Interim summary report EURL-*Salmonella* PT PFGE typing 2018

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15 April 2019

Introduction

This document provides an overview of the results as produced by the participants in the EURL-*Salmonella* Proficiency Test (PT) on PFGE typing - 2018.
The individual laboratory results were sent to each of the participants separately.

Summary results will presented at the EURL-*Salmonella* Workshop in May 2019 (Amersfoort), and the full results will be reported in more detail in the final report on the 23rd *Salmonella* typing study (PT 2018).

Strains

A total of 11 *Salmonella* strains (coded P01–P11) were sent to the participants in the 2018-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 2018"). 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 2018*

Strain codes in 2018 Study Quality PFGE gel image	Corresponding strain codes in previous studies		Strain codes in 2018 Study Provided gel analysis in BN
P01	*S*. Braenderup H9812		002
P02	2013-P5		003
P03	2015-P5		004
P04	2013-P8		006
P05	2014-P6		007
P06	2013-P10		008
P07	2017-P5		009
P08 (a)	2016-P9		011
P09	2017-P10		012 (a)
P10	2014-P9		013
P11	2014-P7		014

(a) 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 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 12 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 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.
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=12), 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

The evaluation of the (optional) analysis of a gel in Bionumerics was included in the study as well. Like last year, a common gel for all participants was used for this, sent by email on 8-11-2018 and named “Provided PFGE gel TRO2018”. This gel image was the TIFF file as sent in by Lab01 for the EURL-*Salmonella* PT on PFGE typing in 2016.

A total of 11 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=11), broken down across the five parameters (see Annex 2), are given in Table 3. The scores per parameter are shown in Figure 2.
Table 4 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”.

Figure 1. Evaluation of the quality of the PFGE images in scores per parameter, 2018 study

