National Institute for Public Health
and the Environment
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Colophon

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Synopsis

21st EURL-*Salmonella* interlaboratory comparison study (2016) on typing of *Salmonella* spp.

The National Reference Laboratories (NRLs) of all 28 European Union (EU) Member States performed well in the 2016 quality control test on *Salmonella* typing. Overall, the EU-NRLs were able to assign the correct name to 99% of the strains tested.

In addition to the standard method for typing *Salmonella* (serotyping), fifteen 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, participants received another ten strains of *Salmonella* to be tested by this method. Thirteen of the fifteen 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 20 *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 Serbia, and EFTA countries Iceland, Norway and Switzerland took part in the 2016 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

Eenentwintigste EURL-*Salmonella* ringonderzoek (2016) voor de typering van *Salmonella* spp.

De Nationale Referentie Laboratoria (NRL's) van de 28 Europese lidstaten scoorden in 2016 goed bij de kwaliteitscontrole op *Salmonella*-typering. Uit de analyse van alle NRL's als groep bleek dat de laboratoria aan 99 procent van de geteste stammen de juiste naam konden geven.

Vijftien 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. Dertien van de vijftien 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 2016 waren dat de kandidaat-lidstaten Macedonië en Servië, 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 2016, the 21st interlaboratory comparison study on the 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 EU-candidate-countries Former Yugoslav Republic of Macedonia (FYROM) and Serbia, 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 (Grimont and Weill, 2007). The laboratories were allowed to send strains for serotyping to another specialised laboratory in their country if this was part of their usual procedure.

Overall, nearly 100% of the strains were typed correctly for the O-antigens, 99% of the strains were typed correctly for the H-antigens and 99% of the strains were correctly named by the participants.
In 2007, criteria for 'good performance' with regard to serotyping were defined (Mooijman, 2007). Using these criteria, 32 participants achieved good results. The 2 participants that did not achieve the level of good performance were no NRLs within the EU, and therefore their participation in a follow-up study including ten additional strains for serotyping was not obligatory.

Fifteen 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. Thirteen (87%) of the fifteen 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. Ten 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.

1 Introduction

This report describes the 21st 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 2016.

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 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 *Salmonella* spp. and to compare the results of the serotyping of *Salmonella* spp. among the NRLs-*Salmonella*. All NRLs performed serotyping of the 20 obligatory strains and all but four of the participants serotyped the optional 21st strain. Any NRLs of EU Member States that do not achieve the defined level of good performance for serotyping have to participate in a follow-up study, in which 10 additional strains have to be serotyped.

For the fourth time, the typing study also included PFGE typing. Fifteen NRLs participated in this part of the study by PFGE typing 10 designated *Salmonella* strains and submitting images for evaluation. Ten of these participants also used a pre-configured database, provided by the EURL-*Salmonella,* to analyse the profiles on their gel in the dedicated computer program BioNumerics.

2 Participants

Country	City	Institute
Austria	Graz	IMED Graz/AGES
Belgium	Brussels	CODA-CERVA
Bulgaria	Sofia	NDRVI
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 for 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	Oeiras	INIAV-Instituto Nacional de Investigação Agrária e Veterinária
Romania	Bucharest	Institute for Diagnosis and Animal Health, Bacteriology Department

Country	City	Institute
Serbia	Belgrade	Institute of Veterinary Medicine of Serbia
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)
United Kingdom	Addlestone	Animal and Plant Health Agency (APHA)
United Kingdom	Belfast	AFBI – Veterinary Sciences Division

3 Materials and methods

3.1 Design of the interlaboratory comparison study

3.1.1 Laboratory codes

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

3.1.2 Protocol and test report

Three weeks before the start of the study, the NRLs received the protocol by email. As usual, 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 data entry were sent to the NRLs in week 45, 2016.
The protocol and test report forms can be found on the EURL-*Salmonella* website:
http://www.eurlsalmonella.eu/Proficiency_testing/Typing_studies

3.1.3 Transport

The parcels containing the strains for serotyping and PFGE typing were sent by the EURL-*Salmonella* in week 45, 2016. All samples were packed and transported as Biological Substance Category B (UN-3373) and transported by a door-to-door courier service.

3.2 Serotyping part of the study

3.2.1 Salmonella *strains for serotyping*

A total of 20 *Salmonella* strains (coded S1–S20) had to be serotyped by the participants. As decided at the 21st EURL-*Salmonella* Workshop in St. Malo (Mooijman, 2016), a less common strain (S21) was additionally included in the study. Testing this strain was optional and results were not included in the evaluation.

The *Salmonella* strains used for the study on serotyping originated from the National *Salmonella* Centre collection 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.
Seven strains (S3, S4, S6, S9, S12, S15, S19) represented serovars included in the EURL-*Salmonella* serotyping studies for the first time.

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

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
S1[a)]	1,4,[5],12	i	-	1,4,[5],12:i:-
S2	6,8,20	e,h	1,2	Newport
S3[b)]	28	z_{10}	e,n,x	Umbilo
S4[b)]	16	k	1,2	Szentes
S5	6,7,14	r	1,5	Infantis
S6[b)]	1,4,12,27	d	e,n,z_{15}	Duisburg
S7	3,{10}{15}{15,34}	y	1,5	Orion
S8	1,4,[5],12	e,h	1,2	Saintpaul
S9[b)]	6,7,14	i	1,2	Augustenborg
S10	1,4,[5],12	e,h	e,n,x	Chester
S11	6,7,14	b	l,w	Ohio
S12[b)]	1,4,[5],12	a	e,n,x	Bispebjerg
S13	1,4,[5],12	i	1,2	Typhimurium
S14	1,9,12	g,m	-	Enteritidis
S15[b)]	1,4,[5],12	e,h	1,5	Reading
S16	6,8	z_{10}	e,n,x	Hadar
S17	6,7,14	f,g	-	Rissen
S18	1,4,[5],12	z_{10}	1,2	Haifa
S19[b)]	6,7,14	y	e,n,z_{15}	Mikawasima
S20	6,7,14	r	1,2	Virchow
S21[c)]	60	r	z	60:r:z

[a)] Typhimurium, monophasic variant as determined by PCR.
[b)] First time represented in an EURL-*Salmonella* serotyping study.
[c)] *Salmonella enterica* subspecies *diarizonae* (optional strain).

3.2.2 *Evaluation of the serotyping results*

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.

3.2.3 *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 presented in Table 3.

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 21st EURL-*Salmonella *typing study*

Strain	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
SF-1	1,4,[5],12	e,h	1,2	Saintpaul
SF-2	1,4,[5],12	e,h	e,n,x	Chester
SF-3	6,7,14	b	l,w	Ohio
SF-4	1,9,12	g,m	-	Enteritidis
SF-5	1,4,[5],12	i	1,2	Typhimurium
SF-6	3,{10}{15}{15,34}	e,h	1,5	Muenster
SF-7	6,8	d	e,n,z_{15}	Herston
SF-8	1,3,19	i	z_6	Taksony
SF-9	6,8	z_{10}	e,n,x	Hadar
SF-10	3,{10}{15}{15,34}	l,v	1,7	Give

3.3 PFGE typing part of the study

3.3.1 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-7 on PFGE typing, organised by the SSI for the Food- and Water-borne Diseases and Zoonoses Laboratories Network (FWD laboratories network) (ECDC, 2016). Background information on the strains is given in Table 4. Additionally, the reference image and its analysis in BioNumerics was kindly provided by SSI. In this way, performance of both the NRLs network and the FWD laboratory network can be compared in the future.

Table 4. Background information on the Salmonella *strains used for PFGE typing in 2016*

Strain code in study 2016 (EURL-*Salmonella*)	Strain code in EQA-6 (ECDC, 2016)	*Salmonella* serovar
P1	Salm 5	Javiana
P2	Salm 7	Stanley
P3	Salm 4	Chester
P4	Salm 10	Infantis
P5	Salm 6	1,4,5,12:i:-
P6	Salm 8	Paratyphi B var. Java
P7	Salm 3	Enteritidis
P8	Salm 2	Poona
P9	Salm 1	Reading
P10	Salm 9	Typhimurium

3.3.2 *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). Annex C of this publication describes the Standard PulseNet protocol *Salmonella* PFGE (PulseNet, 2013).
The PFGE gel images were to be emailed as uncompressed 8-bit grey 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.

3.3.3 *Evaluation of the analysis of the PFGE gel in Bionumerics*

For the second time, the evaluation of the (optional) analysis of the PFGE gel in the bioinformatics software application BioNumerics was included.
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 10 November 2017,
- import the TIFF image and analyse the gel (also see the protocol EURL-*Salmonella* typing study-2016 for further reference),
- 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 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 Results and Discussion

4.1 Technical data interlaboratory comparison study

4.1.1 General

A total of 34 laboratories participated in this study (Chapter 2). These included 29 NRLs-*Salmonella* in the 28 EU Member States, 2 NRLs in EU-candidate countries and 3 NRLs in EFTA countries.

The frequency of serotyping of *Salmonella* at the participating laboratories and the number of strains that were serotyped and PFGE typed in 2016 are summarised in Table 5.

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

Lab code	Serotyping frequency in 2016	No. of strains serotyped in 2016	No. of strains PFGE typed in 2016
8	Daily	150	300
22	Daily	198	
6	Daily	200	
16	Daily	300	22
3	Daily	317	
4	Daily	400	
5	Daily	410	
24	Daily	460	11
12	Daily	500	0
15	Daily	500	30
33	Daily	550	
19	Daily	600	
31	Daily	740	
7	Daily	900	15
23	Daily	1200	200
21	Daily	1300	80
29	Daily	1500	50
10	Daily	1750	100
26	Daily	2500	
32	Daily	3300	150
1	Daily	3500	200
25	Daily	3800	40
18	Daily	4500	
9	Daily	5500	170
13	Thrice a week	150	
14	Thrice a week	400	
17	Twice a week	63	
2	Twice a week	80	
34	Twice a week	190	40
28	Twice a week	208	
30	Twice a week	260	
11	Twice a week	350	

Lab code	Serotyping frequency in 2016	No. of strains serotyped in 2016	No. of strains PFGE typed in 2016
20	Once a week	13	
27	Once a week	3000	
n=34		39789	1408

4.1.2 *Accreditation*

Of the 34 participants, 32 are accredited for serotyping *Salmonella*, mainly according to ISO 17025, and in some cases according to ISO/TR 6579-3. The other two laboratories noted that they were working on their accreditation of *Salmonella* serotyping.
One laboratory is accredited for serotyping of all serovars except *S*. Typhi, and one laboratory is accredited for serotyping *S*. Enteritidis, *S*. Tyhpimurium, *S*. Infantis, *S*. Hadar, and *S*. Virchow; all other laboratories stated that they are accredited for all *Salmonella* serovars.

