23
S-21	9, 12	a	1, 5	Miami	24
S-21	9,12	a	1,5	Miami	25
S-21	9,12	a	1,5	Miami	26
S-21	9,12	a	1,5	Miami	27
S-21	9,12	a	1,5	9,12:a:5	28
S-21	9,12	a	1,5	Miami	29
S-21	9,12	a	1,5	Miami	30
S-21	9,12	a	1,5	9,12:a:1,5	31
S-21	9,12	a	1,5	Miami	32
S-21	1,9,12	a	1,5	1,9,12:a:1,5	33
S-21	9,12	a	1,5	II	34

Annex 5 Details of strains that caused problems in serotyping

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	Lab code
S-2	**6,7**	**l,v**	**1,5**	**Irumu**	**REF**
S-2	6,7	l,z13	1,5	Nessziona	14
S-2	4,12	l,v	1,5	Azteca	21
S-2	4, 12	l,v	1,5	Azteca	26
S-3	**1,3,19**	**d**	**1,5**	**Ahmadi**	**REF**
S-3	1,3,19	d	1,7	Wanatah	7
S-3	3,19	d	1,7	Wanatah	19
S-3	1,3,9	d	5	Ahmadi	31
S-4	**8,20**	**z4,z23**	**[z6]**	**Corvallis**	**REF**
S-4	8,20	l,w	z4,z23	Dabou	5
S-4	8,20	z4	w	Dabou	28
S-6	**6,8**	**z10**	**e,n,x**	**Hadar**	**REF**
S-6	6,8	z10	e,n,z15	Glostrup	5
S-9	**1,13,23**	**m,t**	**-**	**Kintambo**	**REF**
S-9	13,23	m,t	-	Kitambo	8
S-9	13,23	g,m,t	-	Agbeni	10
S-9	13,23	g,t	-	Okatie	13
S-9	13,23	g,t	-	Okatie	15
S-9	13, 23	t	-	SG I: 13, 23: t : -	24
S-9	13,23	g,t	-	Okatie	32
S-10	**6,7**	**y**	**1,2**	**Richmond**	**REF**
S-10	6,7	y	5	Bareilly	28
S-10	6,7,14	y	1,5	Bareilly	33
S-11	**1,13,23**	**z29**	**-**	**Cubana**	**REF**
S-11	1,13,23	z29	e,n,x	1,13,23:z29:e,n,x	23
S-11	1,13,23	z29		1,13,23:z29:	34
S-12	**6,8**	**b**	**1,7**	**Eboko**	**REF**
S-12	6,8	b	1,5	Stourbridge	7
S-12	6,8	b	1,6	Stourbridge	19
S-12	6,8	b	1,2	Skansen	32
S-15	**3,{10}{15}{15,34}**	**e,h**	**l,w**	**Meleagridis**	**REF**
S-15	3,10,[15][15,34]	e,h	l,v	Meleagridis	34
S-18	**6,7,14**	**z10**	**l,w**	**Jerusalem**	**REF**
S-18	6,7	z10	l,v	Jerusalem	7
S-18	6,7	z10	1,6	Eschweiler	19
S-18	6,8	z10	l,w	Jerusalem	21
S-20	**6,7,14**	**g,m,[p],s**	**[1,2,7]**	**Montevideo**	**REF**
S-20	6,7,14,54	g,m,p,s	2,7	Montevideo	31

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

Annex 6 Evaluation of PFGE images per participant and per parameter

Lab code / Parameter	25	14	21	22	8	30	3	16	17	18	20	24	27	1	15	29	Total score per parameter	Average per parameter
Image Acquisition and Running Conditions	1	2	4	3	2	3	4	4	4	4	3	4	4	4	4	4	54	3,4
Cell Suspension	4	4	3	4	4	4	3	4	3	3	4	3	3	3	4	4	57	3,6
Bands	3	3	3	2	3	3	3	4	3	4	4	4	4	4	4	4	55	3,4
Lanes	3	3	4	3	4	3	3	3	3	3	3	4	3	4	4	4	54	3,4
Restriction	4	4	1	4	4	4	4	3	4	4	4	4	4	4	4	4	60	3,8
Gel Background	3	3	3	3	4	3	4	3	4	4	4	3	4	4	4	4	57	3,6
DNA Degradation (smearing in lanes)	3	3	4	4	3	4	4	4	4	4	4	4	4	4	4	4	61	3,8
Total score per participant	21	22	22	23	24	24	25	25	25	26	26	26	26	27	28	28		
Average per participant	3	3,1	3,1	3,3	3,4	3,4	3,6	3,6	3,6	3,7	3,7	3,7	3,7	3,9	4	4		

