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

EURL-*Salmonella* Proficiency Test Typing 2018

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Colophon

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Synopsis

EURL-*Salmonella* Proficiency Test Typing 2018

The National Reference Laboratories (NRLs) of all 28 European Union (EU) Member States performed well in the 2018 quality control test on *Salmonella* typing. Overall, the EU-NRLs were able to assign the correct name to 97% of the strains tested.

In addition to the standard method for typing *Salmonella* (serotyping*)*, twelve 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. Ten of the twelve 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 Proficiency Tests (PTs) 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 Albania, Republic of North Macedonia and Serbia, EFTA countries Iceland, Norway and Switzerland, and Israel took part in the 2018 assessment.

The annual Proficiency Test 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, Proficiency Test

Publiekssamenvatting

EURL-*Salmonella* ringonderzoek typering 2018

De Nationale Referentie Laboratoria (NRL's) van de 28 Europese lidstaten scoorden in 2018 goed bij de kwaliteitscontrole op *Salmonella*-typering. Uit de analyse van alle NRL's als groep bleek dat de laboratoria aan 97 procent van de geteste stammen de juiste naam konden geven.

Twaalf 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. Tien van de twaalf 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). Deze NRL is namens dat land verantwoordelijk 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 2018 waren dat de EU-kandidaat-lidstaten Albanië, Republiek Noord-Macedonië en Servië, de European Free Trade Association (EFTA)-landen IJsland, Noorwegen en Zwitserland, en Israël.

De organisatie van het jaarlijkse ringonderzoek *Salmonella*-typering is in handen van het Europese Unie Referentie Laboratorium voor *Salmonella* (EURL-*Salmonella*). Dit laboratorium is ondergebracht bij het RIVM in Nederland.

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

Contents

Summary

In November 2018, the annual Proficiency Test (PT) 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 for *Salmonella* (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 Albania, Republic of North Macedonia, and Serbia, the EFTA countries Iceland, Norway and Switzerland, and Israel.

All 36 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, 98% of the strains were typed correctly for the O-antigens, 97% of the strains were typed correctly for the H-antigens, and 96% 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, 33 participants achieved good performance in the first stage of the study. One first-time participant could not be evaluated due to its very limited set of antisera available. Two participants that did not achieve the level of good performance participated in a follow-up study including 10 additional strains for serotyping. In the end, all 35 evaluated NRLs achieved good performance.

Twelve 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. Ten of the twelve 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. All of them were able to analyse the PFGE profiles in this computer program, although the assignment of double bands as a single band and the assignment of triple bands as double bands remain well-known difficulties in this analysis.

1 Introduction

This report describes the 2018 Proficiency Test (PT) on typing of *Salmonella* spp. organised by the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*, Bilthoven, the Netherlands) in November 2018.

According to EC Regulation No. 2017/625 (EC, 2017), one of the tasks of the EURL-*Salmonella* is to organise PTs for the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) in the European Union. The main objectives for 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 PTs on typing started in 1995.

A total of 36 laboratories participated in this study. These included 29 NRLs-*Salmonella* in the 28 EU Member States, 3 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 five 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.

For the sixth and final time, the typing study also included PFGE typing. Twelve NRLs participated in this part of the study by PFGE typing 11 designated *Salmonella* strains and submitting images for evaluation. Eleven 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
Albania	Tirana	Food Safety and Veterinary Institute
Austria	Graz	AGES
Belgium	Brussels	Sciensano
Bulgaria	Sofia	NDRVMI
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	German 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	Landspítali University Hospital, Dept. of Clinical Microbiology
Ireland	Celbridge	Central Veterinary Research Laboratory
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
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)
North Macedonia Republic of	Skopje	Faculty of Veterinary Medicine – Food Institute
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	Veterinary Institute 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 – Northern Ireland, 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 36.

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 result forms to report results. Instructions for the completion of these result forms and data-entry were sent to the NRLs on 8 November 2018, but in separate emails for serotyping and for PFGE typing.
The protocol and blank result forms can be found on the EURL-*Salmonella* website:
https://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 5 November 2018. 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 23rd EURL-*Salmonella* Workshop in 2018 (Mooijman, 2018), 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. Three strains (S3, S7, S13) 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 EURL-*Salmonella *PT serotyping 2018*

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
S1	1,9,12	g,m	-	Enteritidis
S2	1,4,12,27	d	1,7	Schwarzengrund
S3 [b)]	4,12,27	r	z_6	Southampton
S4	6,7,14	r	1,5	Infantis
S5	6,8	z_{10}	e,n,x	Hadar
S6	1,4,[5],12	i	1,2	Typhimurium
S7 [b)]	11	b	1,5	Leeuwarden
S8	6,7,14	r	1,2	Virchow
S9	6,8	e,h	1,5	Kottbus
S10	1,4,[5],12,[27]	b	e,n,x	Abony
S11	1,3,19	m,t	-	Cannstatt
S12	1,4,[5],12	f,g	[1,2]	Derby
S13 [b)]	1,9,12	z	1,5	Lawndale
S14	1,4,[5],12	l,v	e,n,z_{15}	Brandenburg
S15	1,4,[5],12	i	1,5	Lagos
S16[a)]	1,4,[5],12	i	-	4,5:i:-
S17	6,7,14	y	1,5	Bareilly
S18	1,4,[5],12	e,h	e,n,x	Chester
S19	6,8	r	l,w	Goldcoast
S20	16	c	l,w	Yoruba
S21[c)]	55	k	z_{39}	55:k:z_{39}

[a)] Typhimurium, monophasic variant as determined by PCR.
[b)] Represented in an EURL-*Salmonella* PT serotyping for the first time.
[c)] *Salmonella enterica* subspecies *salamae* (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 PTs 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.

3.2.3 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 shown 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 EURL*-Salmonella *PT serotyping 2018*

Strain	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
SF-1	1,4,[5],12	f,g	[1,2]	Derby
SF-2	1,9,12	g,m	-	Enteritidis
SF-3	8,20	i	z_6	Kentucky
SF-4	1,4,[5],12	i	1,2	Typhimurium
SF-5	{6,7,14}{54}	g,m,[p],s	[1,2,7]	Montevideo
SF-6	6,7,14	r	1,5	Infantis
SF-7	1,4,[5],12	i	-	4,5:i:-
SF-8	6,8	z_{10}	e,n,x	Hadar
SF-9	6,8,20	e,h	1,2	Newport
SF-10	6,7,14	r	1,2	Virchow

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. All strains have been used before in previous EURL-*Salmonella* PFGE typing studies, and also the reference strain *S.* Braenderup H9812 was included as a test strain. Background information on the strains is given in Table 4.

Table 4. Background information on the Salmonella *strains used for PFGE typing and analysis in 2018*

Strain codes in 2018 Study 'Quality PFGE gel image'	Corresponding strain codes in previous studies	Serovar
P01	*S*. Braenderup H9812	Braenderup
P02	2013-P5	Aberdeen
P03	2015-P5	Manhattan
P04	2013-P8	Poona
P05	2014-P6	Rough
P06	2013-P10	Infantis
P07	2017-P5	Infantis
P08	2016-P9	Reading
P09	2017-P10	Poona
P10	2014-P9	Enteritidis
P11	2014-P7	Enteritidis

3.3.2 *Evaluation of the PFGE gel image*

Participants were asked to test the 11 strains (P01 – P11) using their own routine PFGE method (*Xba*I digestion) and to give details of the method in the 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 results was done on the quality of the PFGE images. Quality grading was performed according to the guidelines as used in the EQAs for the FWD laboratories (based on the PulseNet guidelines, www.pulsenetinternational.org) (Annex 1). To comply with these guidelines the reference strain *S*. Braenderup H9812 must be run in every 6 lanes as a minimum. The guidelines use 7 parameters, which are scored with 1 (poor) to 4 (excellent) points.
In general, an acceptable quality should be obtained for each parameter since a low quality score in just one category can have a high impact on the ability to further analyse the image and compare it to other profiles.

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

The analysis of a PFGE gel in the bioinformatics software application BioNumerics was included in the study as optional. Like in 2017, a common gel image for all participants was used. This TIFF file, named "Provided PFGE gel TRO 2018", was sent by email to the participants on 8 November 2018 and is shown in Annex 2. This image was the TIFF as sent in by Laboratory 01 in the 2016-study on PFGE typing. Strain codes 001, 005, 010, and 015 refer to the *S*. Braenderup standard on this gel image.

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 8 November 2018,
- 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 image 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

4.1.1 General

A total of 36 laboratories participated in this study (Chapter 2). These included 29 NRLs-*Salmonella* in the 28 EU Member States, 3 NRLs in EU-candidate countries, 3 NRLs in EFTA countries, and 1 non-European NRL. One laboratory (Labcode 21) participated for the first time in an EURL-*Salmonella* serotyping study. Because this laboratory only had a limited set of antisera, it was not appropriate to evaluate their results according to the standard procedure as given in 3.2.2. The results were not taken into account in the overall results of the PT serotyping 2018 (n=35).
The frequency of serotyping of *Salmonella* at the participating laboratories and the number of strains that were serotyped and PFGE typed in 2018 are summarised in Table 5.

Table 5. Frequency and number of strains serotyped, and number of strains PFGE typed (for all 36 participants)

Lab code	Serotyping frequency in 2018	No. of strains serotyped in 2018	No. of strains PFGE typed in 2018
17	Daily	70	
16	Daily	140	
32	Daily	295	
31	Daily	354	
4	Daily	400	30
23	Daily	400	
29	Daily	400	
26	Daily	530	32
34	Daily	667	
24	Daily	675	
2	Daily	700	
3	Daily	700	18
14	Daily	800	
35	Daily	900	
9	Daily	1000	
18	Daily	1600	
12	Daily	2000	10
19	Daily	4000	50
8	Daily	4500	100
5	Daily	4800	
6	Daily	5000	130
7	Daily	6000	
11	Daily	6000	450
10	Daily	7500	50
22	Thrice a week	160	
13	Thrice a week	250	PT only
27	Thrice a week	400	
25	Twice a week	6	
36	Twice a week	80	100

Lab code	Serotyping frequency in 2018	No. of strains serotyped in 2018	No. of strains PFGE typed in 2018
33	Twice a week	750	
30	Once a week	60	
28	Once a week	175	
20	Once a week	380	40
1	Once a week	2000	
15	Once a week	2400	
21	Upon request	30	
n=36		56122	1010

4.1.2 Accreditation

Of the 36 participants, 33 are accredited for serotyping *Salmonella*, mainly according to EN ISO/IEC 17025:2005 (or 2017), and in some cases according to EN ISO 15189, or more specifically mentioning CEN ISO/TR 6579-3. One laboratory noted that it is planning to go for accreditation of *Salmonella* serotyping next year, the two other laboratories noted that they were not planning to go for such accreditation.
One laboratory is accredited for serotyping of all serovars except *S*. Typhi. All other laboratories stated that they are accredited for all *Salmonella* serovars.

4.1.3 Transport of samples

All but three participants received their package in the same week sent (week 45 of 2018). The exceptional three packages were delivered within 7 – 11 days after preparation. All packages were received in good condition.

4.2 Serotyping results

4.2.1 General

The 20 obligatory strains were all tested by the *Salmonella* NRLs in the participating countries. Classical serology was used by 32 participants, 2 participants mentioned the combined use of classical serology and Luminex assays, 1 participant used Whole Genome Sequencing and 1 answer was missing.
Details on the number and the source of the sera 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	16
Own preparation	4
Pro-Lab	5
Reagensia	2
Remel	2
Sifin	20
Statens Serum Institute (SSI)	29
Other	3

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	8
2	11
3	13
4	1
5	1

4.2.2 Biochemical testing

Twenty-four participants confirmed the use of biochemical tests.
Ten participants confirmed strain S10 (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.
Twenty-two participants used a variety of biochemical tests (most often malonate and dulcitol) on the optional strain S21, uncommon serovar 55:k:z_{39} (*S. enterica* subsp*. salamae*).

4.2.3 Use of PCR for confirmation

Seventeen laboratories used PCR to confirm strain S16, the monophasic variant of *S*. Typhimurium 1,4,[5],12:i:-, and five of these also used PCR to confirm strain S6, *S*. Typhimurium. The majority of laboratories mentioned using the reference of 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 28 of the 35 participants (80%). This corresponds to 98% of the total number of strains. The H-antigens were typed correctly by 23 of the 35 participants (66%), corresponding to 97% of the total number of strains. As a result, 20 participants (57%) gave the correct serovar names, corresponding to 96% of all strains evaluated.