4.1.3 *Transport of samples*

All but one of the participants received their package in the same week as sent (week 45 of 2016). The remaining parcel was delivered in week 46. All packages were received in good condition.
The participants used a variety of media from various manufacturers for sub-culturing the *Salmonella* strains. Non-selective nutrient agar was the most commonly used medium.

4.2 Serotyping results

4.2.1 *General*

One participant (lab code 16) sent the additional strain S21 to another laboratory for further serotyping or confirmation. Another participant (lab code 10) sent strain S3 to another laboratory, because of a lack of antisera needed for this strain. All other laboratories tested the 20 obligatory strains in their own laboratory.
Details on the number and the source of the sera as used by the participants are summarised in Table 6 and Table 7.

Table 6. Number of laboratories using sera from various manufacturers

Manufacturer	Number of NRLs (n=34)
Biorad	15
Microgen	1
Own preparation	5
Pro-Lab	6
Reagensia	2
Remel	1
Sifin	20
Statens Serum Institute (SSI)	27

Table 7. 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	9
2	12
3	9
4	4

4.2.2 *Biochemical testing*

Twenty-eight participants confirmed the use of biochemical tests. Twenty-one participants used a variety of biochemical tests on the optional strain S21, uncommon serovar 60: r, z (*S. enterica* subsp. *diarizonae*). Eighteen participants confirmed strain S12 (1,4,[5],12;a:e,n,x) to be a *S. enterica enterica* strain (Bispebjerg) by biochemical testing, most often by using malonate.

4.2.3 *Use of PCR for confirmation*

A total of 19 laboratories reported using PCR for the confirmation of serotyped strains. Seventeen of the laboratories use this PCR routinely, and the number of samples tested by PCR in 2016 are summarised in Table 8.

Three laboratories used PCR to confirm all the strains. Sixteen laboratories used PCR to confirm strain S1, the monophasic variant of *S*. Typhimurium 1,4,[5],12:i:-, and seven of these also used PCR to confirm strain S13, *S*. Typhimurium. Strains S12 (1x), S14 (3x), S17 (2x) and S21 (2x) were also reported to have been confirmed using PCR.

Table 8. Number of strains routinely tested by PCR in 2016

Laboratory code	Number of strains tested by PCR in 2016
12	2
22	4
5	13
16	17
24	20
31	22
8	30
28	38
10	80
11	80
20	120
33	148
23	150
29	700
27	750
26	2000
7	Unknown
9 and 21	Not routinely

4.2.4 *Background information on the PCR methods used*

PCR testing is mainly done to confirm monophasic (Typhimurium) strains. Eight laboratories mentioned the following reference:

- EFSA Journal, 2010.

Other references mentioned, sometimes in combination with others, were:

- Aabo et al., 1993;
- Barco et al., 2011;
- Bugarel et al., 2012;
- Lee et al., 2009;
- Park et al., 1993
- Prendergast et al., 2013;
- Tennant et al., 2010.

References regarding molecular serotyping in general were:

- Fitzgerald et al., 2007 and McQuiston et al., 2011.

4.2.5 *Serotyping results per laboratory*

The percentages of correct results per laboratory are shown in Figure 1. 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 typed correctly by 30 of the 34 participants (88%). This corresponds to nearly 100% of the total number of strains. The H-antigens were typed correctly by 28 of the 34 participants (82%), corresponding to 99% of the total number of strains. A total of 24 participants (71%) gave the correct serovar names, corresponding to 99% of all strains evaluated.

