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EURL-*Salmonella* Proficiency Test Typing 2024

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W.F. Jacobs-Reitsma et al.

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Colophon

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Synopsis

EURL-*Salmonella* Proficiency Test Typing 2024

Since 1992, the National Reference Laboratories (NRLs) of the EU Member States are required to take part in an annual quality control, which consists of conducting 'proficiency tests'. For one of these proficiency tests, they have to correctly identify 20 *Salmonella* strains (typing).

In 2024, all NRLs of the 27 EU Member States performed well in this *Salmonella* typing quality control. One non-EU laboratory participating for the first time was evaluated to need extra training first. Overall, the participating laboratories were able to identify 99% of the tested strains correctly.

The laboratories are obliged to use a standard method (serotyping) to type *Salmonella*. Additionally, 24 laboratories voluntarily took part in an extra typing test at DNA level in 2024, using whole genome sequencing (WGS). This more precise method is sometimes needed to trace the source of a contamination.

Each Member State appoints a National Reference Laboratory (NRL) to take part in the proficiency tests. This NRL is responsible for detecting and identifying *Salmonella* in samples of food products or animals on behalf of the Member State. Sometimes, NRLs from countries outside the EU voluntarily take part in the quality control. Six non-EU NRLs took part this time, resulting in a total of 33 laboratories taking part in the proficiency test in 2024.

The European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organises the annual *Salmonella* typing proficiency test. This laboratory is located at RIVM in the Netherlands.

Keywords: *Salmonella*, EURL, NRL, proficiency test, serotyping, molecular typing, WGS, cluster analysis

Publiekssamenvatting

EURL-*Salmonella* ringonderzoek typering 2024

Sinds 1992 zijn de Nationale Referentie Laboratoria (NRL's) van de lidstaten van de Europese Unie verplicht om elk jaar hun kwaliteit te laten toetsen met zogeheten ringonderzoeken. Voor een van de ringonderzoeken moeten zij onder andere aan twintig *Salmonella*-stammen de juiste naam kunnen geven (typering).

In 2024 scoorden alle NRL's van de 27 EU-lidstaten goed bij deze kwaliteitscontrole op typering van *Salmonella*. Een nieuw laboratorium van buiten de EU bleek daarvoor extra training nodig te hebben. Alle deelnemende laboratoria samen konden aan 99 procent van de geteste stammen de juiste naam geven.

De laboratoria zijn verplicht om *Salmonella* met een standaardmethode te typeren (serotypering). Daarnaast deden 24 laboratoria vrijwillig mee aan een extra typering op DNA-niveau, met Whole Genome Sequencing (WGS). Deze preciezere typering kan soms nodig zijn om de bron van een besmetting op te sporen.

Voor de kwaliteitstoetsen wijst elke lidstaat een laboratorium aan, het Nationale Referentie Laboratorium (NRL). Dit NRL is namens dat land verantwoordelijk om *Salmonella* in monsters van levensmiddelen of dieren aan te tonen en te typeren. Soms doen er ook NRL's van landen buiten de EU vrijwillig aan mee. In 2024 waren dat er zes, zodat er in totaal 33 laboratoria deelnamen aan het ringonderzoek van dit jaar.

Het Europese Unie Referentie Laboratorium voor *Salmonella* (EURL-*Salmonella*) organiseert het jaarlijkse ringonderzoek *Salmonella*-typering. Dit laboratorium is gevestigd bij het RIVM in Nederland.

Kernwoorden: *Salmonella*, EURL, NRL, ringonderzoek, serotypering, moleculaire typering, WGS, cluster analyse

Contents

Summary

In November 2024, the annual *Salmonella* typing Proficiency Test (PT) was organised by the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*, Bilthoven, the Netherlands). The PT's main objective was to evaluate whether the typing of *Salmonella* strains by the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) in the European Union was carried out uniformly and whether comparable results were obtained.

A total of 33 laboratories participated in this PT. They included the obligatory 27 NRLs-*Salmonella* in the 27 EU Member States. Six further NRLs from third countries (EU candidate countries, members of the European Free Trade Association (EFTA), and the United Kingdom) participated voluntarily.

All 33 laboratories performed serotyping. The EURL-*Salmonella* selected a total of twenty obligatory *Salmonella* strains plus one optional strain for serotyping. The strains had to be typed according to the method routinely used in each laboratory, following the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007).

Overall, 99,7% of the strains were typed correctly for the O-antigens, 98,8% of the strains were typed correctly for the H-antigens, and 98,8% of the strains were correctly named by the participants.
In 2007, criteria for 'good performance' concerning serotyping were defined. Based on these criteria, the participants' performance was very good, including the four participants that submitted (partial) Whole Genome Sequencing (WGS)-based serotyping results.
All 27 EU NRLs and five non-EU NRLs achieved the defined level of good performance in the first stage of the PT. One non-EU NRL-*Salmonella*, which participated for the first time, did not yet achieve this level. Prior to the next PT Serotyping, the EURL-*Salmonella* organised a dedicated on-site training for this laboratory to help it improve.

The PT Typing 2024 also included an optional part on cluster analysis. A total of 24 participants took part in this WGS-based analysis.
The cluster analysis was mimicking an outbreak situation, with a *Salmonella* Infantis as the reference strain. Raw sequence data on this reference strain, as well as on six other *Salmonella* strains, were made available to the participants via a secure FTP server for 'dry' evaluation. Participants were asked to handle and analyse six 'wet' *Salmonella* strains, to analyse the six 'dry' *Salmonella* strains and to report for each strain whether or not a cluster with the provided reference strain was found.

A total of 24 participants submitted a total of 27 WGS-based cluster analysis results (three participants produced multiple submissions). The participants' cluster analysis results were evaluated by comparing their results to the expected results in the outbreak investigation setting, as pre-defined by the EURL-*Salmonella.*

Out of the 27 submissions, 23 reported the 4 expected strains to be part of the cluster. However, one participant additionally included a ‘strain’ into this cluster consisting of mixed serovars, which should not have passed their quality control (QC) criteria. Consequently, 22 out of the 27 submissions reported the cluster completely as expected.

1 Introduction

This report describes the 2024 Proficiency Test (PT) on typing of *Salmonella* organised by the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*, Bilthoven, the Netherlands) in November 2024.

According to EC Regulation No. 2017/625 (EC, 2017), one of the tasks of the EURL-*Salmonella* is to organise PTs for the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) in the European Union. The main objectives for PTs on typing of *Salmonella* are that the typing should be carried out uniformly in all Member States and that comparable results are obtained. The implementation of PTs on typing started in 1995.

A total of 33 laboratories participated in the PT Typing 2024. They included the obligatory 27 NRLs-*Salmonella* in the 27 EU Member States. Six additional NRLs participated voluntarily: the EU candidate countries Moldova and Serbia, the European Free Trade Association (EFTA) countries Iceland, Norway, and Switzerland, and the United Kingdom.

The main objective of this PT was to evaluate the performance of the EU NRLs in serotyping *Salmonella*. All NRLs performed serotyping of the 20 obligatory strains, and all but two participants serotyped the optional 21st strain. NRLs of EU Member States that would not achieve the defined level of good performance for serotyping would have to participate in a follow-up study.

The PT Typing 2024 also included an optional part on cluster analysis. The cluster analysis involved six 'wet' *Salmonella* strains and allowed participants to choose their own routine WGS method(s) of choice.
The PT was mimicking an outbreak situation, with a *Salmonella* Infantis as the reference strain.
Raw sequence data on this reference strain, as well as on six other *Salmonella* strains, were made available to the participants via a secure FTP server for 'dry' evaluation.
Participants were asked to analyse the six 'wet' *Salmonella* strains and the six 'dry' *Salmonella* strains, and to report for each strain whether or not a cluster with the reference strain was found.
A total of 24 NRLs participated in the cluster analysis and produced a total of 27 submissions.

2 Participants

Table 2.1 displays the list of participants in the EURL-*Salmonella* PT Typing 2024.

*Table 2.1 List of participants in the EURL-*Salmonella *PT Typing 2024*

Country	City	Institute
Austria	Graz	AGES
Belgium	Brussels	Sciensano
Bulgaria	Sofia	National Diagnostic Research Veterinary Institute (NDRVI)
Croatia	Zagreb	Croatian Veterinary Institute
Cyprus	Nicosia	Cyprus Veterinary Services
Czech Republic	Prague	State Veterinary Institute Prague
Denmark	Ringsted	Danish Veterinary and Food Administration (DVFA)
Estonia	Tartu	National Centre for Laboratory Research and Risk Assessment
Finland	Kuopio	Finnish Food Authority
France	Ploufragan	ANSES
Germany	Berlin	German Federal Institute for Risk Assessment (BfR)
Greece	Chalkida	Veterinary Laboratory of Chalkis
Hungary	Budapest	National Food Chain Safety Office, Food Chain Safety Laboratory Directorate
Iceland	Reykjavík	Landspítali University Hospital, Dept. of Clinical Microbiology
Ireland[a)]	Celbridge	DAFM Laboratories
Italy	Legnaro	Istituto Zooprofilattico Sperimentale delle Venezie
Latvia	Riga	Institute of Food Safety, Animal Health and Environment (BIOR)
Lithuania	Vilnius	National Food and Veterinary Risk Assessment Institute
Luxembourg	Dudelange	Laboratoire National de Santé
Malta	Valletta	Malta Public Health Laboratory
Moldova	Chisinau	National Center Animal Health, Plant and Food Safety
Netherlands	Bilthoven	RIVM, Centre for Infectious Diseases Research, Diagnostics and Screening (IDS)
Norway	Ås	Norwegian Veterinary Institute
Poland	Pulawy	National Veterinary Research Institute
Portugal	Oeiras	Instituto Nacional de Investigação Agrária e Veterinária (INIAV)
Romania	Bucharest	Institute for Diagnosis and Animal Health
Serbia	Novi Sad	Scientific Veterinary Institute 'Novi Sad'
Slovak Republic	Dolný Kubín	Veterinary and Food Institute
Slovenia	Ljubljana	UL, Veterinary Faculty, NVI
Spain	Algete-Madrid	Laboratorio Central de Veterinaria

Country	City	Institute
Sweden	Uppsala	National Veterinary Agency (SVA)
Switzerland	Zurich	Institute for Food Safety and Hygiene
United Kingdom	Addlestone	Animal and Plant Health Agency (APHA) Weybridge

a) Also representing the NRL-*Salmonella*-Typing in Northern-Ireland.

3 Materials and methods

3.1 Design of the Proficiency Test (PT)

3.1.1 Laboratory codes

Each participant was randomly assigned a laboratory code: 1-33, plus 63 and 80 for two additional participants in the cluster analysis part.

3.1.2 Protocol and test report

Three weeks before the start of the PT, the NRLs received the protocol by email. Participants used web-based forms to submit their results. Instructions for completing these result forms and data entry were emailed to the NRLs on 5 November 2024, separately for serotyping and for cluster analysis.
The protocol and screenshots of the result forms can be found on the EURL-*Salmonella* website:
https://www.eurlsalmonella.eu/proficiency-testing/typing-studies

3.1.3 Transport

The parcels containing the strains for serotyping and cluster analysis were sent by the EURL-*Salmonella* on 4 November 2024. All samples were packed and transported as Biological Substance Category B (UN 3373) and transported by a door-to-door courier service.

3.2 Serotyping part of the PT

3.2.1 Salmonella strains for serotyping

Participants had to serotype a total of twenty *Salmonella* strains (coded S1–S20). As agreed at the 29th EURL-*Salmonella* Workshop (Mooijman, 2024), a less common strain (S21) was additionally included. Testing this strain was optional and results were not included in the evaluation. Laboratories were allowed to send strains for serotyping to another specialised laboratory in their country if this was part of their usual routine.

The *Salmonella* strains used for the part on serotyping originated from the National *Salmonella* Centre collection in the Netherlands. The strains were serologically verified by the Centre before distribution. Table 3.1 presents the complete antigenic formulas of the 21 serovars in accordance with the most recent White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007) plus published supplements no. 47 (Guibourdenche et al., 2010) and no. 48 (Issenhuth-Jeanjean et al., 2014). However, participants were asked to report only the results as detected and on which the identification of serovar names was based. Nine strains (Table 3.1) represented serovars included in the EURL-*Salmonella* serotyping PTs for the first time.

Table 3.1 Antigenic formulas of the 21 Salmonella *strains according to the White-Kauffmann-Le Minor scheme used in the EURL-*Salmonella *PT Serotyping 2024*

Strain code	O-antigens	H-antigens		Serovar	Origin
		(phase 1)	(phase 2)		
S1	1,9,12	g,m	-	Enteritidis	Human
S2	6,7	g,s,[t]	[1,6]	Menston	Human
S3	6,7,14	r	1,5	Infantis	Human
S4[a)]	13,23	g,[s],t	-	Okatie	Human
S5[a)]	28	z_{10}	l,w	Moroto	Non-human
S6	8,20	z_{10}	z_6	Molade	Human
S7[a)]	3,10	y	e,n,z_{15}	Bolton	Non-human
S8	1,4,[5],12	i	1,2	Typhimurium	Human
S9[a)]	17	l,v	e,n,x	Carmel	Human
S10	1,4,[5],12	i	1,5	Lagos	Human
S11[b)]	1,4,[5],12	i	-	1,4,[5],12:i:-	Human
S12[a)]	9,12	c	z_6	Ridge	Human
S13[a)]	6,7,14	a	e,n,x	Oslo	Human
S14[a)]	11	k	e,n,x,[z_{15}]	Kisarawe	Human
S15	6,7,14	r	1,2	Virchow	Non-human
S16	3,{10}{15}{15,34}	l,v	1,7	Give	Non-human
S17[a)]	[1],6,14,[25]	d	e,n,x	Charity	Non-human
S18[a)]	3,{10}{15}{15,34}	m,t	[1,6]	Southbank	Non-human
S19	6,8	z_{10}	e,n,x	Hadar	Non-human
S20	1,4,12	z	1,7	Indiana	Non-human
S21[c)]	47	k	z_{35}	47:k:z_{35} (IIIb)	Non-human

a) Represented in an EURL-*Salmonella* PT Serotyping for the first time.
b) Monophasic variant of *S*. Typhimurium based on genomic sequences. Phenotypic result: 4,5:i:-.
c) *Salmonella enterica* subspecies *diarizonae* (optional strain).

3.2.2 Evaluation of the serotyping results

The evaluation of deviating serotyping results is presented in Table 3.2.

Table 3.2 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 serovar name	Partly correct
Wrong serovar, or Mixed sera formula	Incorrect

In 2007, the following criteria for 'good performance' in PTs on serotyping were defined (Mooijman, 2007).
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, also occasionally referred to as 'top-5'), 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. Any EU Member State NRLs that fail to meet the criterion of good performance (results with four penalty points or more) have to participate in a follow-up study.

3.3 Cluster analysis part of the PT

3.3.1 Salmonella strains for cluster analysis

A total of six *Salmonella* strains (24SCA01 – 24SCA06) in Heart Infusion (HI) agar transport tubes were sent to the participants in the part on cluster analysis. Background information on these 'wet' strains is presented in Table 3.3A. In addition, raw sequence data (fastq.gz files, md5 checksums) on another seven *Salmonella* strains (24SCA11 – 24SCA16, plus the 24SCA-REF) were made available to the participants via the RIVM secure FTP server for 'dry' evaluation. Background information on the 'dry' strains is presented in Table 3.3B.

Table 3.3A Background information on the 'wet' Salmonella *strains used for cluster analysis in 2024*

Strain code	Serovar	Abbreviation	ST	Origin
24SCA01	Infantis	Inf	32	Human
24SCA02	Infantis	Inf=REF	32	Broilers
24SCA03[a)]	Infantis	Inf	32	Broilers
24SCA04	Infantis	Inf	32	Human
24SCA05[a)]	Infantis	Inf	32	Broilers
24SCA06	Infantis	Inf	32	Broilers

a) Technical duplicates.

Table 3.3B Background information on the 'dry' Salmonella *strains used for cluster analysis in 2024*

Strain code	Serovar	Abbreviation	ST	Origin
24SCA11	Infantis	Inf	32	Broilers
24SCA12[a)]	Mixed strains	Inf+STM		
24SCA13	Infantis	Inf	32	Human
24SCA14	Infantis	Inf=REF	32	Broilers
24SCA15	Infantis	Inf	32	Broilers
24SCA16[b)]	Mixed strains	Inf+Java		
24SCA-REF	Infantis	REF	32	Human

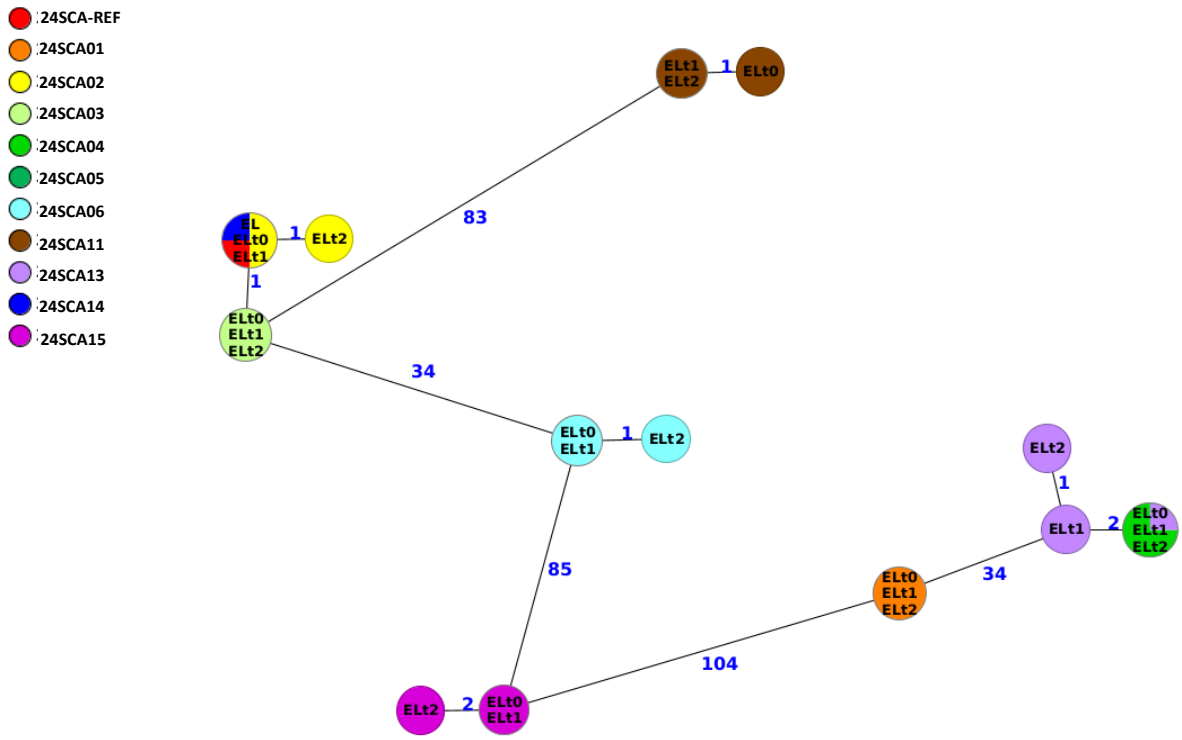
a) Wet-mixed culture of strains 24SCA02 (Inf=REF) and 19SCA03 (*S*. Typhimurium).
b) Wet-mixed culture of strains 24SCA03 (Inf) and 2013S16 (*S*. Paratyphi B, var. Java).