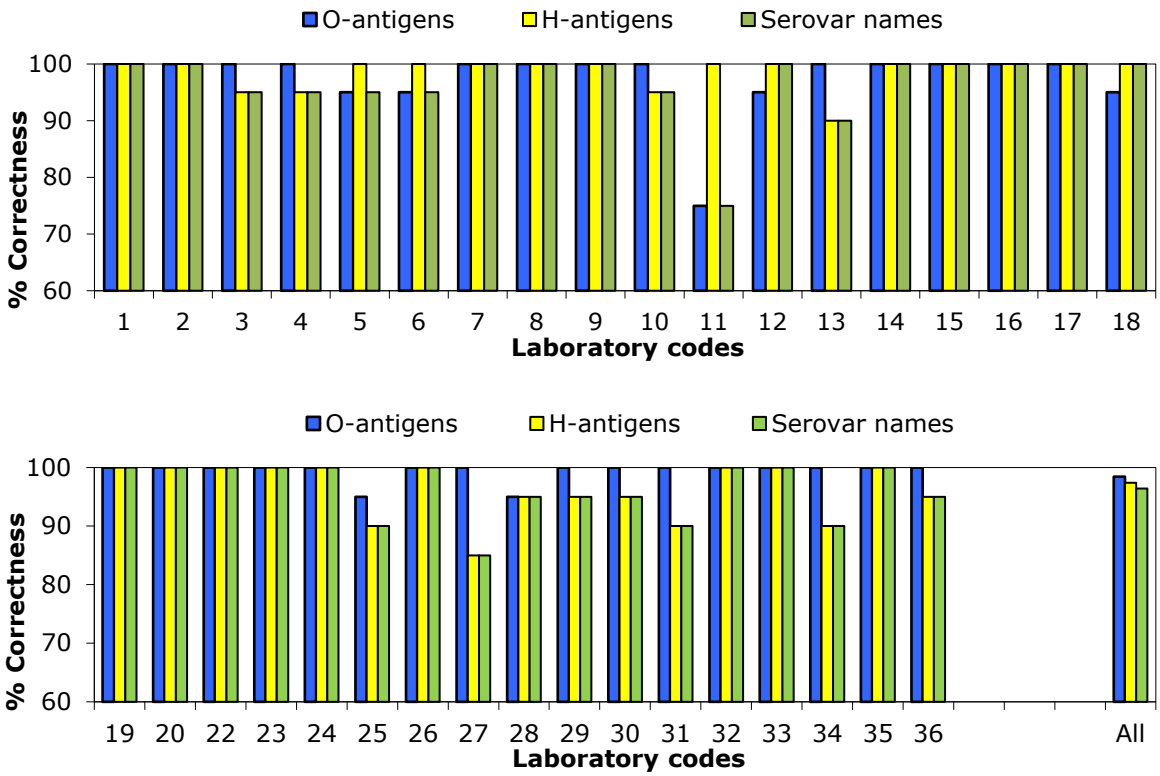

Figure 1. Percentages of correct serotyping results

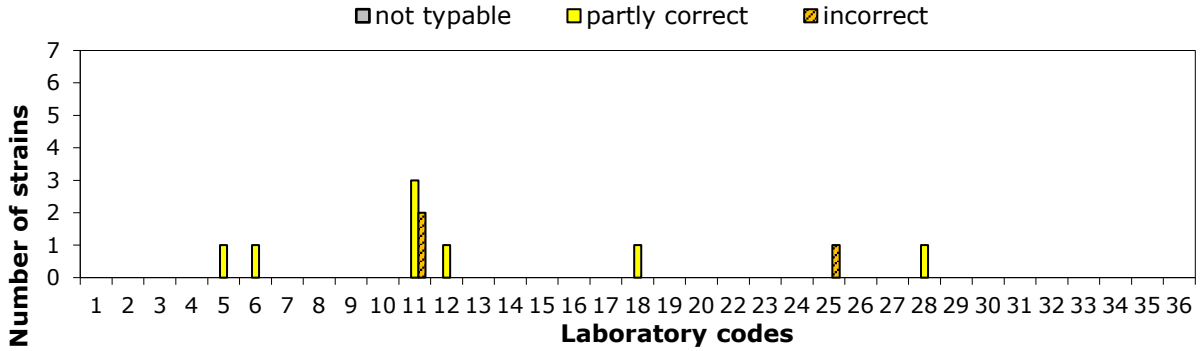

Figure 2. Evaluation of type of errors for O-antigens per NRL

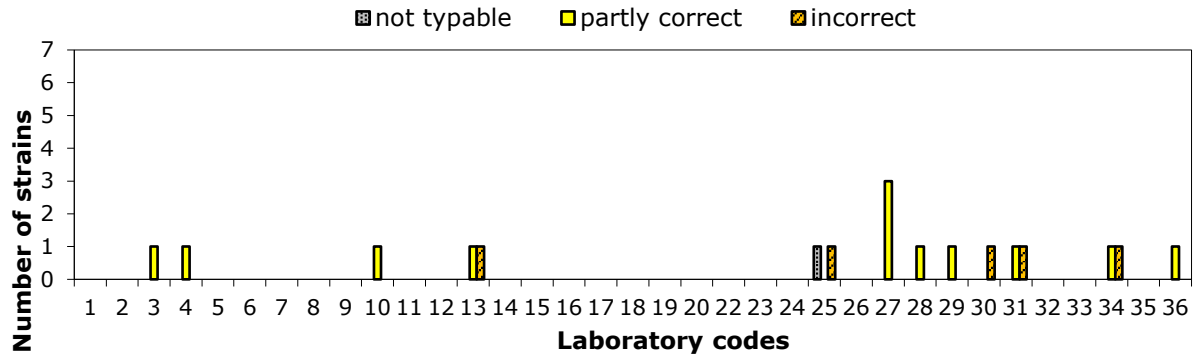

Figure 3. Evaluation of type of errors for H-antigens per NRL

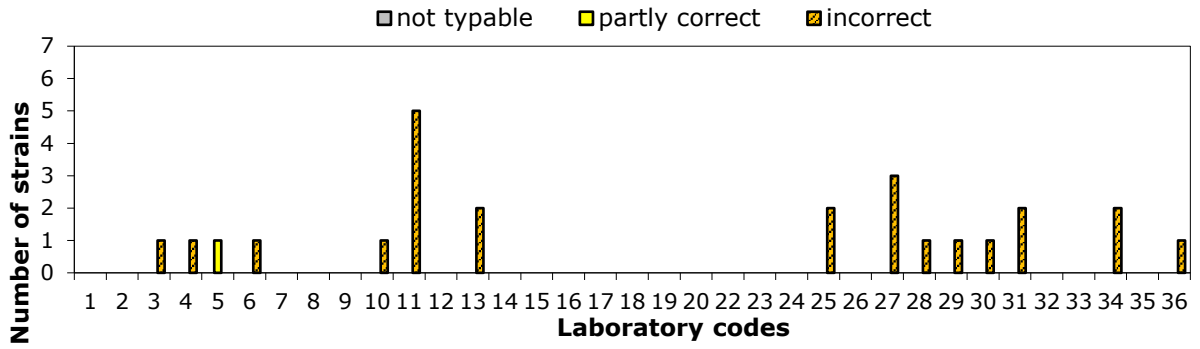

Figure 4. Evaluation of type of errors in the identification of serovar names

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 8 shows the number of penalty points for each NRL and indicates whether the level of good performance was achieved (yes or no). Two participants (Lab 11 and Lab 27) did not meet the level of good performance at the first stage of the study and a follow-up study for these laboratories was organised in March/April 2019.

All participants received their individual laboratory evaluation report on serotyping on 13 February 2019, followed by the interim summary report on 20 February 2019.

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 8. Evaluation of serotyping results per NRL

Lab code	Penalty points	Good performance	Lab code	Penalty points	Good performance
1	0	yes	19	0	yes
2	0	yes	20	0	yes
3	1	yes	21	n.a.	n.a.
4	1	yes	22	0	yes
5	0	yes	23	0	yes
6	1	yes	24	0	yes
7	0	yes	25	2	yes
8	0	yes	26	0	yes
9	0	yes	27	6	NO
10	1	yes	28	1	yes
11	11	NO	29	1	yes
12	0	yes	30	1	yes
13	2	yes	31	2	yes
14	0	yes	32	0	yes
15	0	yes	33	0	yes
16	0	yes	34	2	yes
17	0	yes	35	0	yes
18	0	yes	36	1	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 S16 and S21; these are reported separately in Annex 5.
Apart from some spelling errors in the writing, a completely correct identification was obtained for eleven *Salmonella* serovars: Enteritidis (S1), Southampton (S3), Hadar (S5), Typhimurium (S6), Derby (S12), Lawndale (S13), Brandenburg (S14), Lagos (S15), 1,4,[5],12:i:- (S16), Chester (S18), and Goldcoast (S19).
Details on the strains that caused problems in serotyping are shown in Annex 6. Strain S11, Cannstatt (1,3,19:m,t:-) clearly gave most problems. Nine laboratories did not name this strain correctly, in six cases this was caused by a mistake in the phase 1 H-antigen determination: reporting g,m,t (Kouka) instead of m,t (Cannstatt).
The reported serovar names for strain 1,4,[5],12:i:- (S16) are shown in Annex 5. Seventeen participants used a PCR method to confirm this strain to be a monophasic Typhimurium strain.
Details on the additional and optional strain S21 are given in Annex 5 as well. All but five participants tried to serotype strain S21, a *Salmonella enterica* subsp. *salamae* (II). Only a few laboratories did not have access to the required antisera to finalise this (55:k:z_{39}). Historically, serovar 55:k:z_{39} was named Tranoroa, but this serovar name is withdrawn now from the WKLM scheme (2007). Serovar names were maintained only for subspecies *enterica* serovars. Serovars of the other subspecies of *S. enterica* and those of *S. bongori* nowadays are designated only by their antigenic formula.

4.2.7 *Results follow-up study*

Two participants, one EU NRL and one non-EU NRL, did not achieve the level of good performance in the first part of the PT (Table 8; Lab code 11 and Lab code 27) and both participated in a follow-up study, receiving 10 additional strains for serotyping in week 13, 2019.
Also for the follow-up study, the number of penalty points was determined using the guidelines described in Section 3.2.2. Table 9 shows the results of the follow-up study, both participants achieving the level of good performance.

Table 9. Evaluation of serotyping results per NRL in the follow-up study

Lab code	Penalty points	Good performance
11	0	Yes
27	0	Yes

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

The historical data of the EURL-*Salmonella* PTs 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 remain 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. The rise as seen for the 2018 study is mainly caused by the relatively large number of 7 EU NRLs that made a mistake in typing strain S11 (Cannstatt). However, the number of EU NRLs with a non-Good Performance is low: two in the period 2010 – 2013, only one in the 2014, 2015 and 2018 studies, and none in the 2016 and 2017 studies.

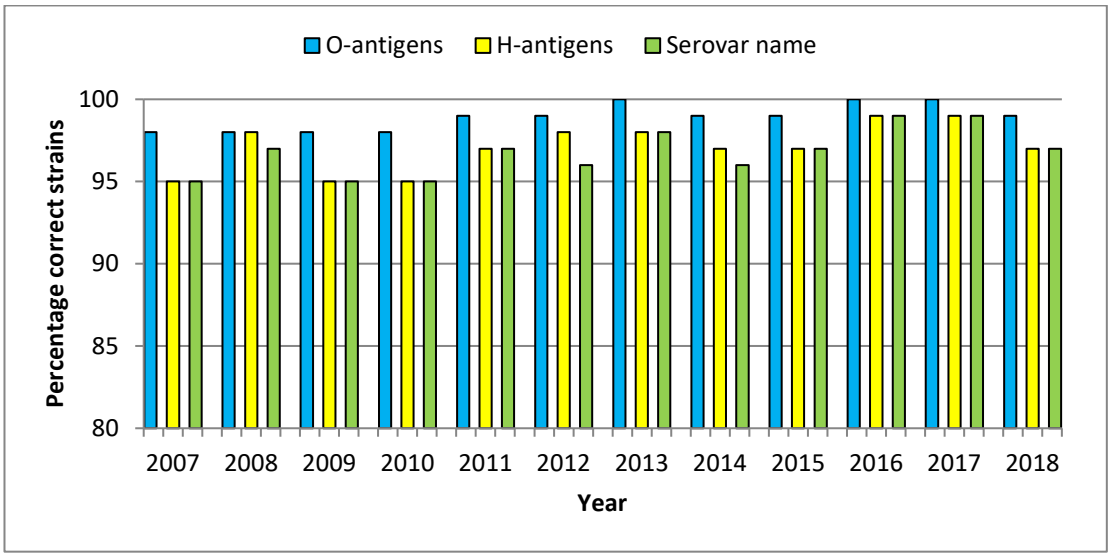

Figure 5. Serotyping results of the EU NRLs in time, based on the percentages of correctly typed strains

