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EURL-*Salmonella* Proficiency Test Serotyping 2025

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W.F. Jacobs-Reitsma et al.

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Synopsis

EURL-*Salmonella* Proficiency Test Serotyping 2025

Since 1992, the National Reference Laboratories (NRLs) of EU Member States have been required to undergo annual quality assessments through proficiency tests. For one of these tests, laboratories must use a standard method (serotyping) to correctly identify 20 *Salmonella* strains.

All 27 EU Member States' NRLs and six non-EU NRLs performed well in this *Salmonella* serotyping quality control in 2025. One of the six non-EU laboratories initially failed to meet the required quality standard but performed well after receiving on-site practical training. Overall, the participating laboratories correctly identified 97% of the strains tested.

Each Member State appoints a National Reference Laboratory (NRL) to participate in the proficiency tests. The NRL is responsible for detecting and identifying *Salmonella* in food and animal samples in its Member State. NRLs from countries outside the EU may also participate voluntarily. Six non-EU NRLs took part in 2025, bringing the total number of participating laboratories to 33.

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

Keywords: *Salmonella*, EURL, NRL, proficiency test, serotyping

Publiekssamenvatting

EURL-*Salmonella* ringonderzoek typering 2025

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 met een verplichte standaardmethode (serotypering) aan twintig *Salmonella*-stammen de juiste naam kunnen geven.

In 2025 scoorden alle NRL's van de 27 EU-lidstaten goed bij deze kwaliteitscontrole op serotypering van *Salmonella*. Net als zes NRL's van landen buiten de EU. Een van deze zes laboratoria heeft hiervoor eerst een praktische training in hun eigen laboratorium gekregen, omdat het de eerste keer onvoldoende kwaliteit had geleverd. Alle deelnemende laboratoria samen konden aan 97 procent van de geteste stammen de juiste naam geven.

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 2025 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

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Summary

From August to October 2025, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*), based in Bilthoven, the Netherlands, organised the annual Proficiency Test (PT) on *Salmonella* serotyping.

The main objective of the PT was to assess whether the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) in the European Union typed *Salmonella* strains consistently and obtained comparable results.

The 2025 PT did not include an optional part on cluster analyses.

A total of 33 laboratories participated in the PT. These comprised the NRLs-*Salmonella* of all 27 EU Member States, whose participation is mandatory, and six NRLs that participated voluntarily: two from EU candidate countries, three from European Free Trade Association (EFTA) countries and one from the United Kingdom.

EURL-*Salmonella* selected 20 mandatory *Salmonella* strains and one less common optional strain for serotyping. Each laboratory typed the strains using its routine method in accordance with the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007).

Overall, the participants correctly typed the O-antigens of 97,5% of the strains and the H-antigens of 98,8% of the strains, and correctly identified the serovar name for 96,9% of the strains.

The criteria for good performance in serotyping were established in 2007. Based on these criteria, the participants performed very well, including the six that submitted partial or additional serotyping results based on whole-genome sequencing (WGS).

All 27 EU NRLs-*Salmonella* and five non-EU NRLs-*Salmonella* met the criteria for good performance initially. One non-EU NRL-*Salmonella* with a limited serotyping scope met the criteria after receiving on-site practical training.

1 Introduction

This report describes the 2025 Proficiency Test (PT) on *Salmonella* serotyping, organised by the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*), based in Bilthoven, the Netherlands, from August to October 2025.

Under Regulation (EU) 2017/625 (EC, 2017), one of the tasks of EURL-*Salmonella* is to organise PTs for the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) in the European Union. The main objectives of PTs on the typing of *Salmonella* are to ensure that typing is carried out uniformly across all Member States and that the results obtained are comparable. PTs on typing were introduced in 1995.

A total of 33 laboratories participated in the 2025 serotyping PT. These included the NRLs-*Salmonella* of all 27 EU Member States, whose participation is mandatory. Six additional NRLs participated voluntarily: two from the EU candidate countries Moldova and Serbia, three from the European Free Trade Association (EFTA) countries Iceland, Norway and Switzerland and one from the United Kingdom.

All NRLs serotyped the 20 mandatory strains and the optional 21st strain. Any NRL from an EU Member State that failed to achieve the defined level of good performance in serotyping would have to participate in a follow-up study.

The 2025 typing PT did not include an optional part on cluster analyses.

2 Participants

Table 2.1 lists the participants in the EURL-*Salmonella* PT Serotyping 2025.

*Table 2.1 List of participants in the EURL-*Salmonella *PT Serotyping 2025*

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 (LABRIS)
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)]	Kildare	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
Slovak Republic	Dolný Kubín	Veterinary and Food Institute

Country	City	Institute
Slovenia	Ljubljana	UL, VF, National Veterinary Institute
Spain	Algete-Madrid	Laboratorio Central de Veterinaria
Sweden	Uppsala	National Veterinary Agency (SVA)
Switzerland	Bern	Institute of Veterinary Bacteriology, University of Bern
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 one of the laboratory codes 1–33. Additional laboratory codes 83 and 86 were assigned to participants 15 and 32, respectively, so that they could report a second set of results based on whole-genome sequencing (WGS). Laboratory 5 participated in the EURL-*Salmonella* serotyping PT for the second year and was invited to resubmit its results under the new laboratory code 55 following hands-on training provided at its laboratory by two EURL-*Salmonella* serotyping experts in early October 2025.

3.1.2 Protocol and test report

Two weeks before the start of the PT, the NRLs received the protocol by email. Participants used a web-based form to submit their results. The NRLs received instructions for completing the result form by email on 12 August 2025.

The protocol and screenshots of the result form are available on the EURL-*Salmonella* website:
https://www.eurlsalmonella.eu/proficiency-testing/typing-studies

3.1.3 Transport

The parcels containing the strains (grown on Heart Infusion agar in transport tubes) were sent by EURL-*Salmonella* on 11 August 2025. All samples were packed as Biological Substance, Category B (UN 3373), and transported by a door-to-door courier service.

3.2 Serotyping

3.2.1 Salmonella *strains for serotyping*

Participants were asked to serotype a total of 20 *Salmonella* strains (coded S1–S20). As agreed at the 30th EURL-*Salmonella* Workshop (Mooijman, 2025), a less common strain (S21) was additionally included. Serotyping this strain was optional and the results were not taken into account in the evaluation.

The *Salmonella* strains originated from the collection of the National *Salmonella* Centre in the Netherlands (RIVM-IDS). The strains were serologically verified by the Centre before distribution. Table 3.1 presents the complete antigenic formulae of the 21 serovars in accordance with the most recent White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007), including published supplements numbers 47 (Guibourdenche et al., 2010) and 48 (Issenhuth-Jeanjean et al., 2014). However, participants were asked to report only the antigens they detected and used as the basis for identifying the serovar.

Three strains, S5 (Limete), S6 (Belem) and S7 (Madelia), represented serovars included in the EURL-*Salmonella* serotyping PTs for the first time.

Table 3.1 Antigenic formulae of the 21 Salmonella *strains used in the EURL-*Salmonella *PT Serotyping 2025, according to the White-Kauffmann-Le Minor scheme*

Strain code	O-antigens	H-antigens		Serovar	Origin
		(phase 1)	(phase 2)		
S1	6,7,14	m,t	[z_{57}]	Oranienburg	Human
S2	6,8	z_{10}	e,n,x	Hadar	Human
S3	16	b	1,2	Hull	Human
S4	1,9,12	g,m	-	Enteritidis	Human
S5[a)]	1,4,12,[27]	b	1,5	Limete	Human
S6[a)]	6,8	c	e,n,x	Belem	Human
S7[a)]	1,6,14,25	y	1,7	Madelia	Human
S8	6,7	k	e,n,x	Singapore	Human
S9	6,7,14	r	1,2	Virchow	Human
S10	3,{10}{15}{15,34}	e,h	1,6	Anatum	Human
S11[b)]	1,4,[5],12	i	-	1,4,[5],12:i:-	Human
S12	1,4,[5],12	f,g	[1,2]	Derby	Human
S13[c)]	1,4,[5],12	b	1,2	Paratyphi B var. Java	Human
S14	1,4,[5],12	r	1,2	Heidelberg	Human
S15	6,7,14	g,m,[p],s	[1,2,7]	Montevideo	Human
S16	1,13,22	z	1,6	Poona	Human
S17	1,4,[5],12	i	1,2	Typhimurium	Human
S18	1,9,12[Vi]	g,p	-	Dublin	Human
S19	6,7,14	r	1,5	Infantis	Human
S20	1,4,12	z	1,5	Kiambu	Human
S21[d)]	52	a	1,5	Uithof	Human

a) Included in an EURL-*Salmonella* PT Serotyping for the first time.
b) Monophasic variant of *S.* Typhimurium, based on genomic sequence analysis. Phenotypic result: 4:i:-.
c) L(+)-tartrate (= d-tartrate)-positive variant based on genomic sequence analysis, often referred to as var. Java.
d) Optional strain.

3.2.2 Evaluation of the serotyping results

In accordance with the protocol for this PT (section 3.1.2), participants were required to use their laboratory's routine serotyping method.

Participants could also use a second serotyping method (e.g. WGS) to analyse the strains and have the results evaluated separately under a second laboratory code. However, only the results obtained using the routine method were used to assess the performance of the NRLs-*Salmonella*.