In preparation of the PT 2024 on cluster analysis, five broiler strains from a research project (Mughini-Gras et al., 2021) and three human surveillance strains were re-cultured from storage (2018/2019 and 2023 respectively). All *Salmonella* strains were submitted for WGS analysis both directly (July 2024) and after sub-culturing 10 times consecutively

on blood-agar and in buffered peptone water (BPW) (August 2024). In addition, several wet-mixed cultures were included in this pre-testing. Subsequently, six 'wet' strains and six 'dry' strains were selected for inclusion in the PT 2024 (see Figure 3.1). One set of wet technical duplicates was included: the shipment tubes of strain 24SCA03 and strain 24SCA05 were both prepared from the same blood-agar plate containing strain 24SCA03.
As in previous years, the PT Cluster Analysis 2024 was mimicking an outbreak situation, with a *Salmonella* Infantis as the reference strain (24SCA-REF). Raw WGS data of this strain (24SCA-REF_R1.fastq.gz and 24SCA-REF_R2.fastq.gz, as well as their md5 checksums) were made available through the RIVM secure FTP server.
For this particular PT 2024, the cgMLST-based cluster definition was set at a maximum of five allelic differences (AD) from the provided reference sequence.

Figure 3.1 shows the WGS pre-test results from the EURL-*Salmonella* for the ten selected and QC-passed strains, and also includes the reference strain (Tables 3.3A and 3.3B). Sequencing was performed in-house, on an Illumina NextSeq platform. Raw data were processed via an in-house developed Juno-assembly pipeline (https://github.com/RIVM-bioinformatics/juno-assembly), which includes the SPAdes 3.15.3 assembler. Cluster analysis was done in Ridom SeqSphere^{+} (version: 9.0.8; 2023-06), using the cgMLST Enterobase v2.0 scheme, and visualised in a minimum spanning tree (MST, Figure 3.1).
Stable and consistent cgMLST analysis results were obtained for all *S*. Infantis strains. Subsequently, the 'wet' strains selected to be included for the PT 2024 were freshly prepared from minus 70°C stocks of July 2024 (Figure 3.1, ELt1).

*Figure 3.1 Minimum spanning tree of the EURL-*Salmonella *(EL) pre-tests and PT 2024 results, (RidomSeqSphere⁺, cgMLST (3002), pairwise ignoring missing values and QC-failing strains excluded)*



EL: One data set, no testing in time;
ELt0: Original WGS data from the stored poultry research and human surveillance *Salmonella* strains;
ELt1: WGS data from initial pre-testing for the PT (July 2024);
ELt2: WGS data after 10x sub-culturing (blood-agar/BPW) for the PT (August 2024).

3.3.2 *Evaluation of the cluster analysis results*

As in previous years, the PT Cluster Analysis 2024 was mimicking an outbreak situation, with a *Salmonella* Infantis as the reference strain (24SCA-REF). For this particular PT 2024, the cgMLST-based cluster definition was set at a maximum of five AD from the provided reference sequence.
Participants were asked to analyse the six 'wet' *Salmonella* strains and the six 'dry' ones, using their own routine WGS method(s) of choice.
Data submission for the WGS results included:

- 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:
  - the raw reads (fastq.gz-files) of strains 24SCA01 - 24SCA06;
  - Recommended but optional: Md5 checksums concerning the downloaded files (24SCA11 - 24SCA16 plus 24SCA-REF) and the participant's uploaded files (24SCA01 - 24SCA06);
  - the distance matrix.

Participants were asked to report per strain (Tables 3.3A and 3.3B):

- whether or not the data passed their quality control (QC) criteria;
- whether or not a clustering match was found with the provided reference strain in the EURL-*Salmonella* PT Typing 2024: 24SCA-REF (*Salmonella* Infantis).

Apart from the reference cluster, any other clusters could be reported optionally. In addition, participants were asked wether their NGS analysis included identification of antimicrobial resistance (AMR) markers and to optionally report on this as well.

Strains 24SCA12 and 24SCA16 were expected not to pass the QC of the participants, because of their characteristics (Table 3.3B). The PT Typing 2024 protocol indicates that participants have to exclude strains from the cluster analysis/distance matrix if the data do not pass their QC.

Based on the PT Typing 2024 protocol and cluster definition, results were expected to indicate that the 'wet' strains 24SCA02 (reference strain), technical duplicate strains 24SCA03 and 24SCA05 (clustering with the reference strain), and the 'dry' strain 24SCA14 (reference strain) form a cluster with the provided reference outbreak strain 24SCA-REF data (see also Figure 3.1).

Evaluation of the participants' cluster analysis results was performed by comparing the participants' results to the expected results in the outbreak investigation setting, as pre-defined by the EURL-*Salmonella.* No specific performance criteria were set for this PT on cluster analysis. As a minimum, it was expected that participants would report any technical duplicate strains to be (part of) one cluster. Also, strains were expected to be excluded from the cluster analysis/distance matrix if the data did not pass the participants' QC.

4 Results and Discussion

4.1 Technical data

4.1.1 General

A total of 33 laboratories participated in this PT (Chapter 2). They included 27 NRLs-*Salmonella* in the 27 EU Member States and six NRLs from third countries: the EU candidate countries Moldova and Serbia, the European Free Trade Association (EFTA) countries Iceland, Norway and Switzerland, and the United Kingdom.

One laboratory (Labcode 33) participated for the first time in an EURL-*Salmonella* serotyping study. Because this non-EU MS laboratory only had a limited set of antisera and a limited scope of serotypes, it was not appropriate to evaluate their results completely according to the standard procedure as presented in section 3.2.2. Their results were excluded from the overall performance results of the PT Serotyping 2024 (n=32).

The frequency of *Salmonella* serotyping at the participating laboratories and the number of strains (approximately) serotyped in 2024 are summarised in Table 4.1.

Table 4.1 Frequency and number of Salmonella *strains serotyped in 2024*

Laboratory code	Serotyping frequency in 2024	No. of strains serotyped in 2024
26	Daily	300
8	Daily	320
11	Daily	320
15	Daily	400
5	Daily	500
20	Daily	500
21	Daily	500
1	Daily	512
13	Daily	550
16	Daily	550
19	Daily	600
7	Daily	900
29	Daily	900
6	Daily	2429
14	Daily	2500
22	Daily	2500
2	Daily	3000
32	Daily	3000
4	Daily	3500
12	Daily	3500
27	Daily	6500
23	Thrice a week	132
28	Thrice a week	233
24	Thrice a week	237
3	Thrice a week	2400

Laboratory code	Serotyping frequency in 2024	No. of strains serotyped in 2024
18	Twice a week	74
17	Twice a week	100
10	Once a week	120
33	Once a week	123
31	Once a week	350
25	Once a week	360
30	Monthly	20
9	-	17
n=33	Overall	37 947

4.1.2 *Accreditation*

Out of the 33 participants, 31 are accredited for serotyping *Salmonella.* Thirty according to EN ISO/IEC 17025, two of them combined with EN ISO 15189. One participant mentioned EN ISO 15189 only.
The one non-EU laboratory not accredited for serotyping is known for this because of its relatively low number of serotyping strains. The one EU NRL currently not accredited for serotyping indicated still to plan re-accreditation for 2025.
Thirty laboratories stated that they are accredited for all *Salmonella* serovars, one of these mentioning the exception of Paratyphi B. Laboratory 33 indicated to be accredited for *S*. Enteritidis, *S*. Typhimurium, *S*. Infantis, *S*. Hadar, and *S*. Virchow only.

4.1.3 *Transport of samples*

All but three participants received their package within two days after shipment on Monday 4 November 2024. One package was received on 8 November, one on 9 November and the final one only arrived by 20 November 2024, due to problems at customs. All laboratories received the packages in good condition.

4.2 Serotyping results

4.2.1 *General*

The twenty obligatory strains were all tested by the NRLs-*Salmonella* in the participating countries. A total of 32 participants used classical serology. Ten of them mentioned the combined use of classical serology and Luminex assays (2), multiplex/real-time PCR (4), or WGS (3). One participant used Whole Genome Sequencing (WGS), supplemented with traditional agglutination using O:6 plus O:8 (strains S6 and S19) and O:22 plus O:23 (strain S4).
Details on the number and the source of the antisera used by the participants are summarised in Tables 4.2 and 4.3.

Table 4.2 Number of laboratories using antisera from various manufacturers

Manufacturer	Number of NRLs (n=32*)
Bio-Rad	13
Pro-Lab	4
Sifin	20
Statens Serum Institut (SSI)	31
Other	2
Own preparation	2

*Missing data from one participant.

Table 4.3 Number of laboratories using antisera from one or more manufacturers and/or in-house prepared sera

Number of manufacturers from which antisera are obtained (including in-house preparations)	Number of NRLs (n=32*)
1	9
2	8
3	13
4	2

*Missing data from one participant.

4.2.2 *Biochemical testing*

Five participants reported they had not used biochemical testing on the strains. A few participants used a standard set of biochemical tests on all the strains. The majority of participants used a variety of tests, or no tests at all, depending on the strain. Strains S2 (Menston), S4 (Okatie), S18 (Southbank), S20 (Indiana), and S21 (47:k:z_{35}) were subject to biochemical tests most often (by 19 – 22 laboratories).
Laboratory 15 routinely tested all 21 strains using MALDI-TOF.

4.2.3 *Use of PCR for confirmation*

Fifteen laboratories used PCR to confirm strain S11, the monophasic variant of *S*. Typhimurium 4,5:i:-, and seven of them also used PCR to confirm strain S8, *S*. Typhimurium. For this, most laboratories indicated the reference 'Tennant et al., 2010' or 'Maurischat et al., 2015'.

4.2.4 *Serotyping results per laboratory*

The evaluation of the type of errors for O- and H-antigens and the identification of the strains are shown in Figures 4.1, 4.2, and 4.3. The percentages of correct results per laboratory are shown in Figure 4.4. The results of participant 33 are excluded from these evaluations.
The O-antigens were all typed correctly by 28 out of the 32 participants (88%). This corresponds to 99,4% of the total number of strains. The H-antigens were typed completely correctly by 29 out of the 32 participants (91%), corresponding to 98,6% of the total number of strains. As a result, 26 participants (81%) reported all serovar names correctly, which corresponds to 98,3% of all strains evaluated.

Figure 4.1 Evaluation of the type of errors for O-antigens, per participant

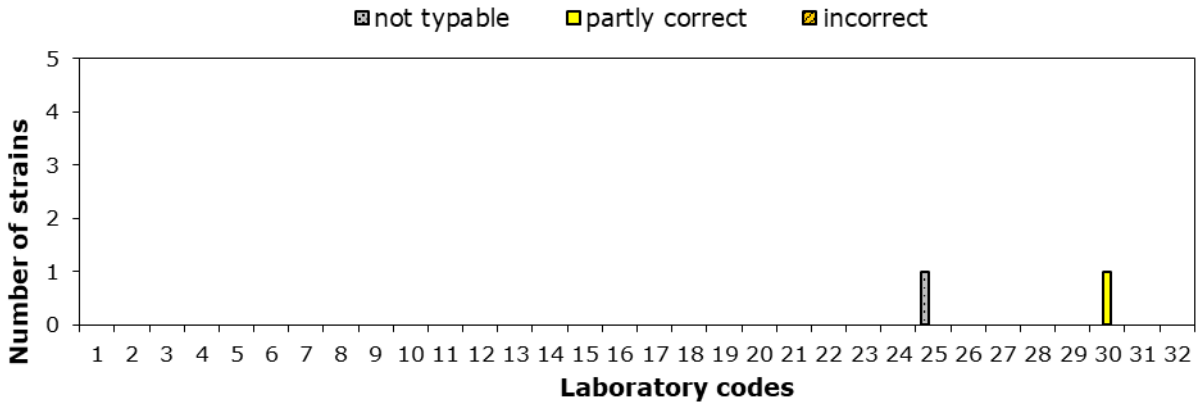
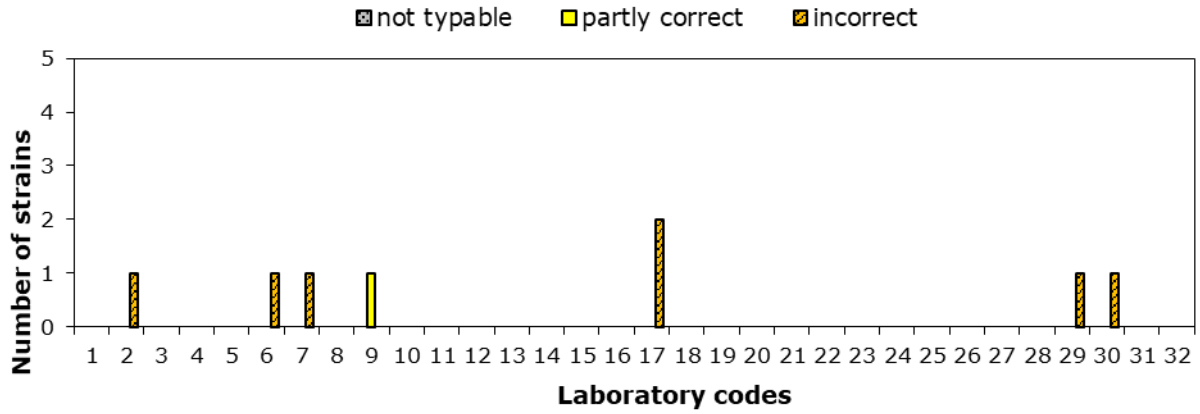


Figure 4.2 Evaluation of the type of errors for H-antigens, per participant



Note: The incorrect result for Laboratory 2 was proven afterwards to have been a typo, made while uploading the data for the H-antigens.

Figure 4.3 Evaluation of the type of errors in the identification of the serovar names, per participant

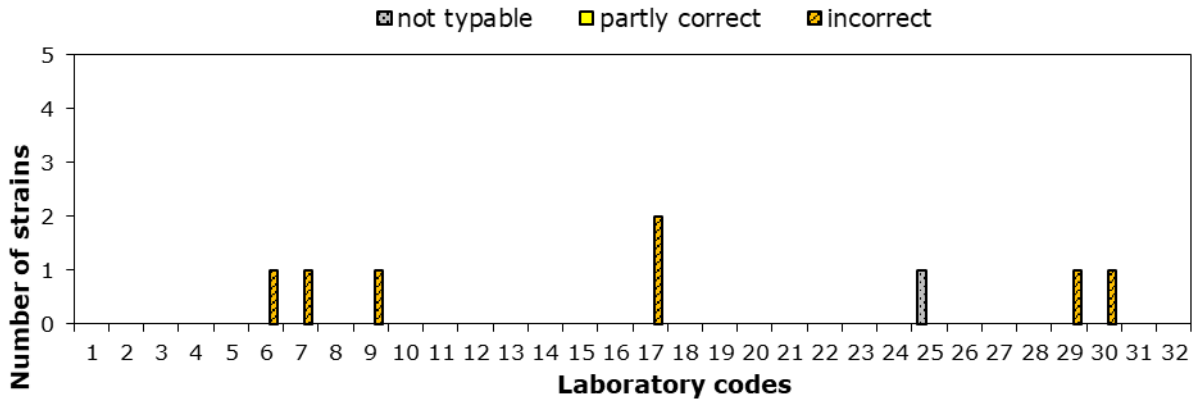
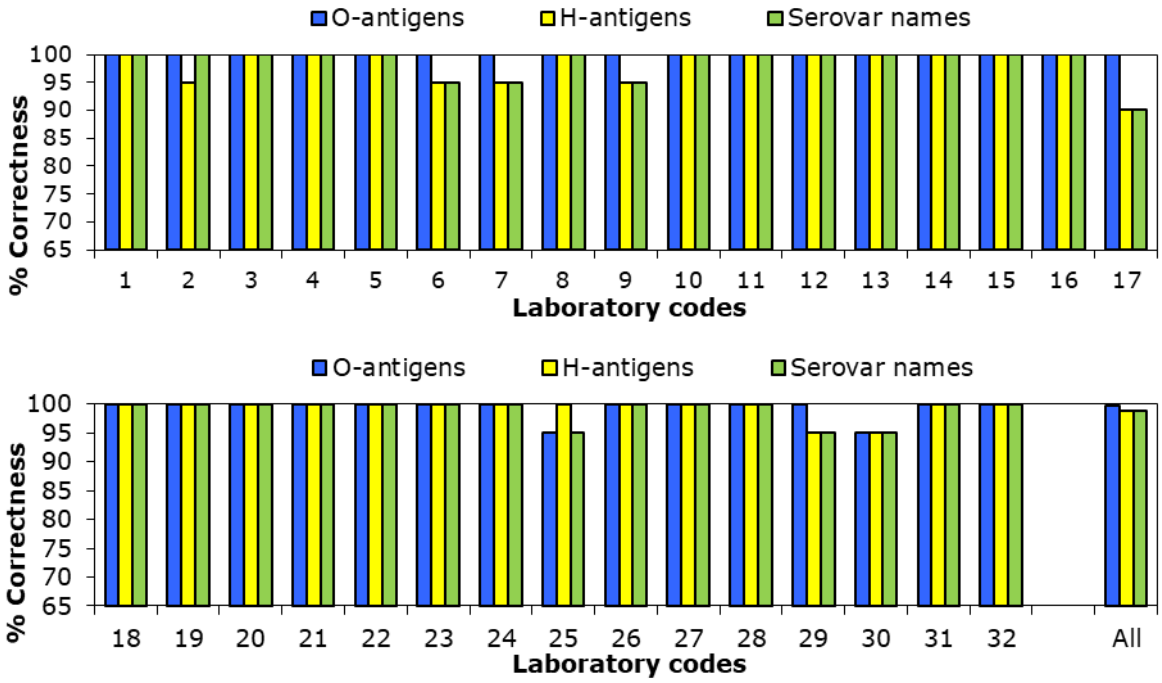


Figure 4.4 Percentages of correct serotyping results, per participant



4.2.5 *Performance of the participants*

The number of penalty points was determined for each NRL using the guidelines described in Section 3.2.2. Table 4.4 shows the number of penalty points for each NRL and indicates whether the level of good performance was achieved (yes or no). Table 4.5 shows the percentages of correctly typed strains, both for EU-MS participants separately and for all participants.

Overall, the participants' performance in the PT Serotyping 2024 was very good. This included the results of Laboratory 9, which has clearly improved on its unsatisfactory performance in the PT Serotyping 2023 (Jacobs-Reitsma et al., 2025a) following the practical and dedicated training at the EURL-*Salmonella* in November 2024.