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

Annex 7 Evaluation of the analysis of the gel in BioNumerics per participant and per parameter

Lab code / Parameter	17	8	14	30	27	1	3	18	20	25	29	15	Total score per parameter	Average per parameter
Position of gel	3	1	3	2	3	3	3	3	3	3	3	3	30	2,5
Strips	2	3	2	3	3	3	3	3	3	3	3	3	31	2,6
Curves	2	2	2	2	2	3	3	3	2	2	3	3	29	2,4
Normalisation	2	3	3	3	3	3	2	3	3	3	3	3	34	2,8
Band assignment	2	3	2	2	2	2	3	2	3	3	2	3	28	2,3
Total score per participant	11	12	12	12	13	14	14	14	14	14	14	15		
Average per participant	2,2	2,4	2,4	2,4	2,6	2,8	2,8	2,8	2,8	2,8	2,8	3		

1=Poor; 2=Fair/Good; 3=Excellent; *Missing value

Annex 8 Examples of PFGE images obtained by the participants

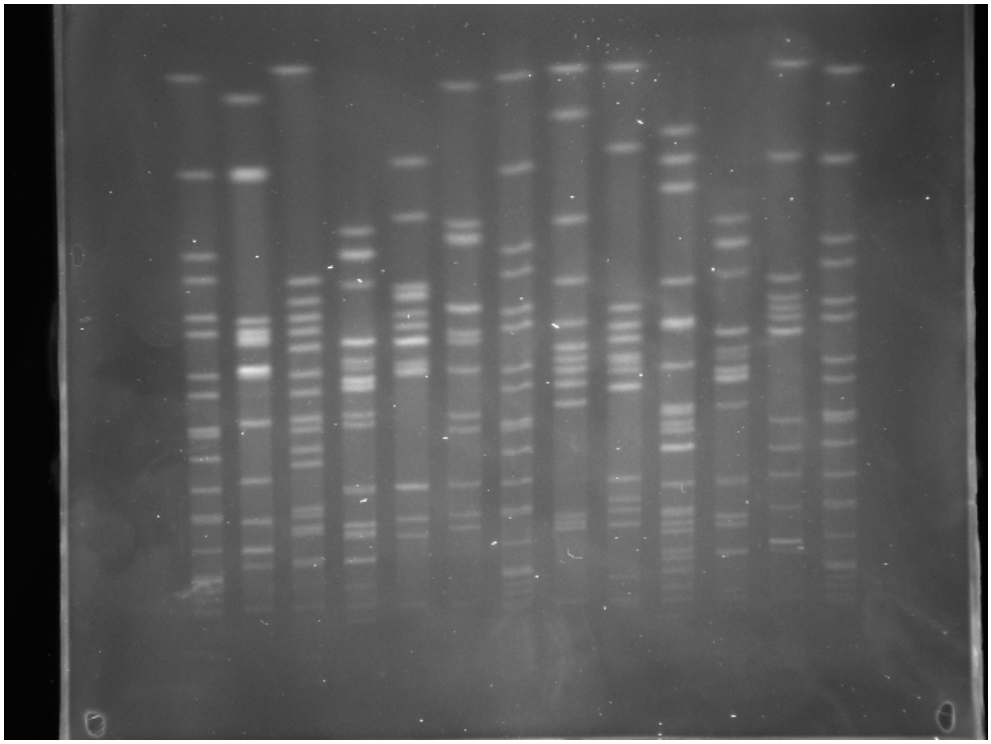


Figure A8.1. Example of a gel (lab code 25) with a generally low score

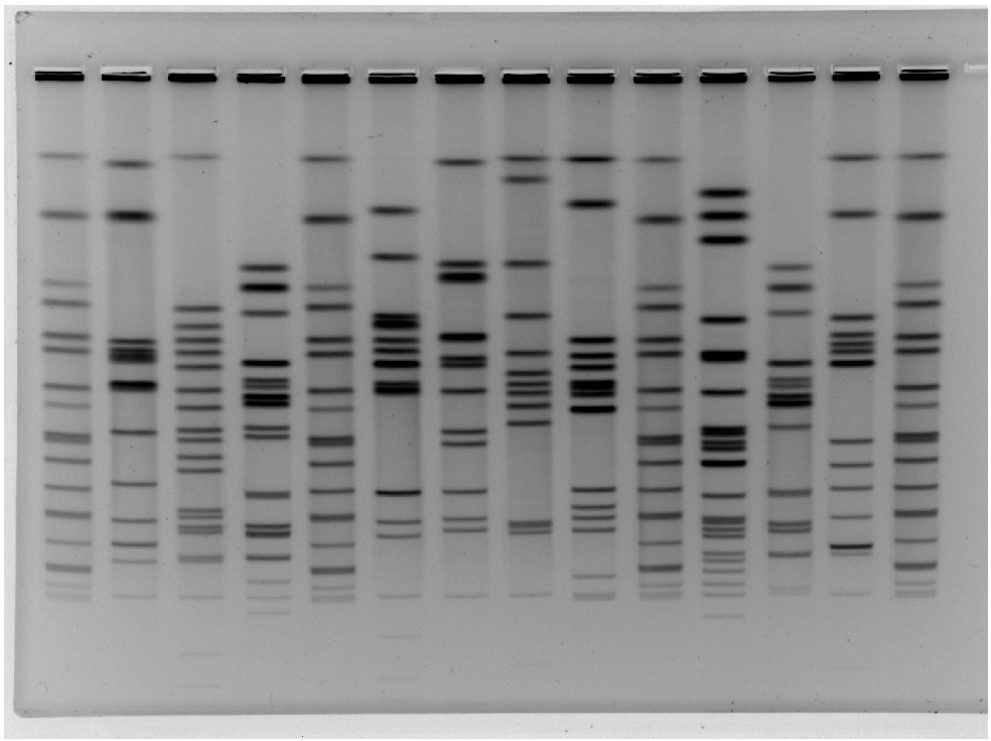


Figure A8.2. Example of a gel (lab code 29) with a generally high score

Annex 9 Example of an individual laboratory evaluation report on PFGE typing results

Individual Laboratory Results Interlaboratory Comparison Study
Salmonella PFGE typing (November 2015), Page 1 of 3

NRL Laboratory code: 8

General comments:

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

