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

22nd EURL-*Salmonella* interlaboratory comparison study (2017) on typing of *Salmonella* spp.

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Colophon

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Synopsis

22nd EURL-*Salmonella* interlaboratory comparison study (2017) on typing of *Salmonella* spp.

The National Reference Laboratories (NRLs) of all 28 European Union (EU) Member States performed well in the 2017 quality control test on *Salmonella* typing. Overall, the EU-NRLs were able to assign the correct name to 98% 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 eleven strains of *Salmonella* to be tested by this method. Eleven 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, EFTA countries Iceland, Norway and Switzerland, and Israel took part in the 2017 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

Tweeëntwintigste EURL-*Salmonella* ringonderzoek (2017) voor de typering van *Salmonella* spp.

De Nationale Referentie Laboratoria (NRL's) van de 28 Europese lidstaten scoorden in 2017 goed bij de kwaliteitscontrole op *Salmonella*-typering. Uit de analyse van alle NRL's als groep bleek dat de laboratoria aan 98 procent van de geteste stammen de juiste naam konden geven.

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 elf extra stammen met deze methode typeren. Elf 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 2017 waren dat de EU-kandidaat-lidstaten Macedonië en Servië, de European Free Trade Association (EFTA)-landen IJsland, Noorwegen en Zwitserland, en Israël.

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 2017, the 22nd 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, the EFTA countries Iceland, Norway and Switzerland, and Israel.

All 35 laboratories performed serotyping. A total of 20 obligatory *Salmonella* strains plus 1 optional *Salmonella* strain were selected by the EURL-*Salmonella* for serotyping. The strains had to be typed according to the method routinely used in each laboratory, following the White-Kauffmann-Le Minor scheme (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, 99% of the strains were typed correctly for the O-antigens, 98% of the strains were typed correctly for the H-antigens, and 98% 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, all 35 participants achieved good results in the first stage of the study, therefore a follow-up was not necessary.

Fifteen participating laboratories also performed additional typing at DNA level using Pulsed Field Gel Electrophoresis (PFGE). The participants received another eleven strains of *Salmonella* to be tested by this method. Eleven to fourteen 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 a common gel in the dedicated software BioNumerics, and all of them were able to analyse the PFGE profiles in this computer program.

1 Introduction

This report describes the 22nd 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 2017.

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 35 laboratories participated in this study. These included 29 NRLs-*Salmonella* in the 28 EU Member States, 2 NRLs in EU-candidate countries, 3 NRLs in EFTA countries, and 1 non-European NRL. 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 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 fifth time, the typing study also included PFGE typing. Fifteen NRLs participated in this part of the study by PFGE typing 11 designated *Salmonella* strains and submitting images for evaluation. Ten of these participants also used a pre-configured database to analyse a common gel for all participants, provided by the EURL-*Salmonella,* in the dedicated computer program BioNumerics.

2 Participants

Country	City	Institute
Austria	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	Ringsted	Danish Veterinary and Food Administration (DVFA) laboratory
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 Chalkida
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
Israel	Kiryat Malachi	Southern Laboratory for Poultry Health
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, NVI
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 35.

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 to report results. Instructions for the completion of these test report forms and data-entry were sent to the NRLs in week 45-2017 for serotyping and in week 47-2017 for PFGE typing.
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* on 30 October 2017. 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 22nd EURL-*Salmonella* Workshop in Zaandam (Mooijman, 2017), 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. Four strains (S4, S11, S16, S18) 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 22nd EURL-*Salmonella *typing study*

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
S1	1,4,[5],12	f,g,s	[1,2]	Agona
S2	6,8	z_{10}	e,n,x	Hadar
S3	3,{10},{15}	r	z_6	Weltevreden
S4 [b)]	28	i	1,5	Cotham
S5	1,9,12	a	e,n,z_{15}	Durban
S6	4,12	e,h	1,7	Kaapstad
S7	1,4,[5],12	i	1,2	Typhimurium
S8	1,9,12	l,z_{13}	e,n,x	Napoli
S9	1,13,22	z	1,6	Poona
S10	6,7,14	r	1,2	Virchow
S11 [b)]	1,13,23	y	l,w	Ordonez
S12	8,20	r,[i]	z_6	Altona
S13	6,7,14	z_{10}	l,w	Jerusalem
S14	6,7,14	r	1,5	Infantis
S15	1,4,[5],12,[27]	d	1,2	Stanley
S16 [b)]	1,4,[5],12,[27]	b	e,n,x	Abony
S17	1,9,12	g,m	-	Enteritidis
S18 [b)]	11	r	e,n,x	Rubislaw
S19 [a)]	1,4,[5],12	i	-	1,4,[5],12:i:-
S20	6,7,14	z_{10}	e,n,z_{15}	Mbandaka
S21 [c)]	50	k	z	50:k:z (IIIb)

[a)] Typhimurium, monophasic variant as determined by PCR.
[b)] Represented in an EURL-*Salmonella* serotyping study for the first time.
[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' in 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, also sometimes referred to as 'top-5'), 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.

3.3 PFGE typing part of the study

3.3.1 Salmonella *strains for PFGE typing*

A total of 11 *Salmonella* strains (coded P01–P11) 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-8 on PFGE typing, organised by the SSI for the Food- and Water-borne Diseases and Zoonoses Laboratories Network (FWD laboratories network) (ECDC, 2018). 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 NRL network and the FWD laboratory network can be compared in the future.
Table 4 also indicates the codes of the test strains as shown in the image sent to the participants for evaluation of their analysis in BioNumerics (file named: "Provided PFGE gel TRO 2017"). Strain codes 001, 005, 010, and 015 refer to the *S*. Braenderup standard.

Table 3. Background information on the Salmonella strains used for PFGE typing and analysis in 2017

Strain code in 2017 Study Quality PFGE gel image (EURL-*Salmonella*)	Strain code in EQA-8 (ECDC/SSI, 2018)		Strain code in 2017 Study Provided gel analysis in BN (EURL-*Salmonella*)
P01	Salm 6		002
P02	Salm 10		003 (a)
P03	Salm 4		004 (a)
P04	Salm 9		006 (b)
P05	Salm 5		007 (b)
P06 (b)	Salm 8		008 (c)
P07 (b)	Salm 1		009 (c)
P08	Salm 7		011
P09	Salm 11		012
P10	Salm 3		013
P11	Salm 2		014

(b) common letters indicate common strains

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 third time, the evaluation of the (optional) analysis of the PFGE gel in the bioinformatics software application BioNumerics was included. New this time was the use of a common gel image for all participants. This TIFF file, called “Provided PFGE gel TRO 2017”, was sent by email to the participants on 22 November 2017 and is shown in Annex 2.
In short, the following actions were to be done:

- start a new database in BioNumerics,
- import the pre-configured database set-up as sent by email on 22 November 2017,
- import the provided common TIFF image and analyse the gel,
- export the analysed data in either XML plus TIF 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 3). 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 35 laboratories participated in this study (Chapter 2). These included 29 NRLs-*Salmonella* in the 28 EU Member States, 2 NRLs in EU-candidate countries, 3 NRLs in EFTA countries, and 1 non-European NRL.

The frequency of serotyping of *Salmonella* at the participating laboratories and the number of strains that were serotyped and PFGE typed in 2017 are summarised in Table 4.

Table 4. Frequency and number of strains serotyped, and number of strains PFGE typed (for all 35 participants)

Lab code	Serotyping frequency in 2017	No. of strains serotyped in 2017	No. of strains PFGE typed in 2017
28	Daily	341	
4	Daily	400	10
33	Daily	411	
25	Daily	600	13
14	Daily	770	
19	Daily	800	
9	Daily	1200	2
18	Daily	1450	
10	Daily	1600	
3	Daily	3500	30
5	Daily	3500	
16	Daily	4000	200
34	Daily	4500	120
15	Daily	5000	
6	Thrice a week	100	
8	Thrice a week	100	
11	Thrice a week	287	
17	Thrice a week	7000	
2	Twice a week	42	
30	Twice a week	70	300
20	Twice a week	150	
21	Twice a week	200	0
13	Twice a week	268	0
12	Twice a week	410	5
27	Twice a week	450	
31	Twice a week	550	
23	Twice a week	600	
29	Twice a week	1200	100
35	Twice a week	7000	840
26	Once a week	36	
24	Once a week	199	
1	Once a week	400	
32	Once a week	400	

Lab code	Serotyping frequency in 2017	No. of strains serotyped in 2017	No. of strains PFGE typed in 2017
7	Once a week	3000	
22	Monthly	80	
n=35		50614	1620

4.1.2 *Accreditation*

Of the 35 participants, 32 are accredited for serotyping *Salmonella*, mainly according to ISO 17025, and in some cases according to ISO 15189, or more specifically ISO/TR 6579-3. The other three 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 Groups A, B, C, D, E, and F. All other laboratories stated that they are accredited for all *Salmonella* serovars.

4.1.3 *Transport of samples*

All but two participants received their package in the same week sent (week 44 of 2017). One parcel was delivered in week 45, and one parcel was delivered in week 47 due to a customs-delay. 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 and blood agar were the most commonly used media.

4.2 Serotyping results

4.2.1 *General*

The 20 obligatory strains were all tested by the *Salmonella* NRLs in the participating countries.
Details on the number and the source of the sera used by the participants are summarised in Table 5 and Table 6.