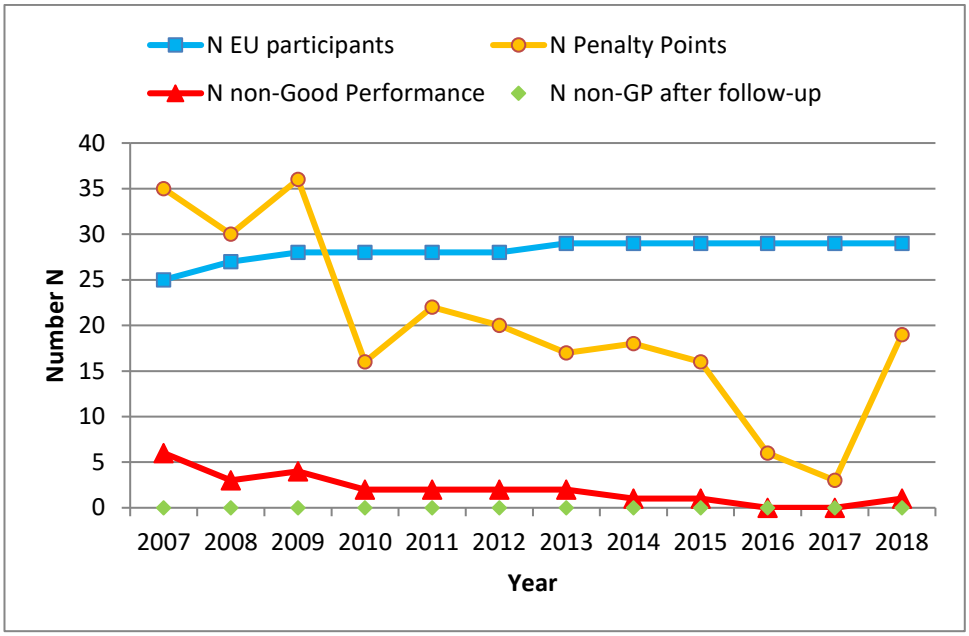

Figure 6. Serotyping results of the EU NRLs in time, based on the number of Penalty Points and non-Good Performance (non-GP)

4.3 PFGE typing results

4.3.1 General

A total of 12 NRLs participated in the study on PFGE typing. Four participants in the 2017 study did not participate in the 2018 study, and one participant was new compared to the 2017 study. Five laboratories have participated in all six PFGE typing studies so far.
Eight participants reported using the Standard PulseNet Protocol *Salmonella* PFGE (PulseNet International, 2013)/the EURL-*Salmonella* SOP (Jacobs et al., 2014). Four participants used this Standard protocol with modifications.
All participants received their individual laboratory evaluation report on PFGE typing on 15 April 2019, together with a report on the overall results. An example of an individual laboratory evaluation report on PFGE typing results is given in Annex 14. The report with the overall results is available on the website: https://www.eurlsalmonella.eu/publications/interlaboratory-comparison-study-reports (accessed 28-2-2020).

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 10-12 respectively.

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

Manufacturer	Number of NRLs
Fermentas	1
Promega	1
Roche Diagnostics	6
Takara	1
Thermofisher Scientific	3

Table 11. Electrophoresis system used by the participants

Electrophoresis system	Number of NRLs
Bio-Rad CHEF Mapper XA	2
Bio-Rad CHEF-DR III System	7
Bio-Rad CHEF-DR II System	2
CHEF Mapper unspecified	1

Table 12. Gel documentation system used by the participants

Gel documentation system	Number of NRLs
BioDoc-It Imaging System/UVP	1
BioRad imager	1
ChemiDoc XRS	1
Cleaver	1
GBox EF (Syngene)	1
GelDoc XR	2
GelDoc XR+	4
GeneGenious (Syngene)	1

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

For staining the gel, one participant used SYBR safe and one used GelRed; all other participants used Ethidium Bromide. The duration of the staining varied between 15 minutes (1x), 20 minutes (3x), 25 minutes (1x) and 30 minutes (7x).
Seven participants used a comb with narrow teeth, and five participants used a comb with wide teeth.

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

The scores per NRL (n=12), 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 12).

Overall, 82% of the scores were Good or Excellent. However, two of the 12 images resulted in a Poor score on at least one of the seven parameters. These two images would therefore be unsuitable for use in interlaboratory database comparison of these PFGE profiles. Lab 20 scored a Poor result for 'Image Acquisition and Running Conditions' and 'Bands', but clearly the 'Band spacing of SB standards does not match global standard' was making this image unsuitable for analysis and comparison; note lane 1 with Lab 20 REF strain and lane 2 with PT strain SB H9812 in Figure A12.1. Lab 13 scored a Poor result for 'Restriction' and 'DNA degradation'. This was, repeatedly and unexplained, applicable to at least four specific test strains, but analysis for five other strains could still have been done.

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 et al., 2014). Thus, the examination of 11 test strains requires the use of the reference strain in at least four lanes. As an improvement to previous studies, no mistakes

were seen on this aspect in 2018. Five participants used the lanes 1, 5, 10, and 15 for the reference strain. Seven participants used the lanes 1, 6, 11, and 15 for this. Unrelated to the lanes used for the reference strain, five participants used a comb with wide teeth, and seven used a comb with narrow teeth.

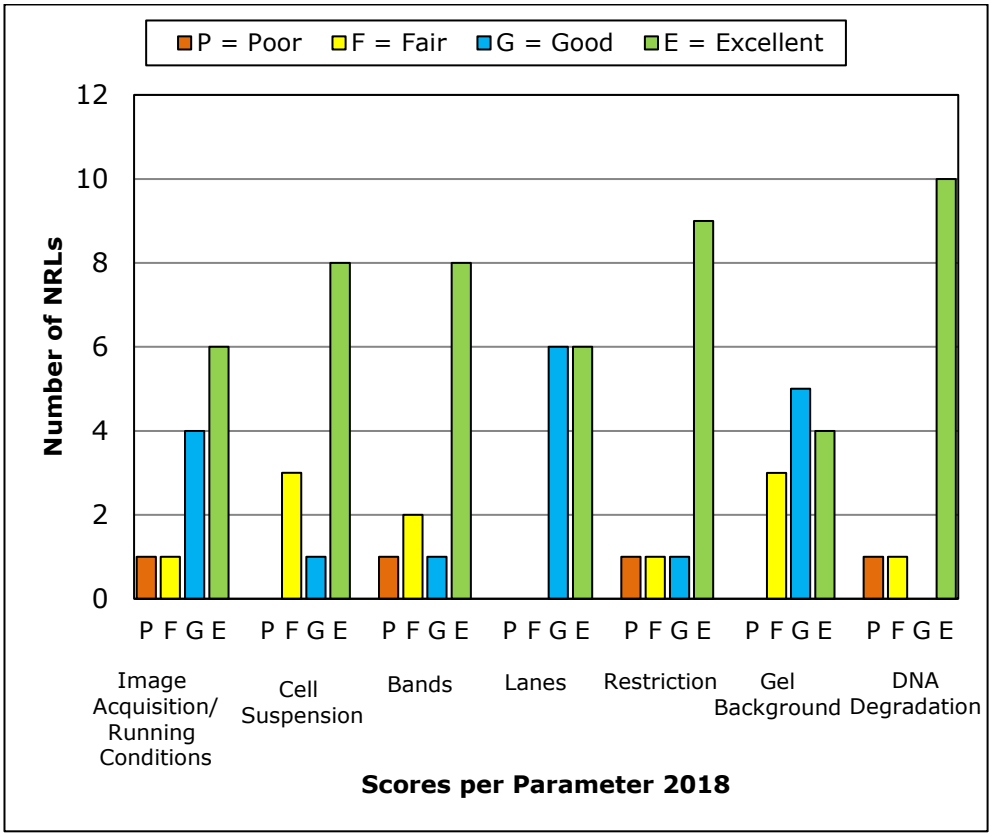

Figure 7. Evaluation of the quality of the PFGE images in scores per parameter, 2018 study

Figure 8 shows the results of the evaluation of the TIFF images from the 2013 – 2018 studies. Improvements over time are clearly visible, however it has to be noted that significant variation between participating laboratories has been found. Also, there has been quite a variety of laboratories, participating to the different studies. Just five laboratories participated in all six PTs on PFGE typing.

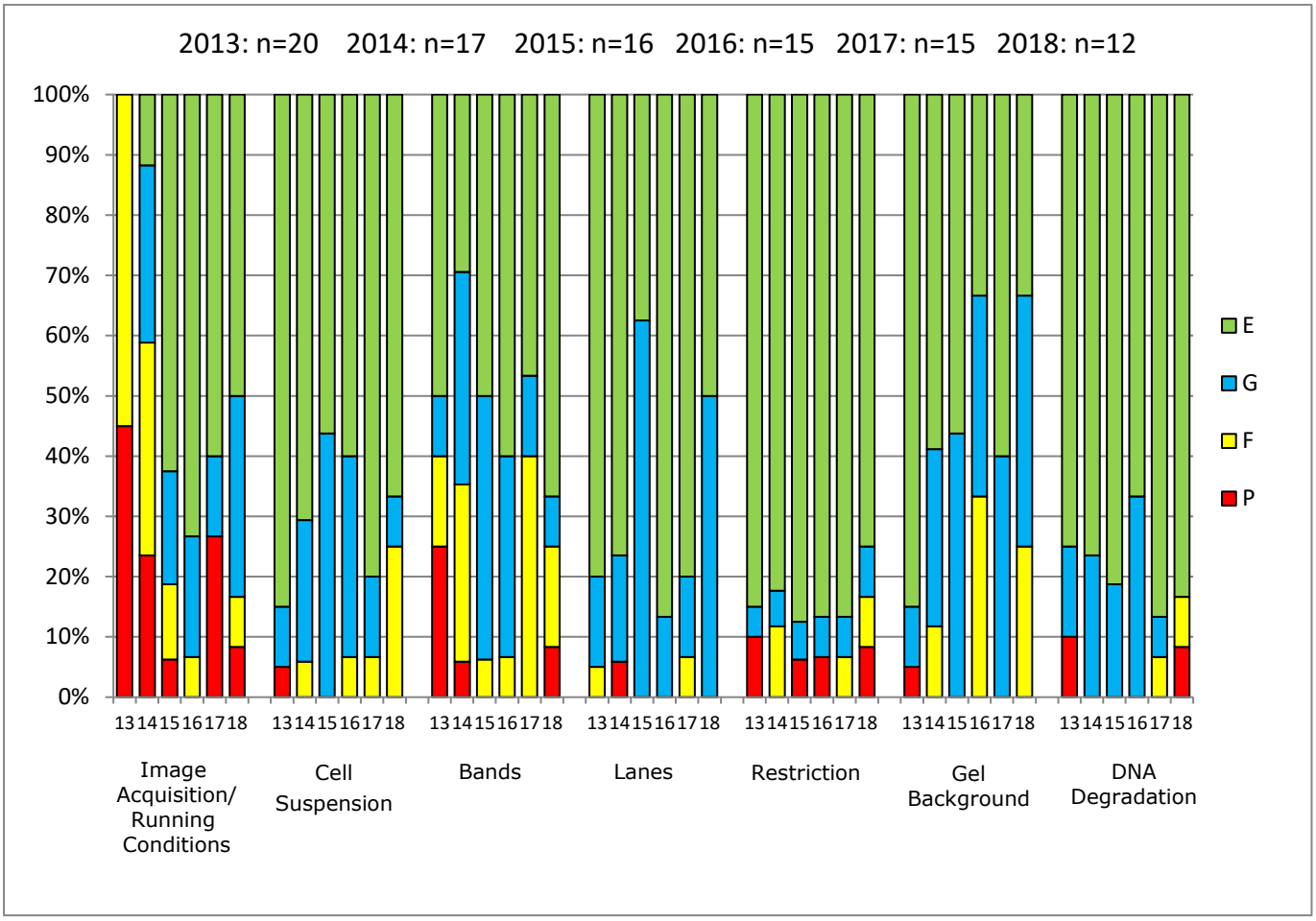

Figure 8. Evaluation of the quality of the PFGE images in scores per parameter, 2013-2018 studies

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 fourth 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 2018', Annex 2).

A total of 11 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. Annex 11 shows the (large) variation in the parameters in BioNumerics, as set by the individual participants for the analysis of the same 'Provided PFGE gel TRO 2018'. Annex 13 shows the comparisons of all participants' results on the analysis of the gel image, per test strain.