Except for Laboratory 33, all participants met the level of good performance. Non-EU MS Laboratory 33 was participating for the first time and clearly did not yet have all materials or the expertise to be able to perform the serotyping properly (see also Appendix 2). Moreover, they did not meet the level of good performance, even by taking into account their limited scope of accreditation (*S*. Enteritidis, *S*. Typhimurium, *S*. Infantis, *S*. Hadar, and *S*. Virchow). Laboratory 33 was contacted for more detailed information exchange, and a dedicated training on-site was organised just before the next EURL-*Salmonella* PT Serotyping in 2025.

All participants received both their individual laboratory evaluation report and the interim summary report on serotyping on 28 February 2025. Appendix 1 shows an example of an individual laboratory evaluation report on serotyping.

Thanks to a very attentive reader, a small but potentially confusing typo was discovered in both the individual Laboratory performance reports and in the Interim summary report. This was updated in Version 2 of the Interim summary report (Jacobs-Reitsma et al., 2025b). The antigenic

formula for Strain S14 (Kisarawe) in Table 1 (Table 3.1 in the current report) was corrected from 11:k:e,n,**z**,[z15] into 11:k:e,n,**x**,[z15]. This typo did not affect the evaluation of the results in any way. All participants were informed on this by email on 30 April 2025.

Table 4.4 Evaluation of serotyping results per NRL

Laboratory code	Number of penalty points	Good performance
1	0	yes
2	0	yes
3	0	yes
4	0	yes
5	0	yes
6	1	yes
7	1	yes
8	0	yes
9	1	yes
10	0	yes
11	0	yes
12	0	yes
13	0	yes
14	0	yes
15	0	yes
16	0	yes
17	2	yes
18	0	yes
19	0	yes
20	0	yes
21	0	yes
22	0	yes
23	0	yes
24	0	yes
25	0	yes
26	0	yes
27	0	yes
28	0	yes
29	1	yes
30	1	yes
31	0	yes
32	0	yes
33	31	NO

Table 4.5 Correctly typed strains per total number of strains tested and per laboratories participating, separately for EU-MS participants and for all participants.

Results	EU-MS participants	All participants*
Total number of participants	27	32
Total number of strains per participant	20	20
O-antigens correct/strains tested	538/540 (99,6%)	638/640 (99,7%)
H-antigens correct/strains tested	533/540 (98,7%)	632/640 (98,8%)
Serovar names correct/strains tested	533/540 (98,7%)	632/640 (98,8%)
O-antigens correct/labs participating	25/27 (93%)	30/32 (94%)
H-antigens correct/labs participating	21/27 (78%)	25/32 (78%)
Serovar names correct/labs participating	21/27 (78%)	25/32 (78%)
Total number of Penalty Points	6	7
Total number 'unsatisfactory performance'	0	0

* Excluding Laboratory 33.

4.2.6 *Serotyping results per strain*

Appendix 2 displays the final naming results reported per strain (S1 – S20) and per laboratory (1-33). A completely correct identification (*Laboratory 33 excluded*) was obtained for 15 *Salmonella* serovars: Enteritidis (S1), Menston (S2), Infantis (S3), Moroto (S5), Typhimurium (S8), Carmel (S9), Lagos (S10), 1,4,[5],12:i:- (S11), Ridge (S12), Oslo (S13), Virchow (S15), Give (S16), Southbank (S18), Hadar (S19), and Indiana (S20).
Appendix 2 also presents the reported serovar names for strain 1,4,[5],12:i:- (S11). Fifteen participants used a PCR method to confirm this strain to be monophasic Typhimurium.

Appendix 3 includes the details on the strains that caused problems in serotyping. The problems all seem to be individual strain/participant cases.

Appendix 4 describes details on the additional and optional strain S21. All but two participants tried to serotype strain S21, a *Salmonella enterica* subsp. *diarizonae* (IIIb). The completely correct serovar name (47:k:z_{35}) was reported by 27 participants.

4.2.7 *Trend analysis of the serotyping results of the EU NRLs*

Historical data for all participants of the EURL-*Salmonella* PTs on the serotyping of *Salmonella* can be found on the EURL-*Salmonella* website: http://www.eurlsalmonella.eu/publications/proficiency-test-reports
The separate historical data on the EU NRLs-*Salmonella* are visualised in Figure 4.5, which presents the percentages of correctly typed strains. Figure 4.6 presents the numbers of penalty points and unsatisfactory performances.
The percentages of correctly typed strains are stable over time, usually indicating better performance for the O-antigens than for the H-antigens (Figure 4.5).
The number of penalty points has clearly declined, from 35 points when this system started in 2007 to as few as 3 points in the 2017, 2020, and 2023 PTs. The rise seen in the 2018 PT was mainly caused by the seven EU NRLs that made a mistake in typing a *S.* Cannstatt strain. The total numbers of penalty points are strongly affected by the system of four

penalty points for one mistake in the 'top-5' *Salmonella* serovars, as is seen in the PT 2021 as well as in the PT 2022 results (Figure 4.6). However, the number of EU NRLs with an initial non-good performance is low: two in the 2010 – 2013 period plus the PT 2022, one in the 2014, 2015, 2018, and 2021 PTs, and none in the 2016, 2017, 2019, 2020, 2023 PTs and the current PT 2024. All follow-up studies organised for these EU NRLs, only occasionally the same ones, resulted in a good performance after all.

*Figure 4.5 Trend in serotyping results of the EU NRLs-*Salmonella*, based on the percentages of correctly typed strains*

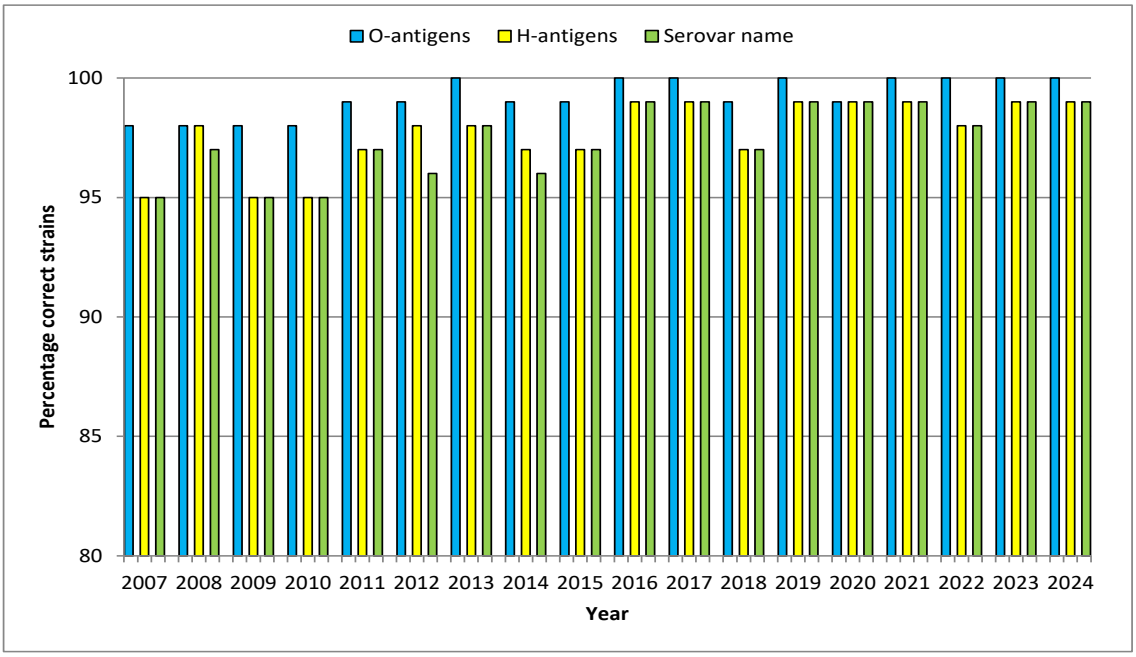
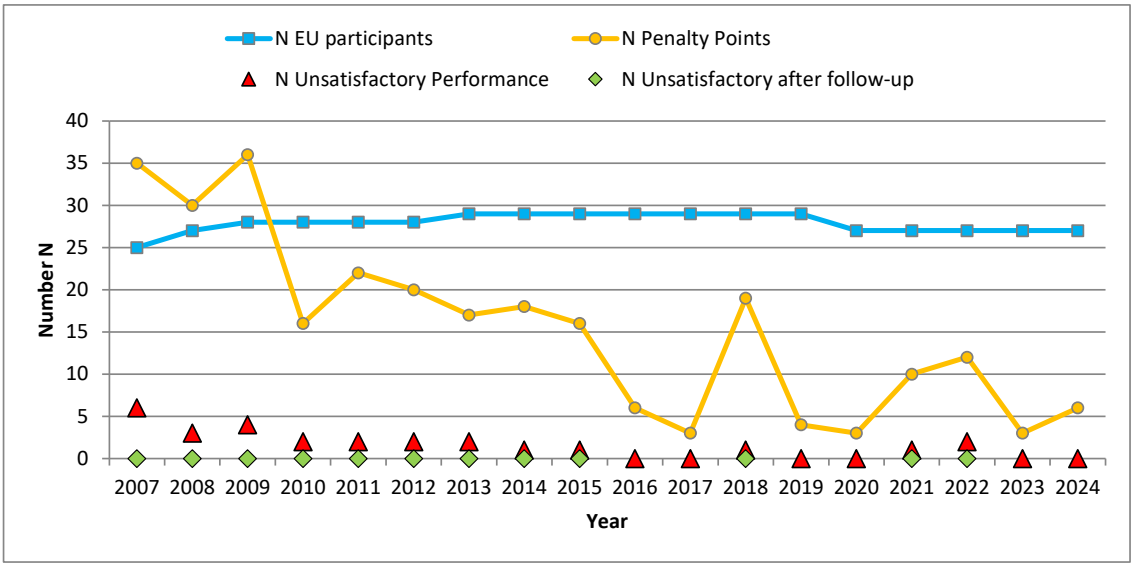


*Figure 4.6 Trend in serotyping results of the EU NRLs-*Salmonella*, based on the number (N) of Penalty Points and Unsatisfactory Performances*



4.3 Cluster analysis results

4.3.1 General

A total of 24 NRLs participated in the cluster analysis; 3 participants provided multiple submissions.
Overall results were presented at the online 30th EURL-*Salmonella* Workshop on 20 May 2025 (https://www.eurlsalmonella.eu/en/workshop-2025) The interim summary report (Jacobs-Reitsma et al., 2025c) was emailed to the participants on 19 May 2025. As in the PT Typing 2023, all relevant information per Laboratory code was tabulated within the Interim summary report.

As a general question, the participants were asked if and how they serotyped the six 'wet' and the six 'dry' strains. Twenty-one participants reported to have serotyped both the 'wet' and the 'dry' strains. Appendix 5 shows these serotyping results, for information purposes only.

4.3.2 Results cluster analysis based on WGS data

Twenty-four participants submitted a total of 27 cluster analysis results based on WGS data. Two participants submitted cgMLST-based as well as SNP-based data results, and one participant submitted cgMLST-based and wgMLST-based data analyses.

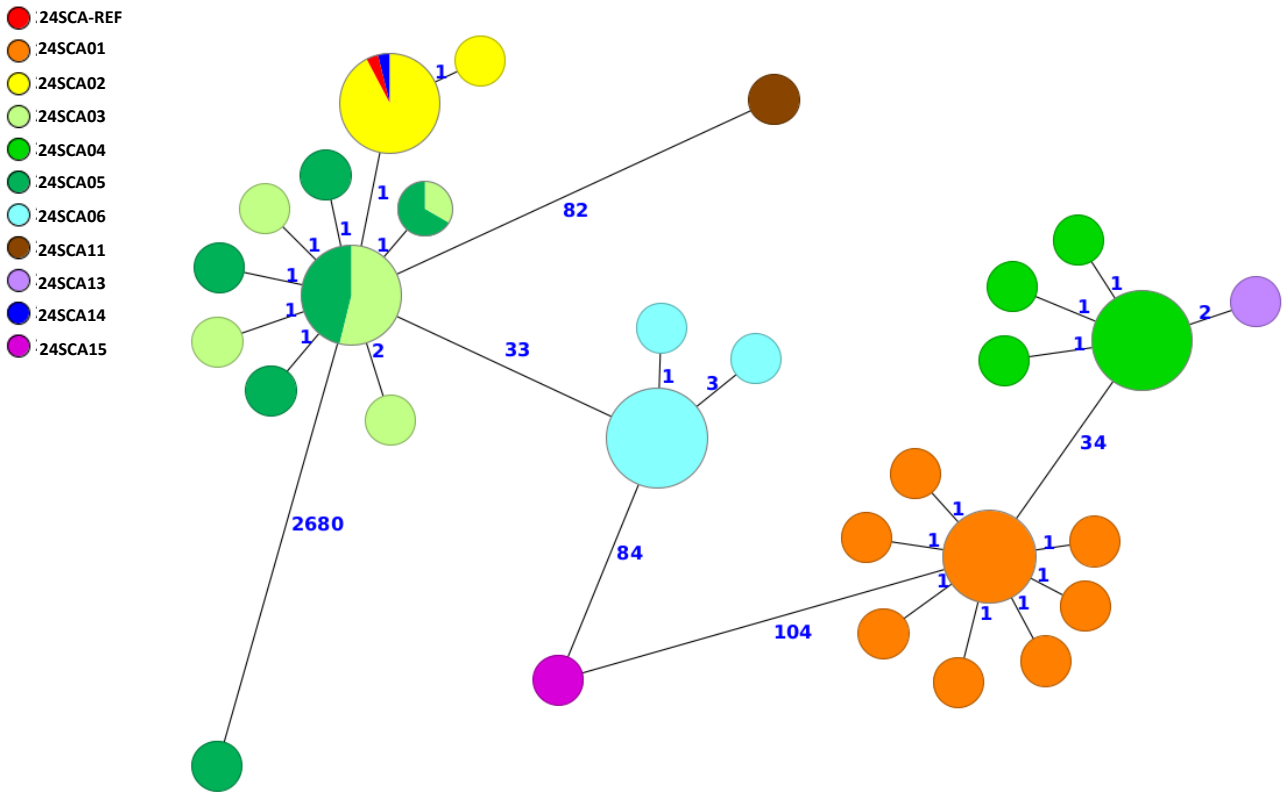
Appendix 6 shows the general details of the wet-lab and dry-lab protocols performed by the participants and the EURL-*Salmonella* (EL). All participants and the EL performed DNA extraction, library preparation, and sequencing in-house, except for Laboratories 63 and 80 (library preparation and sequencing outsourced) and Lab 21 (sequencing outsourced). The most commonly used Illumina platform was the MiSeq (13x), followed by the Illumina NextSeq (9x), MiniSeq (2x) or NovaSeq (1x). Including the EL, 20 submissions were based on cgMLST for data analysis, one on wgMLST, and seven submissions were based on SNP-based analysis (5x reference-based and 2x assembly-based).
Tools used for this analysis varied from in-house pipelines (using the Enterobase scheme) to commercial ones, most often Ridom SeqSphere+ (12x). The most commonly used method for cluster analysis was Minimum Spanning Tree (MST, 10x).

Appendix 7 lists all participants' QC criteria reported for evaluating their WGS data. A variety in naming these QC criteria and in the used thresholds was observed, similar to the previous PTs on cluster analysis (Jacobs-Reitsma et al., 2020, Jacobs-Reitsma et al., 2021, Jacobs-Reitsma et al., 2023a, Jacobs-Reitsma et al., 2023b, Jacobs-Reitsma et al., 2025a). Contamination, coverage, GC%, N50, total length of assembly, and total number of contigs were the most commonly referred parameters.

Fourteen compressed paired-end fastq files (strains 24SCA11 – 24SCA16 plus 24SCA-REF) had to be downloaded for analysis from the secure FTP server. The md5 checksums for these files were also available on the server (Appendix 8). Participants were asked whether they checked the md5sum values after downloading, and 23 participants reported that they had done so. Out of those 23 participants, 19 also

submitted the 'dry' strain md5sum values, which were then checked by the EURL-*Salmonella* again (Appendix 9).
Twenty-two participants submitted the md5sum values of their 'wet' strain analysis before uploading. After downloading by the EURL-*Salmonella*, the md5sums were determined and checked for consistency with the participants' data (Appendix 9).
All participants' raw data (compressed fastq files) for the six 'wet' strains (24SCA01 – 24SCA06) were successfully processed through the EURL-*Salmonella* in-house Juno-assembly pipeline as mentioned before. The *de novo* assembled genomes (fasta files) were analysed in Ridom SeqSphere+, using the cgMLST Enterobase v2.0 and visualised in a MST, which also includes the 'dry' strain data of 24SCA-REF, 24SCA11, 24SCA13, 24SCA14, and 24SCA15 (Figure 4.7). Appendix 10 shows the data per 'wet' strain.

Figure 4.7 MST of the strains from the participants' processed raw data plus the 'dry' strain data (24SCA-REF, 24SCA11, 24SCA13, 24SCA14, 24SCA15) (Ridom SeqSphere+, cgMLST (3002 targets), pairwise ignoring missing values)



Both Figure 4.7 and Appendix 10 show an unexpected result at 2680 AD for strain 24SCA05 reported by Lab 63. This can be explained by Lab 63 reporting serovar Typhimurium instead of Infantis for their strain 24SCA05 (Appendix 5). Most likely, this strain was at some point accidently swapped with the original strain 24SCA05.

An overview of the main QC results on all in-house *de novo* assembled genomes (fasta files) is presented in Table 4.6. Appendix 11 presents detailed data per participant.

Table 4.6 QC results of the in-house de novo *assembled genomes (24SCA01 - 24SCA06), average per participant*

Laboratory Code	Illumina Platform	Average Conta-mination	Average # contigs	Average Largest contig	Average Total length	Average N50	Average Coverage
Lab01	MiSeq	0,97	46	1101740	4820074	411463	103
Lab02	NextSeq	0,97	50	927950	4819345	387637	81
Lab03	NextSeq	0,97	50	929970	4820482	369038	135
Lab04	MiSeq	0,97	50	1058785	4820017	350236	74
Lab05	MiSeq	0,97	49	1098251	4820122	367626	176
Lab07	NextSeq	0,97	50	1098179	4820650	367599	138
Lab11	MiniSeq	0,97	52	868822	4818672	380629	68
Lab12	MiSeq	0,97	85	543387	4816290	143748	97
Lab13	MiSeq	0,97	52	940655	4818937	265559	75
Lab14	MiSeq	0,97	89	700191	4815249	207902	94
Lab16	MiSeq	0,97	46	953141	4821678	327970	74
Lab17	MiSeq	0,97	51	1070286	4811887	369019	109
Lab19	MiSeq	0,97	54	877302	4819588	305439	63
Lab20	NextSeq	1,04	49	1023511	4820365	369621	240
Lab21	NextSeq	0,97	65	507584	4807851	165505	112
Lab23	MiSeq	0,97	51	1073941	4819833	369431	76
Lab24	NextSeq	0,97	47	1105801	4820813	361812	98
Lab27	NextSeq	0,97	219	171462	4796106	57423	111
Lab28	MiSeq	0,97	40	1114415	4822209	423256	152
Lab29	MiSeq	0,97	52	978720	4819641	319186	80
Lab31	MiSeq	0,97	49	1014829	4820115	359945	75
Lab32	MiniSeq	0,97	52	807478	4818326	401158	71
Lab63	NovaSeq	0,83	51	988295	4813974	313526	573
Lab80	NextSeq	0,97	48	1020372	4820244	406532	257
EL	NextSeq	0,95	45	1098233	4800430	394413	168

Participants were asked to report per strain:

- whether or not the data passed their QC criteria;
- whether or not a cluster with the reference strain in the EURL-*Salmonella* PT Typing 2024 (24SCA-REF) was found.