Table 5. Number of laboratories using sera from various manufacturers

Manufacturer	Number of NRLs (n=35)
Biorad	14
Own preparation	3
Pro-Lab	7
Reagensia	2
Remel	2
Sifin	21
Statens Serum Institute (SSI)	30
Other	1

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=35)
1	11
2	11
3	8
4	3
5	2

4.2.2 *Biochemical testing*

Twenty-seven participants confirmed the use of biochemical tests. Twenty-five participants used a variety of biochemical tests on the optional strain S21, uncommon serovar 50: k, z (*S. enterica* subsp. *diarizonae*). Eighteen participants confirmed strain S16 (1,4,[5],12,[27];b:e,n,x) to be an *S. enterica enterica* strain (Abony) by biochemical testing, most often by using malonate or dulcitol.

4.2.3 *Use of PCR for confirmation*

Seventeen laboratories used PCR to confirm strain S19, the monophasic variant of *S*. Typhimurium 1,4,[5],12:i:-, and seven of these also used PCR to confirm strain S17, *S*. Typhimurium. The majority of laboratories mentioned using the following references:

- EFSA Journal, 2010.
- Tennant et al., 2010.

4.2.4 *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 31 of the 35 participants (89%). This corresponds to 99% of the total number of strains. The H-antigens were typed correctly by 28 of the 35 participants (80%), corresponding to 98% of the total number of strains. As a result, 28 participants (80%) also gave the correct serovar names, corresponding to 98% of all strains evaluated.

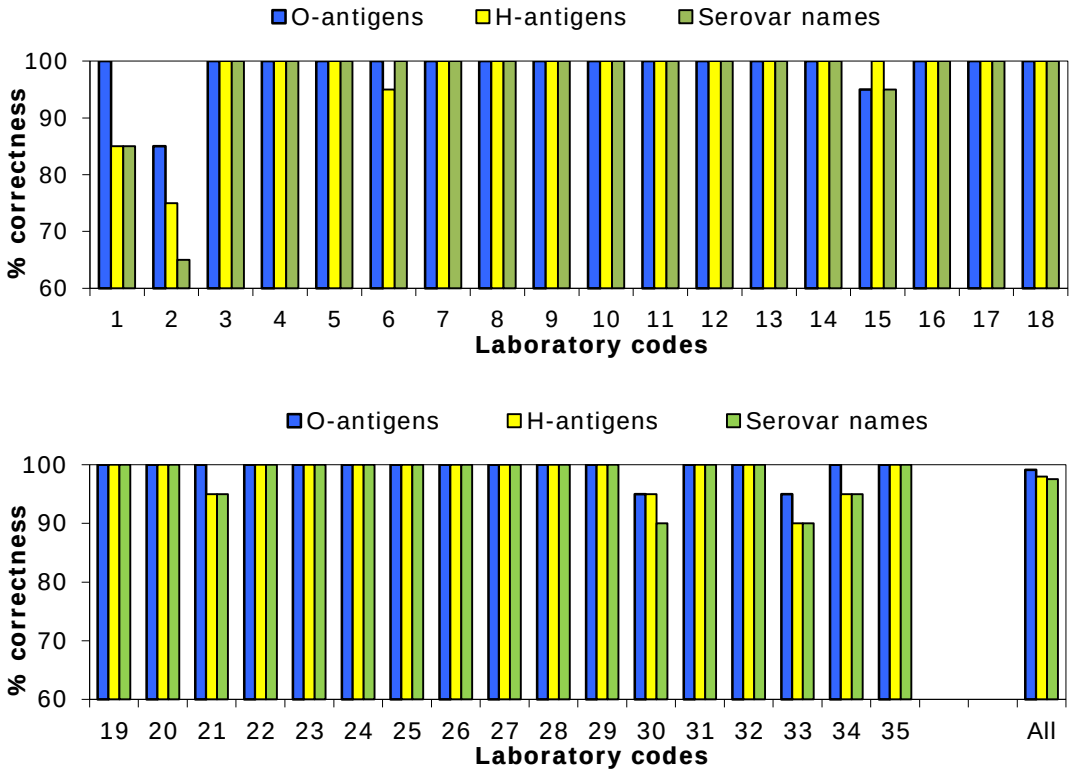

Figure 1. Percentages of correct serotyping results

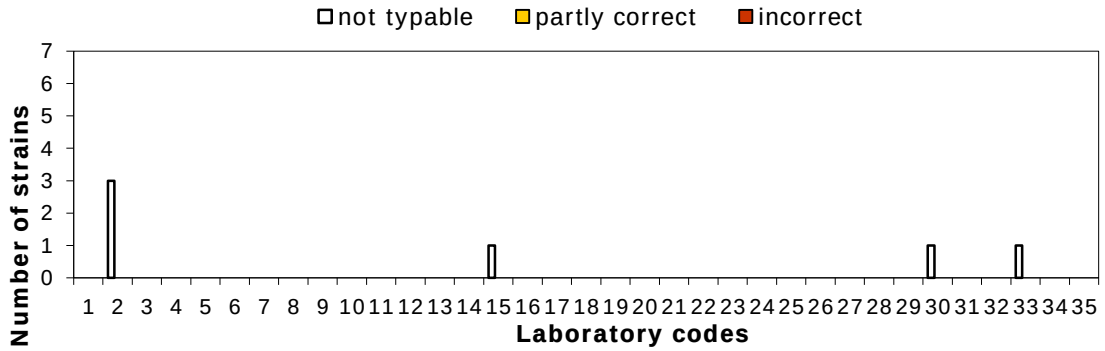

Figure 2. Evaluation of type of errors for O-antigens per NRL

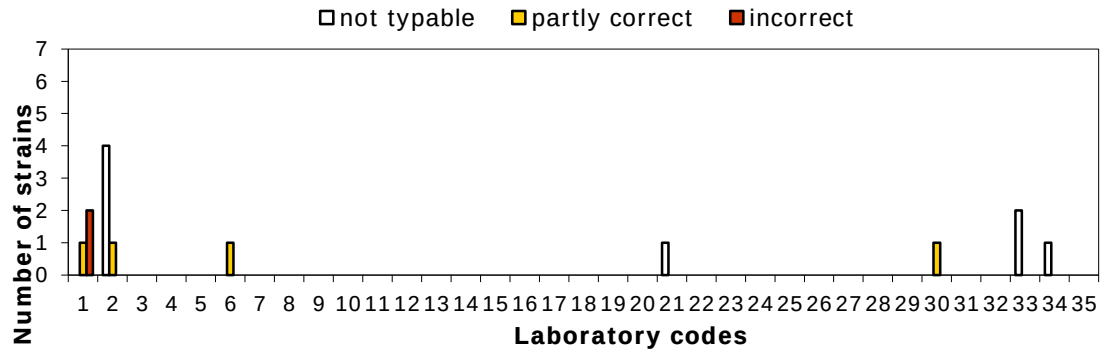

Figure 3. Evaluation of type of errors for H-antigens per NRL

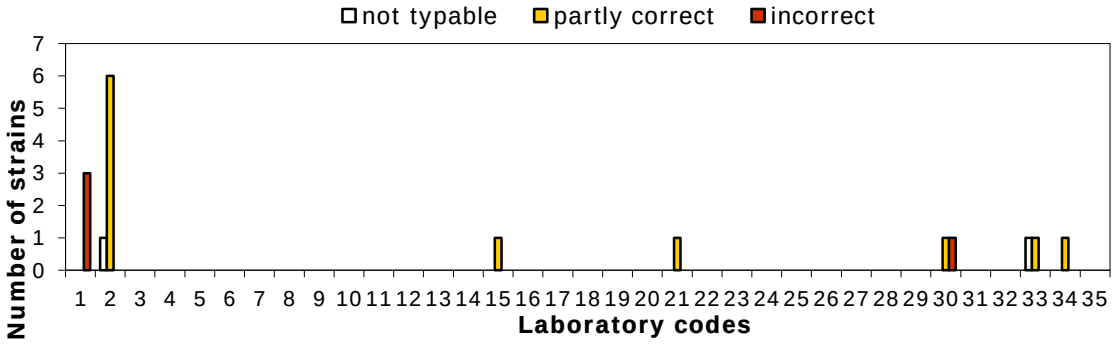

Figure 4. Evaluation of type of errors in the identification of serovar names

4.2.5 *Performance of the participants*

The number of penalty points was determined for each NRL using the guidelines described in Section 3.2.2. Table 7 shows the number of penalty points for each NRL and indicates whether the level of good performance was achieved (yes or no). All participants met the level of good performance at the first stage of the study, therefore a follow-up was not necessary.

All participants received their individual laboratory evaluation report on serotyping on 6 February 2018, followed by the interim summary report on 12 February 2018.