Table 3.2 presents the evaluation of serotyping results that deviated from the expected results.

Table 3.2 Evaluation of serotyping results that deviate from the expected 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 serotyping PTs were defined (Mooijman, 2007).

Penalty points are assigned for the incorrect typing of strains. However, a distinction is made between the 'top-five' *Salmonella* serovars of greatest importance to public health, as identified in EU legislation, and all other serovars:

- Four penalty points: incorrect typing of *S.* Enteritidis, *S*. Typhimurium (including the monophasic variant), *S.* Hadar, *S*. Infantis or *S.* Virchow, or incorrectly assigning the name of one of these five serovars to a different strain;
- One penalty point: incorrect typing of any other *Salmonella* serovar.

The total number of penalty points is calculated for each NRL-*Salmonella*. The criterion for good performance is fewer than four penalty points. NRLs in EU Member States that fail to meet the criterion for good performance (four penalty points or more) are required to participate in a follow-up study.

4 Results and discussion

4.1 Technical data

4.1.1 General

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

Table 4.1 summarises the frequency of *Salmonella* serotyping at the participating laboratories and the approximate number of strains serotyped during the first half of 2025.

Table 4.1 Frequency of serotyping and number of Salmonella *strains serotyped during the first half of 2025*

Laboratory code	Serotyping frequency	No. of strains serotyped
22	Daily	120
27	Daily	120
7	Daily	140
17	Daily	165
8	Daily	200
1	Daily	220
24	Daily	400
16	Daily	407
30	Daily	450
26	Daily	483
14	Daily	500
20	Daily	600
32 (86)	Daily	700 (150)
9	Daily	1 000
15 (83)	Daily	1 200 (1 200)
19	Daily	1 350
12	Daily	1 500
23	Daily	1 600
28	Daily	1 700
2	Daily	3 000
33	Daily	3 300
29	Three times a week	90
21	Three times a week	98
18	Three times a week	110
6	Once a week	35
4	Once a week	52
13	Once a week	60
11	Once a week	106
10	Once a week	130
31	Once a week	220
25	Monthly	15
3	Monthly	23

Laboratory code	Serotyping frequency	No. of strains serotyped
55 (5)	-	27
n=33		20 121

4.1.2 *Accreditation*

The participants' accreditation details are given in Table 4.2.

The non-EU laboratory that was not accredited for serotyping reported that this was due to the relatively low number of strains it serotypes. The EU NRL that was not currently accredited for serotyping reported that it planned to regain accreditation in 2026.

Table 4.2 Details of the participants' accreditation status

Laboratory codes	Accredited method(s)	Serovars covered	According to ISO standard(s)
55 (5)	Classical serology	Enteritidis, Typhimurium, Infantis, Virchow and Hadar	EN ISO/IEC 17025
1, 2, 3, 4, 6, 7, 10, 11, 12, 13, 16, 18, 19, 20, 21, 22, 24, 26, 30 (*n*=19)	Classical serology	All serovars	EN ISO/IEC 17025
8	Classical serology; WGS (using SeqSero)	All serovars	EN ISO/IEC 17025
9	Classical serology; WGS short-read sequencing	All serovars	EN ISO 15189
14	Classical serology; PCR	Classical serology: all serovars PCR: Enteritidis and Typhimurium	EN ISO/IEC 17025
15	Classical serology; Luminex	All serovars	EN ISO/IEC 17025, EN ISO 15189
17	Classical serology; WGS	Classical serology: all serovars; WGS: all serovars from food and feed	EN ISO/IEC 17025
23	Classical serology; Luminex; multiplex PCR	All serovars	EN ISO/IEC 17025
27	Classical serology; PCR STM; API	All serovars	EN ISO/IEC 17025, EN ISO 15189
28	Classical serology and biochemical tests; AMR	All serovars	EN ISO/IEC 17025
31	WGS	All serovars	EN ISO/IEC 17025
32	Classical serology; WGS	All serovars	EN ISO/IEC 17025
33	Serotyping and WGS extraction only	All serovars	EN ISO/IEC 17025
83 (15)	WGS	The 60 most common serovars	EN ISO/IEC 17025, EN ISO 15189
86 (32)	The sequencing method is accredited. Accreditation of the bioinformatic analysis for *Salmonella* is planned for 2026.		

4.1.3 *Transport of samples*

The parcels were shipped to the participants on Monday 11 August 2025, except for one parcel, which was shipped on 18 August 2025 because of an issue with its import permit.

All but four participants received their parcels within two days of shipment. One parcel was received after three days in transit, two parcels were received after seven days and one parcel reached the laboratory after 14 days. All laboratories received the parcels in good condition.

4.2 Serotyping results

4.2.1 *General*

All NRLs-*Salmonella* tested the 20 mandatory strains. Classical serology was routinely used by 31 of the 33 participants. Ten of these participants reported using classical serology in combination with Luminex assays (2), multiplex or real-time PCR (6) or WGS (2). Two participants routinely used whole-genome sequencing (WGS), supplemented by conventional agglutination to confirm certain strains, for example by using O:6 and O:8 (strains S2 and S6), O:25 (strain S7) and O:22 and O:23 (strain S16).

Tables 4.3 and 4.4 summarise details of the different manufacturers of antisera used by the participants.

Table 4.3 Number of laboratories using antisera from each manufacturer or source

Manufacturer or source	Number of laboratories (*n*=32*)
Bio-Rad	14
Pro-Lab	5
Sifin	20
Statens Serum Institut (SSI)	30
Other manufacturers	5
Prepared in-house	1

*Data were missing for one participant.

Table 4.4 Number of laboratories by number of sources from which antisera were obtained, including in-house preparation

Number of antiserum sources, including in-house preparation	Number of laboratories (*n*=32*)
1	9
2	8
3	11
4	3
5	1

*Data were missing for one participant.

4.2.2 *Biochemical testing for confirmation*

Four participants (laboratory codes 3, 6, 25 and 55), as well as the two additional WGS submissions under laboratory codes 83 and 86, reported

that they had not used biochemical testing to confirm the strains. A few participants used a standard set of biochemical tests for all strains. Most participants used a variety of tests, or no tests at all, depending on the strain. Full details are provided in Appendix 3.

4.2.3 Use of PCR for confirmation

Eighteen laboratories used PCR to confirm strain S11, the monophasic variant of *S.* Typhimurium 4,12:i:-. Five of these laboratories also used PCR to confirm strain S17, *S.* Typhimurium.

Five laboratories cited Tennant et al. (2010) or EFSA (2010), while two laboratories used Annex C of EN ISO 6579-4:2025, which also refers to these publications.

Four laboratories cited Maurischat et al. (2015), which is referenced in Annex B (probe-based) and Annex D (agarose-based) of EN ISO 6579-4:2025. In addition, three laboratories mentioned Annex B and one mentioned Annex D.

Other PCR assays were also used, for example to provide additional confirmation of *S.* Enteritidis or *S.* Infantis, or to identify subspecies (see also Appendix 3). Malorny et al. (2003) was cited in relation to tartrate testing to confirm strain S13 (*S.* Paratyphi B var. Java); see also section 4.2.6.

4.2.4 Serotyping results per laboratory

Laboratory codes 83 and 86 were additionally assigned to participants 15 and 32, respectively, to report a second set of results based on WGS. These additional results were not taken into account in the overall performance assessment for this PT.

Laboratory 5, located outside the EU, participated in the EURL-*Salmonella* serotyping PT for the second year and resubmitted its results under the new laboratory code 55 after receiving hands-on training at its laboratory from EURL-*Salmonella*. This laboratory's accreditation covers only the 'top five' serovars, which complicates direct comparison with the other participants and may bias the overall evaluation. Therefore, the results reported under laboratory codes 5 and 55 were excluded from the general overview of the evaluation results for this PT (Figures 4.1–4.4 and Table 4.5).

Figures 4.1, 4.2 and 4.3 show the evaluation of errors in O-antigen typing, H-antigen typing and strain identification, respectively. Figure 4.4 shows the percentage of correct results per laboratory.

All O-antigens were correctly typed by 21 of the 32 participants (66%). This corresponds to 97,5% of all O-antigen results. All H-antigens were correctly typed by 24 of the 32 participants (75%), corresponding to 98,8% of all H-antigen results. Overall, 17 participants (53%) correctly reported all serovar names, corresponding to 96,9% of all serovar identification results.