Table 1. Evaluation tif file according to the Protocol (Annex 1)

Parameter	Evaluation	Comments	Points *
Image Acquisition and Running Conditions	Fair	Bottom of gel not visible. Bottom band of standard may not be 1-1,5 cm from bottom of gel. Gel image not in focus	2
Cell Suspension	Excellent	The cell concentration is approximately the same in each lane.	4
Bands	Good	Slight band distortion in 1 lane but does not interfere with analysis. Bands are slightly fuzzy and/or slanted. A few bands are difficult to see clearly, especially at bottom of gel.	3
Lanes	Excellent	Straight.	4
Restriction	Excellent	Complete restriction in all lanes.	4
Gel Background	Excellent	Clear.	4
DNA Degradation	Good	Minor background (smearing) in a few lanes.	3
Total score:			24

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

Table 2. Evaluation PFGE gel analysis in Bionumerics according to the Protocol (Annex 2)

Parameter	Evaluation	Comments	Points*
Position of gel	Poor	Frame includes wells.	1
Strips	Excellent	All lanes correctly defined.	3
Curves	Good/ Fair	A single curve is not correctly defined.	2
Normalisation	Excellent	All bands assigned correctly in all reference lanes.	3
Band assignment	Excellent	Excellent band assignment in relation to the quality of the gel.	3
Total score:			12