An example of an individual laboratory evaluation report on serotyping results is given in Annex 7. The interim summary report is available on the website: www.eurlsalmonella.eu/publications

Table 7. Evaluation of serotyping results per NRL

Lab code	Penalty points	Good performance	Lab code	Penalty points	Good performance
1	3	yes	19	0	yes
2	0	yes	20	0	yes
3	0	yes	21	0	yes
4	0	yes	22	0	yes
5	0	yes	23	0	yes
6	0	yes	24	0	yes
7	0	yes	25	0	yes
8	0	yes	26	0	yes
9	0	yes	27	0	yes
10	0	yes	28	0	yes
11	0	yes	29	0	yes
12	0	yes	30	1	yes
13	0	yes	31	0	yes
14	0	yes	32	0	yes
15	0	yes	33	0	yes
16	0	yes	34	0	yes
17	0	yes	35	0	yes
18	0	yes			

4.2.6 *Serotyping results per strain*

The results found per strain and per laboratory are given in Annex 4, except for the more complicated strains S19 and S21; these are reported separately in Annex 5.
Apart from some spelling errors, a completely correct identification was obtained for ten *Salmonella* serovars, including all 'top-5' serovars: Hadar (S2), Durban (S5), Kaapstad (S6), Typhimurium (S7), Virchow (S10), Jerusalem (S13), Infantis (S14), Abony (S16), Enteritidis (S17), and 1,4,[5],12:i:- (S19).
Most problems were noted for strains showing a non-typable or a partly typable result, e.g. due to being 'rough' or due to a lack of antisera required. Details of the strains that caused problems in serotyping are shown in Annex 6. Only four strains were incorrectly identified.
Details of the additional and optional strain S21 are given in Annex 5. 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 (50:k:z).

4.2.7 *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 8, in Table A8-1 for EU NRLs only, and in Table A8-2 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 3 points in the 2017 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 and 2017 studies.

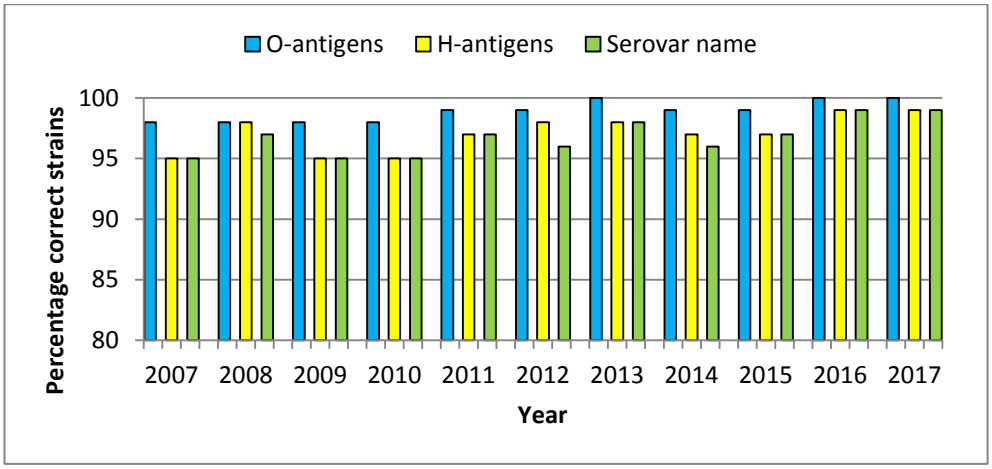

Figure 5. Serotyping results of the EU NRLs in time, based on the percentages of correctly typed strains

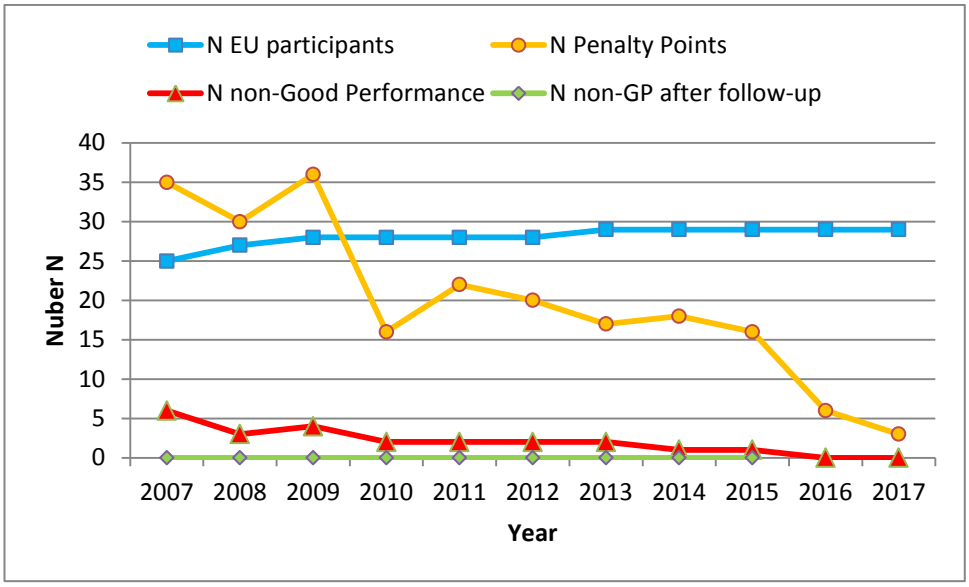

Figure 6. Serotyping results of the EU NRLs in time, based on the number of Penalty Points and non-Good Performance

4.3 PFGE typing results

4.3.1 General

A total of 15 NRLs participated in the fifth study on PFGE typing. Four participants in the 2016-study did not participate in the 2017 study, and another four participants were new compared to the 2016 study. Seven laboratories have participated in all five PFGE typing studies so far.
Nine participants reported using the Standard PulseNet Protocol *Salmonella* PFGE (PulseNet International, 2013)/the EURL-*Salmonella* SOP (Jacobs et al., 2014). Six participants use this Standard protocol with modifications.
All participants received their individual laboratory evaluation report on PFGE typing on 23 July 2018, together with a report on the overall results. An example of an individual laboratory evaluation report on PFGE typing results is given in Annex 12. The report with all the results is available on the website: www.eurlsalmonella.eu/publications

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 Tables 8-10 respectively.

Table 8. 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 9. Electrophoresis system used by the participants

Electrophoresis system	Number of NRLs
Bio-Rad CHEF Mapper XA	1
Bio-Rad CHEF-DR III System	9
Bio-Rad CHEF-DR II System	2
CHEF Mapper unspecified	3

Table 10. Gel documentation system used by the participants

Gel documentation system	Number of NRLs
Chemi Doc XR, Bio-Rad	1
Chemi HR Imaging 410 System	1
Cleaver Scientific Ltd	1
GBox EF (Syngene)	1
GelDoc Universal Hood II	1
GelDoc XR	2
GelDoc XR+	6
GeneGenious (Syngene)	1
UVITEC Alliance 4.7	1

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

For staining the gel, one participant used CYBR Safe and one used GelRed; all other participants used Ethidium Bromide. The duration of the staining varied between 15 minutes (1x) and 60 minutes (1x), but most participants used 20-30 minutes (13x). De-staining was even more diverse, varying between 1 minute and 2 hours; a majority of participants used up to 60 minutes.
Nine participants used a comb with narrow teeth, and six 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 9. The overall scores per parameter are shown in Figure 7.
The quality of the produced PFGE gel images results was generally good, though some variation was noted in results between the laboratories mainly between starters and the more experienced participants (Annex 11).

Overall, 90% of the scores were Good or Excellent. However, four of the 15 images resulted in a Poor score on at least one of the seven parameters. These four images were therefore unsuitable for use in interlaboratory database comparison of these PFGE profiles.

All four images scored a Poor result for “Image Acquisition and Running Conditions” (Figure 7). For three participants (Labs 4, 12, and 19) this was due to the incorrect use of the *S.* Braenderup H9812 reference, a mistake that can easily be avoided in the future.

Using a narrow comb, the reference strain H9812 must be run in every 6 lanes as a minimum; using a wide comb, this reference must be run in every 5 lanes as a minimum (Jacobs-Reitsma et al., 2014). Thus, the examination of 11 test strains requires the use of the reference strain in at least four lanes. Six participants used the lanes 1, 5, 10, and 15 for

the reference strain, five participants used the lanes 1, 6, 11, and 15, and one participant used lanes 1, 6, 10 and 15 for this.

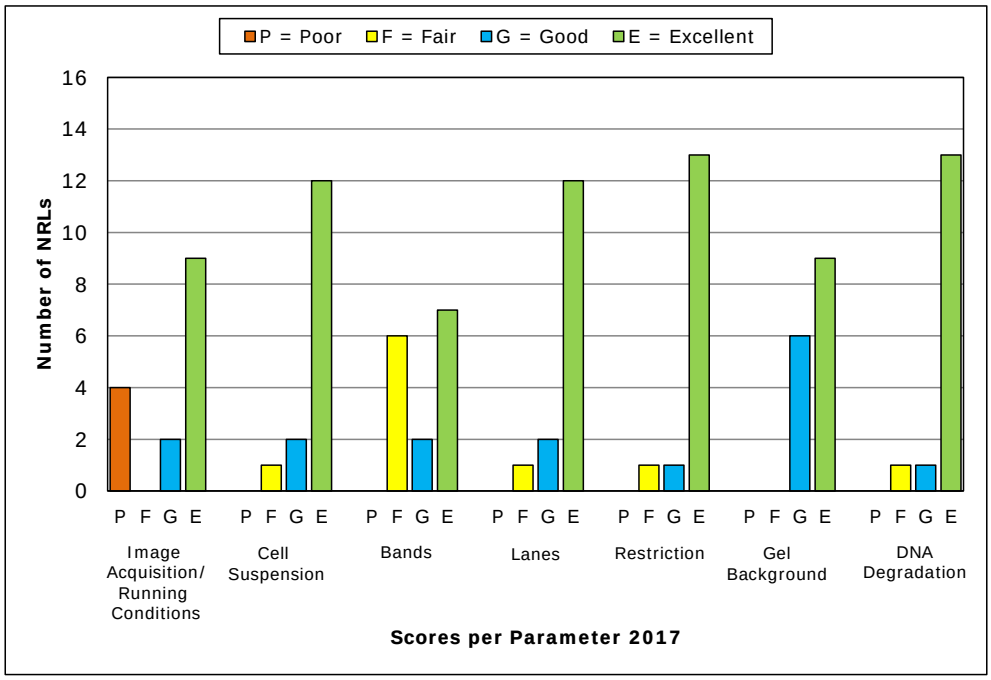

Figure 7. Evaluation of the quality of the PFGE images in scores per parameter, 2017 study

Figure 8 shows the results of the evaluation of the TIFF images from the 2013 – 2017 studies. Improvements over time are clearly visible, however it has to be noted that significant variation between participating laboratories has been found.