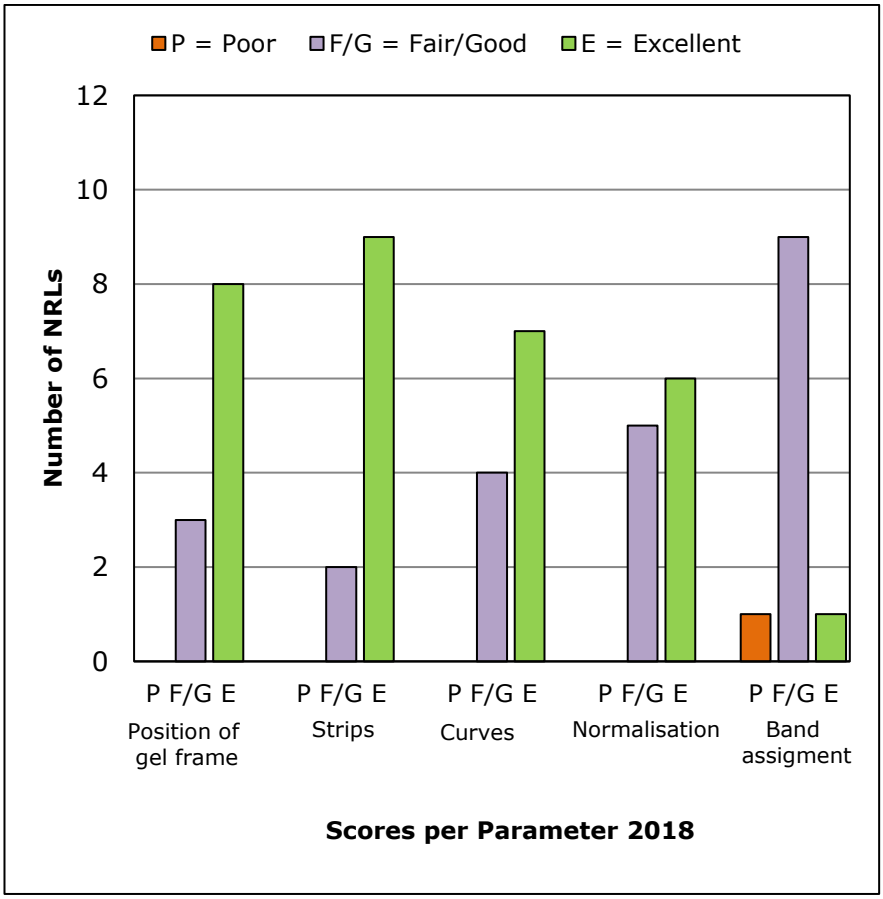

Figure 9. Evaluation of the analysis of the gel in BioNumerics in scores per parameter, 2018 study

Overall, 56% of the scores were Excellent and 42% of the scores were Fair/Good.

Lab 12 and sometimes also Lab 13 tended to assign bands of test strains below 33 kb (Figure 10, black circle and Annex 13), and thereby not following the Protocol, although this minor deviation did not affect the comparison of all participants' results in BioNumerics. This comparison however was affected by the mistake of Lab 03, not assigning the bands at around 40 kb (Figure 10, blue circle and Annex 13), leading to mismatches for all strains concerned (all but strains 011 and 012). Apart from these deviations, 3 strains (codes 003, 004, and 009) were correctly analysed by all participants.
The main problems in the analysis of the same gel image by all participants were the assignment of double bands as single bands (strains 002, 006, 007, 008, and 013) and the assignment of triple bands as double bands (strains 011 and 012), which are well-known difficulties in the analysis of PFGE images. As an example, band assignment results for strain 007 are given in Figure 10. Three times a double band assignment (indicated with purple arrows??), missed once (Labs 03, 04, 20, 26), twice (Labs 08, 11, 12) or three time (Lab 13) lead to a variety of mismatches in the comparison.
One participant (Lab 06) analysed all 10 test strains in the provided gel image in complete agreement with the reference analysis.

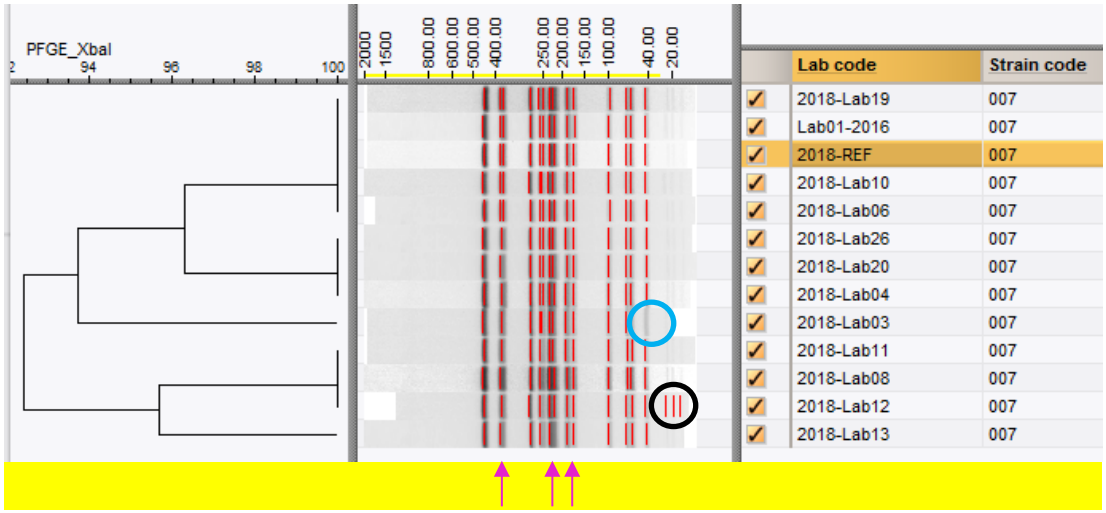

Figure 10. PFGE profiles with band assignment in BioNumerics by 11 participants for strain 007.

Figure 11 shows the overall results from all four studies (2015 – 2018). Correct band assignment remains the most difficult part of the analysis of a gel image in BioNumerics.

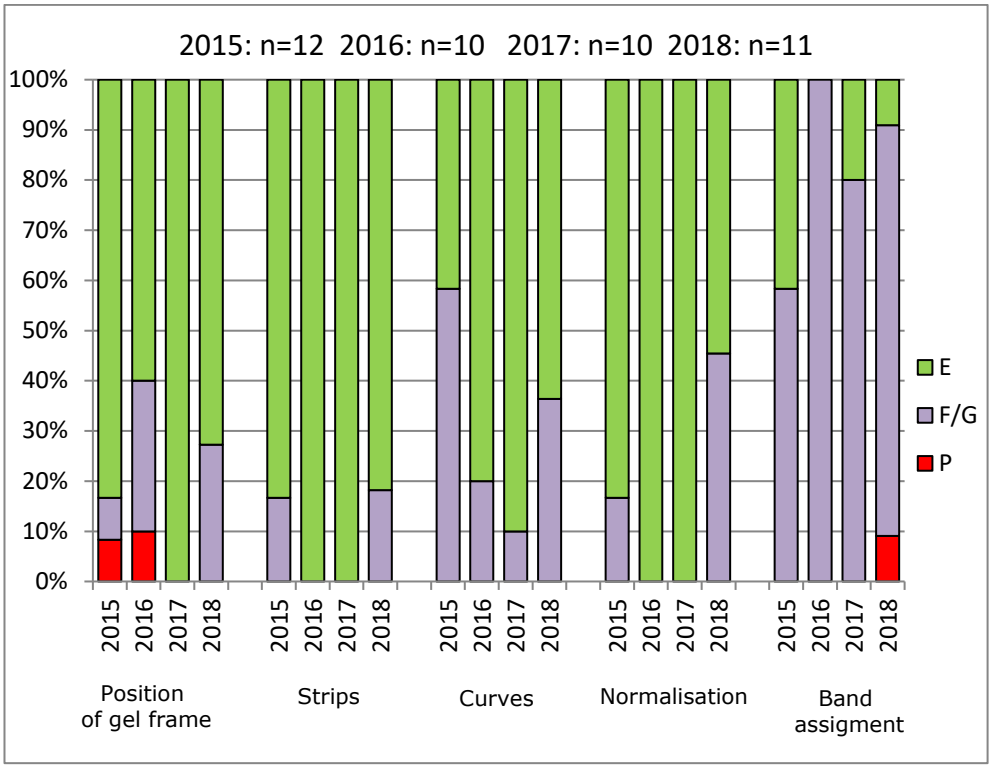

Figure 11. Evaluation of the analysis of the gel in BioNumerics in scores per parameter, 2015-2018 studies (E=Excellent, F/G=Fair/Good, P=Poor)

5 Conclusions

5.1 Serotyping

- Overall results for the 35 evaluated NRLs are:
  - 98% of the strains were typed correctly for the O-antigens.
  - 97% of the strains were typed correctly for the H-antigens.
  - 96% of the strains were correctly named.
- Serotyping of *S*. Cannstatt (1,3,19:m,t:-) caused the most problems in this study (ten participants).
- One EU NRL and one non-EU NRL initially did not achieve the defined level of good performance and both participated in a follow-up study, typing an additional set of 10 strains.
- In the end, all 29 EU NRLs and the 6 non-EU NRLs achieved the defined level of good performance.

5.2 PFGE typing

- Nine of the twelve 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 BioNumerics, and all of them were able to analyse the PFGE profiles in this computer program.
- Assignment of double bands as a single band and assignment of triple bands as double bands remain well-known difficulties in the analysis of PFGE images.

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
n.a.	not applicable
NRL-*Salmonella*	National Reference Laboratory for *Salmonella*
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PT	Proficiency Test
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 Guidelines on quality grading of PFGE images

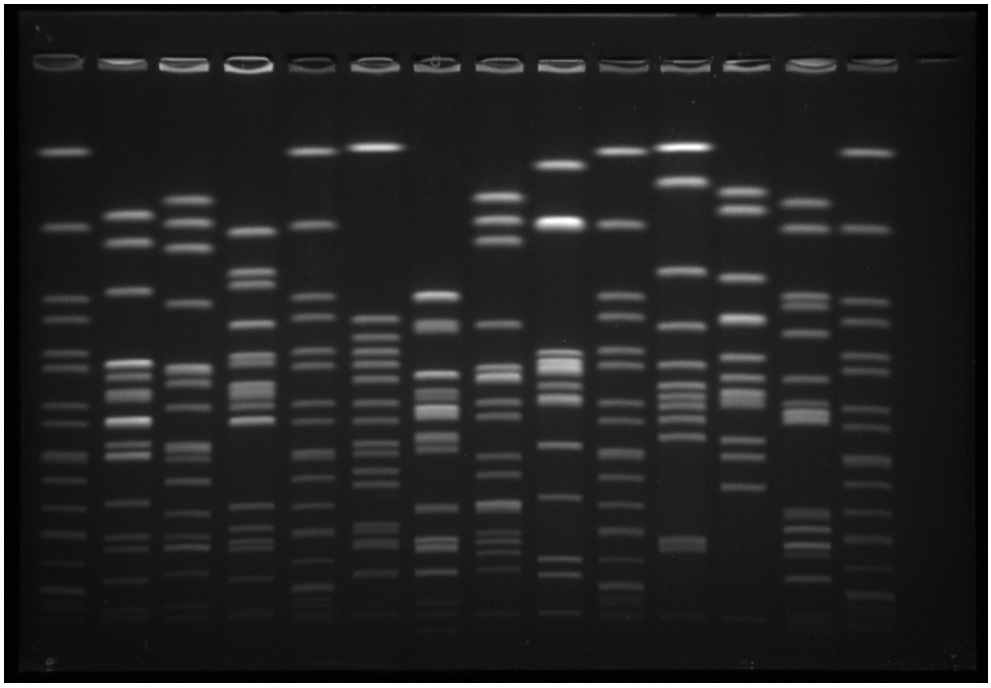
Evaluation of the quality of the PFGE images according to the EQAs for the FWD laboratories (European Centre for Disease Prevention and Control. Seventh external quality assessment scheme for *Salmonella* typing. Stockholm: ECDC; 2016. Available at: https://www.ecdc.europa.eu/en/publications-data/seventh-external-quality-assessment-salmonella-typing (accessed on 28-2-2020).

