Protocol of the 22nd EURL-*Salmonella* Interlaboratory Comparison Study (November 2017) on serotyping and PFGE typing of *Salmonella* strains, for the NRL-*Salmonella* laboratories

Version 2, dated 22-11-2017, with updates on the part of PFGE typing in grey

Introduction

The European Union Reference Laboratory (EURL) - *Salmonella* organises the 22nd interlaboratory comparison study on the typing of *Salmonella* strains amongst the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*).
The main objective of this typing study is to test the performance of the participating laboratories for serotyping and optionally PFGE typing of *Salmonella* spp.
The study will take place in week 44 and onwards. The timetable can be found on page 4 of this protocol.

Like in the last years, all data have to be reported through an electronic result form. The link for this will be sent to you by email, and will also become available at the EURL-*Salmonella* website. **Submission of serotyping data** has to be finalised on **15 December 2017** at the latest.

The study on PFGE typing will use a separate web based test report, and this link will be sent to the participants in a second email. Deadline for the electronic **submission of PFGE typing results** is **22 December 2017** at the latest.

Transportation of the *Salmonella* strains to the laboratories

The strains for the serotyping part and/or the PFGE part of the study will be transported all in one (larger) parcel. The strains will be sent as Biological Substance Category B (UN 3373) with a door-to-door courier to your laboratory.
The shipment of the strains is scheduled for Monday 30 October 2017.

Serotyping

A total number of 20 *Salmonella* strains (coded S1 - S20) have to be serotyped.
An additional *Salmonella* strain (S-21), being a less common *Salmonella* strain, is also included in the package and serotyping of this strain is optional.
The method routinely performed in your laboratory has to be used in the study. Each laboratory is allowed to send strains for serotyping to another reference laboratory in their country, if this is part of the normal routine procedure.
As explained at the recent EURL-*Salmonella* Workshops, please note to be very careful in following the exact instructions of the various manufacturers of the different sera available.

The results for each strain have to be reported with the formula for the O-antigens and H-antigens and the serovar names according to the White-Kauffman-le Minor scheme of 2007 (https://www.pasteur.fr/sites/default/files/veng_0.pdf)
Laboratories have to report only those results, on which the identification of serovar names is based. Definite conclusions can only be based on agglutination with mono-specific antisera. Otherwise it is better to identify the strains by giving the antigenic formula as far as detected.

Examples of preferred reporting:

O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar name
9,12	g,m	-	Enteritidis
4,12	i	2	Typhimurium
4,5,12	i	-	4,5,12:i:-
6,7	-	1,5	6,7:-:1,5
42	g,t	-	42:g,t:-

The evaluation of the serotyping results will be performed by the EURL-*Salmonella* according to Table 1.

Table 1. Evaluation of serotyping results

Results	Evaluation
Auto-agglutination or Incomplete set of antisera (outside range of antisera)	Not typable
Partly typable due to incomplete set of antisera or Part of the formula (for the name of the serovar) or No name serovar	Partly correct
Wrong serovar or mixed sera formula	Incorrect

Hendriksen et al. (J Clin Microbiol 47(9): 2729-2736) reported that colonial form variation may occur with the expression of the O:6_1 antigen by some serogroup C_2 serovars.
Concerning the EURL-*Salmonella* interlaboratory comparison studies on serotyping it was decided to consider the serovar pairs involved (e.g. *S.* Newport/*S.* Bardo and *S.* Hadar/*S.* Istanbul) not as distinct serovars, though they should be reported as actually typed by the participants.
In practice this means that for example a 6,8:z_{10}:e,n,x typed strain has to be reported as Hadar, and a 8:z_{10}:e,n,x typed strain has to be reported as Istanbul, but that either result is considered as correct.

In 2007, criteria for 'good performance' have been defined (Mooijman, 2007). Penalty points are given for strains that are typed incorrectly. A distinction is made between the five most important human health related *Salmonella* serovars (as indicated in EU legislation) and all other strains:

- **4 penalty points**: Incorrect typing of *S*. Enteritidis, *S*. Typhimurium (including the monophasic variant), *S*. Hadar, *S*. Infantis or *S*. Virchow
  or assigning the name of one of these five serovars to another strain.
- **1 penalty point**: Incorrect typing of all other *Salmonella* serovars.

For each NRL-*Salmonella* the total number of penalty points is determined. The NRL meets the criterion of 'good performance' if it has fewer than four penalty points.
A follow-up study will be organised for NRLs with four penalty points or more. All NRLs of the EU Member States not meeting the criterion of 'good performance' have to participate in this follow-up study.

PFGE typing
A total number of 11 *Salmonella* strains will be included in the PGFE typing study, coded P1 - P11.
Participants are asked to test these strains using their own routine PGFE method for this and give details on it in the **electronic test report**.
However, our recommended method can be found in:

- Jacobs W, Kuiling S and van der Zwaluw K, 2014. Molecular typing of *Salmonella* strains isolated from food, feed and animals: state of play and standard operating procedures for pulsed field gel electrophoresis (PFGE) and Multiple-Locus Variable number tandem repeat Analysis (MLVA) typing, profiles interpretation and curation. EFSA supporting publication 2014:EN-703, 74 pp.
  (Available at:
  http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/703e.pdf).