Apart from the reference cluster, any other clusters could optionally be reported.
In addition, and for the first time in the PTs cluster analysis history, participants were asked wether their NGS analysis included identification of AMR markers. Twelve participants submitted results on this topic.

4.3.3 *Evaluation cluster analysis results*

Strains 24SCA12 and 23SCA16 were expected not to pass the QC of the participants, because of their characteristics (Table 3.3B). The PT Typing 2024 Protocol indicated that participants had to exclude strains from the cluster analysis if the data did not pass the QC. Participants' reasons for (not) excluding these strains are presented in Appendix 12.

- Strain 24SCA12 (wet mixture of serovars Infantis and Typhimurium) was reported as not passing the QC criteria by 23

out of the 24 participants, and these 23 participants excluded this strain from their distance matrices.
- Strain 24SCA16 (wet mixture of serovars Infantis and Paratyphi B, var. Java) was reported not passing the QC criteria by all 24 participants and was excluded from all distance matrices.

Appendix 13 shows the participants' distance matrix data per submission, also regarding strains that were (not) excluded from the cluster analysis. The results for Laboratory 3 suggest a mismatch between some of the strain codes and the corresponding data, which also explains the deviating results for the cluster analysis (Table 4.7).

The cluster definition for this particular PT Typing 2024 situation was set at a maximum of five AD from the reference sequence. Based on this cgMLST-based criterium, results were expected to indicate the 'wet' strains 24SCA02 (reference strain), technical duplicate strains 24SCA03 and 24SCA05 (clustering with the reference strain), and the 'dry' strain 24SCA14 (reference strain) to form a cluster with the provided reference outbreak strain 24SCA-REF data (see also Figure 3.1).
Out of the 27 submissions (three participants with multiple submissions), 23 reported those four strains to be part of the cluster. Lab 24 also included the mixed serovars 'strain' 24SCA12 into this cluster. Consequently, 22 out of the 27 submissions reported the cluster completely as expected (Table 4.7).
Technical duplicate strains 24SCA03 and 24SCA05 were reported within one cluster in 24 out of the 27 submissions.

Optionally, any other cluster(s) apart from the reference could be submitted. Out of 21 submissions, 17 correctly reported strains 24SCA04 and 24SCA13 to form a second cluster.
In addition, participants could optionally report the identification of AMR markers. Twelve participants did and predominantly used ResFinder, AMRFinderPlus and PointFinder for this task. Appendix 14 shows detailed data per participant for information purposes only.

Table 4.7 Expected cluster analysis results and the cluster analysis results reported per data analysis method by the 24 participants

	Strain code											
Labcode-method	**24 SCA01**	**24 SCA02**	**24 SCA03**	**24 SCA04**	**24 SCA05**	**24 SCA06**	**24 SCA11**	**24 SCA12**	**24 SCA13**	**24 SCA14**	**24 SCA15**	**24 SCA16**
	Inf	Inf=REF	Inf	Inf	Inf	Inf	Inf	Inf+STM	Inf	Inf=REF	Inf	Inf+Java
Expected	**No**	**Yes**	**Yes**	**No**	**Yes**	**No**	**No**	**n.a.**	**No**	**Yes**	**No**	**n.a.**
1-cgMLST	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
2-cgMLST	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
3-cgMLST	No	No*	Yes	Yes*	No*	Yes*	No	n.a.	No	Yes	No	n.a.
4-cgMLST	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
5-cgMLST	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
7-cgMLST	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
11-cgMLST	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
12-cgMLST[a)]	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
13-cgMLST[b)]	No	Yes	Yes	No	Yes	No	No	n.a.	No	n.a.*	No	n.a.
14-cgMLST	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
16-cgMLST[c)]	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	n.a.*	n.a.
17-cgMLST	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
17-wgMLST	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
19-SNPa[d)]	No	No*	No*	No	Yes	No	No	n.a.	No	No*	No	n.a.
20-cgMLST	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
21-SNPr	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
23-cgMLST	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
23-SNPr	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
24-cgMLST	No	Yes	Yes	No	Yes	No	No	Yes*	No	Yes	No	n.a.
27-SNPr	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
28-cgMLST	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
29-cgMLST	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
31-SNPr	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
32-cgMLST	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
63-SNPr[e)]	No	Yes	Yes	No	n.a.*	No	No	n.a.	No	Yes	No	n.a.
80-cgMLST	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.
80-SNPa	No	Yes	Yes	No	Yes	No	No	n.a.	No	Yes	No	n.a.

*In blue: Deviation from the expected result.
The four clustering strains (24SCA02, 24SCA03, 24SCA05, 24SCA14) are indicated in green. The two strains expected to be excluded (24SCA12, 24SCA16) are indicated in orange.
Yes/No: Whether or not a cluster with the reference strain in the EURL-*Salmonella* PT Typing 2024 (24SCA-REF) was found.
n.a.: not applicable (QC not passed).

a) Comment Lab 12: We would have repeated NGS of strain 24SCA12 as it has an AD=3 to the reference strain, but failed QC.
b) Comment Lab 13: 24SCA12 included *S*. Infantis which clustered with the REF strain but was contaminated with other *Salmonella*. 24SCA14 included *S*. Infantis which clustered with REF strain but was contaminated slightly with *E. coli*. 24SCA16 included *S*. Paratyphi B and some other *Salmonella*. All of them were excluded from the final cluster analysis.
c) Comment Lab 16: Strain 24SCA15: Intraspecies contamination with 37 SNVs, excluded from analysis/distance matrix.
d) Comment Lab 19: Strain 24SCA02, Strain 24SCA03 and 24SCA0314 shared 11 SNPs.
e) Comment Lab 63: Data set passed QC criteria for *Salmonella*, but was excluded from reference-based SNP-analysis as it is a more distant sequence type.

5 Conclusions

5.1 Serotyping

- The overall results for the 32 evaluated participants are:
  - They typed 99,7% of the strains correctly for the O-antigens.
  - They typed 98,8% of the strains correctly for the H-antigens.
  - They named 98,8% of the strains correctly.
- All 27 EU NRLs and five non-EU NRLs achieved the defined level of good performance directly.
- One non-EU NRL-*Salmonella* did not achieve the defined level of good performance. This new participant participated in an on-site practical training prior to the next PT Serotyping.

5.2 Cluster analysis

- The optional WGS cluster analysis was based on the simulation of an outbreak-related request to the NRL network from the EURL-*Salmonella* and/or EFSA/ECDC, including a description of the cluster definition.
- Selection of suitable PT strains included pre-testing the strains by the EURL-*Salmonella*.
  - Six strains were shipped to the participants for 'wet' analysis.
  - Raw WGS data of six strains plus the reference strain were made available through a secure FTP server for 'dry' analysis.
- A total of 24 participants performed cluster analysis and made a total of 27 submissions.
- Out of the 27 submissions, 23 reported the four expected strains to be part of the cluster.
- The two mixed serovars 'strains' were correctly excluded from the cluster analysis, except for strain 24SCA12 by one participant.
- Consequently, 22 out of the 27 submissions reported the cluster completely as expected.

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List of acronyms

AD	Allelic Difference
AMR	Antimicrobial Resistance
ATCC	American Type Culture Collection
BPW	Buffered Peptone Water
CA	Cluster Analysis
cgMLST	core genome Multilocus Sequence Typing
DG-SANTE	Directorate General for Health and Food Safety
EC	European Commission
ECDC	European Centre for Disease prevention and Control
EFSA	European Food Safety Authority
EFTA	European Free Trade Association
EL	EURL-*Salmonella* Laboratory
EU	European Union
EURL-*Salmonella*	European Union Reference Laboratory for *Salmonella*
FTP	file transfer protocol
HI agar	Hearth Infusion agar (in transport tubes)
IDS	Centre for Infectious Diseases Research, Diagnostics and Screening (at RIVM)
Inf	*Salmonella* Infantis
ISO	International Organization for Standardization
Java	*Salmonella* Paratyphi B, var. Java
MALDI-TOF	Matrix Assisted Laser Desorption Ionization Time-Of-Flight mass spectrometry
MLVA	Multiple-Locus Variable number of tandem repeat Analysis
MST	Minimum Spanning Tree
n.a.	not applicable
NGS	Next Generation Sequencing
NRL-*Salmonella*	National Reference Laboratory for *Salmonella*
PCR	Polymerase Chain Reaction
PT	Proficiency Test
QC	Quality Control
REF	Reference
RIVM	National Institute for Public Health and the Environment (Bilthoven, The Netherlands)
SNP	Single Nucleotide Polymorphism
SNPa	assembly-based SNP analysis
SNPr	reference-based SNP analysis
SSI	Statens Serum Institut (Copenhagen, Denmark)
STM	*Salmonella* Typhimurium
TR	Technical Report
wgMLST	whole genome Multilocus Sequence Typing
WGS	Whole Genome Sequencing

Appendix 1 Example of an individual laboratory evaluation report on serotyping results

Page 1 Performance

EURL-*Salmonella* PT Serotyping 2024 Number of penalty points: 0 Evaluation: Good Performance

	Reference Results				**Results NRL lab code:**			**10**
Strain	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
S1	1,9,12	g,m	-	Enteritidis	9,12	g,m	-	Enteritidis
S2	6,7	g,s,[t]	[1,6]	Menston	7	g,s,t	-	Menston
S3	6,7,14	r	1,5	Infantis	6,7	r	1,5	Infantis
S4[a)]	13,23	g,[s],t	-	Okatie	13,23	g,t,s	-	Okatie
S5[a)]	28	z10	l,w	Moroto	28	z10	l,w	Moroto
S6	8,20	z10	z6	Molade	8,20	z10	z6	Molade
S7[a)]	3,10	y	e,n,z15	Bolton	3,10	y	enz15	Bolton
S8	1,4,[5],12	i	1,2	Typhimurium	4,5,12	i	1,2	Typhimurium
S9[a)]	17	l,v	e,n,x	Carmel	17	l,v	enx	Carmel
S10	1,4,[5],12	i	1,5	Lagos	4,5,12	i	1,5	Lagos
S11[b)]	1,4,[5],12	i	-	1,4,[5],12:i:-	4,5,12	i	-	4,5,12:i:-
S12[a)]	9,12	c	z6	Ridge	9,12	c	z6	Ridge
S13[a)]	6,7,14	a	e,n,x	Oslo	7	a	enx	Oslo
S14[a)]	11	k	e,n,x,[z15]	Kisarawe	11	k	enx	Kisarawe
S15	6,7,14	r	1,2	Virchow	7	r	1,2	Virchow
S16	3,{10}{15}{15,34}	l,v	1,7	Give	10	l,v	1,7	Give
S17[a)]	[1],6,14,[25]	d	e,n,x	Charity	14,25	d	enx	Charity
S18[a)]	3,{10}{15}{15,34}	m,t	[1,6]	Southbank	10	m,t	-	Southbank
S19	6,8	z10	e,n,x	Hadar	6,8	z10	enx	Hadar
S20	1,4,12	z	1,7	Indiana	4,12	z	1,7	Indiana
S21[c)]	47	k	z35	47:k:z35 (IIIb)	47	k	z35	47:k:z35

[a)] Represented in an EURL-*Salmonella* PT Serotyping for the first time.
[b)] Monophasic variant of *S*. Typhimurium based on genomic sequences. Phenotypic result: 4,5:i:-.
[c)] *Salmonella enterica* subspecies *diarizonae* (optional strain).

Page 2 Performance
EURL-*Salmonella* PT Serotyping 2024

For back-ground information, reference results are given completely according to the White-Kauffmann-Le Minor scheme (2007).
Participants were asked to report only those results, on which the identification of serovar names was based.

Colour coding:
Blue : remark (e.g. spelling error, or deviations in the results of optional strain S21).
Grey : not typable (e.g. antisera not available, rough strain).
Yellow : partly correct; the naming: no penalty points.
Orange : incorrect; in the naming: 1 penalty point.
Red : incorrect; in the naming: 4 penalty points.

As decided at the 29th EURL-*Salmonella* Workshop (Leiden, NL, 2024), Strain S21 was an additional strain to the study.
Testing of this strain was optional and results were not included in the evaluation (remarks in blue or grey only).
The evaluation of the serotyping results was performed as indicated in Table 1 of the Protocol as sent to the participants.
In addition to that, Good Performance was evaluated on the basis of penalty points as indicated below.

4 penalty points: Incorrect typing of *S*. Enteritidis, *S*. Typhimurium (including monophasic variant), *S*. Hadar, *S*. Infantis or *S*. Virchow or assigning the name of one of these 5 serovars to another serovar.
1 penalty point: Incorrect typing of all other *Salmonella* serovars.
(no penalty points are given in case a strain was non-typable due to auto-agglutination)

Good Performance is defined as < 4 penalty points.

Appendix 2 Serotyping results per strain (S1 – S20) and per laboratory (1 – 33)

Lab: REF	S1 Enteritidis	S2 Menston	S3 Infantis	S4 Okatie	S5 Moroto	S6 Molade	S7 Bolton	S8 Typhimurium	S9 Carmel
1	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
2	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
3	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
4	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
5	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
6	Enteritidis	Menston	Infantis	Okatie	Moroto	Bazenheid[4)]	Bolton	Typhimurium	Carmel
7[1)]	enteritidis	6,7:g,s,t:-[1)]	infantis	13,23:g,s,t,:-[1)]	moroto	molade	bolton	typhimurium	carmel
8	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
9	Enteritidis	Menston	Infantis	Agbeni[4)]	Moroto	Molade	Bolton	Typhimurium	Carmel
10	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
11	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
12	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
13	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
14	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
15	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
16	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
17	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Lamberhurst[4)]	Typhimurium	Carmel
18	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
19	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
20	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
21	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
22	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
23	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
24	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel

Lab:	S1	S2	S3	S4	S5	S6	S7	S8	S9
REF	**Enteritidis**	**Menston**	**Infantis**	**Okatie**	**Moroto**	**Molade**	**Bolton**	**Typhimurium**	**Carmel**
25	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
26	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
27	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
28	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
29	Enteritidis	Menston	Infantis	Okatie	Moroto	Remiremont[4)]	Bolton	Typhimurium	Carmel
30	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
31	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
32	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel
33	Enteritidis	C1[2)]	Virchow[5)]	C2[4)]	C2[4)]	B[4)]	E[2)]	Typhimurium	-[2)]
X	0	0	0	1	0	2	1	0	0

S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	Lab:
Lagos	4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	REF
Lagos	Typhimurium, monophasic	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	1
Lagos	4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	2
Lagos	I 4:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	3
Lagos	Monophasic Typhimurium	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	4
Lagos	4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	5
Lagos	4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	6
lagos	4,5,12:i:-	ridge	oslo	kisarawe	virchow	give	fischerkietz[4)]	3,10:m,t:- [1)]	hadar	indiana	7
Lagos	4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	8
Lagos	Typhimurium monofaza	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	9
Lagos	4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	10
Lagos	4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	11
Lagos	1,4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	12
Lagos	Typhimurium, monophasic	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	13
Lagos	Monophasic Variant S. Typhimurium	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	14
Lagos	4,[5],12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	15
Lagos	monophasic Typhimurium	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	16
Lagos	4,5,12:i:-	Ridge	Oslo	Mannheim[4)]	Virchow	Give	Charity	Southbank	Hadar	Indiana	17
Lagos	4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	18
Lagos	4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	19
Lagos	Monophasic variant (4,5,12:i:-)	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	20
Lagos	4,[5],12:i:- (monophasic Typhimurium)	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	21
Lagos	4,5,12:i:- (mST)	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	22
Lagos	4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	23
Lagos	4,5,12: i: -	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	24
Lagos	4:i:-	Ridge	Olso	Kisarawe	Virchow	Give	Lindern/Charity[2)]	Southbank	Hadar	Indiana	25
Lagos	monophasic Typhimurium	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	26
Lagos	4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	27

S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	Lab:
Lagos	**4,5,12:i:-**	**Ridge**	**Oslo**	**Kisarawe**	**Virchow**	**Give**	**Charity**	**Southbank**	**Hadar**	**Indiana**	**REF**
Lagos	4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	28
Lagos	4,5:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	29
Lagos	Monophasic Salmonella Typhimurium	Ridge	Oslo	Kisarawe	Virchow	Give	Manhattan[4)]	Southbank	Hadar	Indiana	30
Lagos	4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	31
Lagos	4,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	32
C2[4)]	C2[5)]	D[2)]	C1[2)]	B[4)]	Typhimurium[5)]	C1[4)]	Hadar[5)]	Infantis[5)]	B[5)]	Typhimurium[5)]	33
0	0	0	0	1	0	0	2	0	0	0	**X**

[1)]In blue : remark (e.g. spelling error).
[2)]In grey : not typable (e.g. antisera not available, rough strain).
[3)]In yellow : partly correct; in the naming: no penalty points.
[4)]In orange : incorrect; in the naming: 1 penalty point.
[5)]In red : incorrect; in the naming: 4 penalty points.

X = number of deviating laboratories (by penalty points) per strain, *Laboratory 33 excluded.*
NOTE: The Table reflects the raw data submitted by the participants. However, the electronic result form does not allow input in special fonts like 'italic' or 'subscript'.

Results for strain S21 are given in Appendix 4.

Appendix 3 Details per strain that caused problems in serotyping

NOTE: Laboratory 33 excluded.

Strain code	Lab code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
S-2	**REF**	**6,7**	**g,s,[t]**	**[1,6]**	**Menston**
S-2	7	6,7	g,s,t	-	6,7:g,s,t:-
S-3	**REF**	**6,7,14**	**r**	**1,5**	**Infantis**
S-3	2	6,7	r	1,2	Infantis
S-4	**REF**	**13,23**	**g,[s],t**	**-**	**Okatie**
S-4	7	13,23	g,s,t	-	13,23:g,s,t,:-
S-4	9	13,23	g,m,s,t	-	Agbeni
S-6	**REF**	**8,20**	**z10**	**z6**	**Molade**
S-6	6	8,20	z10	1,2	Bazenheid
S-6	29	8,20	z10	l,w	Remiremont
S-7	**REF**	**3,10**	**y**	**e,n,z15**	**Bolton**
S-7	17	3,10	e,h	e,n,z15	Lamberhurst
S-14	**REF**	**11**	**k**	**e,n,x,[z15]**	**Kisarawe**
S-14	17	11	k	l,w	Mannheim
S-17	**REF**	**[1],6,14,[25]**	**d**	**e,n,x**	**Charity**
S-17	7	6,14,25	y	e,n,x	fischerkietz
S-17	25*	6,14	d	e,n,x	Lindern, Charity*
S-17	30	6,8	d	1,5	Manhattan
S-18	**REF**	**3,10**	**m,t**	**-**	**Southbank**
S-18	7	3,10	m,t	-	3,10:m,t:-

*Remark Laboratory 25 regarding Strain S-17: we did not have O25 and O24,25 or control strains in house. Therefore we could not distinguish between Lindern and Charity.

