Protocol

EURL-*Salmonella* Proficiency Test Typing 2023

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EURL Salmonella

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1. Introduction

The European Union Reference Laboratory (EURL) - *Salmonella* organises the 28th 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*.

The serotyping part is compulsory for the NRLs-*Salmonella* in all EU Member States. Optionally, also MLVA may be performed on any applicable strain within the serotyping part of the PT.
A separate cluster analysis part, using NGS data, is optionally available.

The PT will take place in week 45 and onwards. The timetable can be found on page 6 of this protocol.

All data have to be reported through an electronic result form. The link to this form will be sent to the contact persons for serotyping by email in week 45. **Submission of serotyping data** has to be finalised on Friday **15 December 2023** 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 contact persons for cluster analysis in a separate email in week 45 as well. Deadline for the electronic **submission of all NGS cluster analysis results** is Wednesday **31 January 2024** 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 PT will be transported all in one (larger) parcel. The strains, as cultures in Heart Infusion agar transport tubes, will be sent as Biological Substance Category B (UN 3373) with a door-to-door courier to the participating laboratories.
The shipment of the strains is scheduled on Monday 6 November 2023.

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 PT. 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 assure 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 (as detected) for the O-antigens and H-antigens and the serovar names according to the White-Kauffman-Le Minor (WKLM) scheme of 2007 (https://www.pasteur.fr/sites/default/files/veng_0.pdf)
and the supplements published:
-Guibourdenche et al., Supplement 2003–2007 (no. 47) to the White–Kauffmann–Le Minor scheme. Research in Microbiology, 2010, 161, pp. 26–29. https://doi.org/10.1016/j.resmic.2009.10.002
-Issenhuth-Jeanjean et al., Supplement 2008-2010 (no. 48) to the White-Kauffmann-Le Minor scheme. Research in Microbiology, 2014, 165, pp. 526-530. https://doi.org/10.1016/j.resmic.2014.07.004

Laboratories have to report only those results, on which the identification of serovar names is based (also see examples of expected reporting in Table 1). If, based on the results obtained, a definite conclusion on the serovar name cannot be given, then identify the strains by giving the antigenic formula as far as detected.

Table 1. Examples of expected reporting (or not expected reporting)

O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar name*
9	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:-
4,12,27	l,v	e,n,z15	Brandenburg
1,4,[5],12	g,m,s	[1,2]	Hato

In grey: do not take over the notation "as it is" from the WKLM scheme but only report what was tested and detected, e.g. 4:g,m,s:- Hato.

*Please report the serovar name without indicating "*S.*" or "*Salmonella*", to facilitate the overall evaluation of all participants' results.

The evaluation of deviating serotyping results will be performed by the EURL-*Salmonella* according to Table 2.

Table 2. Evaluation of deviating 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, the following criteria for 'good performance' in PTs on serotyping were defined (Mooijman, 2007. The twelfth CRL-*Salmonella* Workshop. RIVM Report no.: 330604006).
Penalty points are given for the incorrect typing of strains, but 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.

4. Optional part on MLVA

Optionally, also MLVA may be performed on any applicable strain within the serotyping part of the PT. The allelic profiles and the schemes used can be additionally submitted in the electronic result form for serotyping.
Results will be evaluated by a comparison among the MLVA participants.

5. Optional part on cluster analysis

The cluster analysis part of the PT typing is optional and is intended to be performed by using NGS.

Like before, the cluster analysis 2023 is mimicking an outbreak situation, with a *Salmonella* Bovismorbificans strain as the reference. NGS data on this strain (fastq.gz-files, md5checksums) will be made available through a sftp server.

Participants will receive 6 *Salmonella* strains (coded 23SCA01 – 23SCA06) for 'wet' elaboration using their own routine method(s).
In addition, NGS data (fastq.gz files, md5checksums) on another 6 *Salmonella* strains (23SCA11 – 23SCA16) will be provided through the sftp server for 'dry' evaluation.

It will be asked to report per strain:
-whether the data passed your Quality Control (QC) criteria or not,
-whether a clustering match with the reference strain was found or not.
Be sure to exclude strains from the cluster analysis if the data did not pass your QC.

For this particular PT 2023 situation, the cgMLST-based cluster definition is set at maximum 5 allelic differences from the reference sequence.

Details on the method(s) used and the outcome of the cluster analysis have to be reported in the **result form**. Additionally, specific NGS data have to be uploaded to the sftp server. Detailed instructions for this will be sent to the contact persons for cluster analysis in week 45, along with the link to the result form.

NGS-based cluster analysis results to be submitted are listed below:

- **Result form**: background information on the wet-lab and dry-lab methods used, including QC criteria; cluster identification in case of an outbreak investigation (cgMLST/wgMLST-based and/or SNP-based).
- **Uploading to the sftp server** according to the instructions to be sent in week 45.
    - **the raw reads** (fastq.gz-files) of strains 23SCA01 -23SCA06. Be sure to name your files to include your laboratory code and strain code in the name, preferably like: 23SCA01Lab01_R1.fastq, 23SCA01Lab01_R2.fastq, etc.
    - Recommended but optional **Md5 checksums** (e.g. as .csv or .txt file) concerning the downloaded files (23SCA11 – 23SCA16 plus 23SCA-REF) and your uploaded files (23SCA01 – 23SCA06). Background information on Md5 checksums can be found in: https://www.eurlsalmonella.eu/sites/default/files/2023-10/20-6-2023_02_IntroWGS_FileIntegrity_vanHoek.pdf (Presentation given at the Joint Training Course of the inter EURLs Working Group on NGS, June 2023 in Bilthoven, the Netherlands).
    - **the distance matrix** (as an .xls or .csv file). Be sure to name the file to include your laboratory code, preferably like: Lab01_Distance_Matrix.xls

Evaluation 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. Also, strains are expected to be excluded from the cluster analysis if the data did not pass your QC.

6. Reporting of the PT results

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 contact persons for serotyping in week 45.
Submission of serotyping data has to be finalised on Friday **15 December 2023** at the latest. If applicable, this also includes any optional MLVA data.

The part on cluster analysis will use a separate result form, and the link to this form will be sent to the contact persons for cluster analysis in a separate email in week 45 as well. Deadline for the electronic **submission of all cluster analysis results** (result form plus additional NGS data as requested) is **31 January 2024** 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 PT, or in case having problems using the electronic result forms, please contact:

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Timetable

EURL-*Salmonella* Proficiency Test Typing 2023
Serotyping and optional part NGS Cluster Analysis

Week	Date	Subject
39	Week of 25 September	Emailing of the link to the registration form for the PT typing. Please **register by 20 October 2023** at the latest.
43	Week of 23 October	Emailing of the protocol 2023.
45	Monday 6 November 2023	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
45	Week of 6 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 NGS Cluster Analysis to the participants in a separate email.
50	Friday 15 December 2023 at the latest	Deadline for completing the electronic submission of **Serotyping** results: **15 December 2023.** After this deadline, the result form for serotyping (and optionally also MLVA results) will be closed.
5	Wednesday 31 January 2024 at the latest	Deadline for completing the electronic submission of **NGS Cluster Analysis** results: **31 January 2024.**
	February 2024	Serotyping: Evaluation of individual laboratory results and Interim summary report.
	April/May 2024	NGS Cluster Analysis: Evaluation of individual laboratory results and Interim summary report.