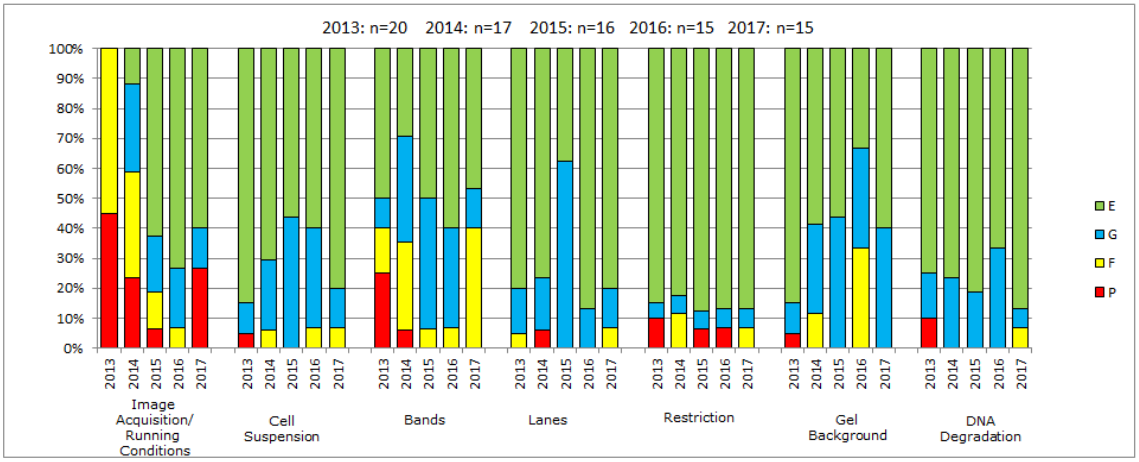

Figure 8. Evaluation of the quality of the PFGE images in scores per parameter, 2013-2017 studies

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

We included the evaluation of the (optional) analysis of a gel in BioNumerics in the study for the third time. The participants all used the pre-configured database provided by the EURL-*Salmonella*, and therefore used identical experimental settings in BioNumerics.

Moreover, all participants analysed the same gel image ("Provided PFGE gel TRO 2017", Annex 2).
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 3), are given in Annex 10. The summarised scores per parameter are shown in Figure 9.

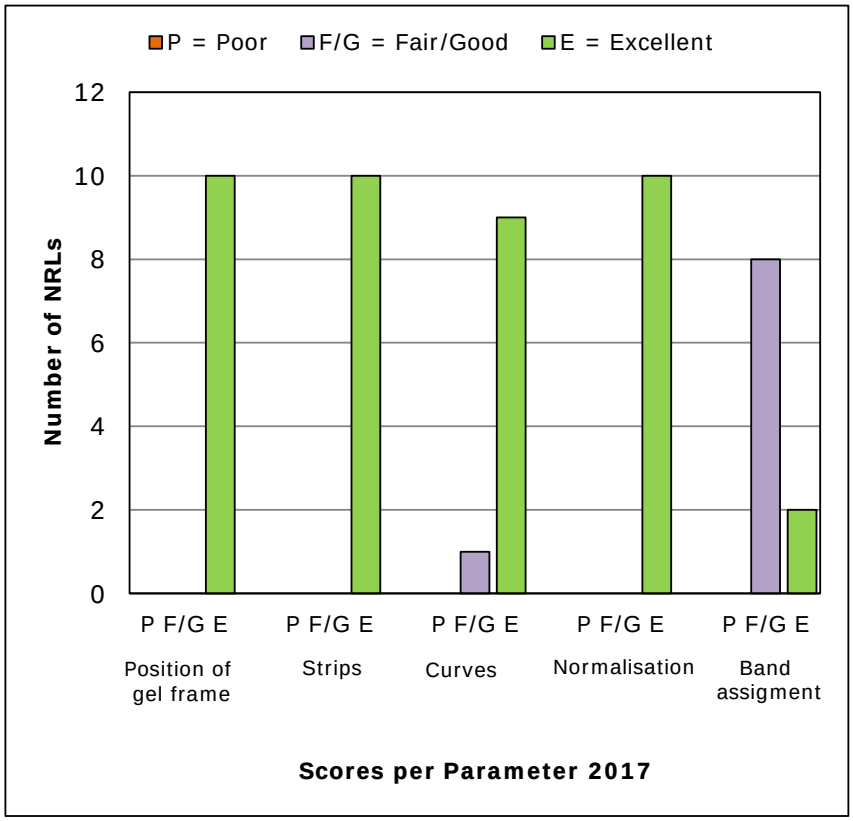

Figure 9. Evaluation of the analysis of the gel in BioNumerics in scores per parameter, 2016 study

Overall, 82% of the scores were Excellent and 18% of the scores were Fair/Good.

Several participants (Labs 18, 20, 25, 35) also tended to assign bands of test strains below 33 kb (Figure 10, black circles), thereby not following the Protocol. Except for this minor deviation, 8 strains (codes 003 – 012) were correctly analysed by all participants. One mistake was noted for strain 002 by one participant (Lab 19).
The main differences were seen in the analysis of strains 013 and 014, all concerning the assignment of double bands as single bands (Labs 3, 16, 18, 19, 25, 29, 35), which is a well-known difficulty in the analysis of PFGE images. As an example, band assignment results for strain 014 are given in Figure 10; specific difficulties with double band assignments are indicated in purple.

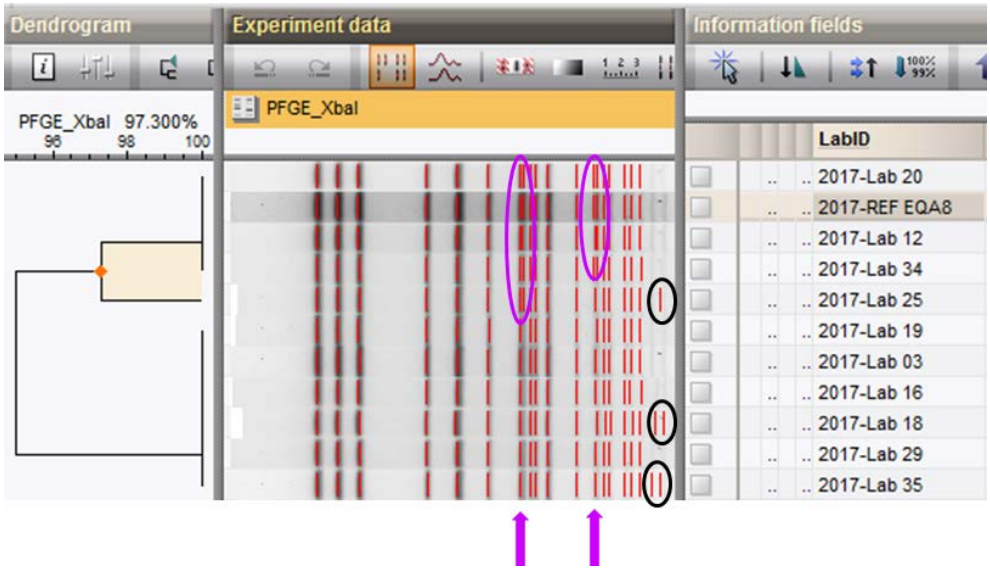

Figure 10. PFGE profiles with band assignment in BioNumerics by 10 participants for strain 014.

Two participants (labs 12 & 34) analysed all 11 test strains in the provided gel image in complete agreement with the reference analysis.

Figure 11 shows the overall results from all three studies (2015 – 2017).