In blue : remark (e.g. spelling error).
In grey : not typable (e.g. antisera not available, rough strain).
In yellow : partly correct; in the naming: no penalty points.
In orange : incorrect; in the naming: 1 penalty point.

Descriptive text explaining the colours used in the Table in Appendix 3:
Strain S-2 reference result: Menston 6,7,:g,s,[t]:[1,6]
Laboratory 7 recorded 6,7:g,s,t:- instead of Menston (remark).

Strain S-3 reference result: Infantis 6,7,14:r:1,5
Laboratory 2 recorded 1,2 instead of 1,5 (incorrect, no penalty point).

Strain S-4 reference result: Okatie 13,23:g,[s],t:-
Laboratory 7 recorded 13,23:g,s,t:- instead of Okatie (remark).
Laboratory 9 recorded g,m,s,t instead of g,[s],t (partly correct) and Agbeni (incorrect, 1 penalty point).

Strain S-6 reference result: Molade 8,20:z10:z6
Laboratory 6 recorded 1,2 instead of z6 (incorrect) and Bazenheid (incorrect, 1 penalty point).
Laboratory 29 recorded l,w instead of z6 (incorrect) and Remiremont (incorrect, 1 penalty point).

Strain S-7 reference result: Bolton 3,10:y:e,n,z15
Laboratory 17 recorded e,h instead of y (incorrect) and Lamberhurst (incorrect, 1 penalty point).

Strain S-14 reference result: Kisarawe 11:k:e,n,x,[z15]
Laboratory 17 recorded l,w instead of e,n,x,[z15] (incorrect) and Mannheim (incorrect, 1 penalty point).

Strain S-17 reference result: Charity [1],6,14,[25]:d:e,n,x
Laboratory 7 recorded y instead of d (incorrect) and fischerkietz (incorrect, 1 penalty point).
Laboratory 25* recorded 6,14 and Lindern, Charity* (not typable).
Laboratory 30 recorded 6,8 instead of [1],6,14,[25] (partly correct), 1,5 instead of e,n,x (incorrect) and Manhattan (incorrect, 1 penalty point).

Strain S-18 reference result: Southbank 3,10:m,t:-
Laboratory 7 recorded 3,10:m,t:- instead of Southbank (remark).

Appendix 4 Details of serotyping results for strain S21

Lab code	O-antigens	H-antigens		Serovar
		(phase 1)	(phase 2)	
REF	**47**	**k**	**z35**	**47:k:z35 (IIIb)**
1	47	k	z35	Salmonella enterica subspecies diarizonae serovar 47:k:z35
2	47	k	z35	47:k:z35
3	47	k	z35	IIIb 47:k:z35
4	47	k	z35	IIIb 47:k:z35
5	47	k	z35	IIIb 47:k:z35
6	47	k	z35	47:k:z35
7	47	k	z35	47:k:z35
8	47	k	z35	Salmonella enterica subsp. diarizonae 47:k:z35
9	47	k	z35	IIIb 47 : k : z35
10	47	k	z35	47:k:z35
11	47	k	z35	47:k:z35
12	47	k	z35	S. IIIb (Salmonella enterica subsp. diarizonae) 47:k:z35
13	OME+[2)]	-[2)]	-[2)]	S. ssp. diarizonae[2)]
14	61[1)]	k	z35	S. enterica subsp. diarizonae 61:k:z35[1)]
15	47	k	z35	IIb 47:k:z35
16	47	k	z35	47: k : z35 (IIIb)
17	[2)]	[2)]	[2)]	[2)]
18	47	k	z35	S. IIIb 47:k:z35 S. enterica subsp. diarizonae
19	47	k	z35	47:k:z35
20	47	k	z35	IIIb 47:k:z35
21	47	k	z35	diarizonae IIIb O47:k:z35
22	47	k	z35	47:k:z35 (IIIb)
23	[2)]	[2)]	[2)]	[2)]
24	47	k	z35	47:k:z35
25	47	k	z35	iiib, 47:k:z35
26	47	k	z35	IIIb (diarizonae)
27	47	k	z35	IIIB 47:K:Z35
28	47	k	z35	47:k:z35
29	47	k	z35	47:k:z35
30	47	k	z35	Lyon IIIb[1)]
31	47	k	z35	47:k:z35
32	47	k	z35	Sub IIIb 47:k:z35 (diarizonae)
33	O:8[1)]	-[2)]	-[2)]	C2[1)]

[1)] In blue: remark, deviations in the results on optional strain S21.
[2)] In grey: not typable (e.g. antisera not available, rough strain).

NOTE: The Table reflects the raw data submitted by the participants. However, the electronic result form does not allow input in special fonts like 'italic' or 'subscript'.

Appendix 5 Serotyping results cluster analysis part

		Strain code					
Lab code	**Serotyping method(s) used**	**24SCA01**	**24SCA02**	**24SCA03**	**24SCA04**	**24SCA05**	**24SCA06**
EL	**In-house pipeline**	**Infantis**	**Infantis**	**Infantis**	**Infantis**	**Infantis**	**Infantis**
1	SISTR	Infantis	Infantis	Infantis	Infantis	Infantis	Infantis
2	Classical serology, SISTR v 1.1.1	Infantis	Infantis	Infantis	Infantis	Infantis	Infantis
3	Seqsero2 (v1.1.1)	Infantis	Infantis	Infantis	Infantis	Infantis	Infantis
5	RIdom SeqSphere+	Infantis, Virchow[1)]	Infantis, Virchow[1)]	Infantis, Virchow[1)]	Infantis, Virchow[1)]	Infantis, Virchow[1)]	Infantis, Virchow[1)]
7	not applicable						
12	Classical serology, Sistr	Infantis	Infantis	Infantis	Infantis	Infantis	Infantis
13	Classical serology	Infantis	Infantis	Infantis	Infantis	Infantis	Infantis
14	SeqSero2	Infantis	Infantis	Infantis	Infantis	Infantis	Infantis
16	NGS, sistr	Infantis	Infantis	Infantis	Infantis	Infantis	Infantis
17	WGS - SeqSero2, SISTR Geno-serotyping	Infantis	Infantis	Infantis	Infantis	Infantis	Infantis
19	Classical serology, SSP and Galaxy sistr	Infantis O7:r:1,5	Infantis O7:r:1,5	Infantis O7:r:1,5	Infantis O7:r:1,5	Infantis O7:r:1,5	Infantis O7:r:1,5
20	Seqsero2 + SISTR1	Infantis	Infantis	Infantis	Infantis	Infantis	Infantis
21	SeqSero 1.2	Infantis	Infantis	Infantis	Infantis	Infantis	Infantis
23	SeqSero_v2	Infantis	Infantis	Infantis	Infantis	Infantis	Infantis
27	MOST, SeqSero2, SISTR	Infantis	Infantis	Infantis	Infantis	Infantis	Infantis
28	WGS SeqSero serotype finder	Infantis	Infantis	Infantis	Infantis	Infantis	Infantis
29	SeqSero2 and Sistr	Infantis	Infantis	Infantis	Infantis	Infantis	Infantis
31	Galaxy Sciensano	Infantis	Infantis	Infantis	Infantis	Infantis	Infantis
32	Based on genome, with sistr software	Intis[1)]	Intis[1)]	Intis[1)]	Intis[1)]	Intis[1)]	Intis[1)]
63	SeqSero2	Infantis	Infantis	Infantis	Infantis	Typhimurium[1)]	Infantis
80	SeqSero2 + Sistr	Infantis	Infantis	Infantis	Infantis	Infantis	Infantis

		Strain code					
Lab code	**Serotyping method(s) used**	**24SCA11**	**24SCA12**	**24SCA13**	**24SCA14**	**24SCA15**	**24SCA16**
EL	**In-house pipeline**	**Infantis**	**failed QC**	**Infantis**	**Infantis**	**Infantis**	**failed QC**
1	SISTR	Infantis	Infantis (but with failed QC status in SISTR)	Infantis	Infantis	Infantis	Contaminated, NT
2	SISTR v 1.1.1	Infantis	QC Fail	Infantis	Infantis	Infantis	QC Fail
3	Seqsero2 (v1.1.1)	Infantis	Lagos[1)]	Infantis	Infantis	Infantis	Paratyphi B var. Java[1)]
5	Ridom SeqSphere+	Infantis, Virchow[1)]	QC failed, >30 cgMLST330 loci missing	Infantis, Virchow[1)]	Infantis, Virchow[1)]	Infantis, Virchow[1)]	QC failed, >30 cgMLST330 loci missing
7	Ridom SeqSphere+	Infantis	NA (Failed QC)	Infantis	Infantis	Infantis	NA (Failed QC)
12	Sistr	Infantis	Infantis[1)]	Infantis	Infantis	Infantis	?
13	SeqSero	Infantis	Infantis[1)]	Infantis	Infantis	Infantis	Paratyphi B[1)]
14	Seqsero2	Infantis	contaminated	Infantis	Infantis	Infantis	contaminated
16	NGS, sistr	Infantis		Infantis	Infantis		
17	SeqSero2, SISTR Geno-serotyping	Infantis	Contaminated	Infantis	Infantis	Infantis	Contaminated
19	SSP and Galaxy sistr	Infantis O7:r:1,5	not applicable	Infantis O7:r:1,5	Infantis O7:r:1,5	Infantis O7:r:1,5	not applicable
20	Seqsero2 + SISTR1	Infantis	/	Infantis	Infantis	Infantis	/
21	SeqSero 1.2	Infantis	-	Infantis	Infantis	Infantis	-
23	SeqSero_v2	Infantis	N/A - failed QC	Infantis	Infantis	Infantis	N/A - failed QC
27	MOST, SeqSero2, SISTR	Infantis	Infantis and Lagos[1)]	Infantis	Infantis	Infantis	Brazzaville|Edinburg|Atento|Leeuwarden & Paratyphi B var. L(+) tartrate+ & Java[1)]
28	SeqSero serotype finder	Infantis	Bradford[1)]	Infantis	Infantis	Infantis	4:r:- [1)]
29	SeqSero2 and Sistr	Infantis	Infantis[1)]	Infantis	Infantis	Infantis	-
31	Galaxy Sciensano	Infantis	Infantis (contaminated)	Infantis	Infantis	Infantis	Paratyphi B III (contaminated)
32	Based on genome, with sistr software	Intis[1)]	Intis[1)]	Intis[1)]	Intis[1)]	Intis[1)]	Paratyphi B Variant Java[1)]
63	SeqSero2	Infantis		Infantis	Infantis	Infantis	

[1)] In blue: Deviation from the expected result.

No data submitted by Participants 4, 11, and 24.

Appendix 6 WGS results cluster analysis part, methods used by the participants

Lab code	Wet lab[a)]	WGS platform[b)]	Data analysis[c)]	Tool for analysis	Method for cluster analysis[d)]
EL	In-In-In	NextSeq	cgMLST	Ridom SeqSphere	MST
1	In-In-In	MiSeq	cgMLST	In-house pipeline with Chewbbaca, scheme from Enterobase	Single linkage
2	In-In-In	NextSeq	cgMLST	inhouse ChewieSnake Pipeline (scheme: Enterobase 3000 loci)	single linkage hierarchical clustering
3	In-In-In	NextSeq	cgMLST	Ridom SeqSphere	MST
4	In-In-In	MiSeq	cgMLST	in-house galaxy	MSTreeV2
5	In-In-In	MiSeq	cgMLST	Ridom SeqSphere	MST
7	In-In-In	NextSeq	cgMLST	Ridom SeqSphere	UPGMA Tree
11	In-In-In	MiniSeq	cgMLST	Ridom SeqSphere	Distance matrix only
12	In-In-In	MiSeq	cgMLST	Ridom SeqSphere	MST
13	In-In-In	MiSeq	cgMLST	Ridom SeqSphere	MST
14	In-In-In	MiSeq	cgMLST	chewBBACA + Grapetree	MSTree V2 - Grapetree's modified version of MST
16	In-In-In	MiSeq	cgMLST	chewBBACA	MST
17-cgMLST	In-In-In	MiSeq	cgMLST	Ridom SeqSphere	NJ
17-wgMLST	In-In-In	MiSeq	wgMLST	chewBBACA	UPGMA
19	In-In-In	MiSeq	SNPa	CSIPhylogeny 1.4	ML
20	In-In-In	NextSeq	cgMLST	Ridom SeqSphere	MST
21	In-In-Out	NextSeq	SNPr	MINTyper 1.0	NJ
23-cgMLST	In-In-In	MiSeq	cgMLST	chewBBACA and chewTree on Galaxy ARIES	chewTree
23-SNPr	In-In-In	MiSeq	SNPr	CSIPhylogeny on https://cge.food.dtu.dk/	ML
24	In-In-In	NextSeq	cgMLST	Ridom SeqSphere	MST

Lab code	Wet lab[a)]	WGS platform[b)]	Data analysis[c)]	Tool for analysis	Method for cluster analysis[d)]
27	In-In-In	NextSeq	SNPr		ML
28	In-In-In	MiSeq	cgMLST	linux command line	NJ
29	In-In-In	MiSeq	cgMLST	chewBBACA and ReporTree	Hierarchical clustering, average linkage
31	In-In-In	MiSeq	SNPr	Galaxy Samtools	ML
32	In-In-In	MiniSeq	cgMLST	Ridom SeqSphere	MST
63	In-Out-Out	NovaSeq	SNPr	bwa, gatk, custom scripts, raxml	ML
80-cgMLST	In-Out-Out	NextSeq	cgMLST	Ridom SeqSphere	MST
80-SNPa	In-Out-Out	NextSeq	SNPa	SNIPPY	ML

a) Wet lab preparations: DNA extraction, library preparation, sequencing. IN: In-house, Out: Outsourced.
b) All Illumina platforms.
c) SNPa: assembly-based, SNPr: reference-based.
d) ML: Maximum Likelihood, MST: Minimum Spanning Tree, NJ: Neighbor Joining.

Appendix 7 WGS results cluster analysis part, QC criteria as listed by the participants

Labcode	Criterium	Tools (if applicable)	Threshold (if applicable)
1	Contamination	Kraken2/Bracken for species confirmation/contamination-check. In house pipeline, VIGAS-P platform (built on IRIDA)	Still no absolute value, but aim for 95% of the reads identified as the species of interest
1	Coverage	Automatically calculated and listed in VIGAS-P (own platform)	Usually aim for minimum 30X coverage
1	GC%	Data from multiQC and from Quast	Not an exact threshold, but will give you an idea if you have sequenced the right species, so more like an indicator of contamination
1	N50	Quast (built in as a tool in the assembly pipeline in VIGAS-P)	Not a real threshold on this, also depending on read length etc but will be evaluated
1	Total length of assembly	Quast (built in as a tool in the assembly pipeline in VIGAS-P)	If this differs too much from what to expect. We do not have an exact threshold for this, but lean towards suggestion from EU-RL AMR+/- 20% of average Salmonella genome or whether the length of one assembly is very different from the rest.
1	Total number of contigs	Quast (built in as a tool in the assembly pipeline in VIGAS-P)	We have no exact threshold for this. We see that number of contigs might be species specific. But for now we lean towards suggestions from EURL AMR less than 500 contigs. But will probably look into it if it's very different from what we use to see for a specific species.
2	# contigs (>= 1000 bp)	Aquamis v. 1.4.2	x ≤ 167.5 (PASS); x > 167.5 (WARNING)
2	Contamination. Parameter: NumContamSNVs (ConFindr v0.7.4)	Aquamis v. 1.4.2	x ≤ 6 (PASS); 6 < x ≤ 7 (WARNING); x > 7 (FAIL)

Labcode	Criterium	Tools (if applicable)	Threshold (if applicable)
2	Coverage	Aquamis v. 1.4.2	x > 40 (PASS); 30 < x ≤ 40 (WARNING); x ≤ 30 (FAIL)
2	Duplication ratio	Aquamis v. 1.4.2	x ≤ 1.002 (PASS); x > 1.002 (WARNING)
2	Fraction of loci found	ChewieSnake 3.2.2	x ≥ 95%
2	GC%	Aquamis v. 1.4.2	51.8 < x ≤ 52.2975 (PASS); x ≤ 51.8, x > 52.2975 (WARNING)
2	N50	Aquamis v. 1.4.2	x > 50423 (PASS); x ≤ 50423 (WARNING)
2	Parameter: Single copy Orthologs	Aquamis v. 1.4.2	x > 0.95 (PASS); x ≤ 0.95 (WARNING)
2	Read Fraction Majority Genus (Genus confirmation & inter genus contamination)	Aquamis v. 1.4.2	x > 0.95 (PASS); x ≤ 0.95 (FAIL)
2	Total length of assembly	Aquamis v. 1.4.2	4627000 < x ≤ 5006000 (PASS); 4351000 < x ≤ 4627000, 5006000 < x ≤ 5326000 (WARNING); x ≤ 4351000, x > 5326000 (FAIL)
3	Contamination	checkm	no more than 4%
3	contamination	Seqsero2	as soon as a notification is given on multiple serovars detected
3	Coverage		>=30
3	GC%	quast	between 51.6%-52.3%
3	N50	quast	>30000
3	Total length of assembly	QUAST	4.4-5.8 Mb
3	Total number of contigs	QUAST	< 300
4	cgMLST loci detected	BLASTn	95% warning; <90% fail
4	Contamination	kraken2 2.1.1	1% warning; 5% fail
4	Contamination same species	ConFindr 0.8.1	>10 SNP warning; >20 SNP fail
4	Coverage	quast 5.2.0	20x warning; 10x fail
4	N50	quast 5.2.0	>20 000

Labcode	Criterium	Tools (if applicable)	Threshold (if applicable)
4	Total length of assembly	quast 5.2.0	4.3 - 5.3 Mb
4	Total number of contigs	quast 5.2.0	<300
5	Contamination	Ridom SeqSphere+ (Mash Screen), Pub MLST (Identify species)	Match identity=1
5	Coverage	Ridom SeqSphere+ (Skesa)	180x
5	GC%	Ridom SeqSphere+	~52-53% is a typical percentage for Salmonella enterica
5	N50	RIdom SeqSphere+	~300kb (samples had around 260kb which is typical for short reads sequencing)
5	Total number of contigs	Ridom SeqSphere+	<100 contigs (samples had between 50 and 70 assembled contigs which is good when using Illumina technology that generates short reads)
7	Contamination	Kmerfinder (DTU Website)	NA
7	Core %	Ridom SeqSphere+	>95%
7	Coverage	Ridom SeqSphere+	>30
7	N50	Ridom SeqSphere+	>15,000
7	Total length of assembly	Ridom SeqSphere+	4.5Mb to 5.4Mb
7	Total number of contigs	Ridom SeqSphere+	<400
11	% of good targets	Statistics implemented in SeqSphere	Cut off = 98% of good targets
11	Base call qualities in the fastQ files (forward & reverse reads)	Statistics implemented in SeqSphere	if the median for any base is less than 20, the result will be “failed”
11	Contamination	Mash Screen	Identity >=0.95, Shared-hashes >=100
11	Coverage	Statistics implemented in SeqSphere	Min = 30 (warning) – optimum = 50 (pass)
11	FastQC adapter content	Statistics implemented in SeqSphere	If more than 10% are present, the result will be “failed”
11	Total length of assembly	Statistics implemented in SeqSphere	Length of contigs assembled < ref genome + 10%

