Protocol of the 20th EURL-*Salmonella* Interlaboratory Comparison Study (November 2015) on serotyping and PFGE typing of *Salmonella* strains, for the NRL-*Salmonella* laboratories

Introduction

The European Union Reference Laboratory (EURL) - *Salmonella* organises the 20th 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 **9 December 2015** 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 2015** 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 26 October 2015.

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 (http://www.pasteur.fr/ip/portal/action/WebdriveActionEvent/oid/01s-000036-089).
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.

At the EURL-Salmonella workshop in Bilthoven in May 2007, the EURL-Salmonella made a proposal for the level of 'good performance' that the NRLs need to achieve during an interlaboratory comparison study on serotyping. 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 10 *Salmonella* strains will be included in the PGFE typing study, coded P1 - P10.
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_PFGE2015.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 PulseNet guidelines (www.pulsenetinternational.org) as shown in Annex 1 of this Protocol. *To comply with these guidelines the reference strain S. Braenderup H9812 must be run in every 6 lanes as a minimum.*

This year we introduce the evaluation of the (optional) **analysis of the gel in Bionumerics** as well.
In this case, use Bionumerics to analyse your gel by creating a local database with correct experiment settings and entry-fields. Pre-configured databases and instructions for use will be sent to the participants in week 44. Analyse the gel in Bionumerics including normalisation and band assignment (also see EFSA supporting publication 2014:EN-703). Prepare the XML export file according to the instructions, including all test strains and reference strains as well as the TIFF image. **Email the XML file** to wilma.jacobs@rivm.nl
Be sure to rename your .xml file to include at least your **laboratory code** in the name, preferably like: Lab01_PFGE2015.xml

Evaluation of the analysis of the gel in Bionumerics will be done according to the guidelines as used in the EQAs for the FWD laboratories (European Centre for Disease Prevention and Control. Fifth external quality assessment scheme for *Salmonella* typing. Stockholm: ECDC; 2014. Available at: http://ecdc.europa.eu/en/publications/Publications/fifth-EQA-salmonella-typing-November-2014.pdf) and shown in Annex 2 of this protocol.

Reporting of the results
Like last years, 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 **9 December 2015** (23:59 h CET) 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 *or* XLM file) is **22 December 2015** (23:59 h CET) 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:

Wilma Jacobs-Reitsma
EURL-*Salmonella*
Laboratory for Zoonoses and Environmental Microbiology (Z&O)
National Institute for Public Health and the Environment (RIVM)
P.O. Box 1
3720 BA Bilthoven
tel. number: +31 30 274 4290
fax. number: +31 30 274 4434
e-mail: wilma.jacobs@rivm.nl

Timetable of the 20th interlaboratory comparison study (2015) on serotyping and optional PFGE typing of *Salmonella* for NRLs-*Salmonella*

Week	Date	Topic
39	21 September	Request for participation PFGE typing (serotyping is obligatory for NRLs)
43	19-23 October	Emailing of the protocol 2015 to the NRLs.
44	26-30 October	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373) by door-to-door courier service. If you did not receive the parcel by **30 October**, please contact the EURL-*Salmonella*.
44	26-30 October	Sending the link and the password for the web based test reports (plus instructions for use) to the participants by email. The PFGE typing part will use a separate web based test report. Sending the pre-configured databases (plus instructions for use) to the PFGE participants.
44	26-30 October	Identification of the strains can start upon arrival of the strains, according to the usual practice of the laboratories.
50	9 December at the latest	Deadline for completing the electronic submission of **serotyping** results: **9 December 2015** (23:59 h CET). After this deadline, the electronic submission form for serotyping results will be closed.
52	22 December at the latest	Deadline for completing the electronic submission of **PFGE typing** results (web-based test report + TIFF file *or* XML file): **22 December 2015** (23:59 h CET).
	December	Data checks at the EURL-*Salmonella*.
	January 2016	Serotyping: Reporting of individual laboratory results and Interim Summary Report.
	March 2016	PFGE typing: Reporting of individual laboratory results.
	Summer 2016	Final report.

ANNEX 1 PulseNet Guidelines on quality grading of PFGE images

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

VERSION:	REPLACED BY:	AUTHORIZED BY:	Page 1 of 2

STANDARD OPERATING PROCEDURE FOR TIFF QUALITY GRADING	CODE: PNQ01 Effective Date: 5 09 2005

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:

VERSION:	REPLACED BY:	AUTHORIZED BY:	Page 2 of 2

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. Fifth external quality assessment scheme for *Salmonella* typing. Stockholm: ECDC; 2014. Available at: http://ecdc.europa.eu/en/publications/Publications/fifth-EQA-salmonella-typing-November-2014.pdf).

Parameters \scores	Excellent	Good	Fair	Poor
Position of gel	Excellent placement of frame, and gel inverted	The image frame is positioned to low		Frame includes wells
		Too much space framed at the bottom of the gel.		Gel not with light bands on dark background
		Too much space framed on the sides of the gel.		
		(Guidelines recommend to frame just beneath the wells)		
Strips:	All lanes correctly defined.	A single lane is not correctly defined	Lanes defined too narrowly (users should include the whole gel lane).	Lanes not defined correctly - Too wide/not following the actual gel lanes
Curves:	1/3 or more of the lane is used for averaging curve thickness	Curves defined either as a very narrow strip or encompassing almost the whole lane		
		(Average thickness is recommended to be reduced/ increased to ~1/3 of the lane)		
Normalisation	All bands assigned correctly in all reference lanes.	Bottom band at 20.5 kb not assigned in some of the reference lanes.		Missing assignments of bands in the reference in lane 5, 10 and 15
				The references were not included in the submitted XML file (follow the XML export guide).
Band assignment	Excellent band assignment in relation to the quality of the gel.	Some double bands are assigned wrongly.		The positions are correct, but double bands assigned at the exact same positions.
			Some shadow bands are assigned	Band assignment not correct, (commonly caused by thickness of the bands/overexposure)
			(Guidelines require control of band assignment after using auto search)	Only used auto search to find bands, no manual corrections.
				(Guidelines require control of band assignment after using auto search).