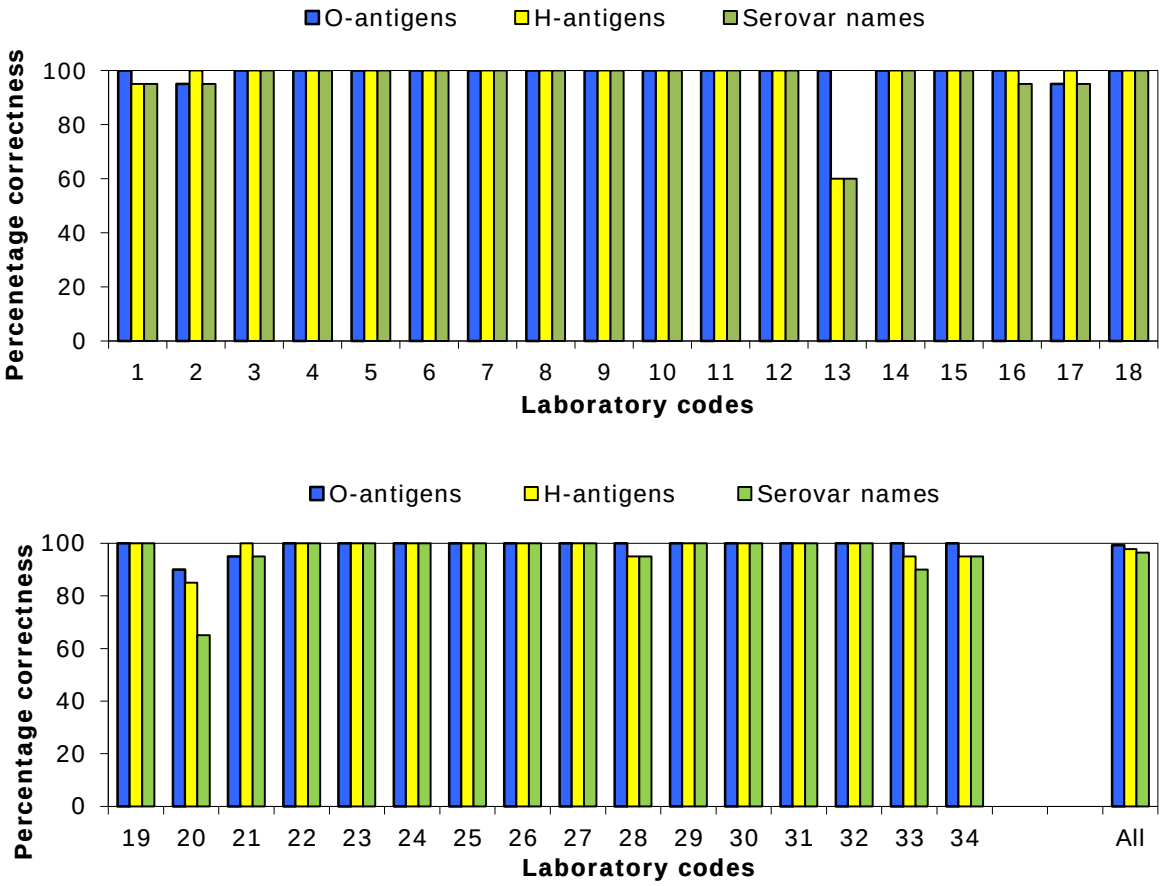

Figure 1. Percentages of correct serotyping results

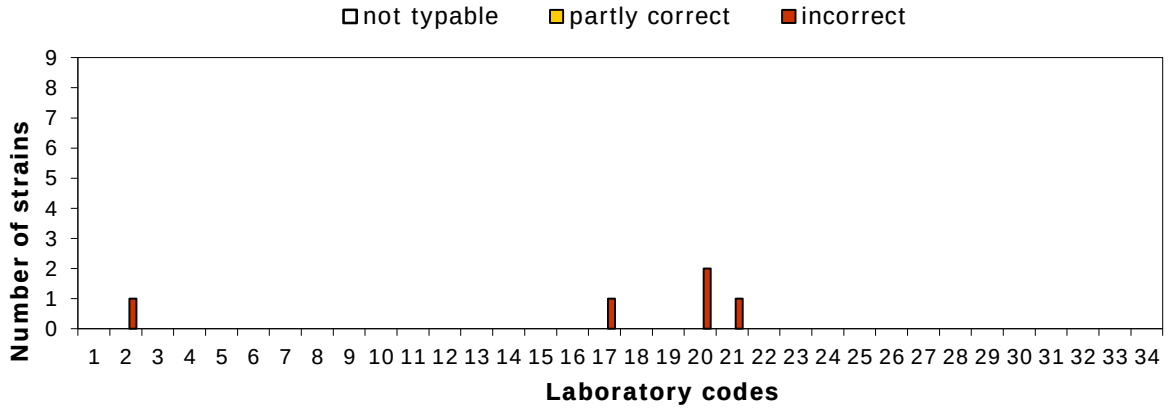

Figure 2. Evaluation of type of errors for O-antigens per NRL

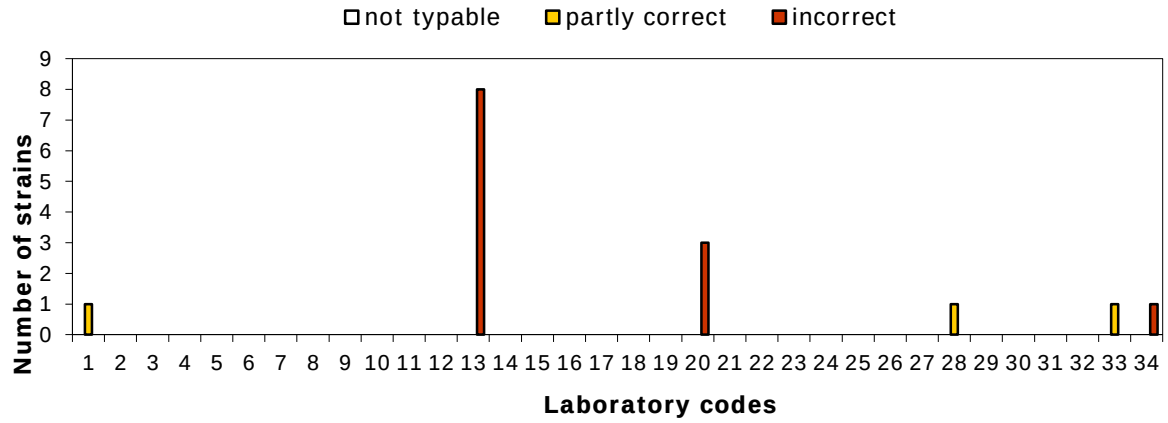

Figure 3. Evaluation of type of errors for H-antigens per NRL

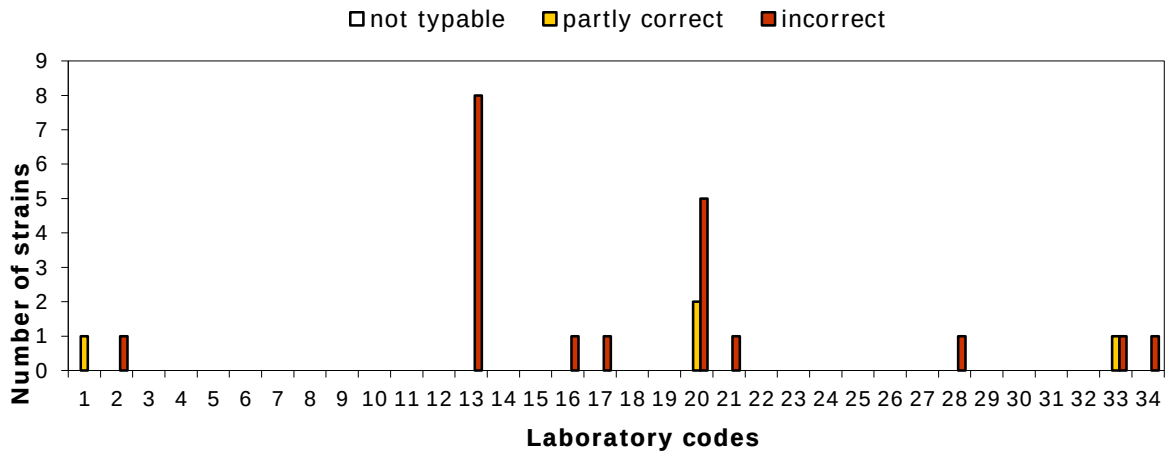

Figure 4. Evaluation of type of errors in the identification of serovar names

4.2.6 Performance of the participants

The number of penalty points was determined for each NRL using the guidelines described in Section 3.2.2. Table 9 shows the number of penalty points for each NRL and indicates whether the level of good

performance was achieved (yes or no). Two participants, both from a non-EU country, did not meet the level of good performance at this stage of the study and, in this case a voluntary, follow-up study was organised.
An example of an individual laboratory evaluation report on serotyping results is given in Annex 6.

Table 9. Evaluation of serotyping results per NRL

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

4.2.7 Serotyping results per strain

The results found per strain and per laboratory are given in Annex 3, except for the more complicated strains S1 and S21; these are reported separately in Annex 4.
A completely correct identification was obtained for ten *Salmonella* serovars: Infantis (S5), Duisburg (S6), Bispebjerg (S12), Typhimurium (S13), Enteritidis (S14), Reading (S15), Hadar (S16), Rissen (S17), Mikawasima (S19), and Virchow (S20).
Most problems occurred with the serovar Umbilo (S3). Six laboratories had difficulties assigning the correct serovar name to this strain, mostly due to problems with the O-antigens. Details of the strains that caused problems in serotyping are shown in Annex 5.
The reported serovar names for strain 1,4,[5],12:i:- (S1) are shown in Annex 4. Nineteen participants used a PCR method to confirm this strain to be a monophasic Typhimurium strain.
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 EU-NRLs had problems serotyping the 'top 5'

serovars, though one NRL reported the 1,4,[5],12:i:- strain as a Typhimurium strain (no PCR confirmation available).

Details of the additional and optional strain S21 are given in Annex 4. All but four participants tried to serotype strain S21, a *Salmonella enterica* subsp. *diarizonae* (IIIb). However, not all laboratories had access to the required antisera to finalise this (60:r:z).

4.2.8 *Results follow-up study*

Two participants, both non-EU-NRLs, did not achieve the level of good performance (Table 9; Lab code 13 and Lab code 20) and one of them participated in a follow-up study, receiving 10 additional strains for serotyping in week 18, 2017. The other laboratory did an extensive internal investigation to find out about any possible mistakes in the serotyping process, but had to decide to await the next interlaboratory study to test the improvements made due to lack of human resources at the time of the follow-up study.
Also for the follow-up study, the number of penalty points was determined using the guidelines described in Section 3.2.2. Table 10 shows the results of the follow-up study for participant 20, which again did not achieve the level of good performance. Unfortunately, the communication on the results and the way these were produced was quite difficult, and thereby hampering the improvement of the serotyping results for the moment.

Table 10. Evaluation of serotyping results per NRL in the follow-up study

Lab code	Penalty points	Good performance
20	9	**No**

4.2.9 *Trend analysis of the serotyping results of the EU NRLs*

The historical data of the EURL-*Salmonella* interlaboratory comparison studies on the serotyping of *Salmonella* are given in Annex 7, in Table A7-1 for EU-NRLs only and in Table A7-2 for all participants per study. The data on the EU-NRLs only are also visualised in Figure 5, showing the percentages of correctly typed strains, and in Figure 6, showing the number of Penalty Points and non-Good Performance in time.
The percentages of correctly typed strains have remained stable over time, usually showing a better performance for the O-antigens than for the H-antigens.
The number of Penalty Points has clearly declined, from 35 points at the start of this system in 2007, to 6 points in the 2016 study. In line with this, the number of EU-NRLs with a non-Good Performance is low: two in the period 2010 – 2013, only one in the 2014 and 2015 studies and none in the 2016 study.

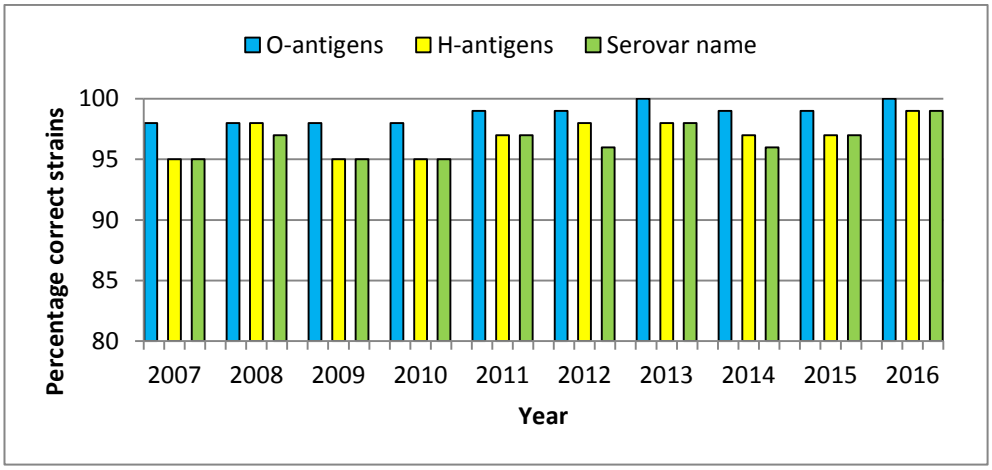

Figure 5. Serotyping results of the EU-NRLs in time, based on the percentages of correctly typed strains

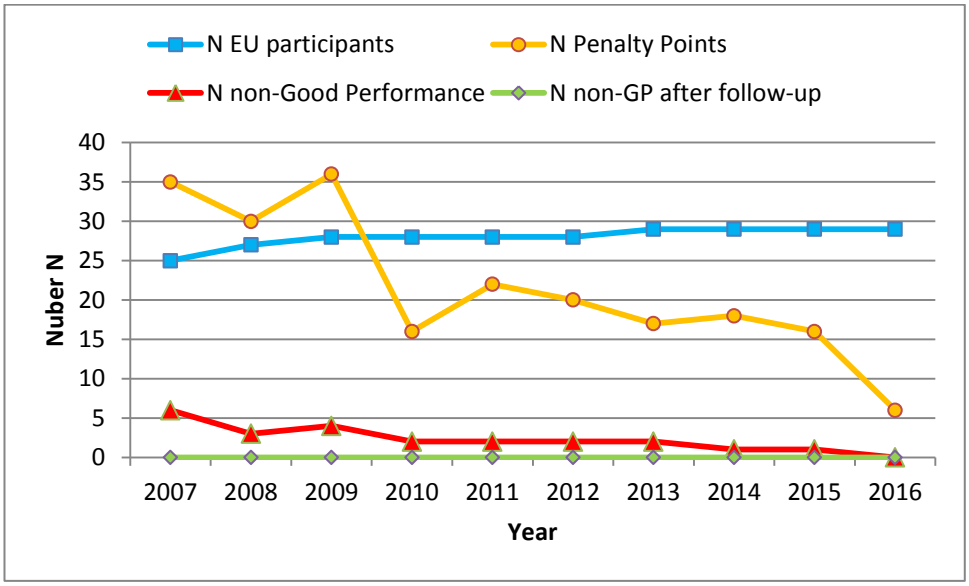

Figure 6. Serotyping results of the EU-NRLs in time, based on the number of Penalty Points and non-Good Performance

4.3 PFGE typing results

4.3.1 General

A total of 15 NRLs participated in the fourth study on PFGE typing. Five participants in the 2015-study did not participate in the 2016 study, and four participants were new, compared to the 2015 study.
Ten participants reported using the Standard PulseNet Protocol *Salmonella* PFGE (PulseNet International, 2013)/the EURL-*Salmonella* SOP (Jacobs et al., 2014). Five participants use this Standard protocol with modifications.