Parameter	Grade [score in points]			
	Poor [1]	**Fair [2]**	**Good [3]**	**Excellent [4]**
Image Acquisition and Running Conditions	- Gel does not fill whole TIFF and band finding is highly affected. - Bottom band of standard not 1–1.5 cm from the bottom of the gel and analysis is highly affected. - Band spacing of standards does not match global standard and analysis is highly affected. - Too few reference lanes included.	- Gel does not fill whole TIFF and band finding is slightly affected. - Wells not included on TIFF. - Bottom band of standard not 1–1.5 cm from the bottom of the gel and analysis is slightly affected. - Band spacing of standards does not match global standard and analysis is slightly affected.	- Gel does not fill whole TIFF but band finding is not affected. - Bottom band of standard not 1–1.5 cm from the bottom of the gel but analysis is not affected.	By protocol, for example: - Gel fills whole TIFF - Wells included on TIFF - Bottom band of standard 1–1.5 cm from the bottom of the gel.
Cell Suspensions	The cell concentrations are uneven from lane to lane, making analysis impossible.	- More than two lanes contain darker or lighter bands than the other lanes. - At least one lane is much darker or lighter than the other lanes, making the gel difficult to analyse.	One or two lanes contain darker or lighter bands than the other lanes.	The cell concentration is approximately the same in each lane.
Bands	- Band distortion making analysis difficult. - Very fuzzy bands. - Many bands too thick to distinguish. - Bands at the bottom of the gel	- Some band distortion (i.e. nicks) in two or three lanes, but still analysable. - Fuzzy bands. - Some bands (four or five) are	- Slight band distortion in one lane, but analysis is not affected. - Bands are slightly fuzzy and/or slanted.	Clear and distinct all the way to the bottom of the gel.

Parameter	Grade [score in points]			
	Poor [1]	**Fair [2]**	**Good [3]**	**Excellent [4]**
	too light to distinguish.	too thick. - Bands at the bottom or top of the gel are light, but analysable.	- A few bands (three or less) are difficult to see clearly (i.e. DNA overload), especially at the bottom of the gel.	
Lanes	'Smiling' or curving affecting analysis.	- Significant 'smiling' - Slight curves on the outside lanes, but still analysable.	- Slight 'smiling' (higher bands in outside lanes than inside). - Slight curving. - Lanes gradually run longer toward the right or left, but still analyzable.	Straight
Restriction	- More than one lane with several shadow bands. - Lots of shadow bands over the whole gel.	- One lane with many shadow bands. - A few shadow bands spread out over several lanes.	- One or two faint shadow bands.	Complete restriction in all lanes.
Gel Background	Lots of debris present, making analysis impossible.	- 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 enhanced making the image look grainy).	- Mostly clear background. - Minor debris not affecting analysis.	Clear
DNA Degradation (smearing in the lanes)	Smearing making several lanes unanalysable.	- Significant smearing in one or two lanes that may or may not make analysis difficult. - Minor background (smearing) in many lanes.	Minor background (smearing) in a few lanes but bands are clear.	Not present

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 2 TIFF image ‘Provided PFGE gel TRO2018’ 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: https://www.ecdc.europa.eu/en/publications-data/seventh-external-quality-assessment-salmonella-typing
(accessed on 28-2-2020)

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.

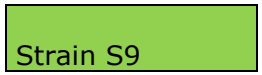
Note that the EFSA supporting publication 2014:EN-703 (recommended SOP) states:
When using the *S*. Braenderup H9812 reference, visible bands of *test* isolates should be marked down to ~33 kb (third band from the bottom of the H9812 reference), but not below (referring to *Band Assignment*).
In *Normalisation*,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

Annex 4 Serotyping results per strain and per laboratory

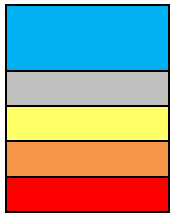
Labcode: REF	S1 Enteritidis	S2 Schwarzengrund	S3 Southampton	S4 Infantis	S5 Hadar	S6 Typhimurium	S7 Leeuwarden	S8 Virchow	S9 Kottbus
1	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
2	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
3	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
4	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
5	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
6	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
7	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
8	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
9	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
10	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Newport
11	Enteritidis	Schwarzengrund	Southampton	Bovismorbificans	Hadar	Typhimurium	Korbol	Bsilla	Ferruch
12	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leewarden	Vircow	Kottbus
13	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Tshiongwe
14	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
15	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
16	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
17	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
18	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
19	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
20	Enteritidis	Schwarzengrud	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
21	Enteritidis	*	*	*	Hadar	Auto-agglutination	*	Virchow	*
22	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
23	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
24	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
25	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Nagoya	Virchow	Kottbus
26	Enteritidis	Schwarzengrund	Sauthampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
27	Enteritidis	Stanley	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Nigeria	Kottbus
28	S. Enteritidis	S. Schwarzengrund	S. Southampton	S. Infantis	S. Hadar	S. Typhimurium	S. Leeuwarden	S. Virchow	S. Newport
29	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
30	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
31	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
32	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Wirchow	Kottbus
33	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
34	Enteritidis	Mons	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
35	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
36	Enteritidis	Schwarzengrund	Southampton	Infantis	Hadar	Typhimurium	Leeuwarden	Virchow	Kottbus
X	0	2	0	1	0	0	2	2	3

Labcode: REF	S10 Abony	S11 Cannstatt	S12 Derby	S13 Lawndale	S14 Brandenburg	S15 Lagos	S17 Bareilly	S18 Chester	S19 Goldcoast	S20 Yoruba
1	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
2	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
3	Abony	Kouka	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
4	Abony	Kouka	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
5	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	6,24:r:l,w	Yoruba
6	Abony	Southbank	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
7	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
8	Abony	Cannatatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
9	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
10	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
11	Abony	Banana	Derby	Lawndale	Brandenburg	Lagos	Tananarive	Chester	Goldcoast	Yoruba
12	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
13	Abony	Kouka	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
14	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
15	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
16	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
17	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
18	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
19	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
20	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
21	*	*	*	*	*	Lagos	*	*	*	*
22	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
23	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
24	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
25	4,12:b:e,n,x	3,19:g,m,t:-	Derby	Lawndale	Brandenburg	Lagos	Lomita	Chester	Goldcoast	Yoruba
26	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
27	Abony	Seftenberg	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
28	S. Abony	S. Cannstatt	S. Derby	S. Lawndale	S. Brandenburg	S. Lagos	S. Bareilly	S. Chester	S. Goldcoast	S. Yoruba
29	Tripoli	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
30	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Lingwala
31	Abony	Kouka	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Shamba
32	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
33	Abony	Cannstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
34	Abony	Kouka	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
35	Abony	Canstatt	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
36	Abony	Kouka	Derby	Lawndale	Brandenburg	Lagos	Bareilly	Chester	Goldcoast	Yoruba
X	1	9	0	0	0	0	2	0	0	2

incomplete set of antisera



Colonial form variation may have played a role, and therefore considered as a correct answer, also see Protocol EURL-*Salmonella* PT typing 2018



remark (eg spelling error)
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 S16 and S21 are given in Annex 5

Annex 5 Details of serotyping results for strains S16 and S21

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	PCR-confirmed	Lab code
S-16	**1,4,[5],12**	**i**	**-**	**1,4,[5],12:i:-**	**Yes**	**REF**
S-16	4,5,12	i	-	4,5,12:i:-	Yes	1
S-16	4	i	-	Typhimurium monophasic variant	Yes	2
S-16	4,5,12	i	-	4,5,12:i:-	No	3
S-16	1,4,5,12	i	-	4,12 : i : -	Yes	4
S-16	4,5,12	i	-	4,5,12:i:-	No	5
S-16	1,4,5,12	i	-	1,4,5,12:i:-	Yes	6
S-16	4,5,12	i	-	4,5,12:i:-	No	7
S-16	4,5,12	i	-	4,5,12:i:-	No	8
S-16	1,4,5,12	i	-	1,4,5,12:i:-	Yes	9
S-16	4,5,12	i	-	Monophasic Typhimurium	Yes	10
S-16	4,5,12	i	-	4,5,12:i:-	No	11
S-16	4	i	-	4:i:-	No	12
S-16	4,5,12	i	-	4,5,12; i; -	No	13
S-16	4,5	i	-	4,5:i:-	Yes	14
S-16	4	i	-	4,5,12:i:-	No	15
S-16	4,5,12	i	-	4,5,12:i:-	Yes	16
S-16	1,4,5,12	i	-	1,4,5,12:i:-	No	17
S-16	1,4,5	i	-	1,4,5;i;- (monophasic ST)	Yes	18
S-16	4,5	i	-	4,5,12:i:-	No	19
S-16	4,5,12	i	-	4,5,12:i:-	No	20
S-16	4,5,12	i	-	4,5,12:i:-	No	21
S-16	4,5,12	i	-	4,5,12:i:-	No	22
S-16	4,5,12	i	-	4,5,12:i:-	Yes	23
S-16	4,5,12	i	-	Typhimurium, monophasic 4,5,12 : i : -	Yes	24
S-16	4,12	i	-	4,12:i:-	No	25
S-16	4,12	i	-	4,5,12:i:-	Yes	26
S-16	4,5,12	i	-	Monophasic Salmonella Typhimurium	No	27
S-16	4,5	i	-	4,5:i:-	No	28
S-16	4,5,12	i	-	4,5,12:i:-	No	29
S-16	4, 5, 12	i	-	4, 5, 12:i:-	No	30
S-16	4,5,12	i	-	4,5,12:i:-	Yes	31
S-16	4,5,12	i	-	4,5,12:i:-	Yes	32
S-16	4,5,12	i	-	4,5,12:i:-	Yes	33
S-16	4,5,12	i	-	4,5,12:i:-	Yes	34
S-16	4,5,12	i	-	4,5,12:i:- Monofasisk Salmonella Typhimurium	Yes	35
S-16	4,5,12	i	-	1,4,5,12:i:-	No	36

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	Lab code
S-21	**55**	**k**	**z39**	**55:k:z39**	**REF**
S-21	55	k	z39	55:k,z39	1
S-21					2
S-21	55	k	z39	55:k:z39	3
S-21	OMF	k	z39	OMF : k : z39 (II)	4
S-21	55	k	z39	55:k:z39	5
S-21	55	k	z39	S.II 55:k:z39	6
S-21	55	k	z39	SG II 55:k:z39	7
S-21	55	k	z39	55:k:z39	8
S-21	55	k	z39	55:k:z39	9
S-21	55	k	z39	SubspII: 55:k:z39	10
S-21	55	k	z39	II 55:k:z39	11
S-21	55	k	z39	II 55:k:z39	12
S-21	55	k	z39	55; k; z39	13
S-21	55	k	z39	55:k:z39	14
S-21	55	z39	k	55:z39,k	15
S-21	55	k	z39	55:k:z39 (II)	16
S-21	55	k	z39	55:k:z39	17
S-21	55	k	z39	55;k;z39	18
S-21	55	k	z39	S. II (Salmonella enterica subsp. salamae) 55:k:z39	19
S-21	55	k	z39	55:k:z39	20
S-21	-	-	-	Incomplete set of antisera	21
S-21					22
S-21	OMF	k		III a arizonae	23
S-21	55	k	z39	Salmonella enterica subsp. salamae serovar 55 : k : z39	24
S-21	-	k	-	-:k:-	25
S-21	55	k	z39	II 55:k:z39	26
S-21	55	k	z39	II	27
S-21	55	k	z39	55:k:z39	28
S-21	55	k	z39	S.enterica subsp. salamae 55:k:z39	29
S-21					30
S-21	55	k	z39	55:k:z39	31
S-21		k			32
S-21	55	k	z39	Tranoroa	33
S-21	-	k	Z35	-:k:z35	34
S-21	55	k	z39	55:k:z39	35
S-21					36

reference result
remark (deviations in the results on optional strain S21)
not typable (antisera not available)

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-2	1,4,12,27	d	1,7	Schwarzengrund	REF
S-2	4,12	d	1,7	Schwarzengrud	20
S-2	4,12	d	1,2	Stanley	27
S-2	4,12,27	d	l,w	Mons	34
S-3	4,12,27	r	z6	Southampton	REF
S-3	1,4	r	z6	Southampton	18
S-4	6,7,14	r	1,5	Infantis	REF
S-4	6,8	r	1,5	Bovismorbificans	11
S-7	11	b	1,5	Leeuwarden	REF
S-7	8	b	1,5	Korbol	11
S-7	11	b	1,5	Leewarden	12
S-7	6,8	b	1,5	Nagoya	25
S-8	6,7,14	r	1,2	Virchow	REF
S-8	6,8	r	1,2	Bsilla	11
S-8	8,6,7	r	1,2	Vircow	12
S-8	6,7	r	1,6	Nigeria	27
S-8	6,7	r	2	Wirchow	32
S-9	6,8	e,h	1,5	Kottbus	REF
S-9	6,8	e,h	1,2	Newport	10
S-9	6,8	e,h	e,n,z15	Tshiongwe	13
S-9	6,8,20	e,h	1,2	S. Newport	28
S-10	1,4,[5],12,27	b	e,n,x	Abony	REF
S-10	4,12	b	e,n,x	4,12:b:e,n,x	25
S-10	4,12	b	z6	Tripoli	29
S-11	1,3,19	m,t	-	Cannstatt	REF
S-11	19	g,m,t	-	Kouka	3
S-11	1,3,19	g,m,t	-	Kouka	4
S-11	3,10	m,t	-	Southbank	6
S-11	4,12	m.t	-	Banana	11
S-11	1,3,19	g,m,t	-	Kouka	13
S-11	3,19	g,m,t	-	3,19:g,m,t:-	25
S-11	3,19	g,t	-	Seftenberg	27
S-11	1,3,19	g,m,t	-	Kouka	31
S-11	3,19	g,m,t	-	Kouka	34
S-11	1,3,19	g,m,t	-	Kouka	36
S-14	1,4,[5],12	l,v	e,n,z15	Brandenburg	REF
S-14	4	l,w	e,n,z15	Brandenburg	18
S-17	6,7,14	y	1,5	Bareilly	REF
S-17	6,8	y	1,5	Tananarive	11