Labcode	Criterium	Tools (if applicable)	Threshold (if applicable)
11	Total number of contigs	Statistics implemented in SeqSphere	<300 = good; between 300-400 = warning; > 400 = rejected
12	Contamination	Mash Screen - Ridom SeqSphere	detection of other species if > 10%
12	Contamination		
12	contig size	Ridom SeqSphere	< 200 bp have to be ignored
12	Coverage	Ridom SeqSphere	>= 50x (unassambled)
12	genome size	Ridom SeqSphere	4.6-5-3 MB
12	minimum percentage good targets (cgMLST)	Ridom SeqSphere	>= 98.0
13	% of good targets in cgMLST	Ridom seqsphere	> 95%
13	Contamination	ConFindr	
13	Contamination	KmerFinder	
13	Contamination	Kraken	
13	Coverage	Ridom seqsphere	> 20
14	Completeness of assembly	CheckM	>98% completeness (based on lineage-specific marker genes)
14	Contamination	Confindr	< 7 single nucleotide variants (SNV)
14	Contamination	true_coverage (module part of the INNUca pipeline)	less than 2 absent or multiple genes (from set of housekeeping genes)
14	Coverage	fastp	30x
14	Q30%	fastp	>70%
14	Total length of assembly	checkM	4.3 – 5.3 Mb
16	Contamination	Kraken and ConFindr	Max of 5% of reads classified as species other than Salmonella with Kraken or more than 9 SNVs with ConFindr
16	Coverage	Quast, samtools	35X
16	GC%	Quast	50-52%

Labcode	Criterium	Tools (if applicable)	Threshold (if applicable)
16	Horizontal coverage	Samtools	More 90% bases covered with a sequencing depth greater than 20X
16	L50	Quast	Max of 15 contigs
16	N50	Quast	150000pb
16	Total length of assembly	Quast	between 4.5Mb and 5.5Mb
16	Total number of contigs	Quast	55 contigs max. (>500 pb)
17	Contamination	ConFindr, Mash, Kraken2, QUAST	No contamination
17	Coverage	QUAST	at least 90%
17	GC%	QUAST, FastQC	52% (plus/minus 1%)
17	L50, LA50, L75, LA75	QUAST	the smaller the better
17	N50	QUAST	the bigger the better
17	Number of misassemblies	QUAST	the smaller the better, under 30
17	Total length of assembly	QUAST	as close to 5 000 000 bp
17	Total number of contigs	QUAST	up to 200
19	Contamination	Kraken; KmerFinder 3.2; SPAdes; QUAST	Species identification; Total length not exceeding 20% genome size
19	N. reads and percentage after filtering	Trimmomatic	
19	N50	SPAdes; QUAST	N50>15000
19	Total length of assembly	SPAdes; QUAST	Total length not exceeding 20% genome size
19	Total number of contigs	SPAdes; QUAST	N.contigs<500
20	Breadth coverage	bbmap	95%
20	Contamination	confinder + species finder	NumContamSNVs>7
20	Depth coverage		50X
20	GC%	Quast	Close to 50 for Salmonella
20	Genome fraction	Quast	>95%

Labcode	Criterium	Tools (if applicable)	Threshold (if applicable)
20	Largest contig	Quast	There is no threshold but an order of size
20	N50	Quast	There is no threshold but an order of size
20	Q30	fastp	>80%
20	Total length of assembly	Quast	between 4,4-5,4 Mb
20	Total number of contigs	Quast	<150
21	N50. All contigs of that length or longer equals at least 50% of the sum of all contigs	CheckM2	30 Kbp
21	Contamination level calculated by the unique genes	CheckM2	>1
21	Kmer	KmerFinder 3.2	Query and Template Coverage
21	The total number of contigs assembled	CheckM2	<500
21	The total size of all contigs in base pairs	CheckM2	<5.3 Mp
23	Contamination	KmerFinder	identification of other species/strains where assembly stats indicate contamination
23	Coverage	Quast and clc workbench	>25
23	GC%	Quast	51-54%
23	N50	Quast	<100.000
23	Total length of assembly	Quast	4.3-5.4 Mbp
23	Total number of contigs	Quast	<500
24	Contamination	Kraken2	QC fails if the majority of reads are from different species than expected
24	Coverage	QualiMap	30
24	GC%	Qualimap	+/-10% of the species's reference genome
24	N50	QUAST	20000

Labcode	Criterium	Tools (if applicable)	Threshold (if applicable)
24	Total length of assembly	QUAST	+/- 10% of the species's reference genome
24	Total number of contigs	QUAST	1500
27	Contamination	KmerID	Less than 75% Salmonella
27	Contamination	SeqSero2	More than one serotype
27	Contamination	Shovill	Assembly > 5.8Mbp
27	Contamination	MOST, SeqSero2, and sistr	Same serovar
27	Coverage	MOST	Depth => 30
27	Total number of contigs	Shovill	Number of contigs => 600
28	Contamination	ConFindr	>10 SNVs is contamination
28	Coverage	Qualimap	>30
28	GC%	Quast	approximately 52%
28	N50	Quast	>15000
28	Total length of assembly	Quast	between 4,5-5,5*10^6
28	Total number of contigs	Quast	<300
29	Contamination	Kraken2	Max 5%
29	Coverage	fastp	Minimum 30x
29	Number of cgMLST alleles	chewBBACA	Minimum 95% (2850 of 3000) loci found
31	Contaminants (Illumina)	Kraken2	1%
31	GC-content deviation	Galaxy FastQC	2%
31	Number of contaminating SNPs	ConFindr	10
32	Core genome genes	Ridom Seqsphere+	Strain missing >5% core genome are excluded
32	Coverage	seqkit toolkit, calculator	at least 30 fold coverage
32	Total length of assembly	seqkit toolkit	Strain with > 20% deviation from the genome size of the NCBI reference strains are discarded
32	Total number of contigs	seqkit toolkit	Strain with > 300 contigs are inspected manually
63	Contamination	Confindr	<7 cSNV; correct Genus

Labcode	Criterium	Tools (if applicable)	Threshold (if applicable)
63	Contamination	Kraken2	<5% non-target species
63	Coverage	picard (+abyss +bwa)	1x >= 90%; >= 30x average
63	N50	Quast	>30kb
63	Total length of assembly	Quast	4.3 - 5.3 M
63	Total number of contigs	Quast	<=300
80	Contamination	Kraken2	appreciation - inter contaminations
80	Contamination	Confidr	5% - intra contaminations
80	Coverage	BBmap	50X
80	GC%	Quast	
80	N50	Quast	
80	Q30	Fastp	80%
80	Total lengh of assembly	Quast	
80	Total number of contigs		max 150 after assembly

Appendix 8 Md5 checksums of the 14 files that had to be downloaded from the SFTP server for further analysis

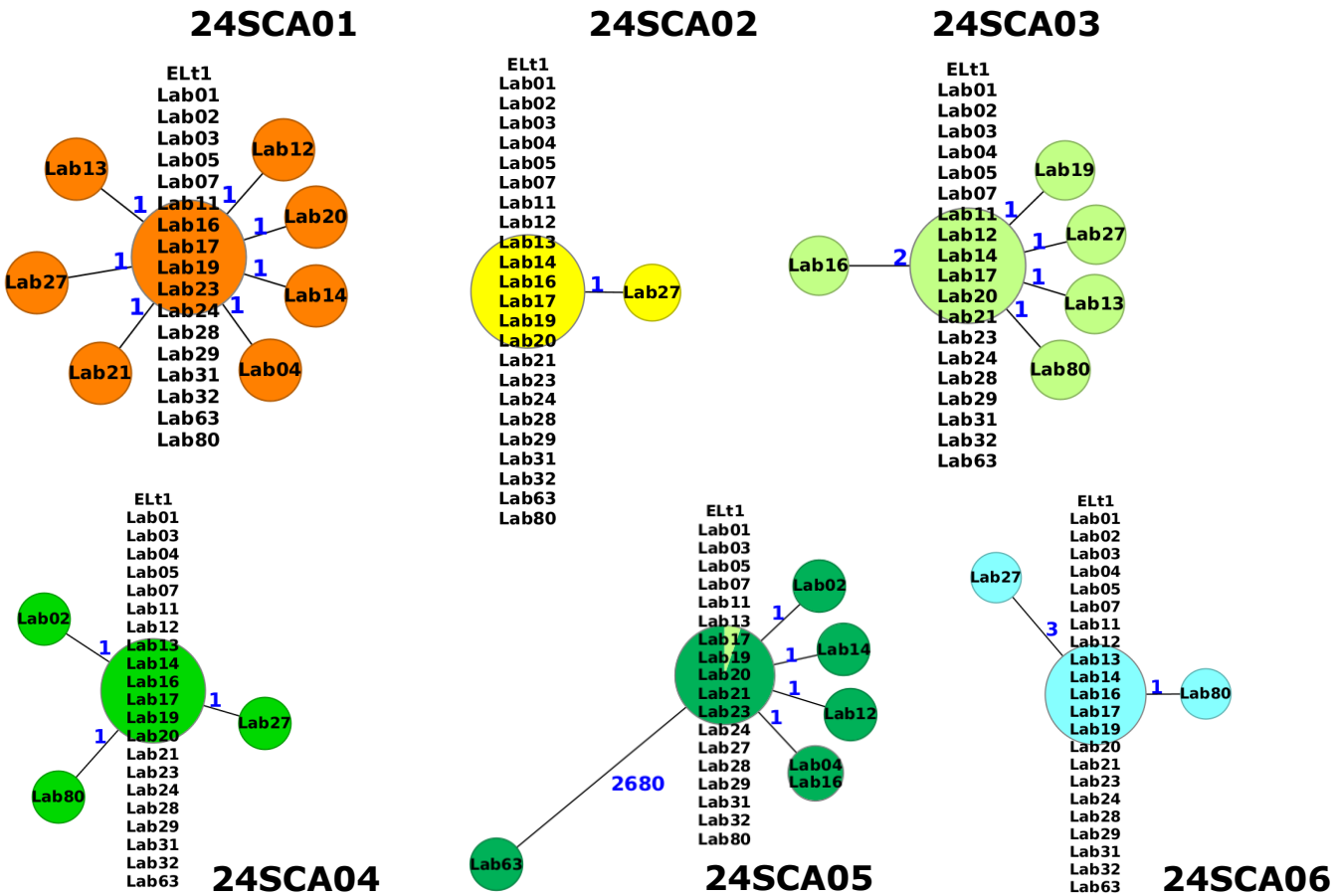
6bf05a577ba9f88494866573553bdb6c	24SCA11_R1.fastq.gz
5a71cd0c82cca8aae46e6f8a1def44e0	24SCA11_R2.fastq.gz
19add44955492a91cccc138439f22c09	24SCA12_R1.fastq.gz
ac240654acd8a1a615d82b5a94f6777c	24SCA12_R2.fastq.gz
2d1b5a7240c8a5ca80a59eff558700a8	24SCA13_R1.fastq.gz
06fc5d9751305b0288d7ab036d680d73	24SCA13_R2.fastq.gz
fdecdbdeb0a5a6000871ea474cbd8534	24SCA14_R1.fastq.gz
5f84a601e9b6f5d63ca2d588cdf70b5e	24SCA14_R2.fastq.gz
02b6951c938daa0b563342d9fcfa9b3f	24SCA15_R1.fastq.gz
44ec5575e2cd84b607994b8d2098be59	24SCA15_R2.fastq.gz
d9e3a11c52344e5317f6e54c79e5a2be	24SCA16_R1.fastq.gz
7b4b4de7c0ea5dab00bbe60316323b08	24SCA16_R2.fastq.gz
383599f27b18687e9ebd88069d40182c	24SCA-REF_R1.fastq.gz
db3b146bb2ecb3dfe9db8450b37fe73b	24SCA-REF_R2.fastq.gz

Appendix 9 Integrity checks of downloaded and uploaded files using md5sums

	'Dry' strains 24SCA11 - 24SCA16			'Wet' strains 24SCA01 - 24SCA06	
Labcode	**Did you check the md5sum values of the downloaded files**	**Lab's md5sum data submitted**	**Check by the EURL-*Salmonella***	**Lab's md5sum data submitted**	**Md5sum determination and check by the EURL-*Salmonella* after downloading**
1	Yes	Yes	OK	Yes	OK
2	Yes	Yes	OK	Yes	OK
3	Yes	Yes	OK	Yes	OK
4	Yes	Yes	OK	Yes	OK
5[a)]	Yes	Yes	No[a)]*	Yes	No[a)]*
7	Yes	Yes	OK[b)]	Yes	OK
11	No*	No*	n.a.	No*	n.a.
12	Yes	Yes	OK	Yes	OK
13	Yes	Yes	OK	Yes	OK
14	Yes	Yes	OK	Yes	OK
16	Yes	Yes	OK	Yes	OK
17	Yes	No*	n.a.	Yes	OK
19	Yes	Yes	OK	Yes	OK
20	Yes	Yes	OK, but mismatch for 24SCA16_R1*	Yes	OK
21	Yes	No*	n.a.	Yes	OK
23	Yes	Yes	OK	Yes	OK
24	Yes	Yes	OK	Yes	OK
27	Yes	Yes	OK	Yes	OK
28	Yes	Yes	OK	Yes	OK
29	Yes	Yes	OK	Yes	OK
31	Yes	No*	n.a.	No*	n.a.
32	Yes	No*	n.a.	Yes	OK
63	Yes	Yes	OK	Yes	OK, but data for strain 24SCA01 only*
80	Yes	Yes	OK	Yes	OK

n.a.: not applicable, OK: matching data.
a): md5sum values of 40 digits were given, therefore these were not comparable to the EURL-*Salmonella* md5sum values of 32 digits.
b): Originally missing data or mismatches for 5/14 files, but all OK after regenerated md5sums by Laboratory 7.
*)In grey : Deviation from the expected result.

Appendix 10 WGS results cluster analysis part, Minimum Spanning Tree per strain



Note: ELt1 24SCA03 was included as ELt1 for strain 24SCA05 (technical duplicates).

Appendix 11 WGS results cluster analysis part, Results QC parameters on the in-house *de novo* assembled genomes, per participant

	Laboratory code: 01		Platform used: MiSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,61	0,45	38	1195314	4593854	52,2	526592	1767806	301	116
24SCA02	99,65	1,21	52	1195749	4915703	52,1	386165	1894388	301	116
24SCA03	99,65	1,21	51	1195749	4918656	52,2	386166	1599562	301	98
24SCA04	99,61	0,45	34	632559	4661012	52,2	397655	1790700	301	116
24SCA05	99,58	1,21	51	1195749	4916168	52,1	386159	1382268	301	85
24SCA06	99,58	1,28	52	1195318	4915048	52,1	386043	1483722	301	91

	Laboratory code: 02		Platform used: NextSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,61	0,45	39	1195858	4592229	52,2	395981	2563706	149	83
24SCA02	99,58	1,21	55	1195211	4916474	52,1	263652	2904110	149	88
24SCA03	99,58	1,21	55	1029087	4915925	52,1	263652	2497670	149	76
24SCA04	99,61	0,45	36	707814	4659619	52,2	526238	2490330	149	80
24SCA05	99,58	1,21	53	719932	4916307	52,1	438261	2943202	149	89
24SCA06	99,59	1,28	59	719795	4915513	52,1	438036	2408422	149	73

	Laboratory code: 03		Platform used: NextSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,56	0,45	39	1195314	4593484	52,2	416100	5194148	151	171
24SCA02	99,59	1,21	52	672994	4916261	52,1	386165	4296896	151	132
24SCA03	99,58	1,21	56	1195318	4917257	52,1	386165	3445596	151	106
24SCA04	99,61	0,45	42	612487	4662167	52,2	253505	4400490	151	143
24SCA05	99,58	1,21	54	708391	4917055	52,1	386166	4108364	151	126
24SCA06	99,58	1,28	54	1195318	4916666	52,1	386124	4273270	151	131

	Laboratory code: 04		Platform used: MiSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,61	0,45	44	1029176	4592624	52,2	263391	1307038	251	71
24SCA02	99,66	1,21	55	1029180	4915231	52,1	263392	1479904	251	76
24SCA03	99,58	1,21	51	1195318	4917043	52,1	386099	1352456	251	69
24SCA04	99,61	0,45	38	708399	4661962	52,2	416245	1489710	251	80
24SCA05	99,58	1,21	57	1195318	4917120	52,1	386165	1384766	251	71
24SCA06	99,65	1,28	53	1195318	4916120	52,1	386124	1450826	251	74

	Laboratory code: 05		Platform used: MiSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,61	0,45	38	1195314	4593373	52,2	397662	3829770	251	209
24SCA02	99,58	1,21	53	1195318	4916370	52,1	386165	3362564	251	172
24SCA03	99,58	1,21	52	1195318	4916246	52,1	386246	3256182	251	166
24SCA04	99,61	0,45	42	612487	4661752	52,2	263391	3538098	251	190
24SCA05	99,59	1,21	54	1195749	4916476	52,1	386165	3141286	251	160
24SCA06	99,58	1,28	53	1195318	4916517	52,1	386124	3089816	251	158

	Laboratory code: 07		Platform used: NextSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,61	0,45	39	1195314	4593636	52,2	397662	3953798	151	130
24SCA02	99,58	1,21	54	1195318	4916512	52,1	386165	4436322	151	136
24SCA03	99,66	1,21	58	1195318	4917653	52,1	386165	5322654	151	163
24SCA04	99,62	0,45	39	612487	4661089	52,2	263391	3592152	151	116
24SCA05	99,65	1,21	56	1195318	4917813	52,1	386165	4438272	151	136
24SCA06	99,58	1,28	53	1195318	4917197	52,1	386043	4793174	151	147

	Laboratory code: 11		Platform used: MiniSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,61	0,45	37	1195859	4590280	52,2	396474	3095764	151	102
24SCA02	99,58	1,21	64	605711	4914227	52,1	263438	1127744	151	35
24SCA03	99,58	1,21	56	1195225	4914865	52,1	438261	2246224	151	69
24SCA04	99,61	0,45	43	612394	4660045	52,2	398631	2084478	151	68
24SCA05	99,58	1,21	61	801847	4917025	52,1	393493	2144960	151	66
24SCA06	99,58	1,28	53	801897	4915592	52,1	393479	2320144	151	71

	Laboratory code: 12		Platform used: MiSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,61	0,45	82	398188	4586285	52,3	101814	1699362	301	112
24SCA02	99,58	1,21	84	560129	4913748	52,2	146789	1827880	301	112
24SCA03	99,58	1,21	91	750168	4913442	52,1	148220	1301488	301	80
24SCA04	99,61	0,45	51	612487	4657937	52,2	213027	1488436	301	96
24SCA05	99,59	1,21	111	516518	4912232	52,2	106045	1475544	301	90
24SCA06	99,58	1,28	93	422832	4914094	52,2	146593	1468284	301	90

