Overall PFGE typing results

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

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Introduction

This document provides an overview of the results as produced by the participants in the EURL-*Salmonella* Interlaboratory Comparison Study on PFGE typing - 2017.
The individual laboratory results were sent to each of the participants separately.

Draft results were presented at the EURL-*Salmonella* Workshop in May 2018 (Uppsala), and the full results will be reported in more detail in the final report on the 22nd *Salmonella* typing study (2017).

Strains

A total of 11 *Salmonella* strains (coded P01–P11) were sent to the participants in the 2017-study on PFGE typing. Background information on the strains is given in Table 1.
Table 1 also indicates the codes of the test strains as shown in the image that was 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 1. Background information on the Salmonella *strains used for PFGE typing in 2017*

Strain code in 2017 Study Quality PFGE gel image (EURL-*Salmonella*)	Strain code in EQA-8 (ECDC/SSI, 2017)		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

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 electronic test report. The PFGE gel images were to be emailed as an uncompressed 8-bit gray scale Tagged Image File Format (TIFF) files to the EURL-*Salmonella*, and had to include the laboratory code in the filename.
A total of 15 participants sent in a PFGE gel image for evaluation.
The evaluation was done on the quality of the PFGE images and quality grading was done according to the PulseNet guidelines (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.
These guidelines use 7 parameters, which are scored with 1 (poor) to 4 (excellent) points.

In general, an acceptable quality should be obtained for each parameter since a low quality score in just one category can have a high impact on the ability to further analyse the image and compare to other profiles.
The scores per NRL (n=15), broken down across the seven parameters (see Annex 1), are given in Table 2. The scores per parameter are shown in Figure 1.

Evaluation of the analysis of the gel in Bionumerics
Like before, the evaluation of the (optional) analysis of a gel in Bionumerics was included in the study as well. New this year was the use of a common gel for all participants, sent by email on 22-11-2017 and named "Provided PFGE gel TRO2017". This gel image was kindly provided by the Statens Serum Institute (SSI) in Kopenhagen, as also used in the 8th EQA for the FWD network.

A total of 10 participants sent in their analysed gel data for evaluation.
In short, this included the following actions by the participants:

- start a new database in Bionumerics,
- import the pre-configured database set-up as sent by email,
- import the provided tif 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 files in a zipped format and properly named to the EURL-*Salmonella*.

Evaluation of the analysis of the gel in Bionumerics was done according to the guidelines as used in the EQAs for the FWD laboratories (Annex 2).
These guidelines use 5 parameters, which are scored with 1 (poor), 2 (fair/good) or 3 (excellent) points.
The scores per NRL (n=10), broken down across the five parameters (see Annex 2), are given in Table 3. The scores per parameter are shown in Figure 2.

Several participants (Labs 18, 20, 25, 35) tended to assign bands of test strains also below 33 kb, which is not to be done according to 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.
In the end, two participants (Lab 12 and lab 34) analysed all 11 test strains in the provided gel image in complete agreement with the reference analysis.

Figure 1. Evaluation of the quality of the PFGE images in scores per parameter, 2017 study