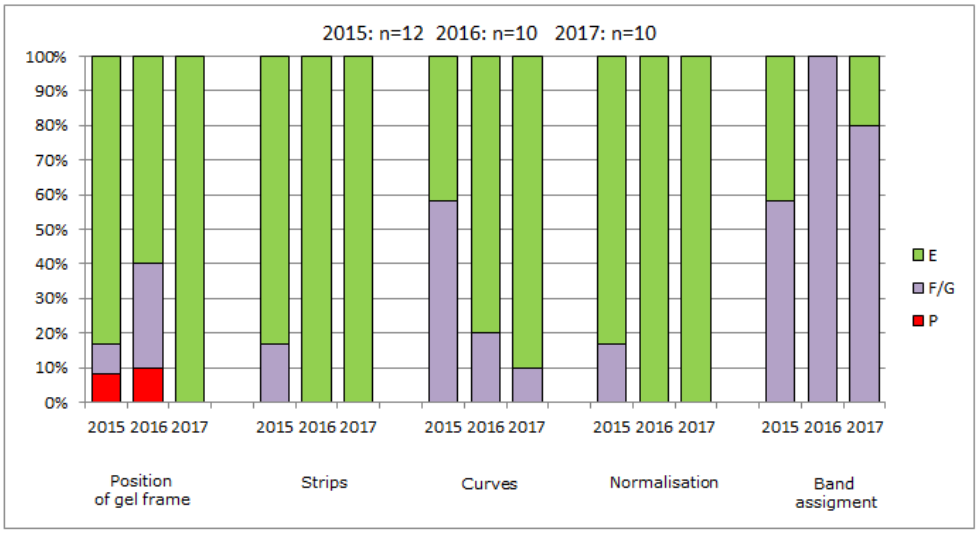

Figure 11. Evaluation of the analysis of the gel in BioNumerics in scores per parameter, 2015-2017 studies

5 Conclusions

5.1 Serotyping

- Overall results for all 35 participating laboratories are:
  - 99% of the strains were typed correctly for the O-antigens.
  - 98% of the strains were typed correctly for the H-antigens.
  - 98% of the strains were correctly named.
- All participants correctly serotyped the 'top 5' strains *S*. Enteritidis, *S*. Hadar, *S*. Infantis, *S*. Typhimurium (including its monophasic variant) and *S*. Virchow.
- All 29 EU-NRLs and all 6 non-EU-NRLs directly achieved the defined level of good performance.

5.2 PFGE typing

- Eleven 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.
- Three participating laboratories should be able to improve their PFGE gel production relatively easily by adjusting the use of the reference strain *S*. Braenderup to the requirements.
- Ten participants also processed a common gel in BioNumerics, and all of them were able to analyse the 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:**

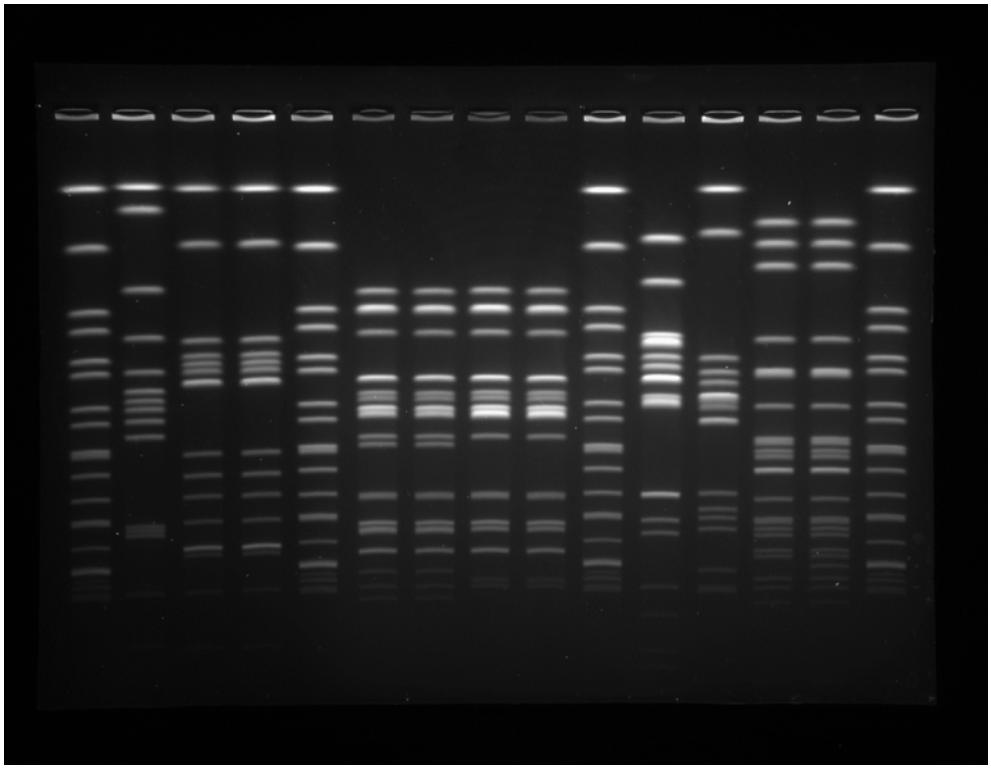
Parameter	TIFF Quality Grading Guidelines			
	Excellent	**Good**	**Fair**	**Poor**
Image Acquisition and Running Conditions	By protocol, for example: - Gel fills whole TIFF - Wells included on TIFF - Bottom band of standard 1-1.5 cm from bottom of gel	- Gel doesn't fill whole TIFF but band finding is 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., ≤3) difficult to see clearly (e.g., DNA overload), especially at bottom of gel	- Some band distortion (e.g., nicks) in 2-3 lanes but still analyzable - Fuzzy bands - Some bands (e.g., 4-5) are too thick - Bands at the bottom of the gel are light, but analyzable	- Band distortion that makes analysis difficult - Very fuzzy bands. - Many bands too thick to distinguish - Bands at the bottom of the gel too light to distinguish
Lanes	Straight	- Slight smiling (higher bands in the outside lanes vs. the inside) - Lanes gradually run longer toward the right or left - Still analyzable	- Significant smiling - Slight curves on the outside lanes - Still analyzable	- Smiling or curving that interferes with analysis

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	- > 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])

Annex 2 TIFF image “Provided PFGE gel TRO2017” to be used by all participants for gel analysis of PFGE images in BioNumerics



Annex 3 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 2-8-2018)

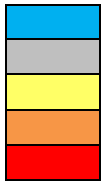
Parameter	Grade [score in points]		
	Poor [1]	Fair [2]	Excellent [3]
Position of Gel Frame	- Wells wrongly included when placing the frame - Gel is not inverted.	- The frame is positioned too low. - Too much space framed at the bottom of the gel. - Too much space framed on the sides of the gel.	Excellent 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 4 Serotyping results per strain and per laboratory

Lab REF	S1 Agona	S2 Hadar	S3 Weltevreden	S4 Cotham	S5 Durban	S6 Kaapstad	S7 Typhimurium	S8 Napoli	S9 Poona	S10 Virchow
1	Derby	Hadar	Ughelli	Cotham	Burban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
2	Agona	Hadar	3,10 : r : -	- : i : 1,5	Durban	Kaapstad	Typhimurium	9 : l,v : e,n,x	- : - : -	Virchow
3	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
4	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
5	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
6	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
7	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
8	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
9	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
10	Agona	Hadar	Weltewreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
11	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
12	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
13	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
14	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
15	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
16	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
17	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
18	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
19	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
20	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
21	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	1,9,12:-:e,n,x	Poona	Virchow
22	S. Agona	S. Hadar	S. Weltevreden	S. Cotham	S. Durban	S. Kaapstad	S. Typhimurium	S. Napoli	S. Poona	S. Virchow
23	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
24	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
25	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
26	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
27	S. Agona	S. Hadar	S. Weltevreden	S. Cotham	S. Durban	S. Kaapstad	S. Typhimurium	S. Napoli	S. Poona	S. Virchow
28	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
29	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
30	Agona	Hadar	Weltevreden		Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
31	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
32	S.Agona	S.Hadar	S.Weltevreden	S.Cotham	S.Durban	S.Kaapstad	S.Typhimurium	S.Napoli	S.Poona	S.Virchow
33	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
34	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
35	Agona	Hadar	Weltevreden	Cotham	Durban	Kaapstad	Typhimurium	Napoli	Poona	Virchow
X	1	0	1	0	0	0	0	0	0	0

Lab REF	S11 Ordonez	S12 Altona	S13 Jerusalem	S14 Infantis	S15 Stanley	S16 Abony	S17 Enteritidis	S18 Rubislaw	S20 Mbandaka	Lab REF
1	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Escanaba	1
2	- : y : l,w	8 : r : -	Jerusalem	Infantis	Stanley	Abony	Enteritidis	11 : - : -	Mbandaka	2
3	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	3
4	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	4
5	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	5
6	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	6
7	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	7
8	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	8
9	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	9
10	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	10
11	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	11
12	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	12
13	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	13
14	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	14
15	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	'o' rough:r:e,n,x	Mbandaka	15
16	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	16
17	Ordonez	Altona	Jeruzalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	17
18	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	18
19	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	19
20	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	20
21	Ordonez	Altona	Jarusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	21
22	S. Ordonez	S. Altona	S. Jerusalem	S. Infantis	S. Stanley	S. Abony	S. Enteritidis	S. Rubislaw	S. Mbandaka	22
23	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	23
24	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	24
25	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	25
26	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	26
27	S. Ordonez	S. Altona	S. Jerusalem	S. Infantis	S. Stanley	S. Abony	S. Enteritidis	S. Rubislaw	S. Mbandaka	27
28	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	28
29	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	29
30	Ordonez	Altona	Jerusalem	Infantis	Eppendorf	Abony	Enteritidis	Ribislaw	Mbandaka	30
31	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	31
32	S.Ordonez	S.Altona	S.Jerusalem	S.Infantis	S.Stanley	S.Abony	S.Enteritidis	S.Rubislaw	S.Mbandaka	32
33	23:-:w	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw		33
34	13,23:-:l,w	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	34
35	Ordonez	Altona	Jerusalem	Infantis	Stanley	Abony	Enteritidis	Rubislaw	Mbandaka	35
X	0	0	0	0	1	0	0	0	1	X



remark (eg spelling errror)
not typable (eg antisera not available, rough)
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 (by penalty points) per strain