	Laboratory code: 13		Platform used: MiSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,61	0,45	37	1081349	4589886	52,2	263436	1430322	251	78
24SCA02	99,58	1,21	58	1195318	4918033	52,1	437073	1860766	251	95
24SCA03	99,58	1,21	63	599661	4916372	52,1	267494	1110746	251	57
24SCA04	99,61	0,45	42	527179	4657966	52,2	194233	1266618	251	68
24SCA05	99,58	1,21	61	1045289	4916230	52,1	183451	1578400	251	81
24SCA06	99,58	1,28	50	1195134	4915133	52,1	247664	1406878	251	72

	Laboratory code: 14		Platform used: MiSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,61	0,45	41	1028492	4593066	52,2	350900	1590424	301	104
24SCA02	99,65	1,21	55	1195318	4915533	52,1	386246	1536546	301	94
24SCA03	99,58	1,21	58	1035348	4915848	52,1	263746	1567772	301	96
24SCA04	99,61	0,45	75	420515	4656677	52,2	123343	1530820	301	99
24SCA05	99,58	1,21	151	276438	4903204	52,2	61227	1204168	301	74
24SCA06	99,58	1,28	154	245034	4907165	52,2	61948	1538628	301	94

	Laboratory code: 16		Platform used: MiSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,61	0,45	37	1195314	4593828	52,2	416100	2522206	151	83
24SCA02	99,58	1,21	50	1081454	4918391	52,2	263438	2716680	151	83
24SCA03	99,58	1,21	53	1081447	4917841	52,1	252601	2374774	151	73
24SCA04	99,61	0,45	36	632559	4664251	52,2	263391	2015220	151	65
24SCA05	99,58	1,21	51	646625	4918853	52,2	386166	2489738	151	76
24SCA06	99,58	1,28	46	1081447	4916906	52,1	386124	1959274	151	60

	Laboratory code: 17		Platform used: MiSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,61	0,45	38	1195952	4593003	52,2	416019	3487092	151	115
24SCA02	99,58	1,21	53	1195318	4915644	52,1	386246	2959974	151	91
24SCA03	99,58	1,21	54	1195318	4917118	52,1	386159	3360188	151	103
24SCA04	99,61	0,45	58	444490	4612048	52,3	253406	3883812	134	113
24SCA05	99,58	1,21	53	1195318	4916464	52,1	386240	3738004	151	115
24SCA06	99,58	1,28	50	1195318	4917043	52,1	386043	3863174	151	119

	Laboratory code: 19		Platform used: MiSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,56	0,47	52	437088	4591316	52,2	152091	938732	151	31
24SCA02	99,58	1,21	53	1081449	4914753	52,1	386166	2368618	151	73
24SCA03	99,58	1,21	64	1195314	4917861	52,1	386166	2413944	151	74
24SCA04	99,61	0,45	38	708445	4660399	52,2	396093	1816784	151	59
24SCA05	99,58	1,21	60	1195318	4916730	52,1	248675	2278930	151	70
24SCA06	99,66	1,28	54	646197	4916470	52,1	263444	2253748	151	69

	Laboratory code: 20		Platform used: NextSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,56	0,83	41	727234	4594201	52,2	398093	6998282	151	230
24SCA02	99,58	1,21	51	1195318	4916832	52,1	386165	9857360	151	303
24SCA03	99,58	1,21	52	1195318	4915872	52,1	386246	8976094	151	276
24SCA04	99,61	0,45	40	632559	4661661	52,2	397660	4915900	151	159
24SCA05	99,58	1,21	54	1195318	4917225	52,1	386165	4497082	151	138
24SCA06	99,58	1,3	53	1195318	4916400	52,1	263398	10794052	151	332

	Laboratory code: 21		Platform used: NextSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,56	0,45	53	476699	4580433	52,2	170813	6203944	75	102
24SCA02	99,58	1,21	70	512189	4906327	52,1	160897	8645340	75	132
24SCA03	99,58	1,21	74	476701	4903132	52,1	170019	6059740	75	93
24SCA04	99,61	0,45	57	383712	4647874	52,2	171377	7775932	75	125
24SCA05	99,58	1,21	65	605813	4905072	52,1	160897	7184280	75	110
24SCA06	99,58	1,28	70	590387	4904269	52,1	159027	7396436	75	113

	Laboratory code: 23		Platform used: MiSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,61	0,45	44	1195314	4593281	52,2	396823	627750	301	41
24SCA02	99,58	1,21	54	1029180	4915931	52,1	263652	938500	301	57
24SCA03	99,58	1,21	59	1195318	4916272	52,1	386165	1310320	301	80
24SCA04	99,61	0,45	38	633197	4660656	52,2	397655	1381722	301	89
24SCA05	99,58	1,21	56	1195318	4916034	52,1	386165	1305664	301	80
24SCA06	99,58	1,28	56	1195318	4916826	52,1	386124	1776284	301	109

	Laboratory code: 24		Platform used: NextSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,61	0,45	38	1195314	4593846	52,2	416019	4439792	151	146
24SCA02	99,58	1,21	48	1195318	4916410	52,1	386165	3188012	151	98
24SCA03	99,58	1,21	55	1145141	4917899	52,1	263392	3063716	151	94
24SCA04	99,61	0,45	39	708399	4662749	52,2	333088	2947602	151	95
24SCA05	99,65	1,21	55	1195318	4917649	52,1	386165	2525596	151	78
24SCA06	99,58	1,28	48	1195318	4916323	52,1	386043	2477690	151	76

	Laboratory code: 27		Platform used: NextSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,56	0,45	185	116409	4571618	52,3	46895	3431202	151	113
24SCA02	99,58	1,24	219	115780	4896378	52,2	40887	2742952	151	85
24SCA03	99,58	1,21	222	144446	4897019	52,2	46714	2882488	151	89
24SCA04	99,56	0,45	63	460101	4655387	52,2	148578	6961018	151	226
24SCA05	99,21	1,21	343	96635	4873258	52,3	28114	2628958	151	81
24SCA06	99,58	1,28	284	95401	4882974	52,3	33351	2354086	151	73

	Laboratory code: 28		Platform used: MiSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,61	0,45	28	1195314	4595403	52,2	526592	2204992	301	144
24SCA02	99,58	1,21	47	1195749	4919202	52,2	438303	2492994	301	153
24SCA03	99,58	1,21	48	1195856	4918356	52,1	386246	2748766	301	168
24SCA04	99,61	0,45	32	708399	4663616	52,2	416107	2322650	301	150
24SCA05	99,58	1,21	45	1195856	4919625	52,2	386246	2527146	301	155
24SCA06	99,58	1,28	42	1195318	4917049	52,1	386043	2307502	301	141

	Laboratory code: 29		Platform used: MiSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,61	0,45	38	960379	4593436	52,2	397662	1462424	251	80
24SCA02	99,58	1,21	57	1029629	4915138	52,1	386246	1492542	251	76
24SCA03	99,58	1,21	58	1195318	4916744	52,1	386141	1451398	251	74
24SCA04	99,62	0,45	37	612487	4659834	52,2	263437	1297356	251	70
24SCA05	99,58	1,21	62	1029516	4916098	52,1	217925	1718820	251	88
24SCA06	99,58	1,28	58	1044990	4916596	52,1	263703	1782512	251	91

	Laboratory code: 31		Platform used: MiSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,61	0,45	41	1044999	4592953	52,2	396100	2341674	151	77
24SCA02	99,65	1,21	54	1029629	4914629	52,1	438209	2010208	151	62
24SCA03	99,58	1,21	53	1195318	4916966	52,1	386246	2449528	151	75
24SCA04	99,61	0,45	40	708399	4662063	52,2	219863	2611802	151	85
24SCA05	99,65	1,21	57	1195318	4917081	52,1	386165	3325476	151	102
24SCA06	99,66	1,28	48	915311	4916998	52,1	333087	1689936	151	52

	Laboratory code: 32		Platform used: MiniSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,61	0,45	43	888195	4589573	52,2	306535	1872766	151	62
24SCA02	99,58	1,21	63	531443	4913739	52,1	306712	1779636	151	55
24SCA03	99,58	1,21	57	801905	4915063	52,1	393471	2870310	151	88
24SCA04	99,61	0,45	36	708281	4660525	52,2	525554	2467502	151	80
24SCA05	99,58	1,21	58	719818	4914879	52,1	438261	2294344	151	70
24SCA06	99,59	1,28	56	1195225	4916175	52,1	436417	2372492	151	73

	Laboratory code: 63		Platform used: NovaSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,56	0,45	37	1195314	4591319	52,2	398093	18181288	151	598
24SCA02	99,65	1,21	58	1195318	4917294	52,1	386165	19379552	151	595
24SCA03	99,58	1,21	55	1195318	4915411	52,1	386165	18732836	151	575
24SCA04	99,61	0,45	42	612487	4659581	52,2	250023	16800056	151	544
24SCA05	99,56	0,37	63	536012	4885103	52,2	197053	21226372	151	656
24SCA06	99,58	1,28	53	1195318	4915135	52,1	263658	15247680	151	468

	Laboratory code: 80		Platform used: NextSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,61	0,45	39	708399	4593277	52,2	416100	6231980	151	205
24SCA02	99,58	1,21	53	1195318	4915868	52,1	436699	8700014	151	267
24SCA03	99,58	1,21	53	1195318	4917430	52,1	436267	11740226	151	361
24SCA04	99,61	0,45	34	632559	4661649	52,2	377697	5722940	151	185
24SCA05	99,58	1,21	55	1195318	4917134	52,1	386384	4326460	151	133
24SCA06	99,58	1,28	52	1195318	4916107	52,1	386043	12793912	151	393

	Laboratory code: EL (t1)		Platform used: NextSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA01	99,56	0,49	36	1195952	4592934	52,2	397667	6075872	151	200
24SCA02	99,66	1,21	51	1195749	4915940	52,1	386165	4890938	151	150
24SCA03	99,65	1,24	51	1195749	4916596	52,1	386165	5783376	151	178
24SCA04	99,61	0,53	40	708399	4660330	52,2	416026	4413050	153	145
24SCA06	99,58	1,28	46	1195318	4916352	52,1	386044	5468162	151	168

EL t1: July 2024.
24SCA05 not listed because it is a technical duplicate of 24SCA03.

	Laboratory code: EL (t1)		Platform used: NextSeq							
Strain	**Completeness**	**Contamination**	**# contigs**	**Largest contig**	**Total length**	**GC (%)**	**N50**	**Input read pairs**	**Read Length**	**Coverage**
24SCA11	99,61	1,28	48	1203467	4919058	52,2	415958	6380200	151	196
24SCA12 [a)]	97,70	19,67*	1683*	94082	5724634*	51,8	6782*	5149300	151	136
24SCA13	99,61	0,45	40	1244845	4660966	52,2	416026	5419736	152	177
24SCA14 [b)]	NA	NA	118	1029761	4926303	52,1	197452	14575596	151	447
24SCA15	99,56	0,64	39	715673	4615984	52,3	245653	5755670	151	188
24SCA16 [c)]	95,77	52,92*	4017*	106170	5802711*	51,9	1751*	4428348	151	115
24SCA-REF [d)]	99,66	1,21	51	1195749	4915940	52,1	386165	4890938	151	150

EL t1: July 2024.
a) Wet-mixed culture of strains 24SCA02 (Inf-REF) and 19SCA03 (*S*. Typhimurium).
b) Original strain data at t=0 (2019), at that time sequenced with an Illumina NovaSeq (outsourced). In general, the NovaSeq generates more sequence data output in comparison to the NextSeq, therefore the two fastq files of this sample were larger than the other fastq files provided for the "dry" part of the PT. We expected it would be included in the analysis by the PT participants since we do not consider fastq file sizes as a QC measure. Also, the pipeline was different from the current Juno pipeline.
c) Wet-mixed culture of strains 24SCA03 (Inf) and 2013S16 (*S*. Paratyphi B, var. Java).
d) Is 24SCA02 at t=1.

Intentional quality control issues (for contamination, # contigs, total length and N50) for strains 24SCA12 and 24SCA16 are highlighted in yellow*.

Appendix 12 Reasons for (not) passing participant's QC and (not) excluding strains 24SCA12 and 24SCA16

Lab code	Strain 24SCA12 passed QC	Reason(s) not passing QC	Strain 24SCA12 excluded from cluster analysis[a)]
Expected	**No**	**Wet mixture of 24SCA02 REF *S.* Infantis and 19SCA03 *S.* Typhimurium**	**Yes**
1	No	Too many contigs (3104 contigs), total length of assembly too long (6,7Mbp), N50 too low (3952 bp). Suspect intra species contamination.	Yes
2	No	Total length (> 5326000); NumContamSNVs (x>7);	Yes
3	No	> 300 contigs, bad N50 (6782), 19,48% contamination, multiple serotypes detected	Yes
4	No	total length > 5.3 Mb, Confindr 37 SNP; number of contigs >300	Yes
5	No	cgMLST good targets percentage <90%	Yes
7	No	Low Core %, high genome size, N50 & Contig counts	Yes
11	No	No contigs =2952, size = 6 Mb = mixed culture	Yes
12	No	percentage good targets too low - 96.6; genome size too high - 5.5 MB	Yes
13	No	Contaminated with other Salmonella, based on MLST2.0 and ConFindr	Yes
14	No	Contamination detected by CheckM is above the quality threshold of 2	Yes
16	No	Intraspecies contamination with 363 SNVs and N50 value is less than 150000pb	Yes
17	No	too many contigs, too big assembly length, wrong GC%, too many missassemblies, too small N50, too large L50	Yes
19	No	N. contigs>500; Total length higher than expected	Yes
20	No	Contamination, total length of assembly, total number of contigs, N50, largest contig	Yes
21	No	checkM2 contamination 4,97; total number of contigs 2360; total length of assembled genome 5,8 Mb; N50 9901; Kmer intraspecies contamination indication	Yes
23	No	Poor assembly, incorrect genome size, possibly mix of strains/serovars	Yes
24	Yes*		No*
27	No	Contamination: multiple serotypes (Infantis, Lagos); large assembly (> 6 Mbp)	Yes
28	No	contamination	Yes
29	No	Did not pass cgMLST loci requirement. Found 94,7%.	Yes
31	No	Contamination	Yes
32	No	too litle core genomes genes/too many contigs	Yes
63	No	Likely a mixture of multiple strains; confindr suggests contamination, assembly too large and too fragmented, N50 too low.	Yes
80	No	Out of the thresholds recommended by EFSA : Too much contigs (4590); length > 5.4Mb; N50=3528 &Contamination detected (Intra specie)	Yes

a) Based on the submitted distance matrix. *) In blue : Deviation from the expected result.

Lab code	Strain 24SCA16 passed QC	Reason(s) not passing QC	Strain 24SCA16 excluded from cluster analysis[a)]
Expected	**No**	**Wet mixture of 24SCA03 *S.* Infantis and 2013S16 *S.* Paratyphi B, var. Java**	**Yes**
1	No	Too many contigs (3043 contigs), total length of assembly too long (6,5Mbp), N50 too low (3965 bp). Suspect intra species contamination.	Yes
2	No	Total length (> 5326000); NumContamSNVs (x>7);	Yes
3	No	only 46% cgMLST targets identified, > 300 contigs, bad N50, 50% contamination with (1751), multiple serotypes detected	Yes
4	No	total length <4.3 Mb, number of contigs >300, N50 too small, <90% cgMLST loci called	Yes
5	No	cgMLST good targets percentage <90%	Yes
7	No	low core %, high genome size, N50 & contig count.	Yes
11	No	No of contigs = 8181, total size = 6.5 Mb = mixed culture	Yes
12	No	percentage good targets too low - 43.0; genome size too high - 6.6 MB	Yes
13	No	% of good cgMLST Targets 88,51 %	Yes
14	No	Contamination detected by CheckM is above the quality threshold of 2	Yes
16	No	Intraspecies contamination with 359 SNVs, N50 value is less than 150000pb and less than 90% bases are covered with a sequencing depth higher than 20X	Yes
17	No	too many contigs, too big assembly length, wrong GC%, too many missassemblies, too small N50, too large L50	Yes
19	No	N. contigs>500; Total length higher than expected; N50<1500	Yes
20	No	Contamination, total length of assembly, total number of contigs, N50, largest contig	Yes
21	No	checkM2 contamination 5,67; total number of contigs 3361; total length of assembled genome 5,9 Mb; N50 6166; Kmer intraspecies contamination indication	Yes
23	No	Poor assembly, incorrect genome size, possibly mix of strains/serovars	Yes
24	No	N50 too small and too many contigs	Yes
27	No	Contamination: multiple serotypes (Java, Paratyphi B var. L(+) tartrate and Brazzaville|Edinburg|Atento|Leeuwarden); large assembly (> 6 Mbp)	Yes
28	No	contamination; N50 to low; nr of contigs to high	Yes
29	No	Did not pass cgMLST loci requirement. Found 37,1%.	Yes
31	No	Contamination	Yes
32	No	too litle core genomes genes/too many contigs	Yes
63	No	Likely a mixture of multiple strains; confindr suggests contamination, assembly too large and too fragmented, N50 too low.	Yes
80	No	Out of the thresholds recommended by EFSA: Too much contigs (8840); length > 5.3Mb; N50=1692 & Contamination detected (Intra species)	Yes

a) Based on the submitted distance matrix.