Annex C of this report in fact describes the Standard PulseNet protocol *Salmonella* PFGE (Standard Operating Procedure for PulseNet PFGE of *Escherichia coli* O157:H7, *Escherichia coli* non-O157 (STEC), *Salmonella* serotypes, *Shigella sonnei* and *Shigella flexneri*. PNL05,

effective date 03-24-2013. Available at: http://www.pulsenetinternational .org/assets/PulseNet/uploads/pfge/PNL05_Ec-Sal-ShigPFGEprotocol.pdf).

In addition, participants are requested to **email their PFGE gel images** as *an uncompressed 8-bit gray scale* ***TIFF file*** to wilma.jacobs@rivm.nl
Be sure to include at least your **laboratory code** in the name of these .tif files, preferably like: Lab99_PFGE2017.tif

The evaluation of the PFGE typing results, after digestion with XbaI, will be done on the quality of the PFGE images and quality grading will be done according to the guidelines as used in the EQAs for the FWD laboratories (European Centre for Disease Prevention and Control. Seventh external quality assessment scheme for *Salmonella* typing. Stockholm: ECDC; 2016) and shown in Annex 1 of this Protocol. These guidelines are considered as an updated version of the PulseNet guidelines that were used in our *Salmonella* typing studies of 2013 - 2015.
To comply with these guidelines the reference strain S. Braenderup H9812 must be run in every 6 lanes as a minimum.

Like before, we also offer the evaluation of the (optional) **analysis of a gel in Bionumerics**, but this year this will be on **a common gel, provided to you** by email on 22-11-2017.
In this case, use Bionumerics to analyse **the gel named "Provided PFGE gel TRO2017"** by creating a new local database with correct experiment settings and entry-fields. The pre-configured database and instructions for use will be sent to the participants in week 47. Analyse the gel in Bionumerics including normalisation and band assignment (also see EFSA supporting publication 2014:EN-703). Prepare the ZIP (Bionumerics 7) or XML export files (Bionumerics 6 or below) according to the instructions, *including all test strains and reference strains*, as well as the TIFF image. **Email all data in a ZIP file** to wilma.jacobs@rivm.nl
Be sure to rename your zip file to include at least your **laboratory code** in the name, preferably like: Lab01_PFGE2017.zip

Evaluation of the analysis of the provided gel in Bionumerics will be done again according to the guidelines as used in the EQAs for the FWD laboratories (European Centre for Disease Prevention and Control. Seventh external quality assessment scheme for *Salmonella* typing. Stockholm: ECDC; 2016) and shown in Annex 2 of this protocol.

Reporting of the results
As usual, all data have to be reported through an electronic result form. The link, also to become available on the EURL-*Salmonella* website, and password for this form will be sent by email to the participants in week 44, along with a short guidance on handling this electronic form.
Submission of serotyping data has to be finalised on **15 December 2017** at the latest.

The study on PFGE typing will use a separate web based test report, and this link will be sent to the participants in a separate email. Deadline for the electronic **submission of PFGE typing results** (web-based test report + TIFF + optional ZIP file) is **22 December 2017** at the latest.

Mind that the electronic result forms are no longer accessible after these deadlines!
In case you foresee problems with the deadline(s), please contact us beforehand.

If you have questions or remarks about this study, or in case having problems using the electronic result forms, please contact:

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Timetable of the 22nd interlaboratory comparison study (2017) on serotyping and optional PFGE typing of *Salmonella* for NRLs-*Salmonella*

Week	Date	Topic
38	18-22 September	Request for participation PFGE typing (serotyping is obligatory for EU NRLs).
42	16-20 October	Emailing of the protocol 2017.
44	30 October- 3 November	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373). If you did not receive the parcel by **3 November**, please contact the EURL-*Salmonella*.
44	30 October- 3 November	Sending the link and the password for the web based test report on serotyping to the participants.
47	22 November	Sending the link for the dedicated web based test report on PFGE typing to the participants in a separate email. The pre-configured database and instructions for use in case of (optional) analysis of the gel in Bionumerics will be included in this email as well.
44	30 October- 3 November	*Upon receipt*: Starting the identification of the strains, according to the usual practice of the laboratory.
50	**15 December 2017** at the latest	Deadline for completing the electronic submission of **serotyping** results: **15 December 2017** (23:59 h CET) After this deadline, the electronic submission form for serotyping results will be closed.
51	**22 December 2017** at the latest	Deadline for completing the electronic submission of **PFGE typing** results: **22 December 2017**
	January 2018	Serotyping: Reporting of individual laboratory results and Interim Summary Report.
	April 2018	PFGE typing: Reporting of individual laboratory results and Summary Report.
	Summer 2018	Final report.

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: 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.