Results for Strains S19 and S21 are given in Annex 5

Annex 5 Details of serotyping results for strains S19 and S21

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	PCR-confirmed	Lab code
S-19	**1,4,[5],12**	**i**	**-**	**1,4,[5],12:i:-**	**yes**	**REF**
S-19	4,5,12	i	-	4,5,12:i:-	no	1
S-19	4.5	i	-	4,5 : i : -	yes	2
S-19	4,5,12	i	-	4,5,12:i:-	no	3
S-19	4	i	-	Typhimurium monophasic variant	yes	4
S-19	4,5,12	i	-	4,5,12:i:-	no	5
S-19	4, 5, 12	i	-	4, 5, 12: i: -	no	6
S-19	4.5	I	-	4,5,12:i:-	no	7
S-19	4,5,12	i	-	4,5,12:i:-	no	8
S-19	1,4,5,12	i	-	1,4,5,12:i:-	yes	9
S-19	4	i	-	S. 4,5,12:i:- (monofasic variant of Typhimurium)	no	10
S-19	4,5,12	i	-	4,5,12:i:-	yes	11
S-19	4,5,12	i	-	4,5,12:i:-	yes	12
S-19	4,5,12	i	-	4,5,12:i:-	no	13
S-19	4,5,12	i	-	monophasic Typhimurium	yes	14
S-19	4,5,12	i	-	4,5,12:i:-	no	15
S-19	4,5,12	i	-	4,5,12:i:-	no	16
S-19	4.5	i	-	Monophasic Typhimurium	yes	17
S-19	4.5	i	-	Monophasic variant of S. Typhimurium	no	18
S-19	4,5,12	i	-	4,5,12 : i : -	yes	19
S-19	4,5,12	i	-	4,5,12:i:-	yes	20
S-19	4,5,12	i	-	4,5,12:i:-	no	21
S-19	1,4,[5],12	i	-	1,4,[5],12:i:-	no	22
S-19	4,5,12	i	-	4,5,12:i:-	yes	23
S-19	4,5,12	i	-	4,5,12:i:-	yes	24
S-19	4,5,12	i	-	4,5,12:i:-	no	25
S-19	4,5,12	i	-	4,5,12:i:-	no	26
S-19	4,5,12	i	-	4,5,12 :i: - . Typhimurium monophasic variant	yes	27
S-19	4,5,12	i	-	Typhimurium, monophasic (4,5,12 : i : -)	yes	28
S-19	4.5	i	-	Monophasic S. Typhimurium 4,5:i:-	yes	29
S-19	4,5,12	i	-	4,5,12:i:-	no	30
S-19	4.5	i	-	4,5:i:-	no	31
S-19	4,5,12	i	-	4,5,12:i:-	yes	32
S-19	4,5,12	i	-	4,5,12:i:-	yes	33
S-19	4,5,12	i	-	4,5,12:i:-	yes	34
S-19	4,5,12	i	-	4,5,12:i:-	no	35

reference
remark (eg spelling errror)

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	Lab code
S-21	**50**	**k**	**z**	**50:k:z (IIIb)**	**REF**
S-21	50	k	z	Hemingford IIIb	1
S-21	61	-	-	61: - : -	2
S-21	50	k	z	50:k:z	3
S-21	50	k	no further	enterica subsp. diarizonae	4
S-21	50	k	z	50:k:z	5
S-21					6
S-21	50	k	z	SIII 50:k:z	7
S-21					8
S-21	50	k	z	50:k:z	9
S-21					10
S-21	50	k	z	50:k:z	11
S-21	50	k	z	IIIb: 50:k:z	12
S-21	50	k	z	50:k:z	13
S-21	-	k	-	-:k:-	14
S-21	50	k	z	50:K:Z sg III b	15
S-21	50	k	z	S. IIIb (Salmonella enterica subsp. diarizonae) 50:k:z	16
S-21	50	k	z	IIIb: 50:k:z	17
S-21	50	k	z	IIIb 50:k:z	18
S-21	61	k	-	61:k:- IIIb	19
S-21	50	k	z	50:k:z	20
S-21	50	k	z	IIIb	21
S-21	50	k	1,5,7	S. enterica subsp. diarizonae /IIIb/	22
S-21	50	k	z	50:k:z	23
S-21	50	k	z	50:k:z	24
S-21	50	k	z	50:k:z	25
S-21	50	k	z	50:k,z	26
S-21	50	k	z	50:k:z. S. enterica subsp. diarizonae	27
S-21	50	k	z	Salmonelle enterica subsp. diarizonae serovar 50 : k z	28
S-21	50	k	z	S. enterica subsp. diarizonae 50:k:z	29
S-21					30
S-21	?	k	z	?:k:z	31
S-21	50	k	z	III a arizonae	32
S-21	50	k	z53	50:k:z53 (IIIb)	33
S-21	50	k	z	S.IIIb 50:k:z	34
S-21	50	k	z	IIIb 50:k:z	35

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

reference
remark (eg spelling errror)

Annex 6 Details of strains that caused problems in serotyping

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	Lab code
S-1	**1,4,[5],12**	**f,g,s**	**[1,2]**	**Agona**	**REF**
S-1	4,12	f,g	-	Derby	1
S-3	**3,{10},{15}**	**r**	**z6**	**Weltevreden**	**REF**
S-3	3,10	r	1,5	Ughelli	1
S-3	3,10	r	-	3,10 : r : -	2
S-3	3,10	r	z6	Weltewreden	10
S-4	**28**	**i**	**1,5**	**Cotham**	**REF**
S-4	-	i	1,5	- : i : 1,5	2
S-4		i	1,5		30
S-5	**1,9,12**	**a**	**e,n,z15**	**Durban**	**REF**
S-5	9,12	a	enz15	Burban	1
S-8	**1,9,12**	**l,z13**	**e,n,x**	**Napoli**	**REF**
S-8	9	l,v	e,n,x	9 : l,v : e,n,x	2
S-8	1,9,12	-	e,n,x	1,9,12:-:e,n,x	21
S-9	**1,13,22**	**z**	**1,6**	**Poona**	**REF**
S-9	-	-	-	- : - : -	2
S-9	12,22	z	6	Poona	32
S-11	**1,13,23**	**y**	**l,w**	**Ordonez**	**REF**
S-11	-	y	l,w	- : y : l,w	2
S-11	23	-	w	23:-:w	33
S-11	13,23	-	l,w	13,23:-:l,w	34
S-12	**8,20**	**r,[i]**	**z6**	**Altona**	**REF**
S-12	8	r	-	8 : r : -	2
S-13	**6,7,14**	**z10**	**l,w**	**Jerusalem**	**REF**
S-13	6,7	z10	l,w	Jeruzalem	17
S-13	6,7	z10	l,w	Jarusalem	21
S-15	**1,4,[5],12,[27]**	**d**	**1,2**	**Stanley**	**REF**
S-15	1,4,12	d	1,5	Eppendorf	30
S-18	**11**	**r**	**e,n,x**	**Rubislaw**	**REF**
S-18	11	-	-	11 : - : -	2
S-18	'o' rough	r	e,n,x	'o' rough:r:e,n,x	15
S-18	11	r	e,n,x	Ribislaw	30
S-20	**6,7,14**	**z10**	**e,n,z15**	**Mbandaka**	**REF**
S-20	6,7	k	enz15	Escanaba	1
S-20	6,7	z10	e,n,x	Mbandaka	6
S-20	spontaneous agglutination				33

reference
remark (eg spelling errror)
not typable (eg antisera not available, rough)
partly correct, in the naming: no penalty points
incorrect, in the naming: 1 penalty point
incorrect, in the naming: 4 penalty points

Annex 7 Example of an individual laboratory evaluation report on serotyping results

Individual Laboratory Results 22nd Interlaboratory Comparison Study *Salmonella* serotyping (November 2017), Page 1 of 2

	Reference Results				**Results NRL labcode:**			**1**
Strain	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
S1	1,4,[5],12	f,g,s	[1,2]	Agona	4.12	f,g	-	Derby
S2	6.8	z10	e,n,x	Hadar	6.8	z10	enx	Hadar
S3	3,{10},{15}	r	z6	Weltevreden	3.10	r	1.5	Ughelli
S4	28	i	1.5	Cotham	28	i	1.5	Cotham
S5	1,9,12	a	e,n,z15	Durban	9.12	a	enz15	Burban
S6	4.12	e,h	1.7	Kaapstad	4.12	e,h	1.7	Kaapstad
S7	1,4,[5],12	i	1.2	Typhimurium	4,5,12	i	1.2	Typhimurium
S8	1,9,12	l,z13	e,n,x	Napoli	9.12	l,z13	enx	Napoli
S9	1,13,22	z	1.6	Poona	13.22	z	1.6	Poona
S10	6,7,14	r	1.2	Virchow	6.7	r	1.2	Virchow
S11	1,13,23	y	l,w	Ordonez	13.23	y	lw	Ordonez
S12	8,20	r,[i]	z6	Altona	8.20	r	z6	Altona
S13	6,7,14	z10	l,w	Jerusalem	6.7	z10	lw	Jerusalem
S14	6,7,14	r	1.5	Infantis	6.7	r	1.5	Infantis
S15	1,4,[5],12,[27]	d	1.2	Stanley	4,5,12	d	1.2	Stanley
S16	1,4,[5],12,[27]	b	e,n,x	Abony	4,5,12	b	enx	Abony
S17	1,9,12	g,m	-	Enteritidis	9.12	g,m	-	Enteritidis
S18	11	r	e,n,x	Rubislaw	11	r	enx	Rubislaw
S19[a)]	1,4,[5],12	i	-	1,4,[5],12:i:-	4,5,12	i	-	4,5,12:i:-
S20	6,7,14	z10	e,n,z15	Mbandaka	6.7	k	enz15	Escanaba
S21	50	k	z	50:k:z (IIIb)	50	k	z	Hemingford IIIb

a) Typhimurium, monophasic variant as determined by PCR.