Appendix 13 Per submission, the participants' distance matrix data for their comparison to the reference strain 24SCA-REF with the test strains

Labcode-method	Strain code												
	24 SCA-REF	**24 SCA01**	**24 SCA02**	**24 SCA03**	**24 SCA04**	**24 SCA05**	**24 SCA06**	**24 SCA11**	**24 SCA12**	**24 SCA13**	**24 SCA14**	**24 SCA15**	**24 SCA16**
	REF	Inf	Inf=REF	Inf	Inf	Inf	Inf	Inf	Inf+STM	Inf	Inf=REF	Inf	Inf+Java
EL-cgMLST	0	130	**0**	**1**	144	**1**	35	84	n.a.	146	**0**	86	n.a.
1-cgMLST	0	131	**0**	**1**	145	**1**	35	87	n.a.	147	**0**	89	n.a.
2-cgMLST	0	132	**0**	**2**	146	**2**	37	88	n.a.	148	**0**	88	n.a.
3-cgMLST	0	35*	130*	**0**	**1***	144*	**1***	84	n.a.	146	**0**	86	n.a.
4-cgMLST	0	138	**0**	**1**	152	**2**	38	94	n.a.	154	**0**	96	n.a.
5-cgMLST	0	129	**0**	**1**	143	**1**	35	83	n.a.	145	**0**	85	n.a.
7-cgMLST	0	130	**0**	**1**	144	**1**	35	84	n.a.	146	**0**	86	n.a.
11-cgMLST	0	130	**0**	**1**	144	**1**	35	84	n.a.	146	**0**	86	n.a.
12-cgMLST[a)]	0	131	**0**	**1**	144	**1**	35	84	n.a.	146	**0**	86	n.a.
13-cgMLST[b)]	0	128	**0**	**1**	142	**1**	35	84	n.a.	146	n.a.*	86	n.a.
14-cgMLST	0	153	**0**	**2**	170	**3**	39	102	n.a.	170	**0**	100	n.a.
16-cgMLST[c)]	0	155	**0**	**3**	170	**3**	41	103	n.a.	171	**0**	n.a.*	n.a.
17-cgMLST	0	132	**0**	**0**	145	**1**	14	88	n.a.	150	**0**	84	n.a.
20-cgMLST	0	130	**0**	**1**	144	**1**	35	84	n.a.	146	**0**	85	n.a.
23-cgMLST	0	124	**1**	**1**	133	**1**	32	86	n.a.	146	**1**	84	n.a.
24-cgMLST	0	130	**0**	**1**	144	**1**	35	84	**1***	146	**0**	86	n.a.
28-cgMLST	0	127	**0**	**1**	141	**1**	35	85	n.a.	143	**0**	86	n.a.
29-cgMLST	0	138	**0**	**2**	149	**3**	37	88	n.a.	152	**2**	93	n.a.
32-cgMLST	0	129	**2**	**1**	144	**1**	35	84	n.a.	146	**0**	85	n.a.
80-cgMLST	0	130	**0**	**2**	145	**1**	35	84	n.a.	146	**0**	85	n.a.
17-wgMLST	0	165	**0**	**1**	189	**1**	26	108	n.a.	197	**0**	103	n.a.
19-SNPa[d)]	0	293	10*	12*	312	**7**	63	177	n.a.	317	15*	195	n.a.
21-SNPr	0	275	**0**	**3**	297	**3**	58	171	n.a.	298	**0**	178	n.a.
23-SNPr	0	279	**3**	**5**	296	**5**	60	167	n.a.	296	**1**	179	n.a.
27-SNPr	0	259	**0**	**3**	282	**3**	55	163	n.a.	282	**0**	171	n.a.
31-SNPr	0	300	**0**	**2**	549	**2**	121	210	n.a.	550	**0**	194	n.a.
63-SNPr[e)]	0	259	**0**	**3**	263	n.a.*	56	160	n.a.	282	**0**	173	n.a.
80-SNPa	0	303	**1**	**5**	954	**3**	344	343	n.a.	954	**0**	320	n.a.

*)In blue : Deviation from the expected result.

The 4 clustering strains (24SCA02, 24SCA03, 24SCA05, 24SCA14) are indicated in green. The 2 strains expected to be excluded (24SCA12, 24SCA16) are indicated in orange.
n.a.: not applicable, strain not included in the laboratory's distance matrix.
Numbers in bold were reported to be clustering with the REF strain.

a) Comment Lab 12: We would have repeated NGS of strain 24SCA12 as it has an AD=3 to the reference strain, but failed QC.
b) Comment Lab 13: 24SCA12 included *S*. Infantis which clustered with the REF strain but was contaminated with other *Salmonella*. 24SCA14 included *S*. Infantis which clustered with REF strain but was contaminated slightly with *E. coli*. 24SCA16 included *S*. Paratyphi B and some other *Salmonella*. All of them were excluded from the final cluster analysing.
c) Comment Lab 16: Strain 24SCA15: Intraspecies contamination with 37 SNVs, excluded from analysis/distance matrix.
d) Comment Lab 19: Strain 24SCA02, Strain 24SCA03 and 24SCA0314 shared 11 SNPs.
e) Comment Lab 63: Data set passed QC criteria for *Salmonella*, but was excluded from reference-based SNP-analysis as it is a more distant sequence type.

Appendix 14 Optional AMR results submissions by twelve participants

Labcode	Tool(s) used	Strain 24SCA01	Strain 24SCA02	Strain 24SCA03
EL	**AMRFinderPlus, PointFinder, ResFinder**	**aac(6')-Iaa, aadA1, dfrA1, parC p.T57S**	**aac(6')-Iaa, aph(3')-Ia, aadA1, sul1, tet(A), dfrA14, gyrA p.D87G, parC p.T57S**	**aac(6')-Iaa, aph(3')-Ia, aadA1, sul1, tet(A), dfrA14, gyrA p.D87G, parC p.T57S**
2[a)]	amrfinder 3.12.8 plus ResFinder 4.6.0	aadA1;dfrA1;sat2	aadA1;aph(3')-Ia;dfrA14;gyrA_D87G;sul1;tet(A)	aadA1;aph(3')-Ia;dfrA14;gyrA_D87G;sul1;tet(A)
3	AMRfinderplus, pointfinder, resfinder	parC p.T57S,dfrA1 (dfrA1_X00926)	gyrA p.D87G parC p.T57S,sul1 (sul1_U12338), sul1 (sul1_EU780013),dfrA14 (dfrA14_DQ388123), dfrA14 (dfrA14_AF393510)	gyrA p.D87G parC p.T57S,sul1 (sul1_U12338),dfrA14 (dfrA14_DQ388123), dfrA14 (dfrA14_AF393510)
4	ResFinder 4.4.2	aac(6')-Iaa, aadA1, dfrA1	aac(6')-Iaa, aadA1, aadA1, aph(3')-Ia, sul1, tet(A), dfrA14	aac(6')-Iaa, aadA1, aadA1, aph(3')-Ia, sul1, tet(A), dfrA14
5	Ridom SeqSphere+, Resfinder	aac(6')-Iaa, aadA1, aac(6')-Iaa, aadA1, dfrA1	aac(6')-Iaa, sul1, dfrA14, tet(A), aph(3')-Ia, chromosomal mutations p.D87G and p.T57S leading to ciprofloxacin and nalidixic acid resistance	aac(6')-Iaa, sul1, dfrA14, tet(A), aph(3')-Ia, chromosomal mutations p.D87G and p.T57S leading to ciprofloxacin and nalidixic acid resistance
11	AMRFinderPlus	aadA1-sat2-dfrA1	aph(3')-Ia - aadA1 - sul1 - tet(A) - dfrA14	aph(3')-Ia - aadA1 - sul1 - tet(A) - dfrA14
12[b)]	NCBI AMR Finder Plus; CGE ResFinder	aadA1, dfrA1	aadA1, sul1, tet(A), dfrA14; qacEdelta1; gyrA_D87G	aadA1, sul1, tet(A), dfrA14; qacEdelta1; gyrA_D87G
16	ResFinder	aac(6')-Iaa, aadA1 and dfrA1	aac(6')-Iaa, aadA1, aph(3')-Ia, sul1, tet(A) and dfrA14	aac(6')-Iaa, aadA1, aph(3')-Ia, sul1, tet(A) and dfrA14
17[c)]	ResFinder, PiontFinder	aadA1, dfrA1, parC (T57S) - trimethoprim	ant(3'')-Ia, aph(3')-Ia, dfrA14, gyrA (D87G), parC (T57S), qacE, sul1, tet(A) - trimethoprim, ciprofloxacin, nalidixic acid, sulfamethoxazole, tetracycline	ant(3'')-Ia, aph(3')-Ia, dfrA14, gyrA (D87G), parC (T57S), qacE, sul1, tet(A) - trimethoprim, ciprofloxacin, nalidixic acid, sulfamethoxazole, tetracycline
19	Resfinder Docker	aac(6')-Iaa; aadA1; dfrA1; parC p.T57S	aac(6')-Iaa; aadA1; aph(3')-Ia; sul1; dfrA14; tet(A); gyrA p.D87G; parC p.T57S	aac(6')-Iaa; aadA1; aph(3')-Ia; sul1; dfrA14; tet(A); gyrA p.D87G; parC p.T57S
21	ResFinder v4.6.0	aac(6')-Iaa; aadA1; dfrA1; parC	aac(6')-Iaa; aadA1; aph(3')-Ia; sul1; tet(A); dfrA14	aac(6')-Iaa; aadA1; aph(3')-Ia; sul1; tet(A); dfrA14; parC; gyrA
31	ResFinder; AbriTAMR run 1.0.19	aac(6')-Iaa, aadA1, parC. pT57S	aac(6')-Iaa; aph(3')-Ia; sul1; tet(A); dfrA14; aadA1, parC. pT57S; gyrAp.D78G	aac(6')-Iaa; aph(3')-Ia; sul1; tet(A); dfrA14;aadA1, parC. pT57S; gyrAp.D78G
63	resfinder	aac(6')-Iaa, aadA1, dfrA1	aac(6')-Iaa, aadA1, aph(3'), dfrA14, sul1, tet(A)	aac(6')-Iaa, aadA1, aph(3'), dfrA14, sul1, tet(A)

Labcode	Tool(s) used for AMR identification	Strain 24SCA04	Strain 24SCA05	Strain 24SCA06
EL	**AMRFinderPlus, PointFinder, ResFinder**	**aac(6')-Iaa, parC p.T57S**	**aac(6')-Iaa, aph(3')-Ia, aadA1, sul1, tet(A), dfrA14, gyrA p.D87G, parC p.T57S**	**aac(6')-Iaa, aph(3')-Ia, aadA1, sul1, tet(A), dfrA14, gyrA p.D87G, parC p.T57S**
2[a)]	amrfinder 3.12.8 plus ResFinder 4.6.0	-	aadA1;aph(3')-Ia;dfrA14;gyrA_D87G;sul1;tet(A)	aadA1;aph(3')-Ia;dfrA14;gyrA_D87G;sul1;tet(A)
3	AMRfinderplus, pointfinder, resfinder	parC p.T57S	gyrA p.D87G parC p.T57S,sul1 (sul1_U12338), sul1 (sul1_EU780013),dfrA14 (dfrA14_DQ388123), dfrA14 (dfrA14_AF393510)	gyrA p.D87G parC p.T57S,sul1 (sul1_U12338), sul1 (sul1_EU780013),dfrA14 (dfrA14_DQ388123), dfrA14 (dfrA14_AF393510)
4	ResFinder 4.4.2	aac(6')-Iaa	aac(6')-Iaa, aadA1, aadA1, aph(3')-Ia, sul1, tet(A), dfrA14	aac(6')-Iaa, aadA1, aadA1, aph(3')-Ia, sul1, tet(A), dfrA14
5	Ridom SeqSphere+, Resfinder	aac(6')-Iaa	aac(6')-Iaa, aadA1, aph(3'), aadA1, sul1, dfrA14, tet(A)	aac(6')-Iaa, sul1, dfrA14, tet(A), aadA1, aph(3')-Ia, sul1
11	AMRFinderPlus	no ARG	aph(3')-Ia - aadA1 - sul1 - tet(A) - dfrA14	aph(3')-Ia - aadA1 - sul1 - tet(A) - dfrA14
12[b)]	NCBI AMR Finder Plus; CGE ResFinder	-	aadA1, sul1, tet(A), dfrA14; qacEdelta1; gyrA_D87G	aadA1, sul1, tet(A), dfrA14; qacEdelta1; gyrA_D87G
16	ResFinder	aac(6')-Iaa	aac(6')-Iaa, aadA1, aph(3')-Ia, sul1, tet(A) and dfrA14	aac(6')-Iaa, aadA1, aph(3')-Ia, sul1, tet(A) and dfrA14
17[c)]	ResFinder, PiontFinder	parC (T57S) - none	ant(3'')-Ia, aph(3')-Ia, dfrA14, gyrA (D87G), parC (T57S), qacE, sul1, tet(A) - trimethoprim, ciprofloxacin, nalidixic acid, sulfamethoxazole, tetracycline	ant(3'')-Ia, aph(3')-Ia, dfrA14, gyrA (D87G), parC (T57S), qacE, sul1, tet(A) - trimethoprim, ciprofloxacin, nalidixic acid, sulfamethoxazole, tetracycline
19	Resfinder Docker	aac(6')-Iaa; parC p.T57S	aac(6')-Iaa; aadA1; aph(3')-Ia; sul1; dfrA14; tet(A); gyrA p.D87G; parC p.T57S	aac(6')-Iaa; aadA1; aph(3')-Ia; sul1; dfrA14; tet(A); gyrA p.D87G; parC p.T57S
21	ResFinder v4.6.0	aac(6')-Iaa	aac(6')-Iaa; aadA1; aph(3')-Ia; sul1; tet(A); dfrA14; parC; gyrA	aac(6')-Iaa; aadA1; aph(3')-Ia; sul1; tet(A); dfrA14; parC; gyrA
31	ResFinder; AbriTAMR run 1.0.19	aac(6')-laa; parC. pT57S	aac(6')-laa; aph(3')-la; sul1; tet(A); dfrA14; aadA1, parC. pT57S; gyrAp.D78G	aac(6')-laa; aph(3')-la; sul1; tet(A); dfrA14; aadA1, parC. pT57S; gyrAp.D78G
63	resfinder	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa, aadA1, aph(3'), dfrA14, sul1, tet(A)

Labcode	Tool(s) used for AMR identification	Strain 24SCA11	24SCA12 (mixed strains Inf+STM)	Strain 24SCA13
EL	**AMRFinderPlus, PointFinder, ResFinder**	**aac(6')-Iaa, aadA1, sul1, tet(A), gyrA p.S83Y, parC p.T57S**	**Failed QC**	**aac(6')-Iaa, parC p.T57S**
2[a)]	amrfinder 3.12.8 plus ResFinder 4.6.0	aadA1;gyrA_S83Y;sul1;tet(A)	QC Fail	-
3	AMRfinderplus, pointfinder, resfinder	gyrA p.S83Y, parC p.T57S,sul1 (sul1_U12338)	blaCARB-2 (blaCARB-2_M69058),gyrA p.D87G, parC p.T57S,sul1 (sul1_U12338),dfrA14 (dfrA14_DQ388123) dfrA14 (dfrA14_AF393510)*	parC p.T57S
4	ResFinder 4.4.2	aac(6')-Iaa, aadA1, aadA1, sul1, tet(A)	QC not passed	aac(6')-Iaa
5	Ridom SeqSphere+, Resfinder	aac(6')-Iaa, aadA1, sul1, tet(A), chromosomal mutations p.S83Y and p.T57S leading to ciprofloxacin and nalidixic acid resistance	aac(6')-Iaa, aadA2b, aph(3')-Ia, sul1, dfrA14, tet(A), floR*	aac(6')-Iaa
11	AMRFinderPlus	aadA1 - sul1 - tet(A)	NA	no ARG
12[b)]	NCBI AMR Finder Plus; CGE ResFinder	aadA1, sul1, tet(A); qacEdelta1; gyrA_S83Y	aadA1, blaCARB-2, floR, tet(A), dfrA14; qacEdelta1; gyrA_D87G;*	-
16	ResFinder	aac(6')-Iaa, aadA1, sul1 and tet(A)		aac(6')-Iaa
17[c)]	ResFinder, PiontFinder	ant(3'')-Ia, gyrA (S83Y), parC (T57S), qacE, sul1, tet(A) - ciprofloxacin, nalidixic acid, sulfamethoxazole, tetracycline	Contaminated	parC (T57S) - none
19	Resfinder Docker	aac(6')-Iaa; aadA1; aph(3')-Ia; sul1; dfrA14; tet(A); gyrA p.D87G; parC p.T57S	Not applicable	aac(6')-Iaa; parC p.T57S
21	ResFinder v4.6.0	aac(6')-Iaa; aadA1; sul1; tet(A)	-	aac(6')-Iaa; parC
31	ResFinder; AbriTAMR run 1.0.19	aac(6')-laa; aph(3')-la; sul1; tet(A); aadA1; parC. pT57S; gyrAp.D78G	aac(6')-laa; aadA2; aph(3')-la; sul1; tet(A); dfrA14; tet(G); blaCARB-2, floR; parC. pT57S; gyrAp.D78G*	aac(6')-laa; parC. pT57S
63	resfinder	aac(6')-Iaa, aadA1, sul1, tet(A)		aac(6')-Iaa

Labcode	Tool(s) used for AMR identification	Strain 24SCA14	Strain 24SCA15	24SCA16 (mixed strains Inf+Java)
EL	**AMRFinderPlus, PointFinder, ResFinder**	**aac(6')-Iaa, aph(3')-Ia, aadA1, sul1, tet(A), dfrA14, gyrA p.D87G, parC p.T57S**	**aac(6')-Iaa, parC p.T57S**	**Failed QC**
2[a)]	amrfinder 3.12.8 plus ResFinder 4.6.0	aadA1;aph(3')-Ia;dfrA14;gyrA_D87G;sul1;tet(A)	-	QC Fail
3	AMRfinderplus, pointfinder, resfinder	gyrA p.D87G parC p.T57S,sul1 (sul1_U12338),dfrA14 (dfrA14_DQ388123), dfrA14 (dfrA14_AF393510)	parC p.T57S	sul1 (sul1_U12338),dfrA14 (dfrA14_DQ388123), dfrA14 (dfrA14_AF393510)*
4	ResFinder 4.4.2	aac(6')-Iaa, aadA1, aadA1, aph(3')-Ia, sul1, tet(A), dfrA14	aac(6')-Iaa	QC not passed
5	Ridom SeqSphere+, Resfinder	aac(6')-Iaa, aadA1, aph(3')-Ia, sul1, dfrA14, tet(A)	aac(6')-Iaa	aadA1, aph(3')-Ia, sul1, dfrA14, tet(A)*
11	AMRFinderPlus	aph(3')-Ia - aadA1 - sul1 - tet(A) - dfrA14	no ARG	NA
12[b)]	NCBI AMR Finder Plus; CGE ResFinder	aadA1, sul1, tet(A), dfrA14; qacEdelta1; gyrA_D87G	-	not applicable
16	ResFinder	aac(6')-Iaa, aadA1, aph(3')-Ia, sul1, tet(A) and dfrA14		
17[c)]	ResFinder, PiontFinder	ant(3'')-Ia, aph(3')-Ia, dfrA14, gyrA (D87G), parC (T57S), qacE, sul1, tet(A) - trimethoprim, ciprofloxacin, nalidixic acid, sulfamethoxazole, tetracycline	parC (T57S) - none	Contaminated
19	Resfinder Docker	aac(6')-Iaa; aadA1; aph(3')-Ia; sul1; dfrA14; tet(A); gyrA p.D87G; parC p.T57S	aac(6')-Iaa; parC p.T57S	Not applicable
21	ResFinder v4.6.0	aac(6')-Iaa; aadA1; aph(3')-Ia; sul1; tet(A); dfrA14	aac(6')-Iaa; parC	-
31	ResFinder; AbriTAMR run 1.0.19	aac(6')-Iaa; aadA1; aph(3')-Ia; sul1; tet(A); dfrA14, parC. pT57S; gyrAp.D78G	aac(6')-laa, parC. pT57S	aac(6')-laa; aph(3')-la; sul1; tet(A); dfrA14; aadA1*
63	resfinder	aac(6')-Iaa, aadA1, aph(3'), dfrA14, sul1, tet(A)	aac(6')-Iaa	

a) AMR markers mdsA;mdsB were present in all isolates but not assigned as intact, since the genes itselves are incomplete as well as the efflux pump they are part of.
b) We would have repeated NGS of strain 24SCA12 as it has an AD=3 to the reference strain, but failed QC.
c) All strains contain the aac(6')-Iaa, the cryptic gene for *Salmonella* sp. Also, not all markers found are important for the *Salmonella* AMR phenotype.
*) In Grey: Deviation from the expected result.

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