4.3.2 Technical data PFGE typing

Details on the manufacturer of the XbaI Enzyme, on the electrophoresis system and on the gel documentation system are summarised in Table 11, Table 12 and Table 13 respectively.

Table 11. Manufacturers of the enzyme XbaI used by the participants

Manufacturer	Number of NRLs
New England BioLabs	2
Promega	2
Roche Diagnostics	6
Thermo Scientific	5

Table 12. Electrophoresis system used by the participants

Electrophoresis system	Number of NRLs
Bio-Rad CHEF Mapper (XA)	3
Bio-Rad CHEF-DR III System	10
Bio-Rad CHEF-DR II System	2

Table 13. Gel documentation system used by the participants

Gel documentation system	Number of NRLs
Chemi Doc XR, Bio-Rad	1
G:Box (Syngene)	1
GelDoc	1
GelDoc XR	2
GelDoc XR+	5
GeneGenious (Syngene)	1
Image Lab 5.2.1	1
Kodak Digital	1
Proxima Geldoc 2000	1
UVP EC3 Chemi HR Imaging System	1

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

One participant used Sybr Safe for staining the gel; all other participants used Ethidium Bromide. The duration of the staining varied between 15 minutes (1x) and 90 minutes (1x), but most participants used 30 minutes (8x). De-staining was even more diverse, varying between 5 minutes and 2 hours, a majority of participants used up to 60 minutes. Eight participants used a comb with narrow teeth, and seven participants used one with wide teeth.

4.3.3 Results on the evaluation of the PFGE gel image

The scores per NRL (n=15), broken down across the seven parameters of evaluation (Annex 1), are given in Annex 8. The overall scores per parameter are shown in Figure 7.

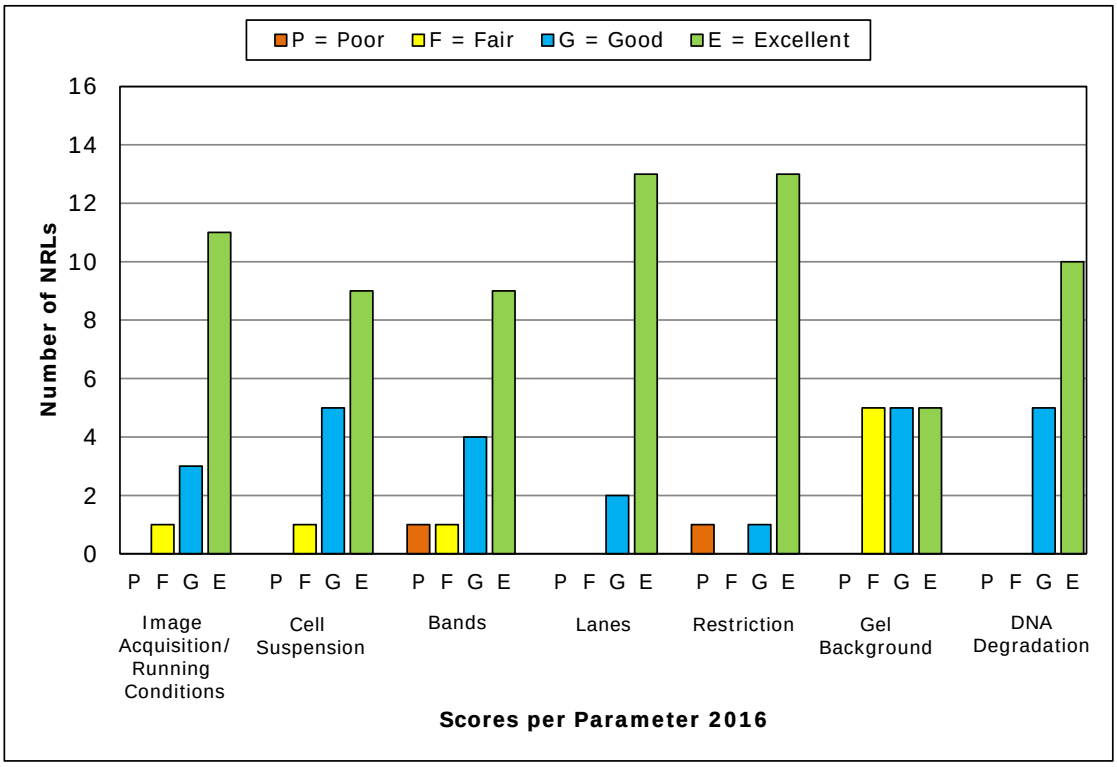

Figure 7. Evaluation of the quality of the PFGE images in scores per parameter, 2016 study

The quality of the produced PFGE gel images results was generally good, though, as in former studies, some variation in results between the participants was seen (Annex 10).
Overall, 90% of the scores were Good or Excellent. However, two of the 15 images resulted in a Poor score on at least one of the seven parameters, one for “Bands” and one for “Restriction” (Figure 7).
This indicates that these two images are not suitable for use in inter-laboratory database comparison of these PFGE profiles.
Most problems were seen in the parameter “Gel background”, with 5 participants scoring only Fair. Fewer problems were seen in the parameters “Lanes” and “DNA degradation”, in which all participants scored Good or Excellent.
Eight out of the 15 participants (53%) scored Good or Excellent for each of the 7 parameters as evaluated.
An example of an individual laboratory evaluation report is given in Annex 11.

Figure 8 shows the results of the evaluation of the TIFF images from the studies 2013 - 2016. Improvements in time are clearly seen in the reduction of red (Poor) results in 2013 and 2014 compared to 2016.

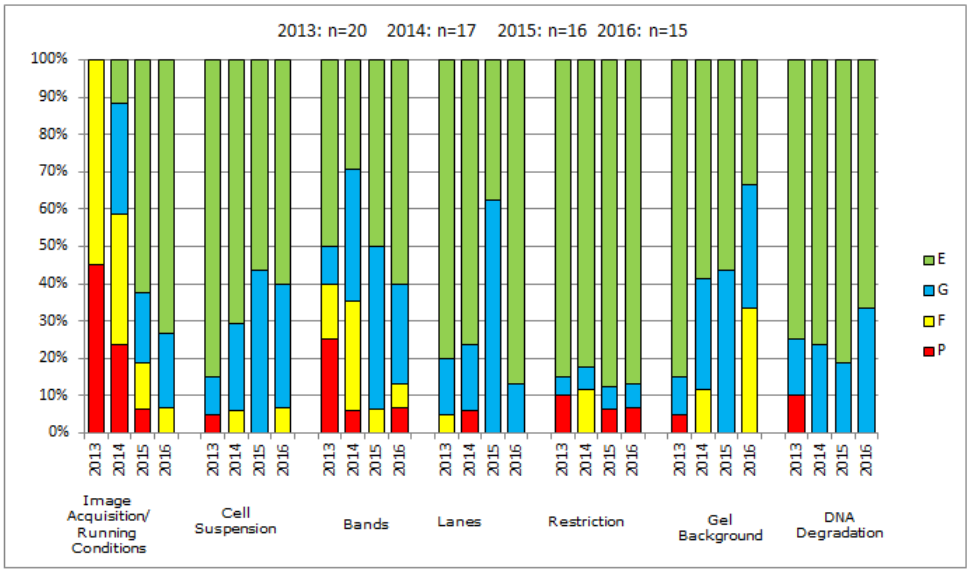

Figure 8. Evaluation of the quality of the PFGE images in scores per parameter, 2013-2016 studies

4.3.4 *Results on the evaluation of the analysis of the gel in BioNumerics*

For the second time the evaluation of the (optional) analysis of the gel in BioNumerics was included in the study as well. The participants all used the pre-configured database provided by the EURL-*Salmonella*, and therefore used identical experimental settings in BioNumerics. A total of 10 participants sent in their analysed gel data for evaluation. The scores per participating NRL, broken down across the five parameters of evaluation (Annex 2), are given in Annex 9. The summarised scores per parameter are shown in Figure 9.

Overall, 68% 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 wells when placing the frame. This will be easy to correct in future analysis.
All ten participants scored a Fair/Good for the parameter “Band assignment”. For all of them this was due to occasionally assigning double bands as single bands; less frequently single bands were assigned as double bands. Three participants were noted to assign bands under 33 kb, thereby not following the protocol. As an example, band assignment results for strain P3 are given in Figure 10.
Figure 11 shows the overall results from both 2015 and 2016.

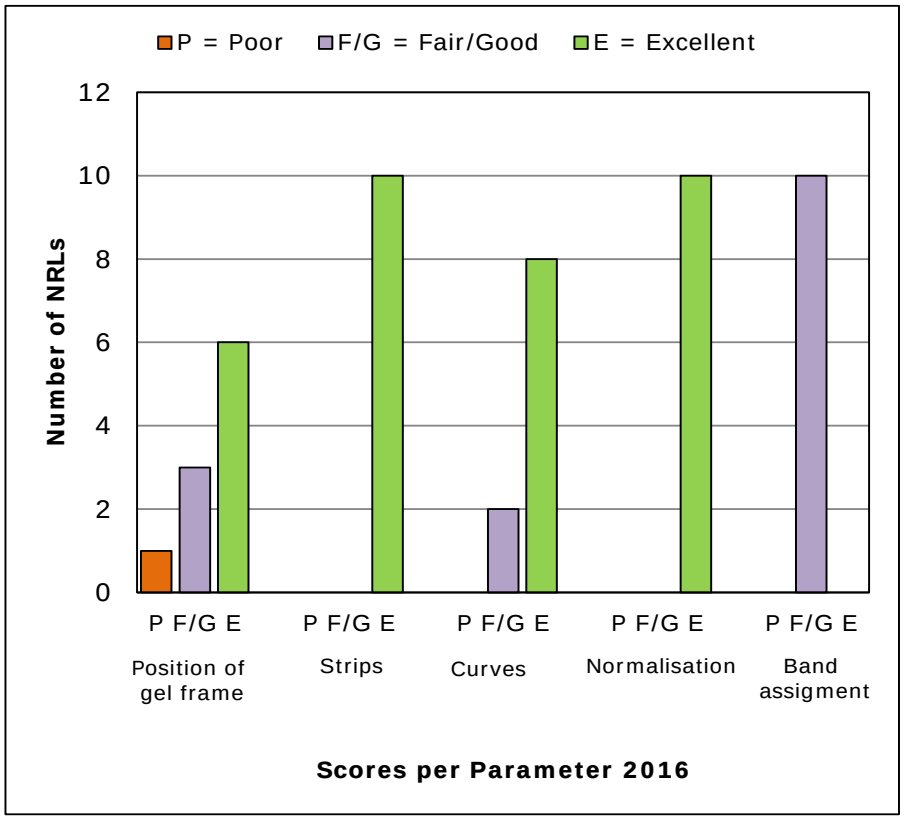

Figure 9. Evaluation of the analysis of the gel in BioNumerics in scores per parameter, 2016 study