Figure 4.1 Evaluation of O-antigen typing errors by participant

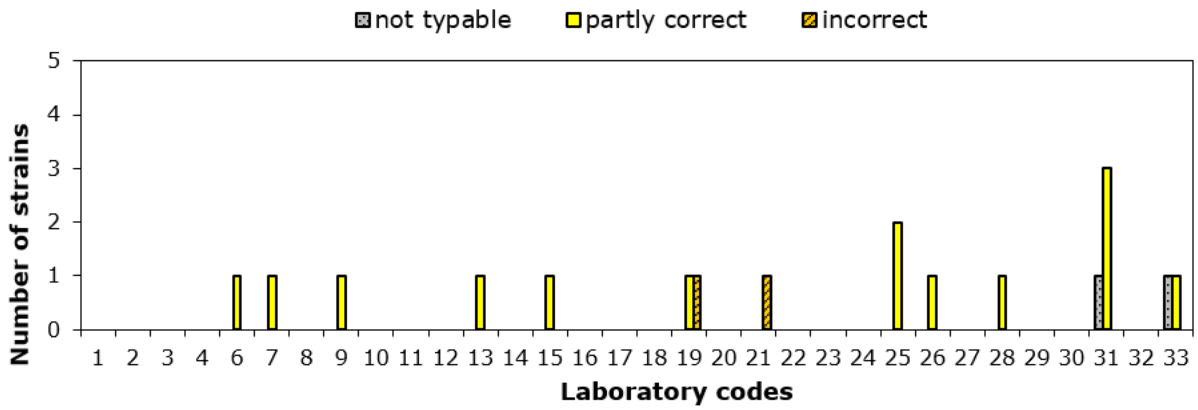


Figure 4.2 Evaluation of H-antigen typing errors by participant

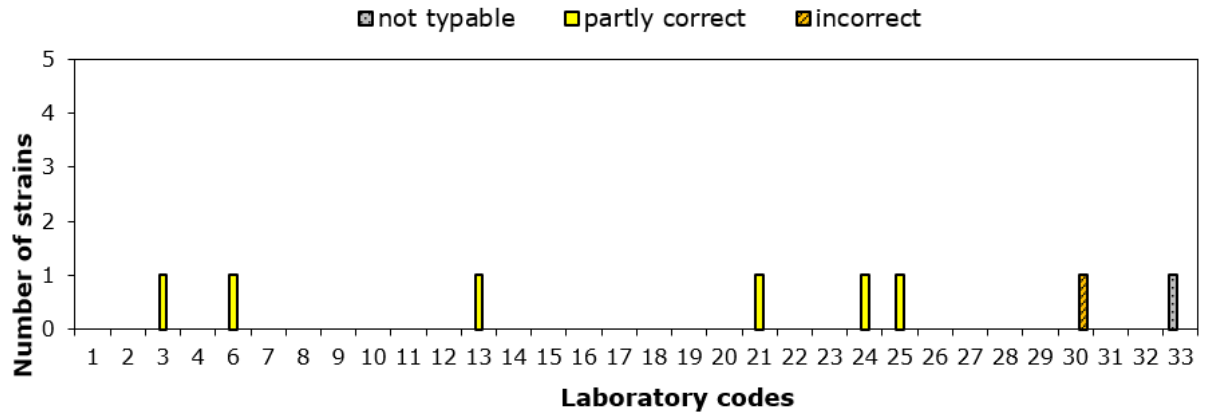


Figure 4.3 Evaluation of serovar identification errors by participant

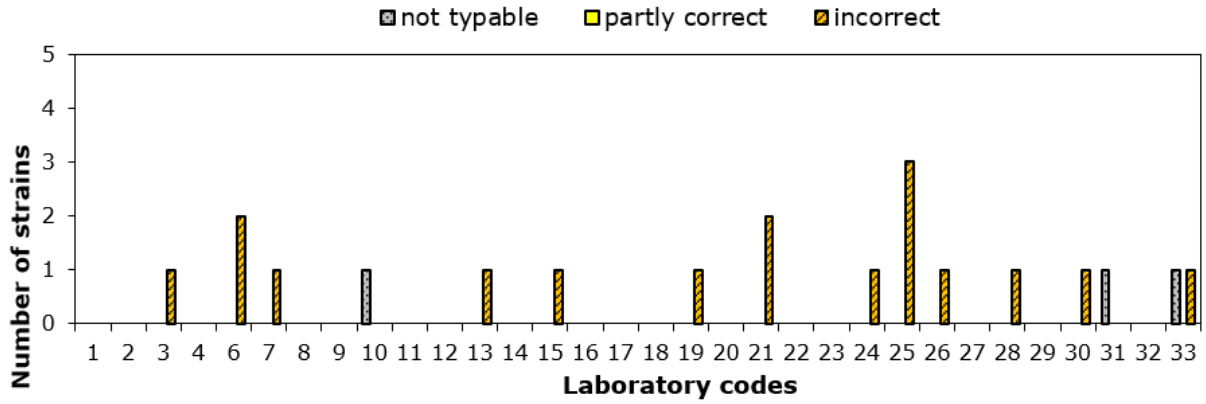
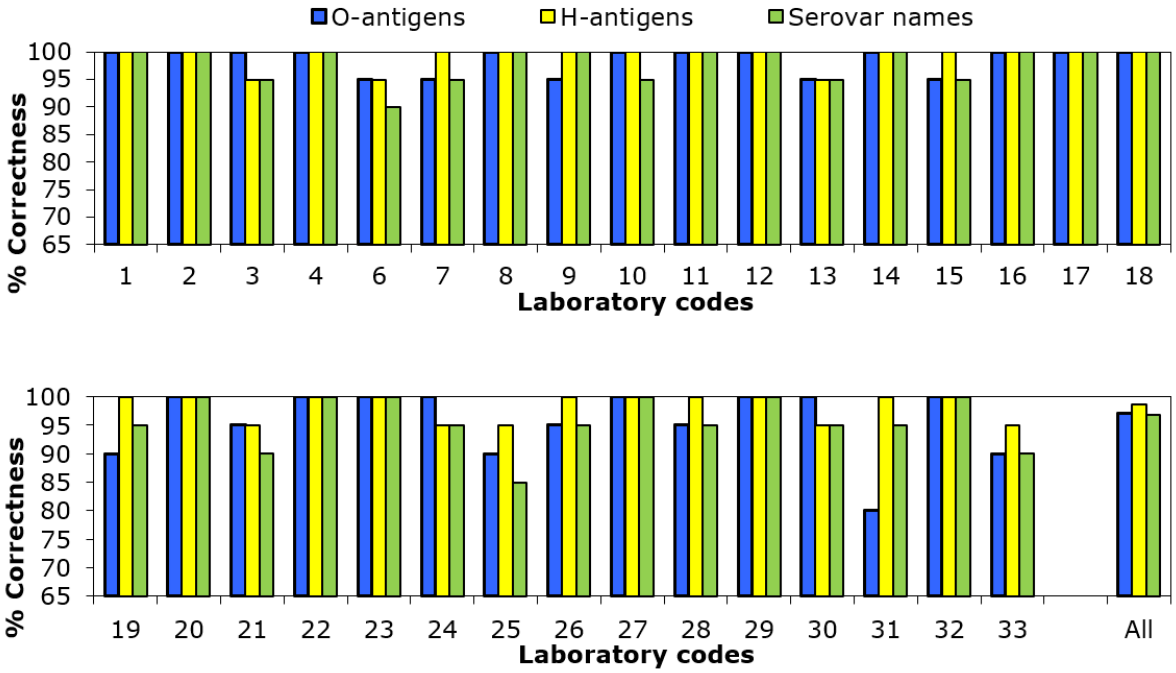


Figure 4.4 Percentage of correct serotyping results by participant



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 4.5 shows the number of penalty points for each NRL and indicates whether or not the criterion for good performance was met. Table 4.6 shows the percentage of correctly typed strains for the EU Member State participants and for all participants.

Overall, the participants performed well in the 2025 serotyping PT, although one strain, S7 (Madelia), resulted in penalty points for a relatively high number of participants (eight).

All participants met the criterion for good performance. Laboratory 55 also met this criterion after receiving dedicated hands-on training, although its assessment covered only a limited serotyping scope (Table 4.5).

All participants received both their individual laboratory evaluation report and the interim summary report (Jacobs-Reitsma et al., 2025) on 21 November 2025. Appendix 1 provides an example of an individual laboratory evaluation report for the serotyping results.

Table 4.5 Evaluation of serotyping performance by NRL

Laboratory code	Number of penalty points	Good performance
1	0	yes
2	0	yes
3	1	yes
4	0	yes
6	2	yes
7	1	yes
8	0	yes
9	0	yes
10	0	yes
11	0	yes
12	0	yes
13	1	yes
14	0	yes
15	1	yes
16	0	yes
17	0	yes
18	0	yes
19	1	yes
20	0	yes
21	2	yes
22	0	yes
23	0	yes
24	1	yes
25	3	yes
26	1	yes
27	0	yes
28	1	yes
29	0	yes
30	1	yes
31	0	yes
32	0	yes
33	1	yes
55*	1	yes

*Limited serotyping scope (‘top five’ only).

Table 4.6 Correct serotyping results by number of strains tested and participating laboratories: EU Member State participants and all participants

Results	EU Member State participants	All participants*
Total number of participants	27	32
Total number of strains per participant	20	20
Correct O-antigen results/strains tested	524/540 (97,0%)	624/640 (97,5%)
Correct H-antigen results/strains tested	533/540 (98,7%)	632/640 (98,8%)
Correct serovar identifications/strains tested	523/540 (96,9%)	620/640 (96,9%)
Participants with all O-antigens correctly typed/participants	18/27 (67%)	21/32 (66%)
Participants with all H-antigens correctly typed/participants	23/27 (85%)	24/32 (75%)
Participants with all serovars correctly identified/participants	14/27 (52%)	17/32 (53%)
Total number of penalty points	14	17
Participants with unsatisfactory performance	0	0

* Excluding the results reported under laboratory codes 5 and 55.

