Protocol

EURL-*Salmonella* Proficiency Test Serotyping 2025

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EURL Salmonella

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1. Introduction

The European Union Reference Laboratory (EURL) - *Salmonella* organises the 30th Proficiency Test (PT) on serotyping 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*.

Note that this year's PT does not contain an optional part on cluster analyses, but only the part on serotyping of *Salmonella*. Participation is compulsory for the NRLs-*Salmonella* in all EU Member States.

The PT will take place in week 33 and onwards. The timetable can be found on page 5 of this protocol.

All data have to be reported through the webbased result form. The link to this form will be sent to the contact persons by email in week 33. **Submission of serotyping results** has to be finalised on Friday **3 October 2025** at the latest.

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

The strains to be serotyped will be provided as cultures in Heart Infusion agar transport tubes and 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 11 August 2025.

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 serotyping method routinely performed in your laboratory has to be used in this PT.

Additionally, participants may also use a second serotyping method (e.g. WGS) to analyse the strains. Please contact the EURL-*Salmonella* by email (wilma.jacobs@rivm.nl) to receive a second Labcode to fill out a separate result form and to obtain a second evalution. Only the results obtained with the routine method will be used to assess the performance of the NRLs-*Salmonella*.

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. Reporting of the PT results

All data have to be reported through the webbased result form. The link to the serotyping result form will be sent by email to the contact persons in week 33.

Submission of serotyping results has to be finalised on Friday **3 October 2025** at the latest.

Mind that the electronic result form is no longer accessible after this deadline!
In case you foresee problems with the deadline, please contact us beforehand.

If you have questions or remarks about this PT, or in case having problems using the result form, please contact:

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Timetable

EURL-*Salmonella* Proficiency Test Serotyping 2025

Week	Date	Subject
25	Week of 16 June	Emailing of the link to the registration form for the PT Serotyping. Please **register by 18 July 2025** at the latest.
31	Week of 28 July	Emailing of the protocol 2025.
33	Monday 11 August 2025	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
33	Week of 11 August	*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.
40	Friday 3 October 2025 at the latest	**Deadline** for completing the electronic submission of serotyping results: **3 October 2025.** After this deadline, the result form will be closed.
	November/December 2025	Evaluation of individual laboratory results and Interim summary report.