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	Lab code
S-17	6,7	e,h	1,5	Lomita	25
S-19	6,8	r	l,w	Goldcoast	REF
S-19	6,24	r	l,w	6,24:r:l,w	5
S-19	6,8	r	l,v	Goldcoast	27
S-20	16	c	l,w	Yoruba	REF
S-20	16	z	1, 7	Lingwala	30
S-20	16	c	e,n,x	Shamba	31

reference strain
remark (e.g. spelling error)
not typable (e.g. antisera not available, rough strain)
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 23rd Interlaboratory Comparison Study *Salmonella* serotyping (November 2018), 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	1,9,12	g,m	-	Enteritidis	9	g,m	-	Enteritidis
S2	1,4,12,27	d	1,7	Schwarzengrund	4,5,12	d	1,7	Schwarzengrund
S3	4,12,27	r	z6	Southampton	4,5,12	r	z6	Southampton
S4	6,7,14	r	1,5	Infantis	6,7	r	1.5	Infantis
S5	6,8	z10	e,n,x	Hadar	6,8	z10	e,n,x	Hadar
S6	1,4,[5],12	i	1,2	Typhimurium	4,5,12	i	1,2	Typhimurium
S7	11	b	1,5	Leeuwarden	11	b	1,5	Leeuwarden
S8	6,7,14	r	1,2	Virchow	6,7	r	1,2	Virchow
S9	6,8	e,h	1,5	Kottbus	6,8	e,h	1,5	Kottbus
S10	1,4,[5],12,[27]	b	e,n,x	Abony	1,4,5,12	b	e,n,x	Abony
S11	1,3,19	m,t	-	Cannstatt	1,3,19	g,m,t	-	Kouka
S12	1,4,[5],12	f,g	[1,2]	Derby	1,4,5	f,g	-	Derby
S13	1,9,12	z	1,5	Lawndale	1,9,12	z	1,5	Lawndale
S14	1,4,[5],12	l,v	e,n,z15	Brandenburg	4,5,12	l,v	e,n,z15	Brandenburg
S15	1,4,[5],12	i	1,5	Lagos	,4,5,12	i	1,5	Lagos
S16[a)]	1,4,[5],12	i	-	4,5:i:-	4,5,12	i	-	1,4,5,12:i:-
S17	6,7,14	y	1,5	Bareilly	6,7	y	1,5	Bareilly
S18	1,4,[5],12	e,h	e,n,x	Chester	4,5,12	e,h	e,n,x	Chester
S19	6,8	r	l,w	Goldcoast	6,8	r	l,w	Goldcoast
S20	16	c	l,w	Yoruba	16	c	l,w	Yoruba
S21[b)]	55	k	z39	55:k:z39				

a) Typhimurium, monophasic variant as determined by PCR.

b) *Salmonella enterica* subspecies *salamae*

Results 23rd Interlaboratory Comparison Study *Salmonella* serotyping (November 2018), 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 23rd EURL-*Salmonella* Workshop (Uppsala, 2018), 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 or grey 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: 1 -> 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**	**23 2018**
No. of participants	25	27	28	28	28	28	29	29	29	29	29	29
No. of strains evaluated	20	20	20	19	19*	20	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%)	575/580 (99%)
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%)	565/580 (97%)
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%)	563/580 (97%)
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%)	24/29 (83%)
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%)	19/29 (66%)
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%)	17/29 (59%)
No. of penalty points	35	30	36	16	22	20	17	18	16	6	3	19
No. of labs not achieving good performance	6	3	4	2	2	2	2	1	1	0	0	1
No. of labs not achieving good performance after follow-up	0	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	23 2018
No. of participants	26	29	31	33	36	31	34	35	34	34	35	36**
No. of strains evaluated	20	20	20	19	19*	20	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%)	689/700 (98%)
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%)	682/700 (97%)
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%)	675/700 (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%)	31/35 (89%)	28/35 (80%)
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%)	23/35 (66%)
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%)	20/35 (57%)
No. of penalty points	36	34	56	37	41	20	20	57	21	21	4	33
No. of labs not achieving good performance	6	4	5	4	4	2	2	2	1	2	0	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)	0	0

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

**1 new participant was not included in the evaluations, because only a limited set of antisera was available.

Annex 9 Evaluation of PFGE images per participant and per parameter

Lab code/ Parameter	**20**	**13**	**12**	**36**	**3**	**11**	**8**	**26**	**4**	**6**	**10**	**19**	**Total score per parameter**	**Average per parameter**
Image Acquisition & Running Conditions	1	3	3	4	3	2	3	4	4	4	4	4	39	3,3
Cell Suspension	2	2	2	3	4	4	4	4	4	4	4	4	41	3,4
Bands	1	3	2	2	4	4	4	4	4	4	4	4	40	3,3
Lanes	3	3	3	4	4	3	3	3	4	4	4	4	42	3,5
Restriction	3	1	4	2	4	4	4	4	4	4	4	4	42	3,5
Gel Background	3	4	2	2	2	4	4	3	3	3	3	4	37	3,1
DNA Degradation (smearing in lanes)	2	1	4	4	4	4	4	4	4	4	4	4	43	3,6
Total score per participant	15	17	20	21	25	25	26	26	27	27	27	28		
Average per participant	2,1	2,4	2,9	3	3,6	3,6	3,7	3,7	3,9	3,9	3,9	4		

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

Annex 10 Evaluation of the analysis of the provided PFGE image in BioNumerics per participant and per parameter

Lab code/ Parameter	**3**	**12**	**11**	**13**	**4**	**8**	**19**	**20**	**6**	**10**	**26**	**Total score per parameter**	**Average per parameter**
Position of gel	3	2	3	3	3	2	2	3	3	3	3	30	2,7
Strips	3	2	3	3	3	3	3	3	2	3	3	31	2,8
Curves	2	2	2	2	3	3	3	3	3	3	3	29	2,6
Normalisation	2	3	2	2	2	3	3	2	3	3	3	28	2,5
Band assignment	1	2	2	2	2	2	2	2	3	2	2	22	2,0
Total score per participant	11	11	12	12	13	13	13	13	14	14	14		
Average per participant	2,2	2,2	2,4	2,4	2,6	2,6	2,6	2,6	2,8	2,8	2,8		

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

Annex 11 Parameters as set by the participants for analysis of the 'Provided PFGE gel TRO 2018' in Bionumerics

Lab code/Parameter	3	4	6	8	10	11	12	13	19	20	26	REF
Strips: Image strip extraction Thickness (pts)	27	29	39	33	37	31	31	29	33	31	35	34
Curves: Averaging thickness (pts)	49	9	13	25	19	49	49	7	11	9	19	9
Background substraction Apply Disk size (%)	10	10	9	15	15	15	10	9	99	99	9	9
Apply least square filtering Cutt off below (%)	1.20	1.10	0.96	0.75	0.75	0.75	1.17	1.10	0.92	1.00	0.95	0.89

Annex 12 Examples of PFGE images obtained by the participants

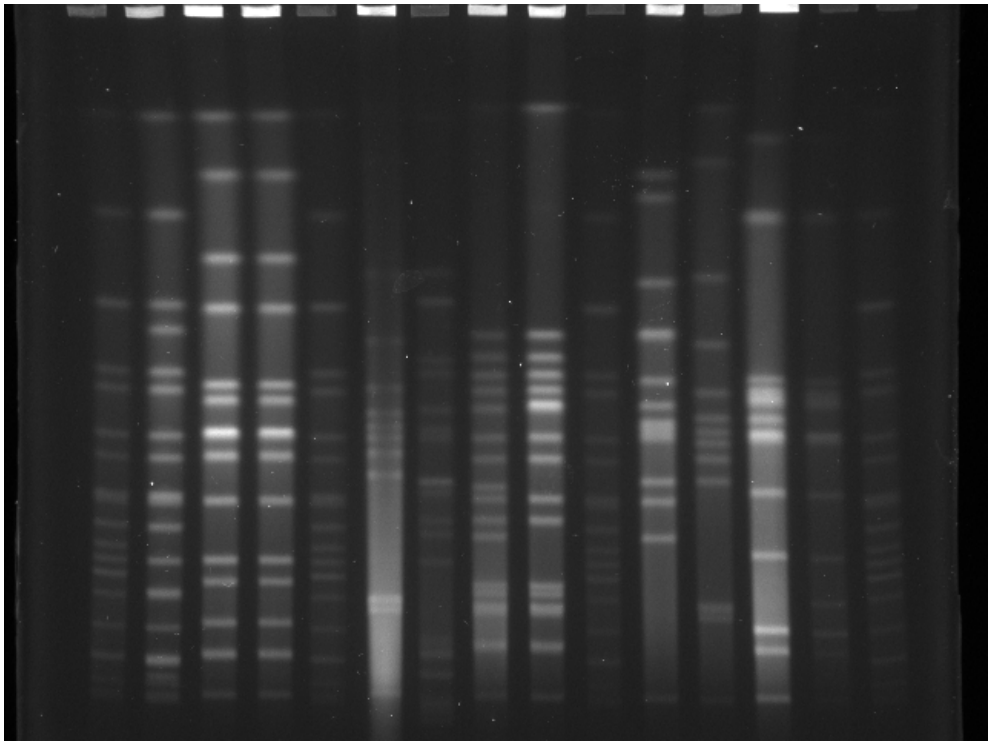


Figure A12.1. Example of a gel (lab code 20) with a generally lower score

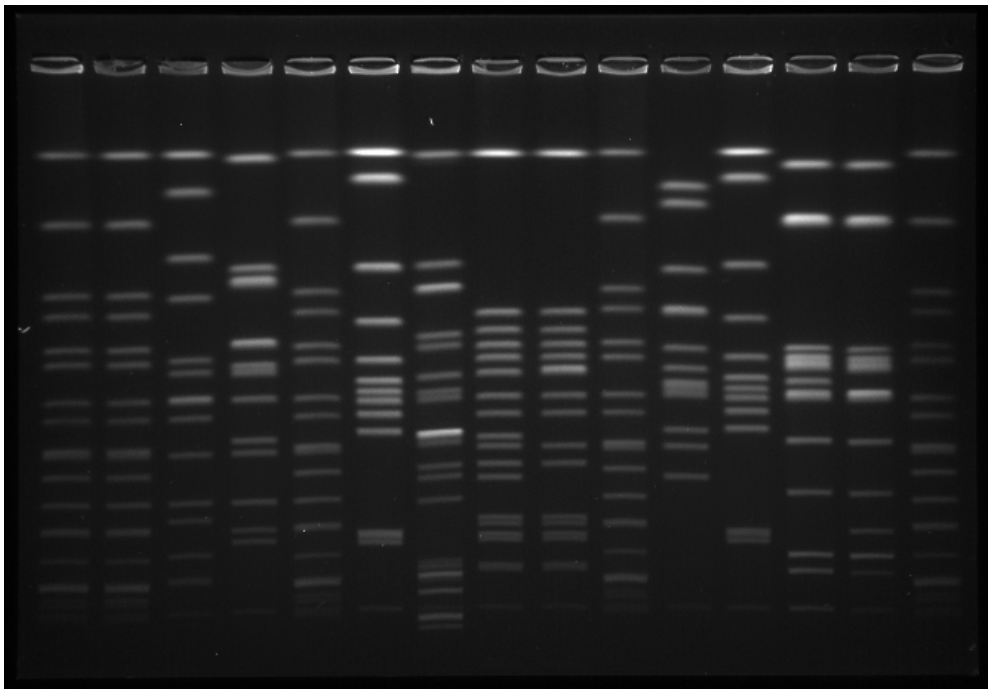
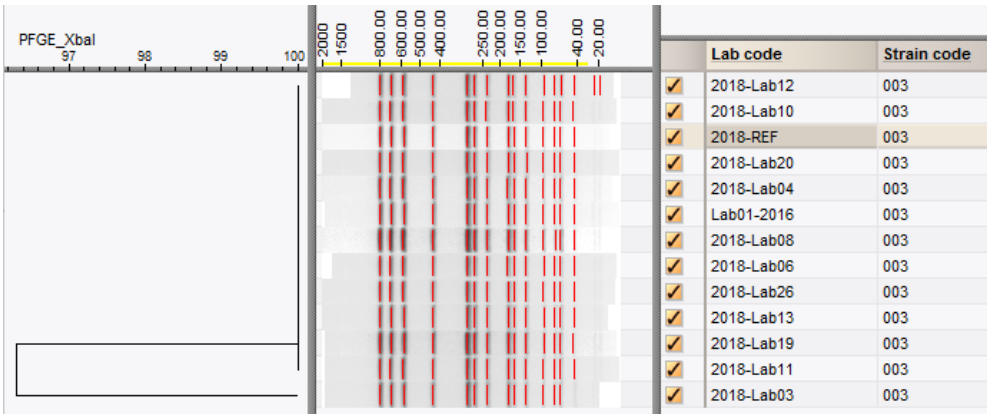