4.2.6 *Serotyping results per strain*

Appendix 2 presents the final serovar names reported for each strain (S1–S20) by each laboratory (codes 1–33 and 55, with codes 83 and 86 included for information only). Excluding laboratory 55, all participants correctly identified 12 *Salmonella* serovars: Hadar (S2), Enteritidis (S4), Virchow (S9), Anatum (S10), 1,4,[5],12:i:- (S11), Derby (S12), Heidelberg (S14), Poona (S16), Typhimurium (S17), Dublin (S18), Infantis (S19) and Kiambu (S20).

Appendix 2 also presents the serovar names reported for strain S11 (1,4,[5],12:i:-). Eighteen participants used PCR to confirm the strain as monophasic *S.* Typhimurium.

Notably, 18 of the 33 submissions for strain S13 identified the strain as Paratyphi B (Appendix 2 and Table 4.7). Five of these 18 submissions reported having tested for d-tartrate, but apparently obtained a negative result. This was unexpected because the strain had tested positive for tartrate during the PT pre-tests, based on WGS analysis using the *in silico* PCR described by Malorny et al. (2003). Recent biochemical testing of this strain at EURL-*Salmonella* produced variable results (EURL-*Salmonella* Newsletter, December 2026).

Paratyphi B var. Tartrate+/var. Java was reported by 15 submissions. Fourteen of these submissions indicated, either directly or indirectly, that strain S13 had been confirmed as d-tartrate positive using one or more of the following methods: biochemical testing, PCR and WGS.

Table 4.7 Serotyping results for strain S13

Laboratory codes*	Serovar name reported	Tartrate confirmation for strain 13	Number of laboratories
3, 4, 6, 7, 10, 11, 18, 21, 22, 23, 24, 25, 30	Paratyphi B	None	13
20	Paratyphi B	Jordan's d-tartrate (neg)	1
16	Paratyphi B	tartrate	1
29	Paratyphi B	tartrate (neg.)	1
2	Paratyphi B	PCR for d-tartrate fermentation	1
31[a)]	Paratyphi B	d-tartrate	1[b)]
9, 83, 86	Paratyphi B var. Java	None	3[b)]
13, 26[c)], 32	Paratyphi B var Java	WGS	3
8, 12	Paratyphi B var. Java	PCR to confirm *S.* Paratyphi B/var. Java	2
28	Paratyphi B var Tartrate +	d-tartrate/PCR	1
1, 14, 15	Paratyphi B var Tartrate +	tartrate	3
19	Paratyphi B var. Java	d-tartrate broth	1
17	Paratyphi B var. Java	Enterotest 24N	1
27	Paratyphi B var. Java	None	1

* Excluding laboratory codes 5/55 and 33.
a) Comment Lab 31: 'Regarding S13. We did d-tartrate test on the strain to determine if the isolate was Paratyphi B var. Java, but the results were not as clear as we would have liked it to be. Due to the deadline, we did not have time to redo the test. In case this was a routine sample in the lab, we would have done the tartrate test again.'
b) WGS was used as the routine method for this PT.
c) *In silico* PCR for d-tartrate gene (Malorny et al., 2003).

Appendix 4 provides details of the strains that caused serotyping problems. The main problem occurred with strain S7, Madelia (1,6,14,25:y:1,7). Nine submissions, including the additional WGS serotyping results submitted under laboratory codes 83 and 86, reported Carrau instead of Madelia. These laboratories may not have had all the necessary O antisera available. In that case, it would have been appropriate to report either 'Carrau or Madelia', as laboratory 31 did, or as much of the antigenic formula as could be determined, as laboratory 30 did.

Appendix 5 provides details of the additional, optional strain S21. All participants attempted to serotype strain S21, a less common serovar of *Salmonella enterica subsp. enterica* (I). After all, the correct serovar identification, Uithof (52:a:1,5), was reported in 32 submissions, including the WGS results submitted under laboratory codes 83 and 86.

4.2.7 Trend analysis of the serotyping results of EU NRLs

Historical data on Salmonella serotyping for all participants in the EURL-Salmonella PTs are available on the EURL-Salmonella website: http://www.eurlsalmonella.eu/publications/proficiency-test-reports

Historical data for the EU NRLs-*Salmonella* participating in these PTs since 2007 are presented in Figure 4.5, which shows the percentage of

correctly typed strains. Figure 4.6 shows the number of penalty points and instances of unsatisfactory performance.

Since 2007, the percentage of correctly typed strains has remained relatively stable, generally showing better performance for O-antigen typing than for H-antigen typing. The 2025 PT was an exception because six participants made errors in the O-antigen typing of strain S7 (1,6,14,25:y:1,7, Madelia) (Figure 4.5).

The number of penalty points has clearly declined, from 35 points when the system was introduced in 2007 to as few as three points in the 2017, 2020 and 2023 PTs. The increase in the 2018 PT was mainly due to seven EU NRLs incorrectly typing an *S.* Cannstatt strain. The increase in the 2025 PT was mainly due to six EU NRLs incorrectly typing strain S7 (*S.* Madelia). The total number of penalty points may also be strongly affected by the four-point penalty assigned for a single incorrect result involving one of the 'top five' *Salmonella* serovars, as demonstrated by the results of the 2021 and 2022 PTs (Figure 4.6).

Nevertheless, the number of EU NRLs with an initial unsatisfactory performance has remained low: two in each PT from 2010 to 2013 and in the 2022 PT; one in each of the 2014, 2015, 2018 and 2021 PTs; and none in the 2016, 2017, 2019, 2020 and 2023–2025 PTs. Only occasionally was the same NRL involved in more than one year. All the follow-up studies organised for these EU NRLs ultimately resulted in good performance (Figure 4.6).

*Figure 4.5 Trend in the percentage of correctly typed strains among EU NRLs-*Salmonella *in EURL-*Salmonella *serotyping PTs since 2007*

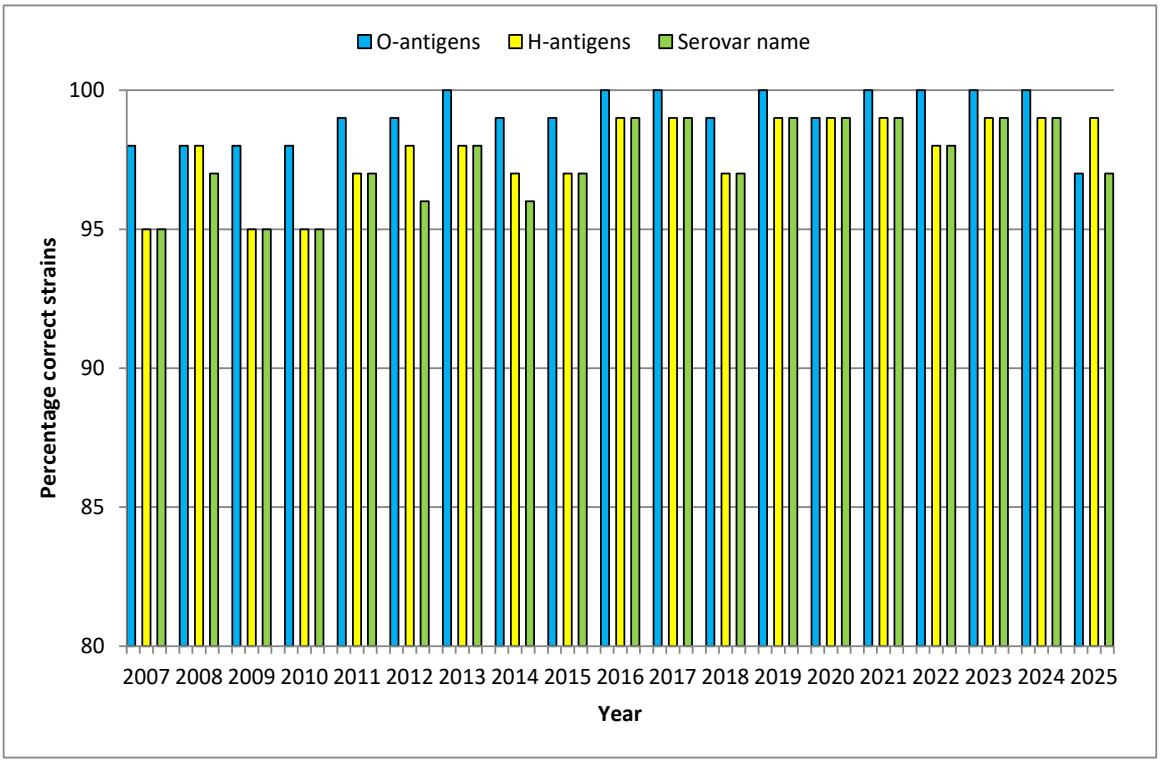
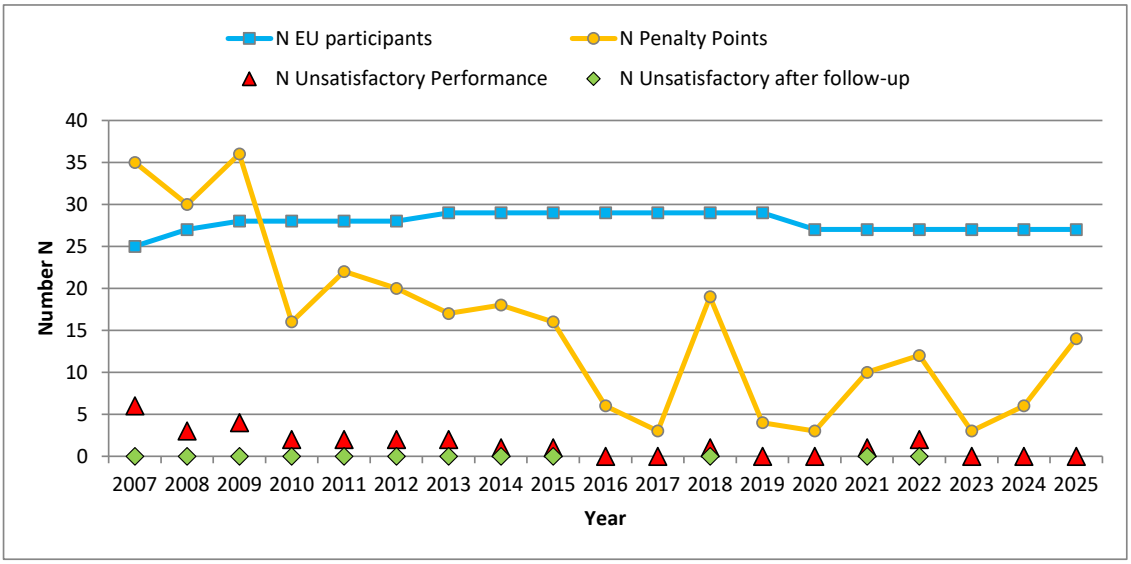