S21 Note that the result 50:k:z for strain S21 was correct, but the named serovar Hemingford would have been 50:d:1,5.

Individual Laboratory Results 22nd Interlaboratory Comparison Study *Salmonella* serotyping (November 2017), Page 2 of 2

For back-ground 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 (eg spelling errror)
not typable (eg serum not available, rough)
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 22nd EURL-*Salmonella* Workshop (Zaandam, 2017), Strain S-21 was an additional strain to the study.
Testing of this strain was optional and results were not included in the evaluation (remarks in blue only).
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: 3 -> Good Performance

EURL-*Salmonella*, Bilthoven, The Netherlands

Annex 8 Historical overview on the results of the EURL-*Salmonella* serotyping studies

Table A8-1. 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	22 2017
No. of participants	25	27	28	28	28	28	29	29	29	29	29
No. of strains evaluated	20	20	20	19	19*	20	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%)	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%)	572/580 (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%)	572/580 (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%)	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%)	24/29 (83%)
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%)	24/29 (83%)
No. of penalty points	35	30	36	16	22	20	17	18	16	6	3
No. of labs not achieving good performance	6	3	4	2	2	2	2	1	1	0	0
No. of labs not achieving good performance after follow-up	0	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 A8-2. 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	22 2017
No. of participants	26	29	31	33	36	31	34	35	34	34	35
No. of strains evaluated	20	20	20	19	19*	20	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%)	694/700 (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%)	686/700 (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%)	683/700 (98%)
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%)	31/35 (89%)
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%)	28/35 (80%)
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%)	28/35 (80%)
No. of penalty points	36	34	56	37	41	20	20	57	21	21	4
No. of labs not achieving good performance	6	4	5	4	4	2	2	2	1	2	0
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)	0

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

Annex 9 Evaluation of PFGE images per participant and per parameter

Lab code / Parameter	**21**	**19**	**4**	**18**	**12**	**34**	**13**	**16**	**29**	**9**	**20**	**25**	**30**	**3**	**35**	**Total score per parameter**	**Average per parameter**
Image Acquisition & Running Conditions	1	1	1	3	1	4	4	4	4	4	4	4	3	4	4	46	3,1
Cell Suspension	2	3	4	3	4	4	4	4	4	4	4	4	4	4	4	56	3,7
Bands	3	2	3	2	4	2	2	2	2	4	4	4	4	4	4	46	3,1
Lanes	2	4	4	4	3	4	4	4	4	4	4	3	4	4	4	56	3,7
Restriction	2	4	4	4	4	3	4	4	4	4	4	4	4	4	4	57	3,8
Gel Background	3	3	3	3	4	3	4	4	4	3	4	4	4	4	4	54	3,6
DNA Degradation (smearing in lanes)	2	4	4	4	4	4	4	4	4	4	3	4	4	4	4	57	3,8
Total score per participant	15	21	23	23	24	24	26	26	26	27	27	27	27	28	28		
Average per participant	2,1	3	3,3	3,3	3,4	3,4	3,7	3,7	3,7	3,9	3,9	3,9	3,9	4	4		

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

Annex 10 Evaluation of the analysis of the gel in BioNumerics per participant and per parameter

Lab code / Parameter	18	3	16	19	20	25	29	35	12	34	Total score per parameter	Average per parameter
Position of gel	3	3	3	3	3	3	3	3	3	3	30	3,0
Strips	3	3	3	3	3	3	3	3	3	3	30	3,0
Curves	2	3	3	3	3	3	3	3	3	3	29	2,9
Normalisation	3	3	3	3	3	3	3	3	3	3	30	3,0
Band assignment	2	2	2	2	2	2	2	2	3	3	22	2,2
Total score per participant	13	14	14	14	14	14	14	14	15	15		
Average per participant	2,6	2,8	2,8	2,8	2,8	2,8	2,8	2,8	3	3		

1=Poor; 2=Fair/Good; 3=Excellent.

Annex 11 Examples of PFGE images obtained by the participants

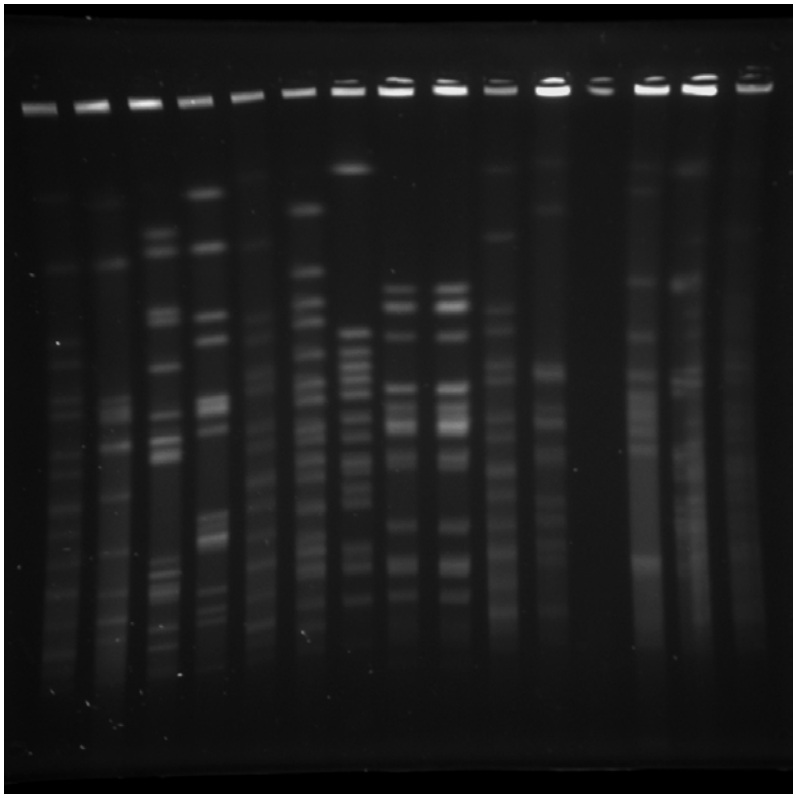


Figure A11.1. Example of a gel (lab code 21) with a generally lower score

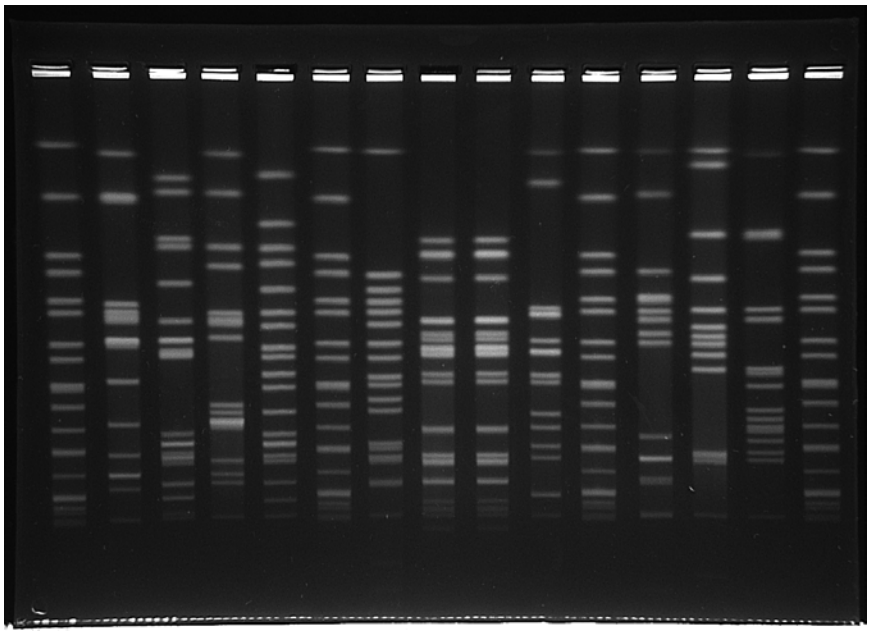


Figure A11.2. Example of a gel (lab code 3) with a generally high score

Annex 12 Example of an individual laboratory evaluation report on PFGE typing results