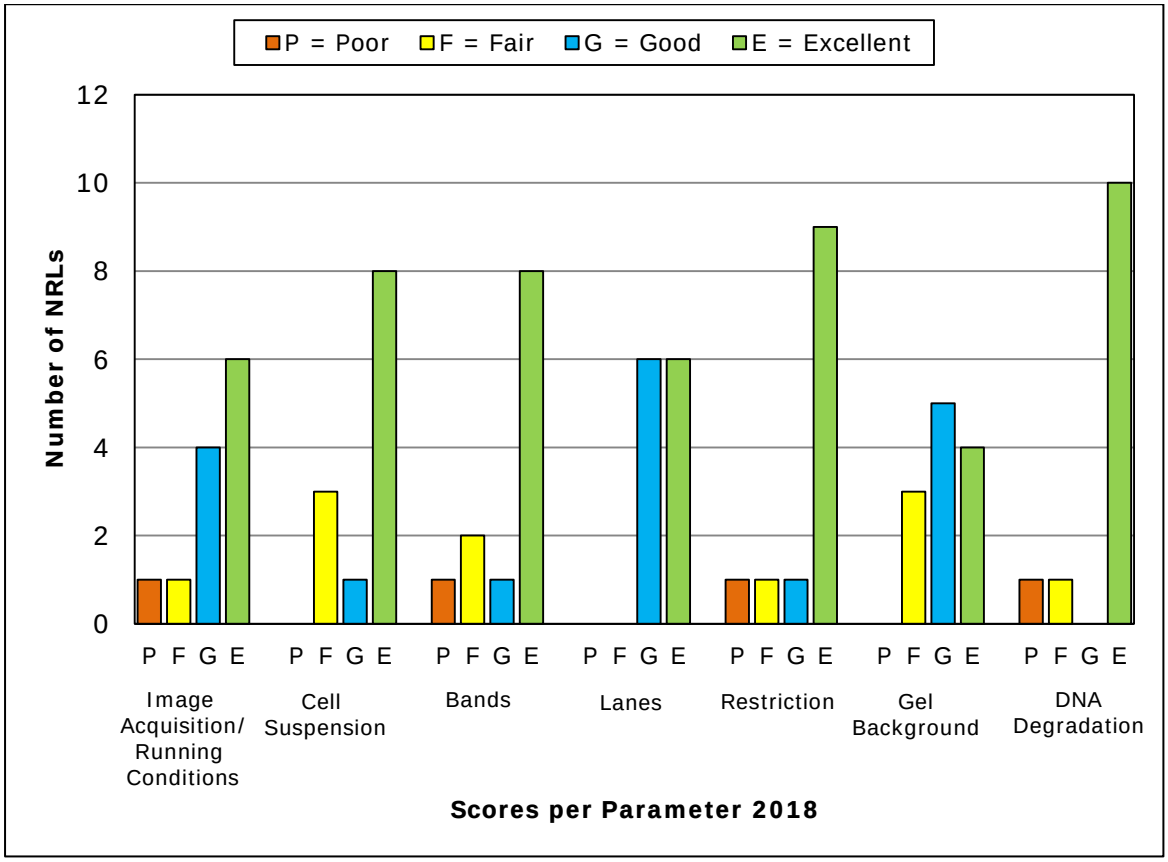

Figure 2. Evaluation of the analysis of the gel in Bionumerics in scores per parameter, 2018 study

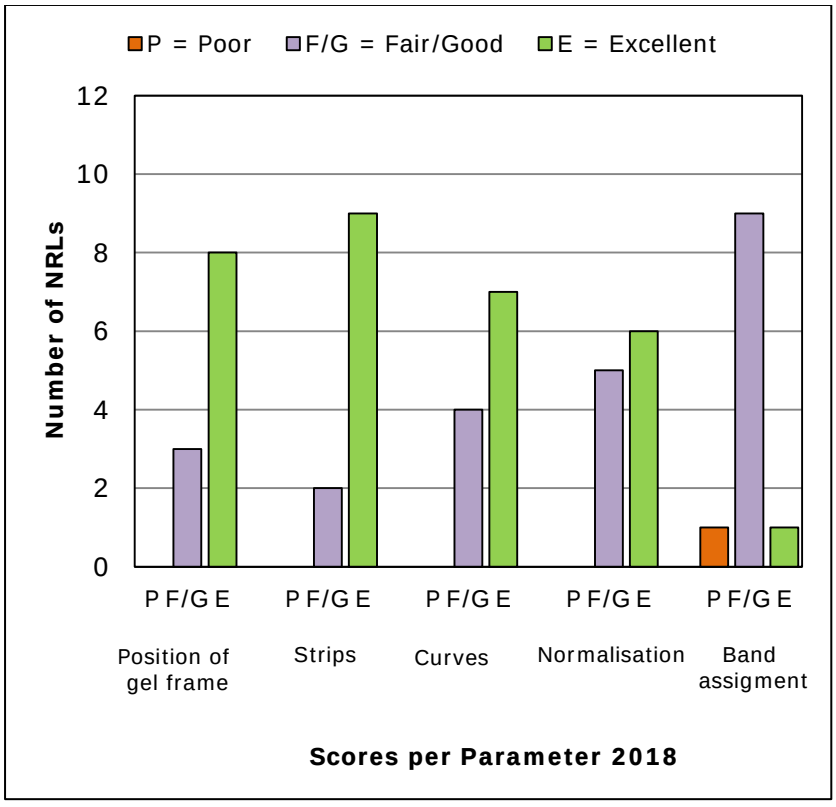

Table 2. Evaluation of the PFGE images per participant and per parameter

Lab code / Parameter	20	13	12	36	3	11	8	26	4	6	10	19	Total score per parameter	Average per parameter
Image Acquisition and 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.

Table 3. 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.

Table 4. 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 1 PulseNet 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: http://ecdc.europa.eu/en/publications/Publications/salmonella-typing-seventh-external-quality-assessment.pdf).

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 too light to distinguish.	- Some band distortion (i.e. nicks) in two or three lanes, but still analysable. - Fuzzy bands. - Some bands (four or five) are too thick. - Bands at the bottom or top of the gel are light but still analysable.	- Slight band distortion in one lane, but analysis is not affected. - Bands are slightly fuzzy and/or slanted. - A few bands (three or less) are difficult to see clearly (i.e. DNA overload) especially at the bottom of the gel.	Clear and distinct all the way to 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 towards the right or left, but still analysable.	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 (i.e. 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

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.