*Figure 4.6 Trends in the number of penalty points and instances of unsatisfactory performance among EU NRLs-*Salmonella *in EURL-*Salmonella *serotyping PTs since 2007*



5 Conclusions

5.1 Serotyping

- For the 32 fully evaluated participants, the overall results were as follows:
  - 97,5% of the O-antigen results were correct.
  - 98,8% of the H-antigen results were correct.
  - 96,9% of the serovar identifications were correct.
- All 27 EU NRLs and five non-EU NRLs met the defined criterion for good performance at the initial evaluation.
- One non-EU NRL-*Salmonella* with a limited accreditation scope met the defined criterion for good performance after receiving on-site practical training.

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List of acronyms and abbreviations

AMR	antimicrobial resistance
DG SANTE	Directorate-General for Health and Food Safety
EC	European Commission
EFSA	European Food Safety Authority
EFTA	European Free Trade Association
EU	European Union
EURL-*Salmonella*	European Union Reference Laboratory for *Salmonella*
IDS	Centre for Infectious Diseases Research, Diagnostics and Screening
IEC	International Electrotechnical Commission
ISO	International Organization for Standardization
MS	Member State
n.a.	not applicable
NRL-*Salmonella*	National Reference Laboratory for *Salmonella*
ONPG	o-nitrophenyl-β-D-galactopyranoside
PCR	polymerase chain reaction
PT	proficiency test
qPCR	quantitative polymerase chain reaction
REF	reference
RIVM	National Institute for Public Health and the Environment (Bilthoven, the Netherlands)
SSI	Statens Serum Institut (Copenhagen, Denmark)
TR	technical report
TSI	triple sugar iron agar
WGS	whole-genome sequencing

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

Performance

EURL-*Salmonella* PT Serotyping 2025 Number of penalty points: 0 Evaluation: Good performance

	Reference results				**Results* lab code:**			**1**
Strain	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
S1	6,7,14	m,t	[z57]	Oranienburg	7	m,t	-	Oranienburg
S2	6,8	z10	e,n,x	Hadar	6,8	z10	x	Hadar
S3	16	b	1,2	Hull	16	b	2	Hull
S4	1,9,12	g,m	-	Enteritidis	9	g,m	-	Enteritidis
S5[a)]	1,4,12,[27]	b	1,5	Limete	4	b	5	Limete
S6[a)]	6,8	c	e,n,x	Belem	6,8	c	x	Belem
S7[a)]	1,6,14,25	y	1,7	Madelia	14,25	y	7	Madelia
S8	6,7	k	e,n,x	Singapore	7	k	x	Singapore
S9	6,7,14	r	1,2	Virchow	7	r	2	Virchow
S10	3,{10}{15}{15,34}	e,h	1,6	Anatum	3	h	6	Anatum
S11[b)]	1,4,[5],12	i	-	1,4,[5],12:i:-	4	i	-	Typhimurium monophasic variant
S12	1,4,[5],12	f,g	[1,2]	Derby	4	f,g	-	Derby
S13[c)]	1,4,[5],12	b	1,2	Paratyphi B var. Java	4	b	2	Paratyphi B (Java)
S14	1,4,[5],12	r	1,2	Heidelberg	4	r	2	Heidelberg
S15	6,7,14	g,m,[p],s	[1,2,7]	Montevideo	7	g,m,s	-	Montevideo
S16	1,13,22	z	1,6	Poona	13,22	z	6	Poona
S17	1,4,[5],12	i	1,2	Typhimurium	4	i	2	Typhimurium
S18	1,9,12[Vi]	g,p	-	Dublin	9	g,p	-	Dublin
S19	6,7,14	r	1,5	Infantis	7	r	5	Infantis
S20	1,4,12	z	1,5	Kiambu	4	z	5	Kiambu
S21[d)]	52	a	1,5	Uithof	52	a	5	Uithof

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:i:-. c) L(+)-tartrate (= d-tartrate)-positive variant based on genomic sequences, often referred to as var. Java. d) Optional strain.
* Method used in this PT: classical serology.

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 30th EURL-*Salmonella* Workshop (20 May 2025, online), Strain S21 was an additional strain to the PT.
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 the 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 by strain (S1-S20) and laboratory

Laboratory codes 5 and 55 refer to the same participant. Codes 83 and 86 represent additional WGS submissions and are included for information only.

Note: The tables contain the raw data submitted by the participants. However, the electronic results form did not support special formatting such as italics or subscripts.

Details of the strains that caused serotyping problems, as indicated by the colour coding, are explained further in Appendix 4.

Results for the optional strain S21 are provided in Appendix 5.

Lab code:	S1	S2	S3	S4	S5	S6	S7	S8	S9
REF	**Oranienburg**	**Hadar**	**Hull**	**Enteritidis**	**Limete**	**Belem**	**Madelia**	**Singapore**	**Virchow**
1	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
2	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
3	Othmarscher[4)]	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
4	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
6	Othmarschen[4)]	Hadar	Hull	Enteritidis	Limete	Belem	Carrau[4)]	Singapore	Virchow
7	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Carrau[4)]	Singapore	Virchow
8	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
9	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
10	6,7:m,t:- [2)]	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
11	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
12	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
13	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Carrau[4)]	Singapore	Virchow
14	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
15	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Carrau[4)]	Singapore	Virchow

Lab code:	S1	S2	S3	S4	S5	S6	S7	S8	S9
REF	**Oranienburg**	**Hadar**	**Hull**	**Enteritidis**	**Limete**	**Belem**	**Madelia**	**Singapore**	**Virchow**
16	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
17	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
18	Oranienburg	Hadar	Hull	Enteritidis	Limete	Santiago[1)]	Madelia	Singapore	Virchow
19	Oranienburg	Hadar[1)]	Skansen[4)]	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
20	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
21	Othmarschen[4)]	Hadar	Skansen[4)]	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
22	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
23	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
24	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
25	Oranienburg	Hadar	Hull	Enteritidis	Limete	Santiago[4)]	Gatow[4)]	Escanaba[4)]	Virchow
26	Oranienburg	Hadar	Hull	Enteritidis	Limete	Santiago[1)]	Carrau[4)]	Singapore	Virchow
27	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
28	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem[1)]	Carrau[4)]	Singapore	Virchow
29	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
30[1)]	6,7:m,t:-[1)]	hadar	hull	enteritidis	mono[4)]	belem	14,25:y:1,7[1)]	singapore	virchow
31	Oranienburg	Hadar[1)]	Hull	Enteritidis	Limete	Belem[1)]	Carrau or Madelia[2)]	Singapore	Virchow
32	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem	Madelia	Singapore	Virchow
33	Oranienburg	Hadar	Hull	Enteritidis	Limete	Santiago[1)]	Carrau[4)]	Singapore	Virchow
55[1)]	7:m,t[2)]	Hadar	-[2)]	Enteritidis	4[2)]	8,6[2)]	-[2)]	7[2)]	Virchow
83[1)]	Oranienburg	Hadar	Hull	Enteritidis	Limete	Belem[1)]	Carrau[4)]	Singapore	Virchow
86[1)]	Oranienburg	Hadar[1)]	Hull	Enteritidis	Limete	Santiago or Belem[2)]	Carrau[4)]	Singapore	Virchow
X	3	0	2	0	1	1	8	1	0

[1)] In blue : remark (e.g. a spelling error).
[2)] In grey : not typable (e.g. antisera not available or a rough strain).
[3)] In yellow : partly correct; no penalty points for the serovar name.
[4)] In orange : incorrect; one penalty point for the serovar name.
[5)] In red : incorrect; four penalty points for the serovar name.
X = number of laboratories with deviations resulting in penalty points for each strain. Laboratory codes 55, 83 and 86 were excluded.