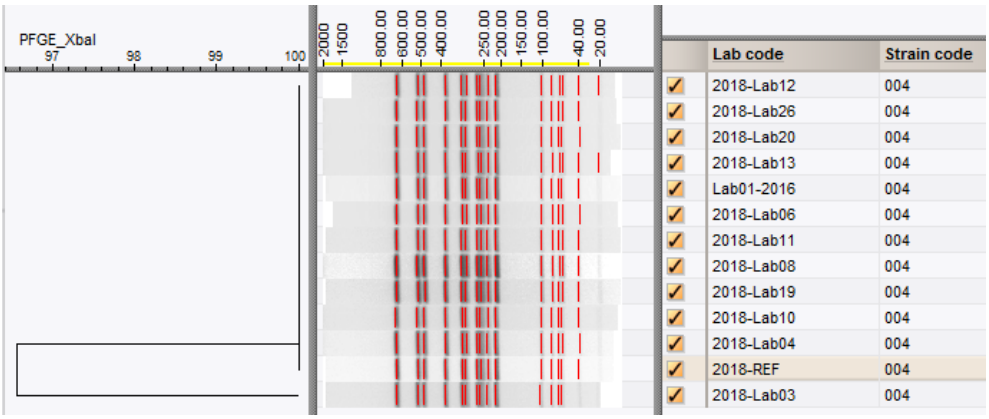
Figure A12.2. Example of a gel (lab code 19) with a generally high score

Annex 13 Comparison of all participants' results for analysis of the 'Provided PFGE gel TRO 2018' in Bionumerics, per test strain.

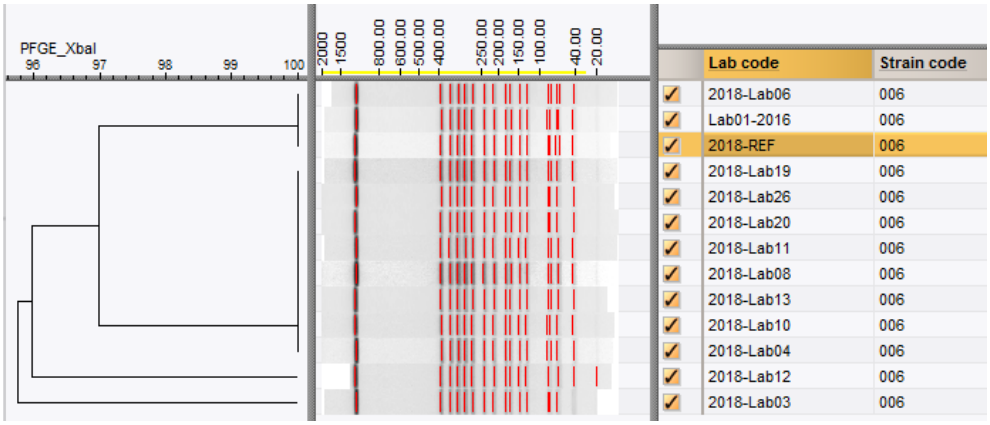
Lab code	Strain code
2018-Lab06	002
2018-REF	002
2018-Lab26	002
2018-Lab11	002
Lab01-2016	002
2018-Lab13	002
2018-Lab20	002
2018-Lab19	002
2018-Lab10	002
2018-Lab12	002
2018-Lab08	002
2018-Lab04	002
2018-Lab03	002



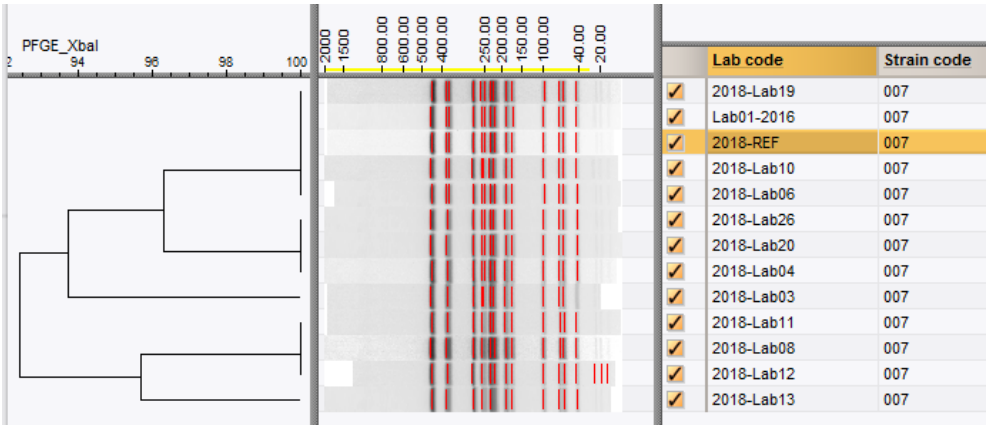
Lab code	Strain code
2018-Lab12	003
2018-Lab10	003
2018-REF	003
2018-Lab20	003
2018-Lab04	003
Lab01-2016	003
2018-Lab08	003
2018-Lab06	003
2018-Lab26	003
2018-Lab13	003
2018-Lab19	003
2018-Lab11	003
2018-Lab03	003



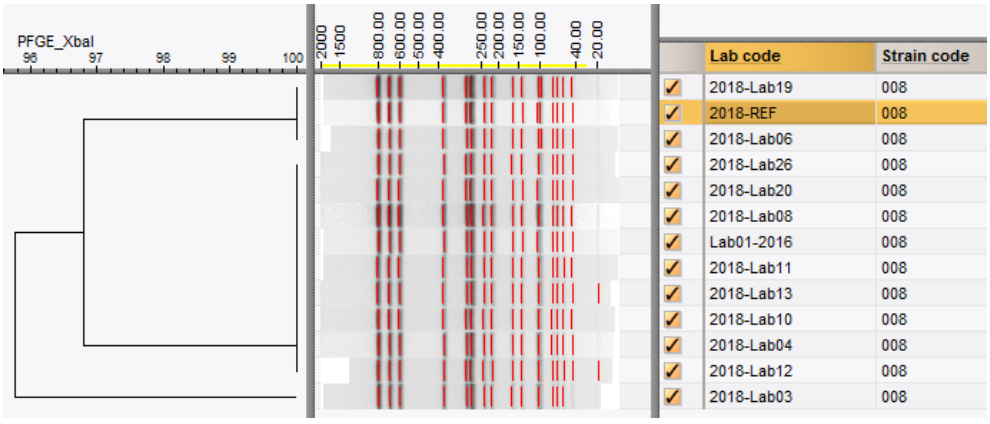
Lab code	Strain code
2018-Lab12	004
2018-Lab26	004
2018-Lab20	004
2018-Lab13	004
Lab01-2016	004
2018-Lab06	004
2018-Lab11	004
2018-Lab08	004
2018-Lab19	004
2018-Lab10	004
2018-Lab04	004
2018-REF	004
2018-Lab03	004



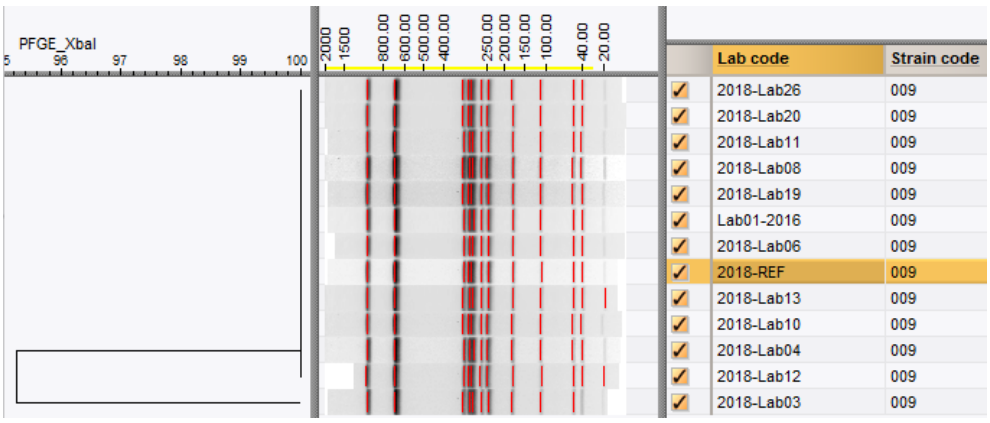
Lab code	Strain code
2018-Lab06	006
Lab01-2016	006
2018-REF	006
2018-Lab19	006
2018-Lab26	006
2018-Lab20	006
2018-Lab11	006
2018-Lab08	006
2018-Lab13	006
2018-Lab10	006
2018-Lab04	006
2018-Lab12	006
2018-Lab03	006



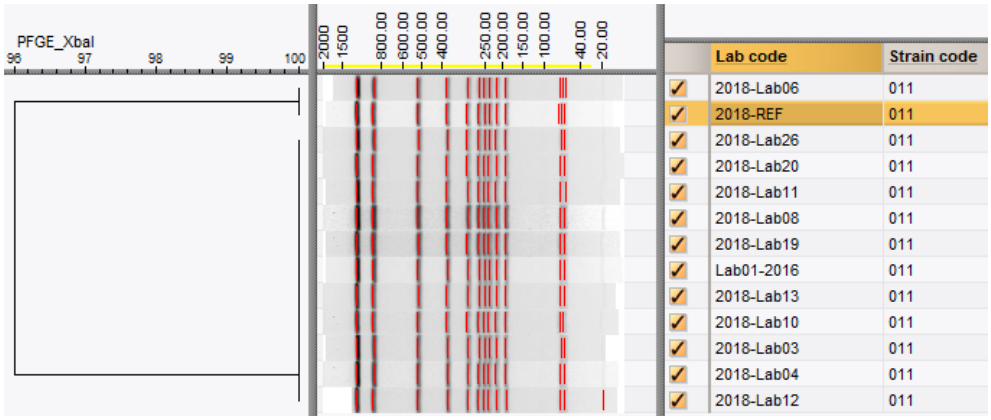
Lab code	Strain code
2018-Lab19	007
Lab01-2016	007
2018-REF	007
2018-Lab10	007
2018-Lab06	007
2018-Lab26	007
2018-Lab20	007
2018-Lab04	007
2018-Lab03	007
2018-Lab11	007
2018-Lab08	007
2018-Lab12	007
2018-Lab13	007



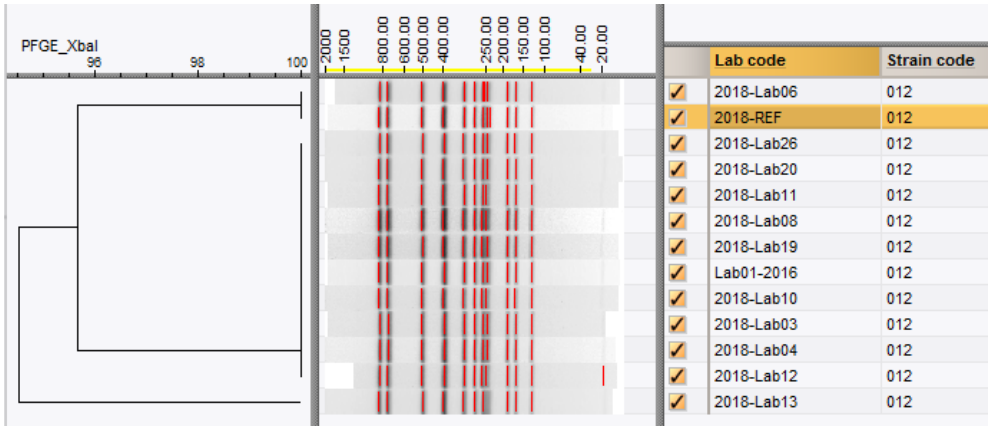
Lab code	Strain code
2018-Lab19	008
2018-REF	008
2018-Lab06	008
2018-Lab26	008
2018-Lab20	008
2018-Lab08	008
Lab01-2016	008
2018-Lab11	008
2018-Lab13	008
2018-Lab10	008
2018-Lab04	008
2018-Lab12	008
2018-Lab03	008