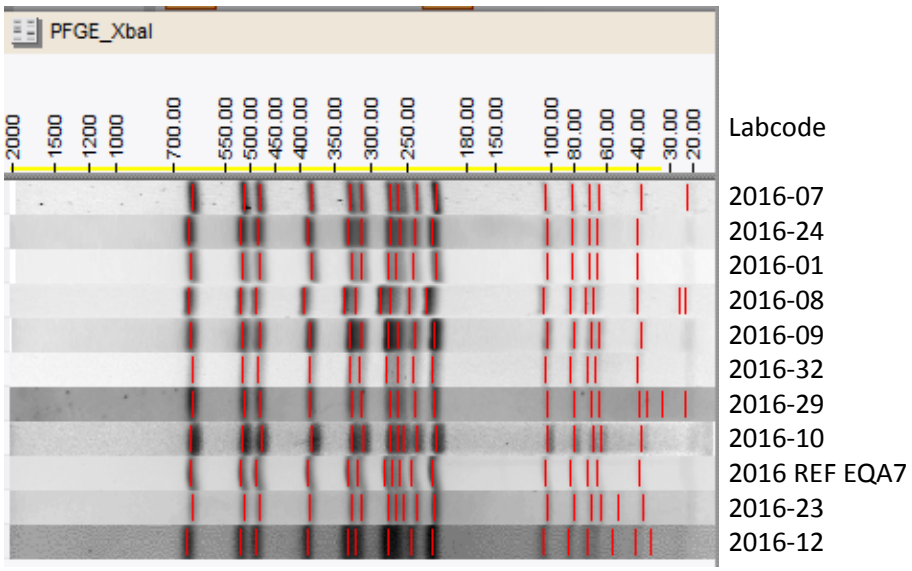

Figure 10. PFGE profiles with band assignment in BioNumerics by 10 participants for strain P3.

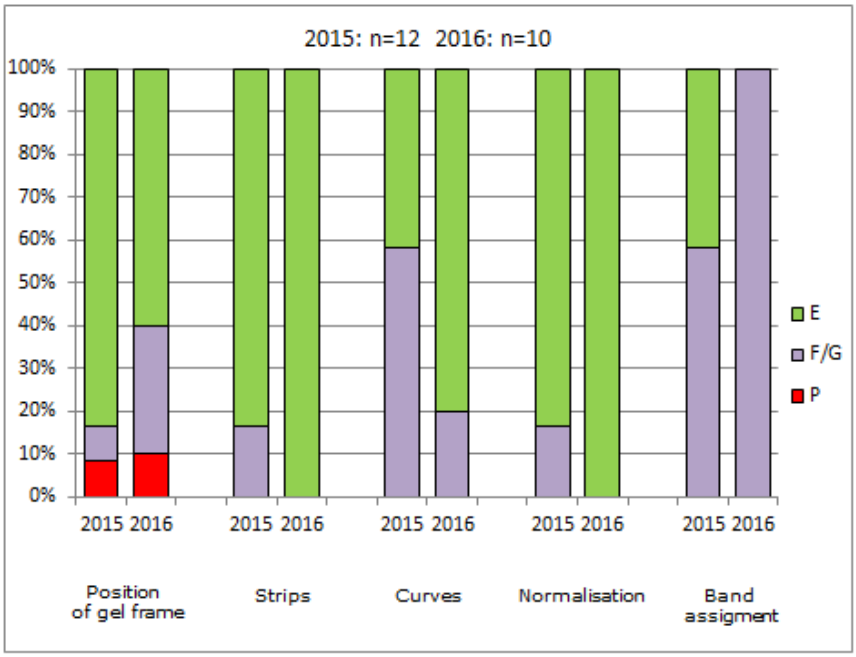

Figure 11. Evaluation of the analysis of the gel in BioNumerics in scores per parameter, 2015-2016 studies

5 Conclusions

5.1 Serotyping

- Overall results for all 34 participating laboratories are:
  - Nearly 100% of the strains were typed correctly for the O-antigens.
  - 99% of the strains were typed correctly for the H-antigens.
  - 99% of the strains were correctly named.
  - Serotyping of *S*. Umbilo caused the most problems in this study (six participants).
- All participants correctly serotyped the 'top 5' strains *S*. Enteritidis, *S*. Hadar, *S*. Infantis, *S*. Typhimurium and *S*. Virchow.
- All 29 EU-NRLs directly achieved the defined level of good performance.
- Two non-EU-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. Only one non-EU-NRL participated, but was not able to improve itself.

5.2 PFGE typing

- Thirteen (87%) of the fifteen 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.
- Ten participants also processed their gel in BioNumerics, and all of them were able to analyse their PFGE profiles in this computer program.

List of abbreviations

BN	BioNumerics
DG-SANTE	Directorate General for Health and Food Safety
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 on quality grading of PFGE images

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

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. Seventh external quality assessment scheme for Salmonella typing. Stockholm: ECDC; 2016. Available at: http://ecdc.europa.eu/en/publications/Publications/salmonella-typing-seventh-external-quality-assessment.pdf
(accessed on 17-1-2018)

Parameter	Grade [score in points]		
	Poor [1]	Fair [2]	Excellent [3]
Position of Gel Frame	- Wells wrongly included when placing the frame - Gel is not inverted.	- The 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.	Excellent placement of frame and gel is inverted.
Strips	Lanes incorrectly defined.	- Lanes are defined too narrowly (or widely). - Lanes are defined outside profile. - A single lane is not correctly defined.	All lanes correctly defined.
Curves	Curve set so that artefacts will cause wrong band assignment.	Curve extraction is defined either too narrowly or including almost the whole lane.	1/3 or more of the lane is used for averaging curve extraction.
Normali-zation	- Many bands not assigned in the reference lanes. - The references were not included when submitting the data. - Assignment of band(s) in reference lane(s) to incorrect size(s).	- Bottom bands <33kb are not assigned in some or all of the reference lanes. - Some bands wrongly assigned in reference lane(s).	All bands correctly assigned in all reference lanes
Band Assignment	Incorrect band assignment making inter-laboratory comparison impossible.	- Few double bands assigned as single bands or single bands assigned as double bands. - Few shadow bands are assigned. - Few bands are not assigned.	Excellent band assignment with regard to the quality of the gel.

Note that the EFSA supporting publication 2014:EN-703 (recommended SOP) states:
When using the *S*. Braenderup H9812 reference, visible bands of *test* isolates should be marked down to ~33 kb (third band from the bottom of the H9812 reference), but not below (referring to *Band Assigment*). In *Normalisation*, all bottom bands (also < 33 kb) in all *reference* lanes are assigned.

Annex 3 Serotyping results per strain and per laboratory

Lab REF	S2 Newport	S3 Umbilo	S4 Szentes	S5 Infantis	S6 Duisburg	S7 Orion	S8 Saintpaul	S9 Augustenborg	S10 Chester
1	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
2	Newport	Djibouti	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
3	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
4	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
5	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
6	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
7	Newport	Umbilo	Szentes	Infantis	Duisdurg	Orion	Saintpaul	Augustenborg	Chester
8	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
9	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
10	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
11	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
12	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
13	Cremieu	Moroto	Maumee	Infantis	Duisburg	Langensalza	Chester	Stuttgart	Chartres
14	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	SaintPaul	Augustenborg	Chester
15	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
16	Newport	Luckenwalde	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
17	Newport	Djibouti	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
18	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
19	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
20	8:e,h:2	Albert	OMC:k:2	Infantis	Duisburg	Muenster	Sandiego	Aberden	Chester
21	Newport	Telhashomer	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
22	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
23	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
24	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
25	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
26	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
27	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
28	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Oritamerin	Chester
29	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
30	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
31	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Chester
32	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenburg	Chester
33	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Augustenborg	Sandiego
34	Newport	Umbilo	Szentes	Infantis	Duisburg	Orion	Saintpaul	Norton	Chester

Lab REF	S2 Newport	S3 Umbilo	S4 Szentes	S5 Infantis	S6 Duisburg	S7 Orion	S8 Saintpaul	S9 Augustenborg	S10 Chester
X	1	6	1	0	0	2	2	4	2

S11 Ohio	S12 Bispebjerg	S13 Typhimurium	S14 Enteritidis	S15 Reading	S16 Hadar	S17 Rissen	S18 Haifa	S19 Mikawasima	S20 Virchow	Lab REF
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	1
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	2
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	3
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	4
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	5
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	6
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	7
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	8
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	9
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haïfa	Mikawasima	Virchow	10
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	11
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	12
Adime	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	13
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	14
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	15
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	16
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	17
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	18
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	19
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Istanbul	Rissen	Shubra	Mikawasima	Virchow	20
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	21
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	22
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	23
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	24
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	25
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	26
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	27
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	28
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	29
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	30
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	31
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	32

S11 Ohio	S12 Bispebjerg	S13 Typhimurium	S14 Enteritidis	S15 Reading	S16 Hadar	S17 Rissen	S18 Haifa	S19 Mikawasima	S20 Virchow	Lab REF
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa		Virchow	33
Ohio	Bispebjerg	Typhimurium	Enteritidis	Reading	Hadar	Rissen	Haifa	Mikawasima	Virchow	34
1	0	0	0	0	0	0	1	0	0	**X**

remark
partly correct (no penalty points)
incorrect (1 penalty point)
incorrect (4 penalty points)