Lab code:	S10	S11	S12	S13	S14
REF	Anatum	4,12:i:-	Derby	Paratyphi B var. Java	Heidelberg
1	Anatum	Typhimurium monophasic variant	Derby	Paratyphi B (Java)	Heidelberg
2	Anatum	4,12:i:-	Derby	Paratyphi B	Heidelberg
3	Anatum	Typhimurium monofaza	Derby	Paratyphi B	Heidelberg
4	Anatum	4,12:i:-	Derby	Paratyphi B	Heidelberg
6	Anatum	4,5,12:i:-	Derby	Paratyphi B	Heidelberg
7	Anatum	4,12:i:-	Derby	Paratyphi B	Heidelberg
8	Anatum	Typhimurium, monophasic	Derby	Paratyphi B var. Java	Heidelberg
9	Anatum	I 4,[5],12:i:-	Derby	Paratyphi B var. Java	Heidelberg
10	Anatum	4,12:i:-	Derby	Paratyphi B	Heidelberg
11	Anatum	4:i:- monophasic Typhimurium	Derby	Paratyphi B	Heidelberg
12	Anatum	4,12:i:- (mST)	Derby	Paratyphi B var. Java	Heidelberg
13	Anatum	4,12:i:-	Derby	Paratyphi B var. Java	Heidelberg
14	Anatum	4,12:i:-	Derby	Paratyphi B var. Java	Heidelberg
15	Anatum	Monophasic Typhimurium	Derby	Paratyphi B var Tartrate +	Heidelberg
16	Anatum	4,12:i:-	Derby	Paratyphi B	Heidelberg
17	Anatum	4,5,12:i:-	Derby	Paratyphi B var. Java	Heidelberg
18	Anatum	Monophasic variant of Typhimurium (4,12:i:-)	Derby	Paratyphi B	Heidelberg
19	Anatum	4,12:i:-	Derby	Paratyphi B var. Java	Heidelberg
20	Anatum	4,12:i:-	Derby	Paratyphi B	Heidelberg
21	Anatum	4,12; i; -	Derby	Paratyphi B	Heidelberg
22	Anatum	4,12:i:-	Derby	Paratyphi B	Heidelberg
23	Anatum	4:i:-	Derby	Paratyphi B	Heidelberg
24	Anatum	4:i: -	Derby	Paratyphi B	Heidelberg
25	Anatum	Monophasic *Salmonella* Typhimurium	Derby	Paratyphi B	Heidelberg
26	Anatum	Typhimurium, monophasic (4,12:i:-)	Derby	Paratyphi B var Java	Heidelberg
27	Anatum	4,12:i:-	Derby	Paratyphi B var. Java	Heidelberg
28	Anatum	4,5,12:i:-	Derby	Paratyphi B dT+	Heidelberg

Lab code:	S10	S11	S12	S13	S14
REF	**Anatum**	**4,12:i:-**	**Derby**	**Paratyphi B var. Java**	**Heidelberg**
29	Anatum	4,12:i:-	Derby	Paratyphi B	Heidelberg
30[1)]	anatum	4,12:i:-	derby	paratypi B2[1)]	heidelberg
31	Anatum	4:i:-	Derby	Paratyphi B**	Heidelberg
32	Anatum	4:i:-	Derby	Java[1)]	Heidelberg
33	Anatum	4,12:i:-	Derby	Not tested[2)]	Heidelberg
55[1)]	3[2)]	Typhimurium monophasic	4,8,6:x[4)]	4[2)]	4[2)]
83[1)]	Anatum	Monophasic Typhimurium	Derby	Paratyphi B var Tartrate +	Heidelberg
86[1)]	Anatum	Monophasic Typhimurium	Derby	Java[1)]	Heidelberg
X	0	0	0	0	0

[1)] In blue : remark (e.g. a spelling error).
[2)] In grey : not typable (e.g. antisera not available or a rough strain).
[3)] In yellow : partly correct; no penalty points for the serovar name.
[4)] In orange : incorrect; one penalty point for the serovar name.
[5)] In red : incorrect; four penalty points for the serovar name.
** Laboratory 31 remark for strain S13: 'We did d-tartrate test on the strain to determine if the isolate was Paratyphi B var. Java, but the results were not as clear as we would have liked it to be. Due to the deadline, we did not have time to redo the test. In case this was a routine sample in the lab, we would have done the tartrate test again.'
X = number of laboratories with deviations resulting in penalty points for each strain. Laboratory codes 55, 83 and 86 were excluded.

Lab:	S15	S16	S17	S18	S19	S20
REF	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
1	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
2	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
3	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
4	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
6	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
7	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
8	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
9	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
10	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
11	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
12	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
13	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
14	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
15	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
16	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
17	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
18	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
19	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
20	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
21	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
22	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
23	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
24	Menston[4)]	Poona	Typhimurium	Dublin	Infantis	Kiambu
25	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
26	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
27	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
28	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
29	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu

Lab:	S15	S16	S17	S18	S19	S20
REF	**Montevideo**	**Poona**	**Typhimurium**	**Dublin**	**Infantis**	**Kiambu**
30[1)]	montevideo	poona	typhimurium	dublin	infantis	kiambu
31	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
32	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
33	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
55[1)]	Montevideo	-[2)]	Typhimurium	Dublin	Infantis	4[2)]
83[1)]	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
86[1)]	Montevideo	Poona	Typhimurium	Dublin	Infantis	Kiambu
X	1	0	0	0	0	0

[1)] In blue : remark (e.g. a spelling error).
[2)] In grey : not typable (e.g. antisera not available or a rough strain).
[3)] In yellow : partly correct; no penalty points for the serovar name.
[4)] In orange : incorrect; one penalty point for the serovar name.
[5)] In red : incorrect; four penalty points for the serovar name.
X = number of laboratories with deviations resulting in penalty points for each strain. Laboratory codes 55, 83 and 86 were excluded.

Appendix 3 Details of confirmation tests

The confirmation tests used by participants are shown in the table below, except in the cases described below.

Participants with laboratory codes 3, 6, 25 and 55, as well as the additional WGS submissions under codes 83 and 86, reported that they had not used confirmation tests.

The participant with laboratory code 21 reported using biochemical confirmation but did not specify which test or tests were used.

Laboratory code 22 used malonate for strain S15 only.

Laboratory code 30 used PCR only to confirm the monophasic variant of *S.* Typhimurium in strain S11 and *S.* Typhimurium in strain S17.

An empty cell in the table indicates that the laboratory reported no confirmation test for the relevant strain.
Table notes:

a) Routine method: WGS.
b) Standard set: malonate, ONPG, mannitol, sorbose phosphate, sucrose, urea, TSI and lysine decarboxylase.
c) 'We did not have O-25 and O-24,25 or control strains in house for agglutination.'

PCR[1)] PCR to confirm *S*. Typhimurium and its monophasic variant.
PCR[2)] PCR for subspecies identification (Lee et al., 2009).

Lab code	**Oranienburg (S1)** 6,7,14:m,t:[z57]	**Hadar (S2)** 6,8:z10:e,n,x	**Hull (S3)** 16:b:1,2	**Enteritidis (S4)** 1,9,12:g,m:-
1	Dulcitol, malonate, ONPG, sorbitol			
2	Dulcitol, malonate, ONPG, salicin			
4	Dulcitol, malonate	Dulcitol, malonate	Dulcitol, malonate	Dulcitol, malonate
7	PCR[1)]	PCR[1)]	PCR[1)]	PCR[1)]
8	Dulcitol, malonate, salicin			
9[a)]		Serological agglutination test		
10	Malonate			
11	API 20E			
12	PCR[2)]			
13				PCR
14	ONPG, lysine decarboxylase, Tartrate	ONPG, lysine decarboxylase	ONPG, lysine decarboxylase	ONPG, lysine decarboxylase, PCR
15	Malonate			
16	Dulcitol, malonate, ONPG, salicin, sorbitol, lactose			PCR to confirm *S*. Enteritidis
17	Enterotest 24N			
18	Malonate	Malonate	Malonate	
19	Dulcitol, malonate, ONPG	Dulcitol, malonate, ONPG	Dulcitol, malonate, ONPG	Dulcitol, malonate, ONPG
20	Dulcitol, malonate, d-tartrate			
23	Dulcitol, malonate, ONPG, salicin, sorbitol, lactose			
24	Dulcitol, malonate, salicin, sorbitol, Galacturonate + Mucate			
26	Malonate			
27	Malonate, ONPG, sorbitol			
28	Inosite, d-tartrate	Inosite, d-tartrate	Inosite, d-tartrate	Glucose

Lab code	**Oranienburg (S1)** 6,7,14:m,t:[z57]	**Hadar (S2)** 6,8:z10:e,n,x	**Hull (S3)** 16:b:1,2	**Enteritidis (S4)** 1,9,12:g,m:-
29	Gelatinase (neg.) and tartrate (pos.)			
31[a)]	Malonate	Agglutination with O-6 and O-8		
32	Standard set[b)]	Standard set[b)]	Standard set[b)]	Standard set[b)]
33	Dulcitol, Malonate, ONPG, Sorbitol			