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NRL Laboratory code: **35**

General comments: None

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

Parameter	Evaluation	Comments	Points*
Image Acquisition and Running Conditions	Excellent	Gel fills whole TIFF. Wells included on TIFF. Bottom band of standard 1-1,5 cm from bottom of gel.	4
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	Excellent	Clear.	4
DNA Degradation (smearing in the lanes)	Excellent	Not present.	4
Total score:			28

* 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	Excellent	Excellent placement of frame, and gel is inverted.	3
Strips	Excellent	All lanes correctly defined.	3
Curves	Excellent	1/3 or more of the lanes is used for averaging curve extraction.	3
Normalisation	Excellent	All bands assigned correctly in all reference lanes.	3
Band assignment	Fair/Good	Bands under 33 kb are assigned (not to be done according to the Protocol). Few double bands assigned as single bands.	2
Total score:			14

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

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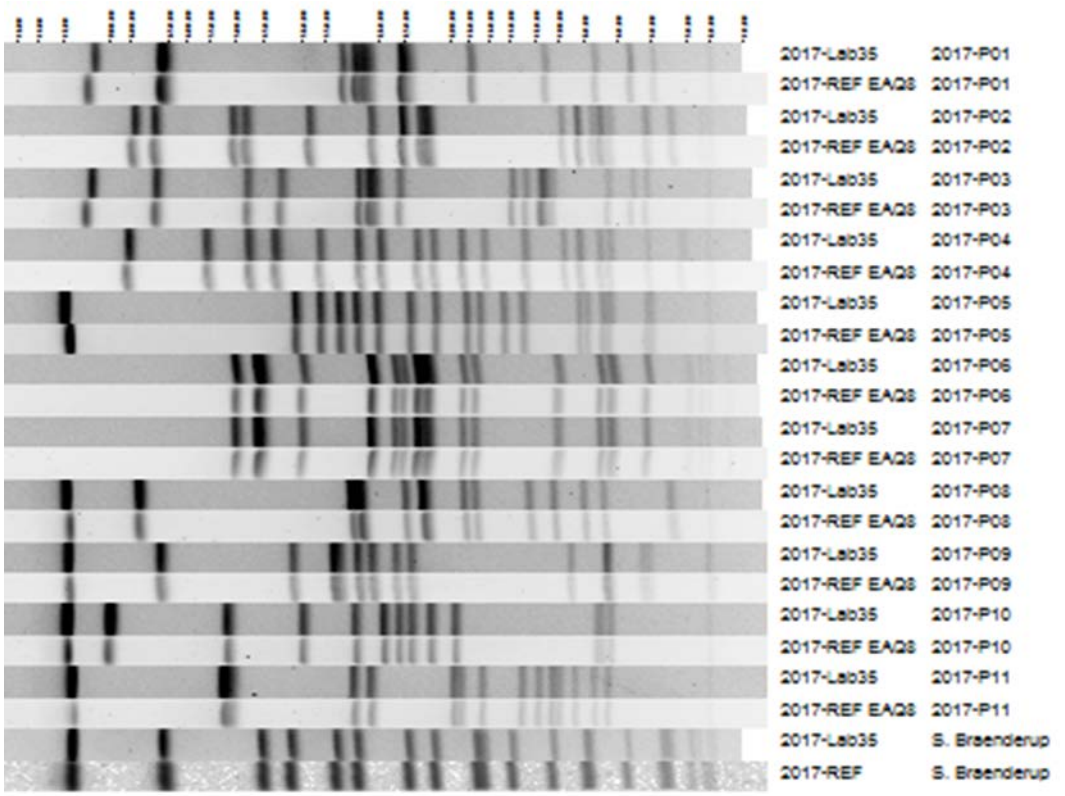

Figure 1. Comparison of your PFGE profiles with the reference profiles

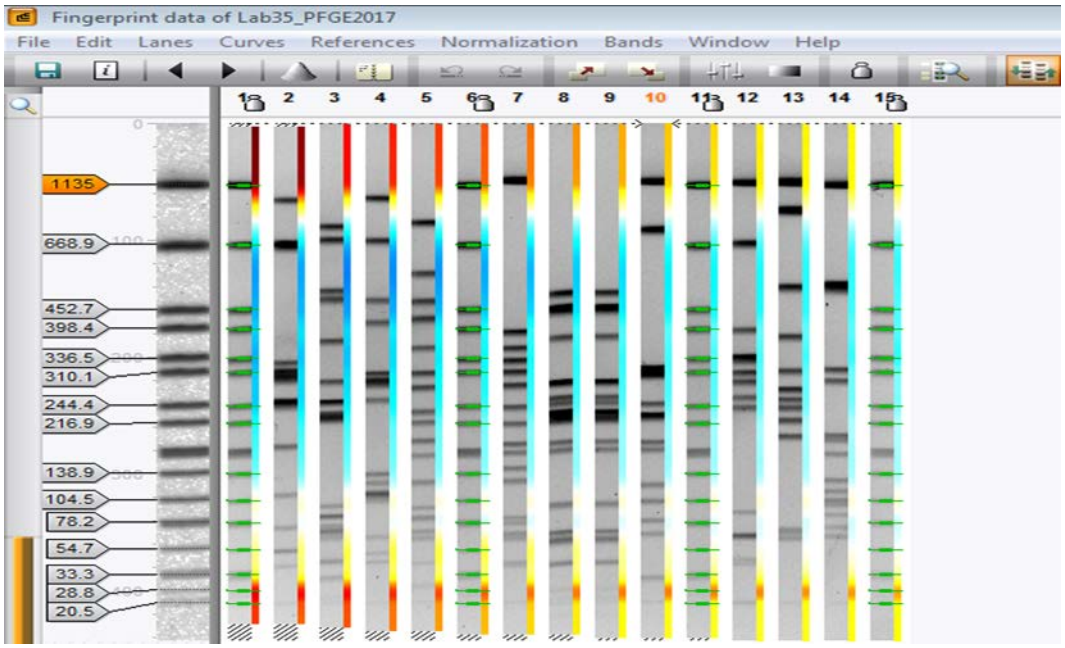

Figure 2. Display of the "Distortion bar" option in Bionumerics of your gel Darker colours indicate critical normalisation.

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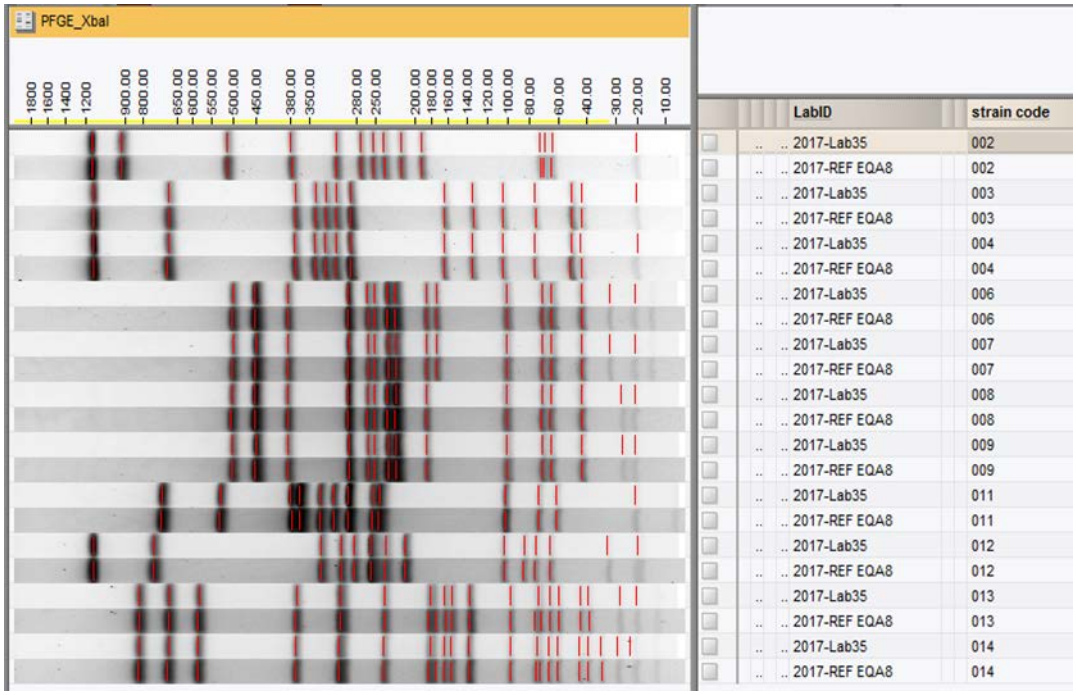

LabID	strain code
2017-Lab35	002
2017-REF EQA8	002
2017-Lab35	003
2017-REF EQA8	003
2017-Lab35	004
2017-REF EQA8	004
2017-Lab35	006
2017-REF EQA8	006
2017-Lab35	007
2017-REF EQA8	007
2017-Lab35	008
2017-REF EQA8	008
2017-Lab35	009
2017-REF EQA8	009
2017-Lab35	011
2017-REF EQA8	011
2017-Lab35	012
2017-REF EQA8	012
2017-Lab35	013
2017-REF EQA8	013
2017-Lab35	014
2017-REF EQA8	014

Figure 3. Comparison of your analysis in Bionumerics with the reference analysis of the Provided PFGE gel TRO2017

EURL-*Salmonella*, Bilthoven, The Netherlands

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