X = number of deviating laboratories per strain

Results for Strains S1 and S21 are given in Annex 4

Annex 4 Details of serotyping results for strains S1 and S21

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	PCR-confirmed	Lab code
S-1	**1,4,[5],12**	**i**	**-**	**1,4,[5],12:i:-**	**yes**	**REF**
S-1	4,5	i	2	Typhimurium	no	1
S-1	4,5,12	i	-	1,4,5,12:i:-	no	2
S-1	4,5,12	i	-	4,5,12:i:-	no	3
S-1	4,5,12	i	-	4,5,12: i : - . Typhimurium monophasic variant.	no	4
S-1	4,5,12	i	-	4,5,12:i:-	yes	5
S-1	4, 5, 12	i	-	4,5,12:i:-	no	6
S-1	4,12	i	-	4,12 : i : -	yes	7
S-1	4,5,12	i	-	4,5,12:i:-	yes	8
S-1	1,4,5,12	i	-	1,4,5,12:i:-	yes	9
S-1	4,5,12	i	-	4,5,12 : i : -	yes	10
S-1	4,5,12	i	-	4,5,12:i:-	yes	11
S-1	4,5,12	i	-	Typhimurium, monophasic (4,5,12:i:-)	yes	12
S-1	4,5,12	i	-	4,5,12:i:-	no	13
S-1	4,5,12	i	-	4,5,12:i:-	no	14
S-1	4,5	i	-	4,5:i:-	no	15
S-1	4	i	-	Typhimurium monophasic variant	yes	16
S-1	4,5,12	i	-	4,5,12:i:-	no	17
S-1	4,5,12	i	-	4,5,12:i:- (Typhimurium-like monophasic variant)	no	18
S-1	4,5,12	i	-	4,5,12:i:-	no	19
S-1	4,5	i	-	4,5:i:- S. Typhimurium monophasic	yes	20
S-1	1,4,5,12	i	-	1,4,5,12:i:-	yes	21
S-1	1,4,5,12	i	-	1,4,5,12:i:-	yes	22
S-1	1, 4, 5	i	-	Monophasic S. Typhimurium	yes	23
S-1	4,5,12	i	-	1,4,[5],12:i:-	yes	24
S-1	4,5,12	i	-	4,5,12:i:-	no	25

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	PCR-confirmed	Lab code
S-1	4,5,12	i	-	4,5,12:i:-	yes	26
S-1	4,5	i	-	4,5,12:i:-	yes	27
S-1	4,5,12	i	-	4,5,12:i:-	yes	28
S-1	1,4,5,12	i	-	Monophasic variant S.Typhimurium	yes	29
S-1	1,4,5,12	i	-	1,4,5,12:i:-	no	30
S-1	4,5,12	i	-	monofasisk subspI=4,5:i:-	yes	31
S-1	4,5,12	i	-	4,5,12:i:-	no	32
S-1	4,5,12	i	-	monophasic Typhimurium	yes	33
S-1	4,5,12	i	-	4,5,12:i:-	no	34

Reference
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	**60**	**r**	**z**	**60:r:z**	**REF**
S-21	60	r	z	S. IIIb 60 : r : z	1
S-21	-	-	-		2
S-21	60	r	z	Salmonella enterica subsp. diarizonae (III b) 60:r:z	3
S-21	60	r	z	S. enterica subsp. diarizonae 60:r:z	4
S-21	60	r	z	III b diarizonae	5
S-21					6
S-21	60	r	-	60 : r : - IIIb	7
S-21	60	r	z	60:r:z	8
S-21	60	r	z	S. IIIb 60:r:z	9
S-21				Salmonella Subspecies II (salamae)	10
S-21	60	r	z	60:r:z	11
S-21	60	r	z	Salmonella enterica subsp.diarizonae ser. 60 : r : z	12
S-21					13
S-21	60	r	-	60:r:-	14
S-21	?	r	z	?:r:z	15
S-21	60	r	-	60 : r : - (enterica subsp. diarizonae)	16
S-21	60	r	z	S. enterica subsp. diarizonae /IIIb/	17
S-21	60	r	z	SGIIIb 60:r:z	18
S-21	60	r	z	60:r:z	19
S-21	OMG	r	-	OMG:r:-	20
S-21	60	r	z53	60:r:z53	21
S-21	60	r	z	60:r:z	22
S-21	60	r	z	S. enterica subsp. diarizonae 60:r:z	23
S-21	60	r	z	IIIb 60:r:z	24
S-21	60	r	z	60:r:z	25
S-21	60	r	z	IIIb 60:r:z	26
S-21	60	r	z	SIII 60:r:z	27
S-21	-	r	-	-:r:-	28
S-21	60	r	z	subsp. Diarizonae	29
S-21	60	r	z	60:r:z	30

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	Lab code
S-21	60	r	z	S.SubspIIIb=60:r:z	31
S-21	60	r	z	60:r:z	32
S-21					33
S-21	60	r	z	III b	34

S-21: *Salmonella enterica* subspecies *diarizona* (IIIb), optional strain

reference
remark

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	**6,8,20**	**e,h**	**1**	**Newport**	**REF**
S-2	6,8	h	6	Cremieu	13
S-2	8	e,h	2	8:e,h:2	20
S-3	**28**	**z10**	**e,n,x**	**Umbilo**	**REF**
S-3	17	z10	e,n,x	S. Djibouti	2
S-3	28	z10	w	Moroto	13
S-3	28	z10	e,n,x	Luckenwalde	16
S-3	17	z10	e,n,x	S. Djibouti	17
S-3	4	z10	e,n,x	S. Albert	20
S-3	11	z10	e,n,x	S. Telhashomer	21
S-4	**16**	**k**	**1,2**	**Szentes**	**REF**
S-4	16	k	6	Maumee	13
S-4	OMC	k	2	OMC:k:2	20
S-7	**3,{10}{15}{15,34}**	**y**	**1,5**	**Orion**	**REF**
S-7	10	y	w	Langensalza	13
S-7	3,10	e,h	5	S. Muenster	20
S-8	**1,4,[5],12**	**e,h**	**1,2**	**Saintpaul**	**REF**
S-8	4,5,12	e	x	Chester	13
S-8	4,5	e,h	e,n,z15	S. Sandiego	20
S-9	**6,7,14**	**i**	**1,2**	**Augustenborg**	**REF**
S-9	7	i	z6	Stuttgart	13
S-9	11	i	2	S. Aberden	20
S-9	6,7	i	1,5	Oritamerin	28
S-9	6,7	i	l,w	Norton	34
S-10	**1,4,[5],12**	**e,h**	**e,n,x**	**Chester**	**REF**
S-10	4,12	h	w	Chartres	13
S-10	4,12	e,h	e,n,z15	Sandiego	33
S-11	**6,7,14**	**b**	**l,w**	**Ohio**	**REF**
S-11	6,7	b	6	Adime	13
S-16	**6,8**	**z10**	**e,n,x**	**Hadar**	**REF**
S-16	8	z10	e,n,x	S. Istanbul	20
S-18	**1,4,[5],12**	**z10**	**1,2**	**Haifa**	**REF**
S-18	4,5	z	2	S. Shubra	20
S-19	**6,7,14**	**y**	**e,n,z15**	**Mikawasima**	**REF**
S-19	6,7	y	-		33

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 Example of an individual laboratory evaluation report on serotyping results

Individual Laboratory Results 21st Interlaboratory Comparison Study *Salmonella* serotyping (November 2016)
Page 1 of 2

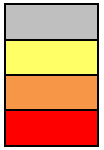
	Reference Results				**Results NRL labcode:**			**34**
Strain	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
S1[a)]	1,4,[5],12	i	-	1,4,[5],12:i:-	4,5,12	i	-	4,5,12:i:-
S2	6,8,20	e,h	1,2	Newport	6,8	e,h	1,2	Newport
S3	28	z10	e,n,x	Umbilo	28	z10	e,n,x	Umbilo
S4	16	k	1,2	Szentes	16	k	1,2	Szentes
S5	6,7,14	r	1,5	Infantis	6,7	r	1,5	Infantis
S6	1,4,12,27	d	e,n,z15	Duisburg	4	d	e,n,z15	Duisburg
S7	3,{10}{15}{15,34}	y	1,5	Orion	10	y	1,5	Orion
S8	1,4,[5],12	e,h	1,2	Saintpaul	4,5	e,h	1,2	Saintpaul
S9	6,7,14	i	1,2	Augustenborg	6,7	i	l,w	Norton
S10	1,4,[5],12	e,h	e,n,x	Chester	4	e,h	e,n,x	Chester
S11	6,7,14	b	l,w	Ohio	6,7	b	l,w	Ohio
S12	1,4,[5],12	a	e,n,x	Bispebjerg	4	a	e,n,x	Bispebjerg
S13	1,4,[5],12	i	1,2	Typhimurium	4,5	i	1,2	Typhimurium
S14	1,9,12	g,m	-	Enteritidis	9,12	g,m	-	Enteritidis
S15	1,4,[5],12	e,h	1,5	Reading	4,5	e,h	1,5	Reading
S16	6,8	z10	e,n,x	Hadar	6,8	z10	e,n,x	Hadar
S17	6,7,14	f,g	-	Rissen	6,7	f,g	-	Rissen
S18	1,4,[5],12	z10	1,2	Haifa	4,5	z10	1,2	Haifa
S19	6,7,14	y	e,n,z15	Mikawasima	6,7	y	e,n,z15	Mikawasima
S20	6,7,14	r	1,2	Virchow	6,7	r	1,2	Virchow
S21[b)]	60	r	z	60:r:z	60	r	z	III b

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

Individual Laboratory Results 21st Interlaboratory Comparison Study *Salmonella* serotyping (November 2016)
Page 2 of 2

For detailed information, reference results are given completely according to the White-Kauffmann-le Minor scheme (2007).
Participants were asked to report only those results, on which the identification of serovar names was based.

Colour coding:



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

As decided at the 21st EURL-*Salmonella* Workshop (St. Malo, 2016), Strain S-21 was an additional strain to the study.
Testing of this strain was optional and results were not included in the evaluation.
The evaluation of the serotyping results was performed as indicated in Table 1 of the Protocol as sent to the participants.
In addition to that, Good Performance was evaluated on the basis of penalty points as indicated below.
(as decided at the 12th CRL-*Salmonella* Workshop, Bilthoven, 2007).