Lab code	**Limete (S5)** 1,4,12,[27]:b:1,5	**Belem (S6)** 6,8:c:e,n,x	**Madelia (S7)** 1,6,14,25:y:1,7	**Singapore (S8)** 6,7:k:e,n,x
1	Dulcitol, Malonate, ONPG, Sorbitol			
2	Dulcitol, Malonate, ONPG, Salicine			
4	Dulcitol, Malonate	Dulcitol, Malonate	Dulcitol, Malonate	Dulcitol, Malonate
7	PCR[1)]	PCR[1)]	PCR[1)]	PCR[1)]
8	Dulcitol, Malonate, Salicine			
9[a)]		Serological agglutination	Serological agglutination	
10				
11	API 20E			
12	PCR[2)]			
13			WGS	
14	ONPG, lysine decarboxylase, Tartrate	ONPG, lysine decarboxylase	ONPG, lysine decarboxylase	ONPG, lysine decarboxylase
15	Malonate			
16	Dulcitol, malonate, ONPG, salicin, sorbitol, lactose			
17				
18	Malonate	Malonate	Malonate	Malonate
19	Dulcitol, malonate, ONPG	Dulcitol, malonate, ONPG	Dulcitol, malonate, ONPG	Dulcitol, malonate, ONPG
20	Dulcitol, malonate, d-tartrate			
23				
24	Dulcitol, malonate, salicin, sorbitol, galacturonate and mucate			
26	Malonate			
27	Malonate, ONPG, sorbitol			
28	Inosite, d-tartrate	Inosite, d-tartrate	Inosite, d-tartrate	Inosite, d-tartrate
29	Gelatinase (neg.) and tartrate (pos.)			

Lab code	**Limete (S5)** 1,4,12,[27]:b:1,5	**Belem (S6)** 6,8:c:e,n,x	**Madelia (S7)** 1,6,14,25:y:1,7	**Singapore (S8)** 6,7:k:e,n,x
31[a)]	Malonate	Agglutination O-6 & O-8	See note[c)]	
32	Standard set[b)]	Standard set[b)]	Standard set[b)]	Standard set[b)]
33	Dulcitol, malonate, ONPG, sorbitol			

Lab code	**Virchow (S9)** 6,7,14:r:1,2	**Anatum (S10)** 3,{10}{15}{15,34}:e,h:1,6	**4,12:i:- (S11)** 1,4,[5],12:i:-	**Derby (S12)** 1,4,[5],12:f,g:[1,2]
1			PCR[1)]	
2			PCR[1)]	
4	Dulcitol, malonate	Dulcitol, malonate	Dulcitol, malonate	Dulcitol, malonate
7	PCR[1)]	PCR[1)]	PCR[1)]	PCR[1)]
8			PCR[1)], Dulcitol, malonate, salicin	
9[a)]				
10			PCR[1)]	
11				
12			PCR[1)]	
13			PCR[1)]	
14	ONPG, lysine decarboxylase	ONPG, lysine decarboxylase	ONPG, lysine decarboxylase, PCR[1)]	ONPG, lysine decarboxylase
15			PCR[1)]	
16			PCR[1)]	
17			PCR[1)]	
18		Malonate		
19	Dulcitol, malonate, ONPG	Dulcitol, malonate, ONPG	Dulcitol, malonate, ONPG	Dulcitol, malonate, ONPG
20			PCR[1)]	
23			PCR[1)]	
24			PCR[1)]	
26			PCR[1)]	
27			PCR[1)]	
28	Inosite, d-tartrate	Inosite, d-tartrate	Inosite, d-tartrate	Inosite, d-tartrate
29				
31[a)]				
32	Standard set[b)]	Standard set[b)]	PCR[1)], Standard set[b)]	Standard set[b)]
33				

Lab code	**Paratyphi B var. Java (S13)** 1,4,[5],12:b:1,2	**Heidelberg (S14)** 1,4,[5],12:r:1,2	**Montevideo (S15)** 6,7,14:g,m,[p],s:[1,2,7]
1	d-tartrate		
2	PCR for d-tartrate fermentation		
4	Dulcitol, malonate	Dulcitol, malonate	Dulcitol, malonate
7	PCR[1)]	PCR[1)]	PCR[1)]
8	Tartrate PCR		
9[a)]			
10			
11			
12	PCR to confirm *S.* Paratyphi B/var. Java		
13	WGS		
14	ONPG, lysine decarboxylase, Tartrate	ONPG, lysine decarboxylase	ONPG, lysine decarboxylase
15	Tartrate		Malonate
16	Tartrate		Dulcitol, malonate, ONPG, salicin, sorbitol, lactose
17	Enterotest 24N		
18	Malonate		
19	Dulcitol, malonate, ONPG	Dulcitol, malonate, ONPG	Dulcitol, malonate, ONPG
20	Dulcitol, malonate, d-tartrate		
23			
24			
26	WGS: in silico PCR[4)] for the d-tartrate gene		
27			
28	Inosite, d-tartrate, PCR	Inosite, d-tartrate	Malonate, Inosite, d-tartrate
29	Tartrate (neg.)		
31[a)]	d-tartrate		
32	Standard set[b)] and WGS to confirm the result	Standard set[b)]	Standard set[b)]
33			

Lab code	**Poona (S16)** 1,13,22:z:1,6	**Typhimurium (S17)** 1,4,[5],12:i:1,2	**Dublin (S18)** 1,9,12[Vi]:g,p:-	**Infantis (S19)** 6,7,14:r:1,5
1				
2				
4	Dulcitol, malonate	Dulcitol, malonate	Dulcitol, malonate	Dulcitol, malonate
7	PCR[1)]	PCR[1)]	PCR[1)]	PCR[1)] and qPCR
8				
9[a)]				
10				
11				
12				
13				
14	ONPG, lysine decarboxylase	ONPG, lysine decarboxylase, PCR[1)]	ONPG, lysine decarboxylase	ONPG, lysine decarboxylase
15				
16		PCR[1)]		
17		PCR[1)]		
18	Malonate			
19	Dulcitol, malonate, ONPG	Dulcitol, malonate, ONPG	Dulcitol, malonate, ONPG	Dulcitol, malonate, ONPG
20				
23		PCR[1)]		
24				
26				
27				
28	Inosite, d-tartrate	Inosite, d-tartrate	Inosite, d-tartrate	Inosite, d-tartrate
29				
31[a)]	Agglutination O-22 & O-23			
32	Standard set[b)]	Standard set[b)]	Standard set[b)]	Standard set[b)]
33				

Lab code	**Kiambu (S20)** 1,4,12:z:1,5	**Uithof (S21)** 52:a:1,5
1	Dulcitol, malonate, ONPG, sorbitol	Dulcitol, malonate, ONPG, sorbitol
2		
4	Dulcitol, malonate	Dulcitol, malonate
7	PCR[1)]	PCR[1)] Dulcitol, malonate
8	Dulcitol, malonate, salicin	Dulcitol, malonate, salicin
9[a)]		
10		
11	API 20E	
12		
13		
14	ONPG, lysine decarboxylase, Tartrate	ONPG, lysine decarboxylase
15	Malonate	
16	Dulcitol, malonate, ONPG, salicin, sorbitol, maltose, rhamnose, lactose	Dulcitol, malonate, ONPG, salicin, sorbitol, maltose, rhamnose, lactose
17		Enterotest 24N
18	Malonate	Malonate, EPC (biochemical test)
19	Dulcitol, malonate, ONPG	Dulcitol, malonate, ONPG
20	Dulcitol, malonate, d-tartrate	
23	Dulcitol, malonate, ONPG, salicin, sorbitol, lactose	Dulcitol, malonate, ONPG, salicin, sorbitol, lactose
24	Galacturonate	
26	Malonate	
27		
28	Inosite, d-tartrate	Inosite, d-tartrate
29		
31[a)]	Malonate	
32	Standard set[b)]	Standard set[b)]
33	Dulcitol, malonate, ONPG, sorbitol	Dulcitol, malonate, ONPG, sorbitol

Appendix 4 Details of serotyping problems by strain

Note: Laboratory codes 5 and 55 were excluded.

The text below the table explains the colour coding.

