Protocol EURL-*Salmonella* Proficiency Test Typing 2020

1. Introduction

The European Union Reference Laboratory (EURL) - *Salmonella* organises the 25th Proficiency Test (PT) on typing of *Salmonella* strains amongst the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*).
The main objective of this typing PT is to test the performance of the participating laboratories for serotyping of *Salmonella* spp.
A cluster analysis part, using PFGE and/or MLVA and/or WGS data, is optionally available as a second pilot study.

The PT will take place in week 45 and onwards. The timetable can be found on page 5 of this protocol.

All data have to be reported through an electronic result form. The link to this form will be sent by email in week 45. **Submission of serotyping data** has to be finalised on **11 December 2020** at the latest.

The part on cluster analysis will use a separate result form, and the link to this form will be sent to the participants in a separate email in week 45 as well. Deadline for the electronic **submission of all cluster analysis results** is **29 January 2021** at the latest.

2. Transportation of the *Salmonella* strains to the laboratories

The strains for the serotyping part and optionally the cluster analysis 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 2 November 2020.

3. 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* serovar, 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.
If working with *Salmonella* antisera, please note to be very careful in following the exact instructions of the various manufacturers of the different antisera 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.
If, based on the results as obtained, a definite conclusion on the serovar name cannot be given, then 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:-
4	i	-	4:i:-
6,7	-	1,5	6,7:-:1,5
42	g,t	-	42:g,t:-

*Please report the serovar name without indicating "*S.*" or "*Salmonella*".

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* PTs 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. Nevertheless, typing should include testing for the presence of O:6 antigen.
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.

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 (results with four penalty points or more) have to participate in a follow-up study.

4. Second pilot on cluster analysis

The cluster analysis part of the PT typing is optional and can be performed up to the choice of the participant by PFGE and/or MLVA and/or WGS (or any combination of these methods).
Note that PFGE is no longer performed at the EURL-*Salmonella* and evaluation of PFGE results will only be based on comparing the results as send in by the PFGE participants.

The pilot cluster analysis 2020 is mimicking an outbreak situation, with a monophasic *Salmonella* Typhimurium ST34, MLVA type 3-14-13-NA-211 as the reference strain. WGS data on this strain (fastq-files) will be made available through a secure ftp server.

Participants are asked to analyse 10 *Salmonella* strains (coded SCA01 – SCA10) using their own routine method(s) of choice, and to report per strain if a clustering match with the reference strain was found.
For this particular PT2020 situation, the cluster definition is set at maximum 6 allelic differences from the reference sequence (WGS). For MLVA, the cluster definition is set at no loci with a different number of repeats.

Details on the method(s) used and the outcome of the cluster analysis have to be reported in the **electronic result form**. Additionally, specific data for PFGE and WGS have to be send by email or have to be uploaded to a secure ftp server. Detailed instructions for this will be sent to the participants in week 45, along with the link to the result form.

Results to be submitted are listed below.

PFGE:

- **Electronic result form**: protocol used, position of the lanes, potential cluster identification in case of an outbreak situation.
- **Email the PFGE gel image** as *an uncompressed 8-bit gray scale TIFF file* to wilma.jacobs@rivm.nl . Be sure to include your laboratory code in the name of the .tif file, preferably like: Lab01_PFGE2020.tif
- Prepare the ZIP (Bionumerics 7) or XML export files (Bionumerics 6 or below), from the analysis in BioNumerics, *including all test strains and reference strains*, *as well as the TIFF image*. **Email these BN analysis data in a ZIP file** to wilma.jacobs@rivm.nl . Be sure to rename your zip file to include your laboratory code in the name, preferably like: Lab01_PFGE2020.zip
- **Important** for the PFGE analysis in BioNumerics:
    - Please create a new local database with correct experiment settings and entry-fields for proper inter-participants comparison. A pre-configured database and instructions for use will be sent to the participants in week 45.
    - please already rename your PFGE gel image (preferably like Lab01_PFGE2020.tif) *before* importing it into the pre-configured database.

MLVA:

- **Electronic result form**: scheme/loci used, the allelic profile, cluster identification in case of an outbreak situation.

WGS:

- **Electronic result form**: background information on the wet-lab and dry-lab methods used, cluster identification in case of an outbreak investigation (SNP-based and/or cgMLST/wgMLST-based)
- **Upload the raw reads** (fastq-files) to the secure ftp server according to the instructions to be sent in week 45. Be sure to name your files to include your laboratory code and strain code in the name, preferably like: Lab01_SCA01_R1.fastq, Lab01_SCA01_R2.fastq, etc.
- **Email the distance matrix** (preferably as an .xls or .csv file) to wilma.jacobs@rivm.nl. Be sure to name the file to include your laboratory code, preferably like: Lab01_Distance_Matrix.xls

Evaluation (per methodology) of the participants' cluster analysis results will be done by comparing the participants' results to the expected results in an outbreak investigation setting, as pre-defined by the EURL-*Salmonella.*
As a minimum, it will be expected to have any technical duplicate strains be reported as (part of) one cluster.

5. Reporting of the PT results

As usual, all data have to be reported through an electronic result form. The link to the serotyping result form will be sent by email to the participants in week 45. **Submission of serotyping data** has to be finalised on **11 December 2020** at the latest.

The part on cluster analysis will use a separate result form, and the link to this form will be sent to the participants in a separate email in week 45 as well. Deadline for the electronic **submission of all cluster analysis results** (result form plus additional PFGE and/or WGS data as requested) is **29 January 2021** 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

EURL- *Salmonella* Proficiency Test Typing 2020

Serotyping and optional part PFGE and/or MLVA and/or WGS Cluster Analysis

Week	Date	Subject
39	Week of 21 September	Emailing of the link to the registration form for the typing study. Please **register by 16 October 2020** at the latest.
43	Week of 19 October	Emailing of the protocol 2020.
45	2 November	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
45	Week of 2 November	*Upon receipt*: Starting the identification of the strains, according to the usual practice of the laboratory. Sending the link for the result form on serotyping to the participants. Sending the link for the result form on PFGE and/or MLVA and/or WGS Cluster Analysis to the participants in a separate email.
50	11 December 2020 at the latest	Deadline for completing the electronic submission of **serotyping** results: **11 December 2020** After this deadline, the result form for serotyping will be closed.
	29 January 2021 at the latest	Deadline for completing the electronic submission of **PFGE/MLVA/WGS Cluster Analysis** results: **29 January 2021**
	February 2021	Serotyping: Evaluation of individual laboratory results and Interim Summary Report.
	April/May 2021	PFGE/MLVA/WGS Cluster Analysis: Evaluation of individual laboratory results and Summary Report.
	Summer 2021	Final report.