4 penalty points: Incorrect typing of *S*. Enteritidis, *S*. Typhimurium (including monophasic variant), *S*. Hadar, *S*. Infantis or *S*. Virchow or assigning the name of one of these 5 serovars to another serovar.
1 penalty point: Incorrect typing of all other *Salmonella* serovars.
(no penalty points are given in case a strain was non-typable due to auto-agglutination)

Good Performance is defined as < 4 penalty points.

Number of penalty points for your laboratory in this study: 1 -> Good Performance

EURL-Salmonella, Bilthoven, The Netherlands

Annex 7 Historical overview on the results of the EURL-*Salmonella* serotyping studies

*Table A7-1. 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	21 2016
No. of participants	25	27	28	28	28	28	29	29	29	29
No. of strains evaluated	20	20	20	19	19*	20	20	20	20	20
O-antigens correct/strains	490/500 (98%)	529/540 (98%)	551/560 (98%)	530/532 (99%)	527/532 (99%)	554/560 (99%)	579/580 (100%)	575/580 (99%)	577/580 (99%)	578/580 (100%)
H-antigens correct/strains	477/500 (95%)	528/540 (98%)	532/560 (95%)	520/532 (98%)	518/532 (97%)	547/560 (98%)	570/580 (98%)	563/580 (97%)	564/580 (97%)	576/580 (99%)
Names correct/strains	473/500 (95%)	521/540 (97%)	529/560 (95%)	518/532 (97%)	463/476 (97%)	539/560 (96%)	567/580 (98%)	559/580 (96%)	564/580 (97%)	573/580 (99%)
O-antigens correct/labs	17/25 (68%)	19/27 (70%)	21/28 (75%)	26/28 (93%)	26/28 (93%)	23/28 (82%)	28/29 (97%)	25/29 (86%)	27/29 (93%)	27/29 (93%)
H-antigens correct/labs	14/25 (56%)	18/27 (67%)	12/28 (43%)	20/28 (71%)	20/28 (71%)	18/28 (64%)	21/29 (72%)	19/29 (66%)	18/29 (62%)	25/29 (86%)
Names correct/labs	13/25 (52%)	14/27 (52%)	13/28 (46%)	18/28 (64%)	21/28 (75%)	16/28 (57%)	20/29 (69%)	16/29 (55%)	17/29 (59%)	23/29 (79%)
No. of penalty points	35	30	36	16	22	20	17	18	16	6
No. of labs not achieving good performance	6	3	4	2	2	2	2	1	1	0
No. of labs not achieving good performance after follow-up	0	0	0	0	0	0	0	0	0	0

*2 strains: only O and H antigens evaluated, not the naming of those serovars

*Table A7-2. 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	21 2016
No. of participants	26	29	31	33	36	31	34	35	34	34
No. of strains evaluated	20	20	20	19	19*	20	20	20	20	20
O-antigens correct/strains	510/520 (98%)	568/580 (98%)	603/620 (97%)	616/627 (98%)	670/684 (98%)	612/620 (99%)	678/680 (100%)	679/700 (97%)	676/680 (99%)	675/680 (99%)
H-antigens correct/strains	497/520 (96%)	568/580 (98%)	581/620 (94%)	598/627 (95%)	657/684 (96%)	605/620 (98%)	666/680 (98%)	660/700 (94%)	660/680 (97%)	665/680 (98%)
Names correct/strains	493/520 (95%)	560/580 (97%)	578/620 (93%)	593/627 (95%)	586/612 (96%)	597/620 (96%)	662/680 (97%)	658/700 (94%)	659/680 (97%)	656/680 (96%)
O-antigens correct/labs	18/26 (69%)	22/29 (76%)	23/31 (74%)	29/33 (88%)	31/36 (86%)	24/31 (77%)	32/34 (94%)	29/35 (83%)	31/34 (91%)	30/34 (88%)
H-antigens correct/labs	15/26 (58%)	21/29 (72%)	14/31 (45%)	22/33 (67%)	25/36 (69%)	19/31 (61%)	24/34 (71%)	22/35 (63%)	21/34 (62%)	28/34 (82%)
Names correct/labs	14/26 (54%)	17/29 (59%)	15/31 (48%)	20/33 (61%)	25/36 (69%)	17/31 (55%)	23/34 (68%)	20/35 (57%)	19/34 (56%)	24/34 (71%)
No. of penalty points	36	34	56	37	41	20	20	57	21	21
No. of labs not achieving good performance	6	4	5	4	4	2	2	2	1	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)	0	1 (n=1)

*2 strains: only O and H antigens evaluated, not the naming of those serovars

Annex 8 Evaluation of PFGE images per participant and per parameter

Lab code / Parameter	**34**	**16**	**29**	**23**	**10**	**25**	**7**	**8**	**12**	**21**	**9**	**24**	**32**	**1**	**15**	**Total score per parameter**	**Average per parameter**
Image Acquisition & Running Conditions	4	3	3	4	3	4	2	4	4	4	4	4	4	4	4	55	3,7
Cell Suspension	2	3	3	3	4	4	4	3	4	4	4	4	3	4	4	53	3,5
Bands	2	1	3	3	3	3	4	4	4	4	4	4	4	4	4	51	3,4
Lanes	4	4	4	4	4	3	4	4	3	4	4	4	4	4	4	58	3,9
Restriction	1	4	4	3	4	4	4	4	4	4	4	4	4	4	4	56	3,7
Gel Background	2	4	2	2	2	2	3	4	3	3	3	3	4	4	4	45	3,0
DNA Degradation (smearing in lanes)	3	3	3	4	4	4	4	3	4	3	4	4	4	4	4	55	3,7
Total score per participant	18	22	22	23	24	24	25	26	26	26	27	27	27	28	28		
Average per participant	2,6	3,1	3,1	3,3	3,4	3,4	3,6	3,7	3,7	3,7	3,9	3,9	3,9	4	4		

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

Annex 9 Evaluation of the analysis of the gel in BioNumerics per participant and per parameter

Lab code / Parameter	**12**	**7**	**9**	**23**	**29**	**1**	**8**	**10**	**24**	**32**	**Total score per parameter**	**Average per parameter**
Position of gel	1	2	2	2	3	3	3	3	3	3	25	2,5
Strips	3	3	3	3	3	3	3	3	3	3	30	3,0
Curves	2	3	3	3	2	3	3	3	3	3	28	2,8
Normalisation	3	3	3	3	3	3	3	3	3	3	30	3,0
Band assignment	2	2	2	2	2	2	2	2	2	2	20	2,0
Total score per participant	11	13	13	13	13	14	14	14	14	14		
Average per participant	2,2	2,6	2,6	2,6	2,6	2,8	2,8	2,8	2,8	2,8		