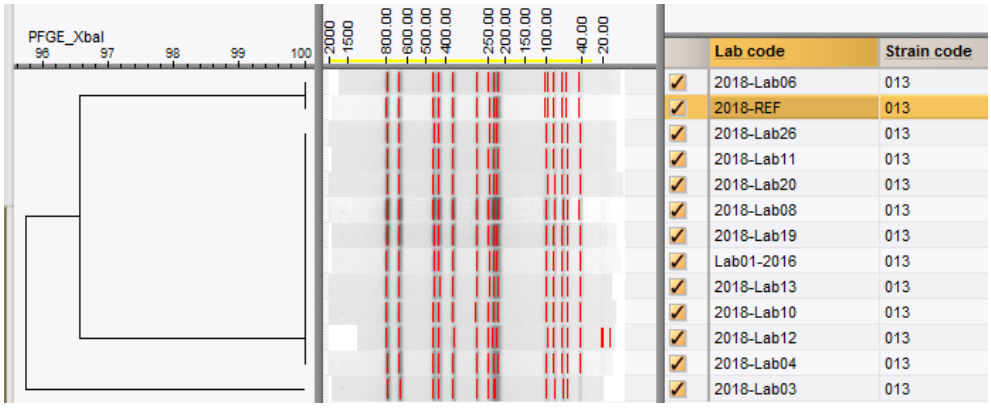
Lab code	Strain code
2018-Lab26	009
2018-Lab20	009
2018-Lab11	009
2018-Lab08	009
2018-Lab19	009
Lab01-2016	009
2018-Lab06	009
2018-REF	009
2018-Lab13	009
2018-Lab10	009
2018-Lab04	009
2018-Lab12	009
2018-Lab03	009



Lab code	Strain code
2018-Lab06	011
2018-REF	011
2018-Lab26	011
2018-Lab20	011
2018-Lab11	011
2018-Lab08	011
2018-Lab19	011
Lab01-2016	011
2018-Lab13	011
2018-Lab10	011
2018-Lab03	011
2018-Lab04	011
2018-Lab12	011



Lab code	Strain code
2018-Lab06	012
2018-REF	012
2018-Lab26	012
2018-Lab20	012
2018-Lab11	012
2018-Lab08	012
2018-Lab19	012
Lab01-2016	012
2018-Lab10	012
2018-Lab03	012
2018-Lab04	012
2018-Lab12	012
2018-Lab13	012



Lab code	Strain code
2018-Lab06	013
2018-REF	013
2018-Lab26	013
2018-Lab11	013
2018-Lab20	013
2018-Lab08	013
2018-Lab19	013
Lab01-2016	013
2018-Lab13	013
2018-Lab10	013
2018-Lab12	013
2018-Lab04	013
2018-Lab03	013

Annex 14 Example of an individual laboratory evaluation report on PFGE typing results

Individual Laboratory Results Interlaboratory Comparison Study
Salmonella PFGE typing (November 2018), Page 1 of 3

NRL Laboratory code: **13**

General comments:
Your TIFF file was not sent as an uncompressed 8-bit grey scale, which was requested. We had to convert your TIFF file before our evaluation in BioNumerics.
Many lanes with failing results, this makes the evaluation of the image quite difficult.
In routine, it might still be possible to analyse results for strains P01, P02, P06, P07, P08.

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

Parameter	Evaluation	Comments	Points*
Image Acquisition and Running Conditions	Good	Bottom band of standard not 1-1,5 cm from bottom of gel but analysis not affected.	3
Cell Suspension	Fair	>2 lanes contain darker or lighter bands than the other lanes, making the gel difficult to analyse	2
Bands	Good	Bands are slightly fuzzy.	3
Lanes	Good	Light curving.	3
Restriction	Poor	More than 1 lane with several shadow bands.	1
Gel Background	Excellent	Clear.	4
DNA Degradation (smearing in the lanes)	Poor	Smearing making several lanes unanalysable.	1
Total score:			17

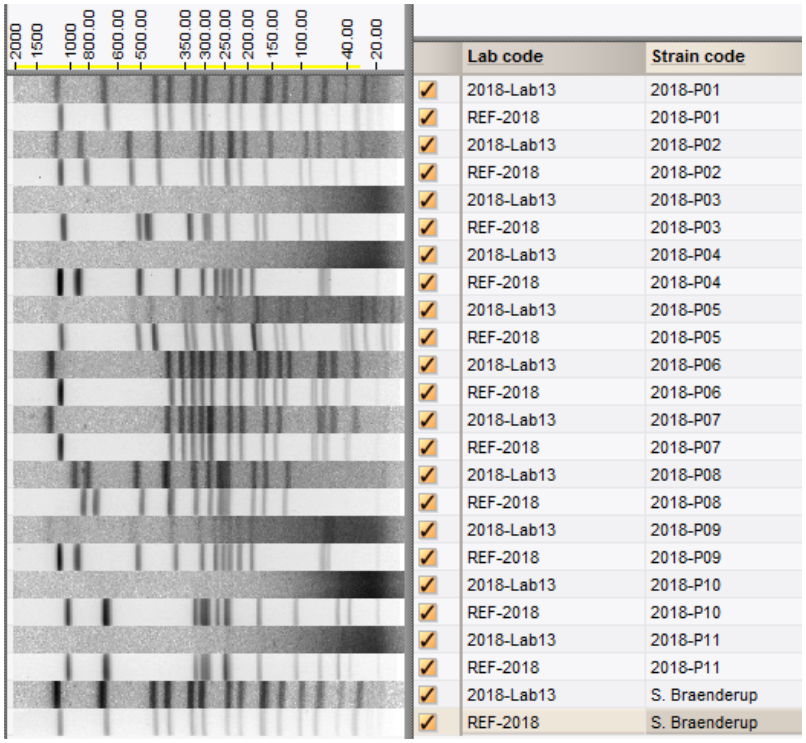
* 1=Poor, 2=Fair, 3= Good, 4= Excellent. At maximum 4 points per parameter

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	Fair/Good	Curve extraction is defined too narrowly.	2
Normalisation	Fair/Good	One bottom band < 33 kb is not assigned in one of the reference lanes.	2
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:			12

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

Individual Laboratory Results Interlaboratory Comparison Study
Salmonella PFGE typing (November 2018), Page 2 of 3



	Lab code	Strain code
✓	2018-Lab13	2018-P01
✓	REF-2018	2018-P01
✓	2018-Lab13	2018-P02
✓	REF-2018	2018-P02
✓	2018-Lab13	2018-P03
✓	REF-2018	2018-P03
✓	2018-Lab13	2018-P04
✓	REF-2018	2018-P04
✓	2018-Lab13	2018-P05
✓	REF-2018	2018-P05
✓	2018-Lab13	2018-P06
✓	REF-2018	2018-P06
✓	2018-Lab13	2018-P07
✓	REF-2018	2018-P07
✓	2018-Lab13	2018-P08
✓	REF-2018	2018-P08
✓	2018-Lab13	2018-P09
✓	REF-2018	2018-P09
✓	2018-Lab13	2018-P10
✓	REF-2018	2018-P10
✓	2018-Lab13	2018-P11
✓	REF-2018	2018-P11
✓	2018-Lab13	S. Braenderup
✓	REF-2018	S. Braenderup

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

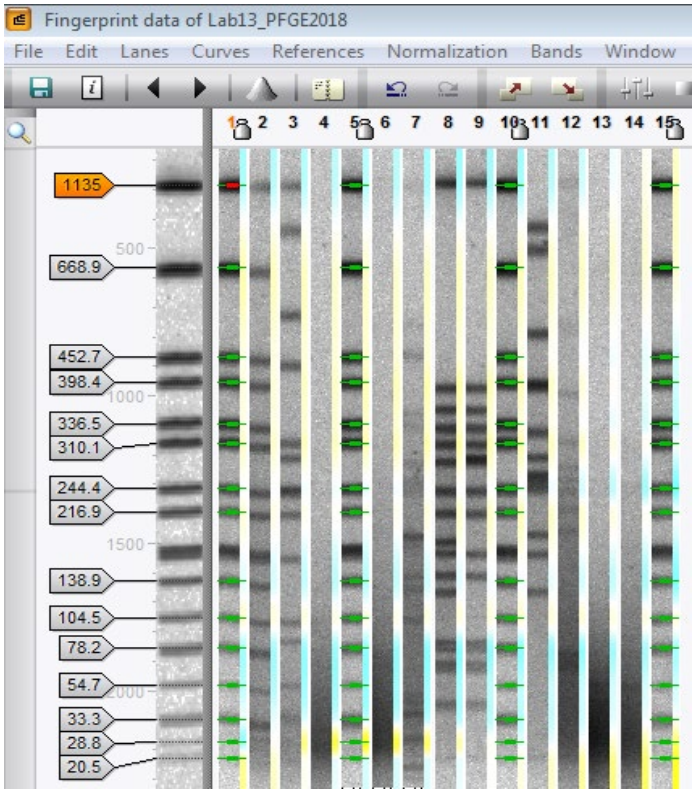
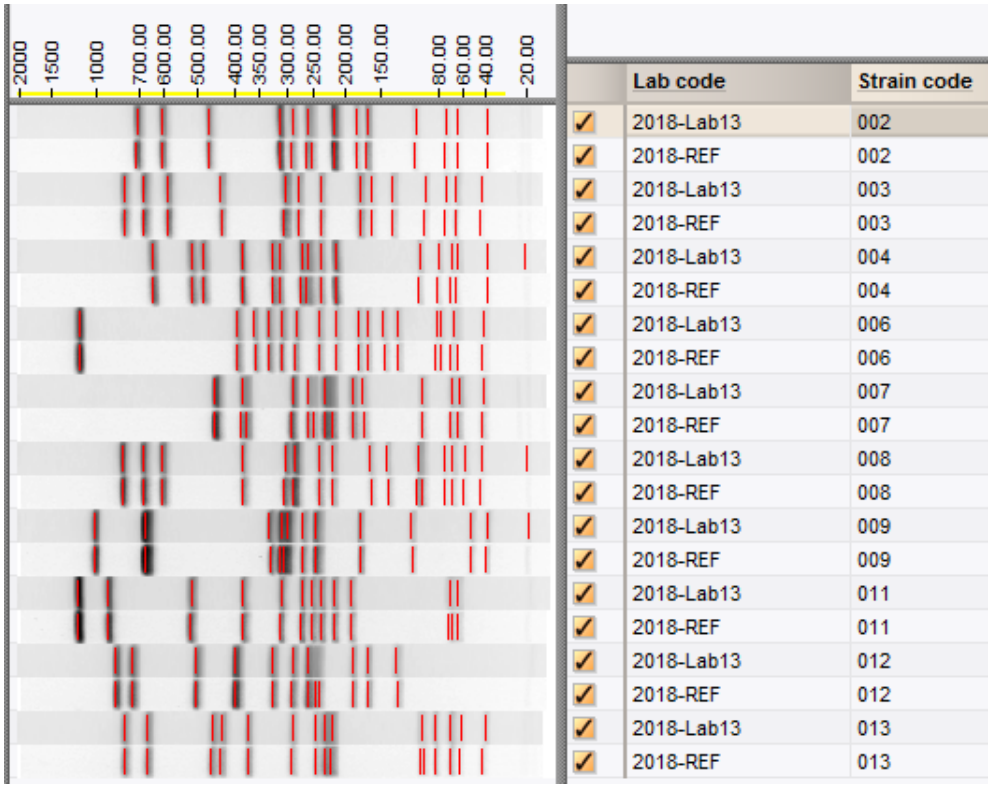


Figure 2. Display of the "Distortion bar" option in Bionumerics of your gel Darker colours indicate critical normalisation.

Individual Laboratory Results Interlaboratory Comparison Study
Salmonella PFGE typing (November 2018), Page 3 of 3



	Lab code	Strain code
✓	2018-Lab13	002
✓	2018-REF	002
✓	2018-Lab13	003
✓	2018-REF	003
✓	2018-Lab13	004
✓	2018-REF	004
✓	2018-Lab13	006
✓	2018-REF	006
✓	2018-Lab13	007
✓	2018-REF	007
✓	2018-Lab13	008
✓	2018-REF	008
✓	2018-Lab13	009
✓	2018-REF	009
✓	2018-Lab13	011
✓	2018-REF	011
✓	2018-Lab13	012
✓	2018-REF	012
✓	2018-Lab13	013
✓	2018-REF	013

Figure 3. Comparison of your analysis in Bionumerics with the reference analysis of the Provided PFGE gel TRO2018

EURL-*Salmonella*, Bilthoven, The Netherlands

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