

Protocol of the 18th EURL-*Salmonella* Interlaboratory Comparison Study (November 2013) on serotyping, phage typing and PFGE typing of *Salmonella* strains, for the NRL-*Salmonella* laboratories

Introduction

The European Union Reference Laboratory (EURL) - *Salmonella* organises the eighteenth 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 phage typing and PFGE typing of *Salmonella* spp. The start of the study has to be postponed with 1 or 2 weeks and now will take place in week 46/47 and onwards. The *updated* timetable can be found on the last page of this protocol.

Like in the last year 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 and phage typing data** has to be finalised on **20 December 2013** at the latest.

The data on phage typing will be forwarded by the EURL-*Salmonella* to Public Health England (PHE, London, United Kingdom) for further analyses.

The pilot 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** is **17 January 2014** at the latest.

Transportation of the *Salmonella* strains to the laboratories

The strains for the serotyping part and/or the phage typing 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 now scheduled for either Monday 11 November or Monday 18 November 2013. Participants will be notified on the definite date as soon as possible.

Serotyping

A total number of 20 *Salmonella* strains (coded S1 - S20) have to be serotyped.

An additional *Salmonella* strain (S-21), being a non-*S. enterica* subsp. *enterica* 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 EURL-*Salmonella* Workshops in 2012 and 2013, 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
4,5,12	i	1,2	Typhimurium
4,12	i	-	4,12:i:-

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	Abbreviation
Auto-agglutination or Incomplete set of antisera (outside range of antisera)	Not typable	NT
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	-

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 *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.

Phage typing

A total number of 20 *Salmonella* strains are included in the phage typing study:

- 10 strains of *S. Enteritidis* coded E1 - E10
- 10 strains of *S. Typhimurium* coded T1 - T10

The evaluation of the phage typing results will be done in collaboration with the *Salmonella* Reference Service of PHE, London, UK.

Pilot study PFGE typing

A total number of 10 *Salmonella* strains will be included in the PGFE typing study, coded P1 - P10. The general reference strain *S. Braenderup* H9812 is added to the package as well, for convenience of the participants when needed.

As this is the first time a PGFE typing study is included as a pilot, the 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. Also, participants are requested to email their PFGE gel images as a TIFF file to wilma.jacobs@rivm.nl. Be sure to include at least your laboratory code in the name of these .tif files.

The evaluation of the PFGE typing results, after digestion with XbaI, will be done on the quality of the PFGE images only (no evaluation of gel analysis in Bionumerics yet) and quality grading will be done according to the PulseNet guidelines (www.pulsenetinternational.org).

Reporting of the results

Like last year 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 46/47, along with a short guidance on handling this electronic form.

Submission of serotyping and phage typing data has to be finalised on **20 December 2013** (23:59 h CET) at the latest.

The pilot 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** is **17 January 2014** (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, 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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**Updated Timetable of the 18th interlaboratory comparison study (2013)
on serotyping and optional phage typing/PFGE typing of *Salmonella*
for NRLs-*Salmonella***

Week	Date	Topic
44	29 October	Emailing of the protocol 2013 to the NRLs.
46/47	11/18 November	Mailing 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 15/22 November , please contact the EURL- <i>Salmonella</i> .
46/47	11/18 November	Sending the link and the password for the web based test reports to the participants by email. The pilot study on PFGE typing will use a separate web based test report.
46/47	11/18 November	Identification of the strains can start upon arrival of the strains, according to the usual practice of the laboratories.
51	20 December at the latest	Deadline for completing the electronic submission of serotyping and phage typing results: 20 December 2013 (23:59 h CET) After this deadline, the electronic submission form for serotyping and phage typing results will be closed.
3	17 January at the latest	Deadline for completing the electronic submission of PFGE typing results: 17 January 2014 (23:59 h CET)
	December/January	Data checks at the EURL- <i>Salmonella</i> .
	February 2014	Serotyping and phage typing: Reporting of individual laboratory results and Interim Summary Report.
	April 2014	PFGE typing: Reporting of individual laboratory results and Interim Summary Report.
	Summer 2014	Final report.