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

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

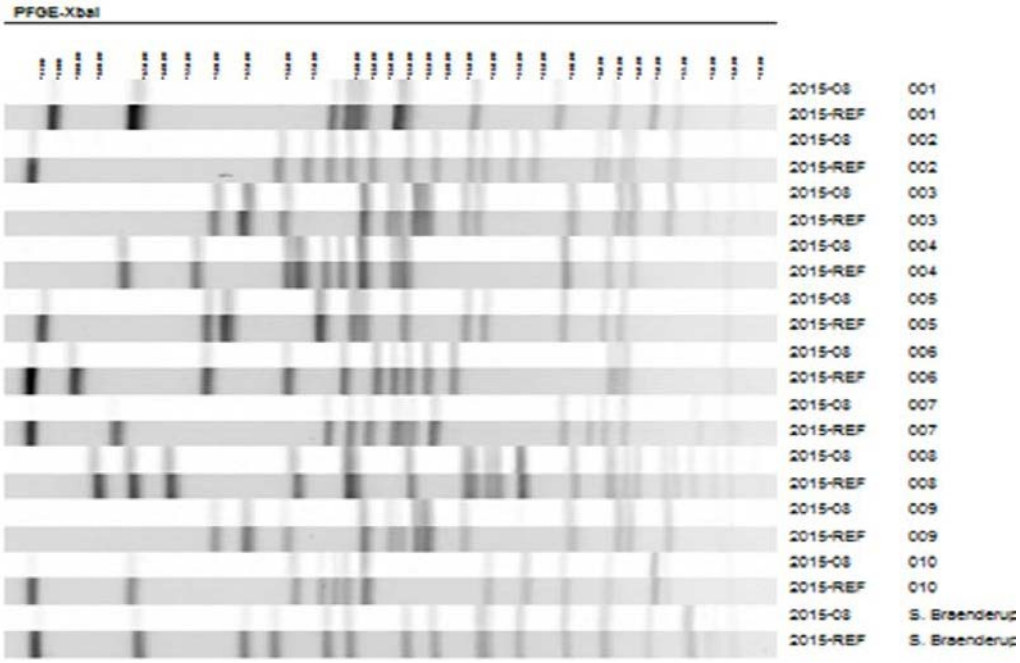
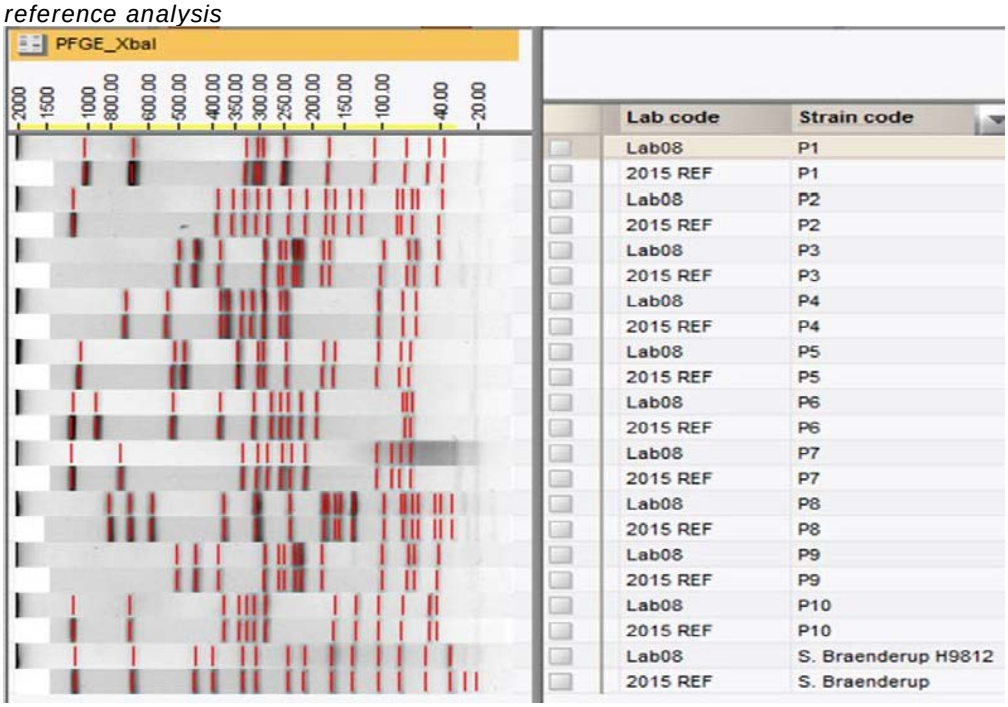


Figure 2. Comparison of your PFGE gel analysis in Bionumerics with the reference analysis



Lab code	Strain code
Lab08	P1
2015 REF	P1
Lab08	P2
2015 REF	P2
Lab08	P3
2015 REF	P3
Lab08	P4
2015 REF	P4
Lab08	P5
2015 REF	P5
Lab08	P6
2015 REF	P6
Lab08	P7
2015 REF	P7
Lab08	P8
2015 REF	P8
Lab08	P9
2015 REF	P9
Lab08	P10
2015 REF	P10
Lab08	S. Braenderup H9812
2015 REF	S. Braenderup

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Figure 3. Display of the "Distortion bar" option in Bionumerics of your gel Darker colours indicate critical normalisation.

EURL-*Salmonella*, Bilthoven, The Netherlands

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