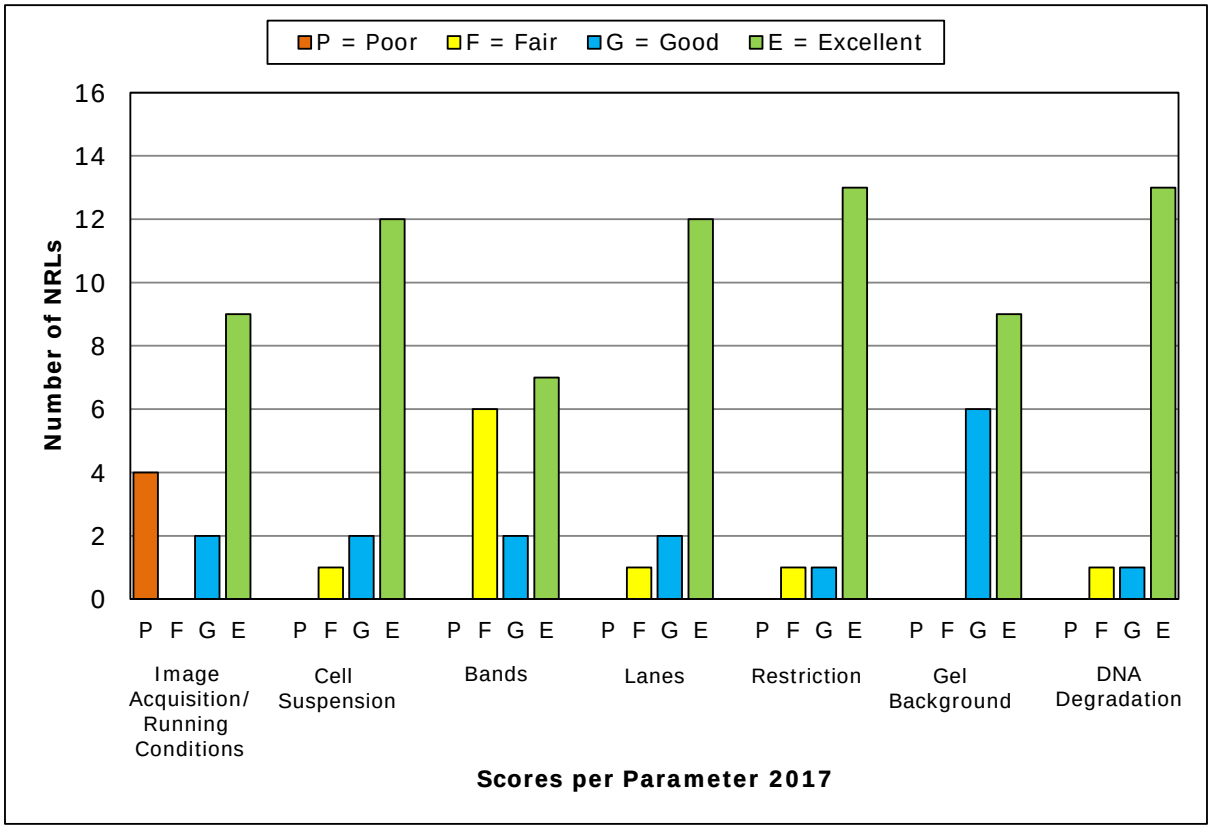

Figure 2. Evaluation of the analysis of the gel in Bionumerics in scores per parameter, 2017 study

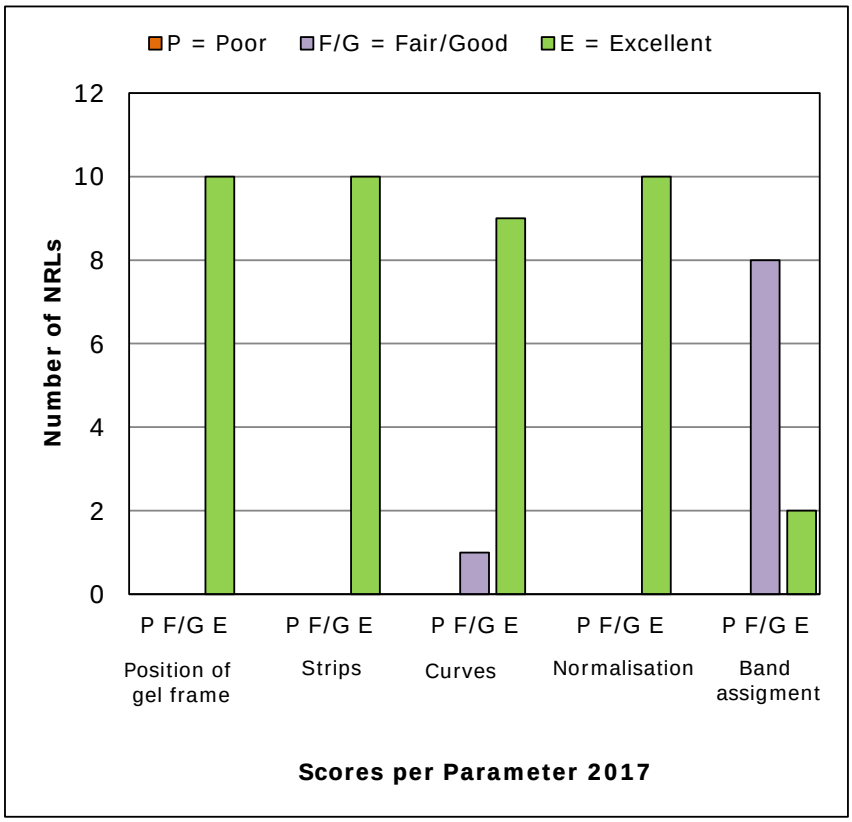

Table 2. Evaluation of the PFGE images per participant and per parameter

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 and 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.

Table 3. Evaluation of the analysis of the provided PFGE image 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 1 PulseNet Guidelines on quality grading of PFGE images

Evaluation on the quality of the PFGE images according to the PulseNet guidelines (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

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Restriction	Complete restriction in all lanes	- One to two faint shadow bands on gel	- One lane with many shadow bands - A few shadow bands spread out over several lanes	- Greater than 1 lane with several shadow bands - Lots of shadow bands over the whole gel
Gel Background	Clear	- Mostly clear background - Minor debris present that doesn't affect analysis	- Some debris present that may or may not make analysis difficult (e.g., auto band search finds too many bands) - Background caused by photographing a gel with very light bands (image contrast was "brought up" in photographing gel-makes image look grainy)	- Lots of debris present that may or may not make analysis difficult (i.e., auto band search finds too many bands)
DNA Degradation (smearing in the lanes)	Not present	- Minor background (smearing) in a few lanes but bands are clear	- Significant smearing in 1-2 lanes that may or may not make analysis difficult - Minor background (smearing) in many lanes	- Significant smearing in >2 lanes that may or may not make analysis difficult - Smearing so that a lane is not analyzable (except if untypeable [thiourea required])

5. FLOW CHART:

6. BIBLIOGRAPHY:

7. CONTACTS:

8. AMENDMENTS:

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ANNEX 2 Evaluation of gel analysis of PFGE images in Bionumerics

Evaluation of gel analysis of PFGE images in Bionumerics according to the EQAs for the FWD laboratories (European Centre for Disease Prevention and Control. Seventh external quality assessment scheme for *Salmonella* typing. Stockholm: ECDC; 2016. Available at: http://ecdc.europa.eu/en/publications/Publications/salmonella-typing-seventh-external-quality-assessment.pdf).

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.
Normalization	- 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.