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

Annex 10 Examples of PFGE images obtained by the participants

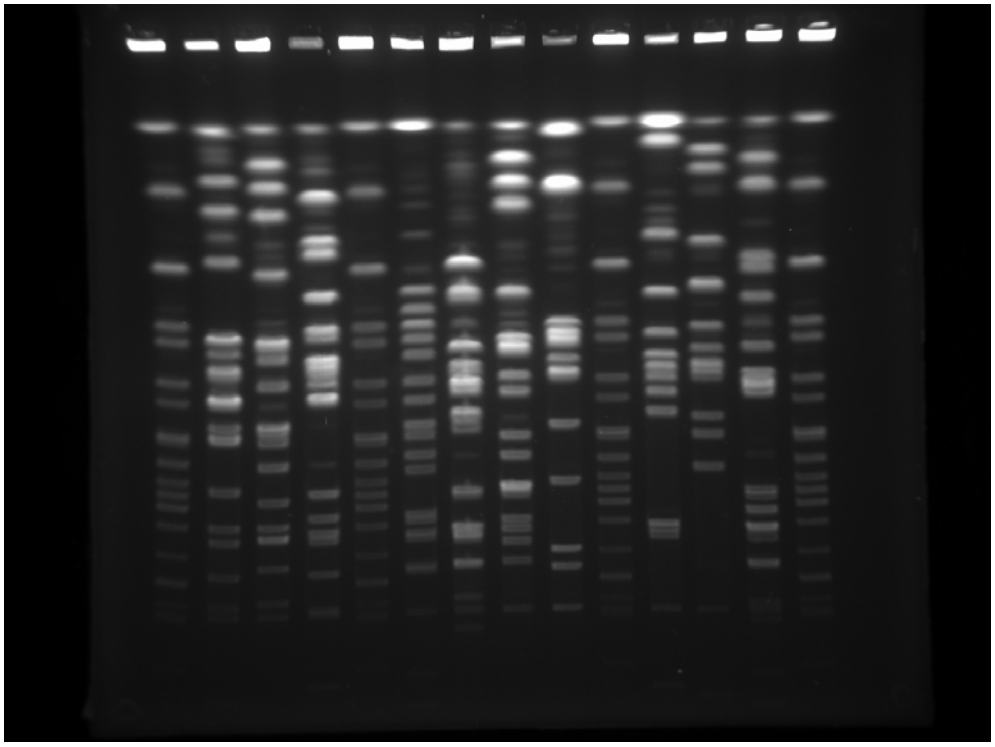


Figure A10.1. Example of a gel (lab code 34) with a generally lower score

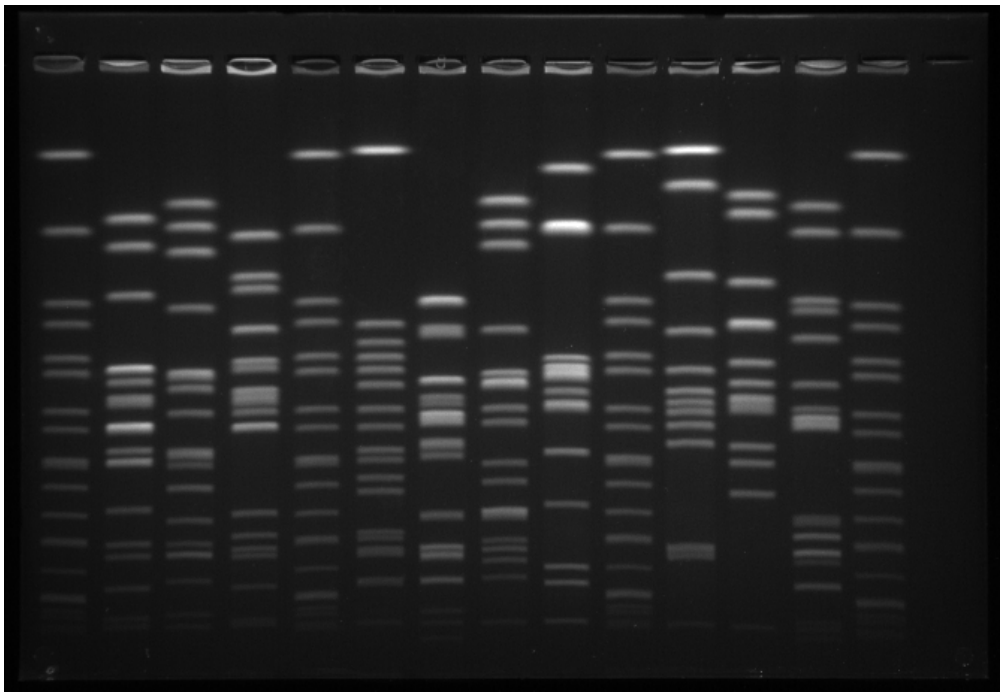


Figure A10.2. Example of a gel (lab code 15) with a generally high score

Annex 11 Example of an individual laboratory evaluation report on PFGE typing results

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NRL Laboratory code: **7**

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	Wells not included on TIFF.	2
Cell Suspension	Excellent	The cell concentration is approximately the same in each lane.	4
Bands	Excellent	Clear and distinct all the way to the bottom of the gel.	4
Lanes	Excellent	Straight.	4
Restriction	Excellent	Complete restriction in all lanes.	4
Gel Background	Good	Mostly clear background. Minor debris present that does not affect analysis.	3
DNA Degradation	Excellent	No DNA degradation visible (no smearing).	4
Total score:			25

* 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 frame	Fair	The frame seems to be positioned correctly, but the wells are not visible on the image, so not able to judge	2
Strips	Excellent	All lanes correctly defined.	3
Curves	Excellent	1/3 or more of the lanes is used for averaging curve thickness.	3
Normalisation	Excellent	All bands assigned correctly in all reference lanes.	3
Band assignment	Fair	Bands under 33 kb are assigned (not to be done according to the Protocol). Few double bands assigned as single bands.	2
Total score:			13

* 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

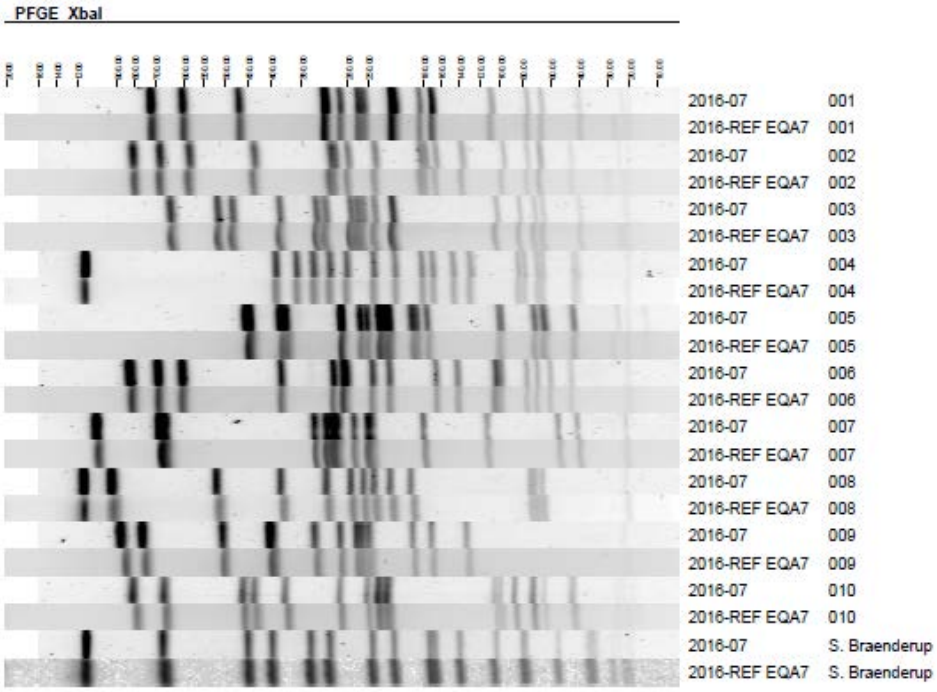

Figure 2. Comparison of your PFGE gel analysis in Bionumerics with the reference analysis

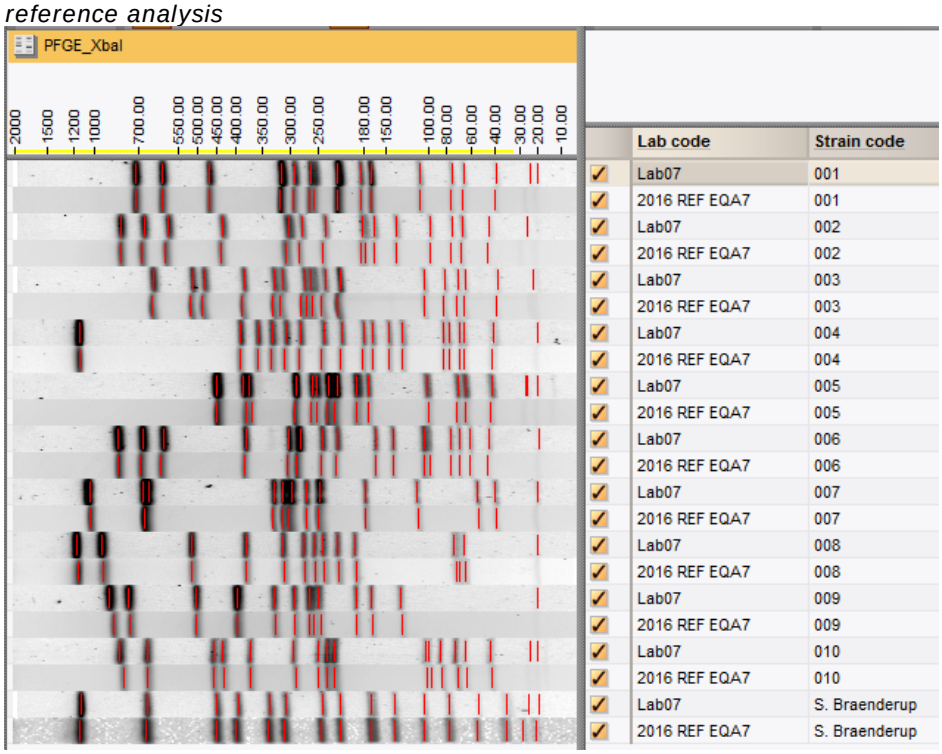

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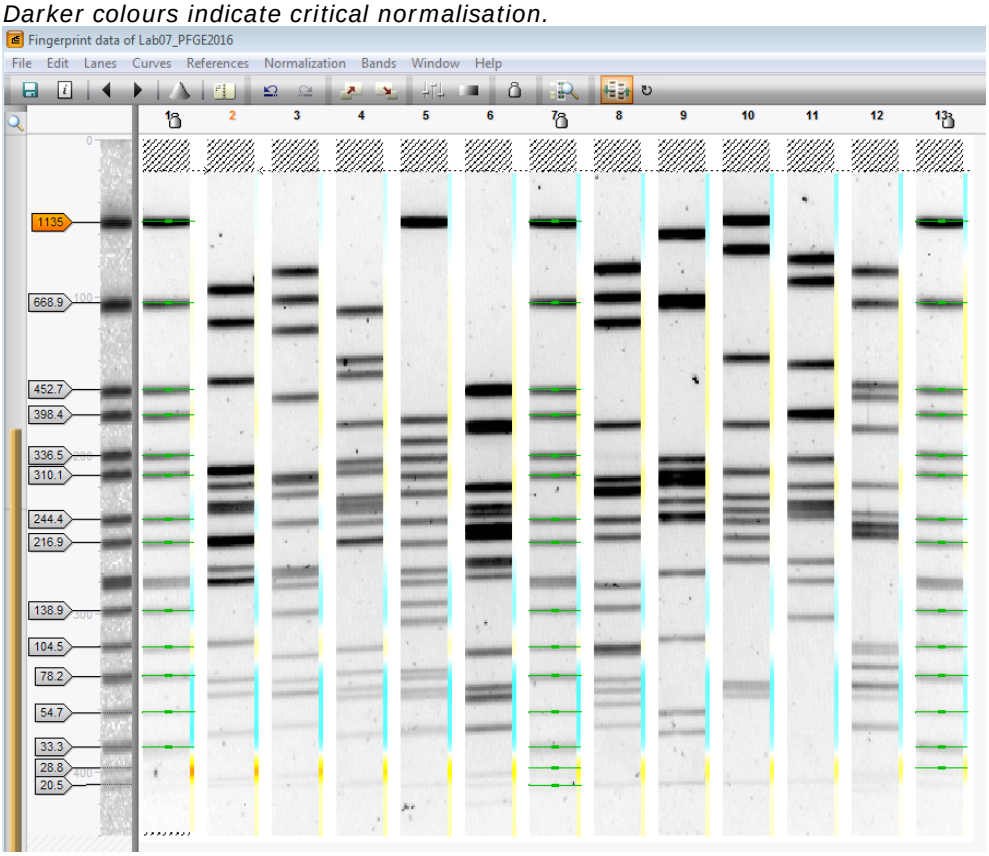
Figure 3. Display of the "Distortion bar" option in Bionumerics of your gel. Darker colours indicate critical normalisation.



EURL-*Salmonella*, Bilthoven, The Netherlands

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