Strain code	Lab code	O-antigens	H-antigens		Serovar
			(phase 1)	(phase 2)	
S1	**REF**	**6,7,14**	**m,t**	**[z57]**	**Oranienburg**
S1	3	6,7	g,m,t	-	Othmarscher
S1	6	6,7	g,m	-	Othmarschen
S1	10[a)]	6,7	m,t	-	6,7:m,t:-
S1	21	6,7	g,m,t	-	Othmarschen
S1	30	6,7	m,t	-	6,7:m,t:-
S2	**REF**	**6,8**	**z10**	**e,n,x**	**Hadar**
S2	19	8	z10	e,n,x	Hadar
S2	31	8	z10	e,n,x	Hadar
S2	86	8	z10	e,n,x	Hadar
S3	**REF**	**16**	**b**	**1,2**	**Hull**
S3	13	16	b	1,2,5	Hull
S3	19	6,8	b	1,2	Skansen
S3	21	6,8	b	1,2	Skansen
S5	**REF**	**1,4,12,[27]**	**b**	**1,5**	**Limete**
S5	30	4,12	l,w	5	mono
S6	**REF**	**6,8**	**c**	**e,n,x**	**Belem**
S6	18	8	c	e,n,x	Santiago
S6	25	8,20	c	e,n,x	Santiago
S6	26	8	c	e,n,x	Santiago
S6	28	8	c	x	Belem
S6	31	8	c	e,n,x	Belem
S6	33	8	c	e,n,x	Santiago
S6	83	8	c	e,n,x	Belem
S6	86	8	c	e,n,x	Santiago or Belem
S7	**REF**	**1,6,14,25**	**y**	**1,7**	**Madelia**
S7	6	6,14	y	1,7	Carrau
S7	7	14,24	y	1,7	Carrau
S7	13	14	y	1,7	Carrau
S7	15	6,14	y	1,7	Carrau
S7	25	6,7	y	1,7	Gatow
S7	26[b)]	6,14	y	1,7	Carrau
S7	28	14,24	y	1,7	Carrau
S7	30	14,25	y	1,7	14,25:y:1,7
S7	31[c)]	6,14	y	1,7	Carrau or Madelia
S7	33	6,14,24	y	1,7	Carrau
S7	83	6,14	y	1,7	Carrau
S7	86	6,14	y	1,7	Carrau
S8	**REF**	**6,7**	**k**	**e,n,x**	**Singapore**

Strain code	Lab code	O-antigens	H-antigens		Serovar
			(phase 1)	(phase 2)	
S8	25	6,7	k	e,n,z15	Escanaba
S13	**REF**	**1,4,[5],12**	**b**	**1,2**	**Paratyphi B var. Java**
S13	30	4,5,12	b	1,2	paratypi B2
S13	32	4	b	1,2	Java
S13	33	-	-	-	Not tested
S13	86	4	b	1,2	Java
S15	**REF**	**6,7,14**	**g,m,[p],s**	**[1,2,7]**	**Montevideo**
S15	24	7	g,s	-	Menston
S16	**REF**	**1,13,22**	**z**	**1,6**	**Poona**
S16	9	13	z	1,6	Poona
S16	31	13	z	1,6	Poona
S16	86	13	z	1,6	Poona

a) S1: 'We can't write the serovar name, because we haven't z57 and z64 sera.'
b) S7: 'Not straight forward O-antigen (O25?).'
c) S7: 'We did not have O-25 and O-24,25 or control strains in house. Therefore, we could not distinguish between Carrau and Madelia.'

Colour coding
In blue : remark (e.g. a spelling error).
In grey : not typable (e.g. antisera not available or a rough strain).
In yellow: partly correct; no penalty points for the serovar name.
In orange: incorrect; one penalty point for the serovar name.

Explanation of the colour-coded results

Strain S1 reference result: Oranienburg 6,7,14:m,t:[z57]
Laboratories 3 and 21 reported g,m,t rather than m,t (partly correct) and Othmarschen (incorrect, one penalty point).

Laboratory 6 reported g,m rather than m,t (partly correct) and Othmarschen (incorrect, one penalty point).

Laboratory 10 reported the formula 6,7:m,t:- rather than Oranienburg; this was evaluated as not typable because of comment a) by this laboratory.

Laboratory 30 reported the formula 6,7:m,t:- rather than Oranienbrug; this was recorded as a remark.

Strain S2 reference result: Hadar 6,8:z10:e,n,x
Laboratories 19, 31 and 86 reported 8 rather than 6,8 (partly correct) but still reported Hadar as the serovar name (remark).

Under the PT protocol, a strain with the antigenic formula 8:z10:e,n,x should have been reported as 'Istanbul'. A strain reported as 'Hadar' would be expected to have the O-antigen formula 6,8. The laboratories were therefore asked whether they had performed a separate check for O:6.

Strain S3 reference result: Hull 16:b:1,2
Laboratory 13 reported 1,2,5 rather than 1,2 (partly correct).

Laboratories 19 and 21 reported 6,8 rather than 16 (incorrect) and Skansen (incorrect, one penalty point).

Strain S5 reference result: Limete 1,4,12,[27]:b:1,5
Laboratory 30 reported l,w rather than b (incorrect) and mono (incorrect, one penalty point).

Strain S6 reference result: Belem 6,8:c:e,n,x
Laboratories 28, 31 and 83 reported 8 rather than 6,8 (partly correct) but still reported Belem as the serovar name (remark).
Under the PT protocol, a strain with the antigenic formula 8:c:e,n,x should have been reported as 'Santiago'. A strain reported as 'Belem' would be expected to have the O-antigen formula 6,8. The laboratories were therefore asked whether they had performed a separate check for O:6.

Laboratories 18, 26 and 33 reported 8 rather than 6,8 (remark) and Santiago rather than Belem (remark).
Under the PT protocol, a strain with the antigenic formula 8:c:e,n,x should indeed have been reported as 'Santiago'. However, the laboratories were asked whether they had performed a separate check for O:6.

Laboratory 25 reported 8,20 rather than 6,8 (partly correct) and Santiago rather than Belem (incorrect, one penalty point).

Laboratory 86 reported 8 rather than 6,8 and 'Santiago or Belem' as the serovar name; both results were evaluated as not typable.

Strain S7 reference result: Madelia 1,6,14,25:y:1,7
Laboratory 25 reported 6,7 rather than 1,6,14,25 (partly correct) and Gatow rather than Madelia (incorrect, one penalty point).

Laboratories 6, 15, 26, 83 and 86 reported 6,14 rather than 1,6,14,25 (partly correct) and Carrau rather than Madelia (incorrect, one penalty point).

Laboratory 33 reported 6,14,24 rather than 1,6,14,25 (partly correct) and Carrau rather than Madelia (incorrect, one penalty point).

Laboratories 7 and 28 reported 14,24 rather than 1,6,14,25 (partly correct) and Carrau rather than Madelia (incorrect, one penalty point).

Laboratory 13 reported 14 rather than 1,6,14,25 (partly correct) and Carrau rather than Madelia (incorrect, one penalty point).

Laboratory 30 reported the antigenic formula 14,25:y:1,7 rather than Madelia; this was recorded as a remark.

Laboratory 31 reported 6,14 rather than 1,6,14,25 and 'Carrau or Madelia' as the serovar name; both results were evaluated as not typable because of comment c) by this laboratory.

Strain S8 reference result: Singapore 6,7:k:e,n,x
Laboratory 25 reported e,n,z15 rather than e,n,x (partly correct) and Escanaba (incorrect, one penalty point).

Strain S13 reference result: Paratyphi B var. Java 1,4,[5],12:b:1,2
Laboratories 32 and 86 reported 'Java' (remark).

Laboratory 30 reported ‘paratypi B2’ (remark).

Laboratory 33 reported ‘Not tested’ (not typable).

Strain S15 reference result: Montevideo 6,7,<u>14</u>:g,m,[p],s:[1,2,7]
Laboratory 24 reported g,s rather than g,m,[p],s (partly correct) and Menston (incorrect, one penalty point).

Strain S16 reference result: Poona <u>1</u>,13,22:z:1,6
Laboratories 9, 31 and 86 reported 13 rather than <u>1</u>,13,22 (partly correct) but correctly reported Poona as the serovar name.

Appendix 5 Serotyping results for strain S21

Lab code	O-antigens	H-antigens		Serovar
		(phase 1)	(phase 2)	
REF	52	a	1,5	Uithof
1	52	a	5	Uithof
2	52	a	1,5	Uithof
3	52	a	1,5	Uithof
4	52	a	1,5	Uithof
6	52	a	1,5	Uithof
7	52	a	1,5	Uithof
8[a)]	-[2)]	a	5	*S.* ssp.I (-:a:5)[2)]
9	52	a	1,5	Uithof
10	52	a	1,5	Uithof
11	52	a	1,5	Uithoff[1)]
12	52	a	1,5	Uithof
13	52	a	1,5	Uithof
14	52	a	1,5	Uithof
15	52	a	1,5	Uithof
16	52	a	5	Uithof
17	52	a	1,5	Uithof
18	52	a	1,5	Uithof
19	52	a	1,5	Uithof
20	52	a	1,5	Uithof
21	52	a	1,5	Uithof
22	52	a	1,5	Uithof
23	52	a	1,5	Uithof
24	52	a	5	Uithof
25	48[1)]	a	1,5,7[1)]	Hisingen[1)]
26	52	a	1,5	Uithof
27	52	a	1,5	Uithof
28	52	a	1,5	Uithof
29	OME+[2)]			-[2)]
30	52	a	1,5	uithof[1)]
31	52	a	1,5	Uithof
32	52	a	1,5	Uithof
33	52	a	1,5	Uithof
55	-[2)]	-[2)]	-[2)]	-[2)]
83	52	a	1,5	Uithof
86	52	a	1,5	Uithof

a) Comment Laboratory 8: 'The strain was not reactive by any of our O polyvalent antisera. We made the WGS and sent the sequences to SeqSero and received result Uithof (52:a:1,5). We do not have the monovalent O52, so we could not receive the result by classical serology and would use also in routine the WGS and SeqSero if needed.'

[1)] In blue: remark indicating a deviation in the results for optional strain S21.

[2)] In grey: not typable (e.g. antisera not available or a rough strain).

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