Interim Summary Report

EURL-*Salmonella* Proficiency Test Serotyping 2024

Version 2

W.F. Jacobs-Reitsma, A.J. Verbruggen, and K.A. Mooijman
RIVM, Bilthoven, The Netherlands

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This document, Z&O letter report 2025-0038 dated 30 April 2025, cancels and replaces the first version of the Interim Summary Report EURL-*Salmonella* Proficiency Test Serotyping 2024 in Z&O letter report 2025-0023 dated 28 February 2025.

The antigenic formula for Strain S14 (Kisarawe) in Table 1 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.

This investigation was co-funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the granting authority European Health and Digital Executive Agency (HaDEA). Neither the European Union nor the granting authority can be held responsible for them.

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1. Introduction

This interim summary report describes the overall results on the serotyping part of the Proficiency Test (PT) on typing of *Salmonella*, organised by the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*, Bilthoven, the Netherlands) in November 2024. Results regarding the part on Cluster Analysis will be reported separately.
A total of 33 laboratories participated in the PT 2024. These included 27 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella)* in the 27 EU Member States and 6 NRLs from third countries (EU candidate Member States, members of the European Free Trade Association (EFTA), and the United Kingdom).
The main objective of this PT was to evaluate the performance of the NRLs in serotyping *Salmonella*.

2. Materials and Methods

2.1. *Salmonella* strains for serotyping

A total of 20 *Salmonella* strains (coded S1 - S20) had to be serotyped by the participants. 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.
The *Salmonella* strains used for the PT on serotyping originated from the National *Salmonella* Centre collection in the Netherlands. The strains were verified by the Centre before distribution. 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) are shown in Table 1. However, participants were asked to report only the results as detected and on which the identification of serovar names was based. Nine strains (Table 1) represented serovars included in the EURL-*Salmonella* serotyping PTs for the first time.

2.2 Laboratory codes

Each participant was randomly assigned a laboratory code 1 - 33.

Table 1. Antigenic formulas of the 21 Salmonella *strains according to the White-Kauffmann-LeMinor scheme used in the EURL-*Salmonella *PT Serotyping 2024*

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

[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).

2.3 Transport

The parcels containing the strains for typing were sent by the EURL-*Salmonella* on Monday 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.

2.4 Evaluation of the serotyping results

The evaluation of deviating serotyping results is described in Table 2.

Table 2. Evaluation of deviating serotyping results

Results	Evaluation
Auto-agglutination or, Incomplete set of antisera (outside range of antisera)	Not typable
Partly typable due to incomplete set of antisera or, Part of the formula (for the name of the serovar) or, No name serovar	Partly correct
Wrong serovar or, Mixed sera formula	Incorrect

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 sometimes 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 4 penalty points.
All EU Member State NRLs not meeting the criterion of good performance (scoring four penalty points or more) have to participate in a follow-up study, in which 10 additional strains have to be serotyped.

3. Results

3.1 Serotyping results of the NRLs-*Salmonella*

3.1.1. General comments on this year's evaluation

As decided at the 29th EURL-*Salmonella* Workshop (Mooijman, 2024), Strain S21 was an additional strain to the study. Testing of this strain was optional and results were not included in the evaluation.
Non-EU MS Laboratory 33 was participating in the EURL-*Salmonella* PT Serotyping for the first time and this laboratory clearly did not yet have all materials and expertise to be able to perform properly. Therefore, Laboratory 33 is excluded from the general overview on this PT's evaluation of results (Figures 1-4, Table 4, Annex B).

3.1.2. Serotyping results per laboratory

The evaluations of the type of errors for O- and H-antigens and for the identification of the strains are shown in Figures 1, 2, and 3.
The percentages of correct results per laboratory are shown in Figure 4.
The number of penalty points was determined for each NRL using the guidelines described in section 2.4. Table 3 shows the number of penalty points for each NRL and indicates whether the level of good performance was achieved (yes or no).

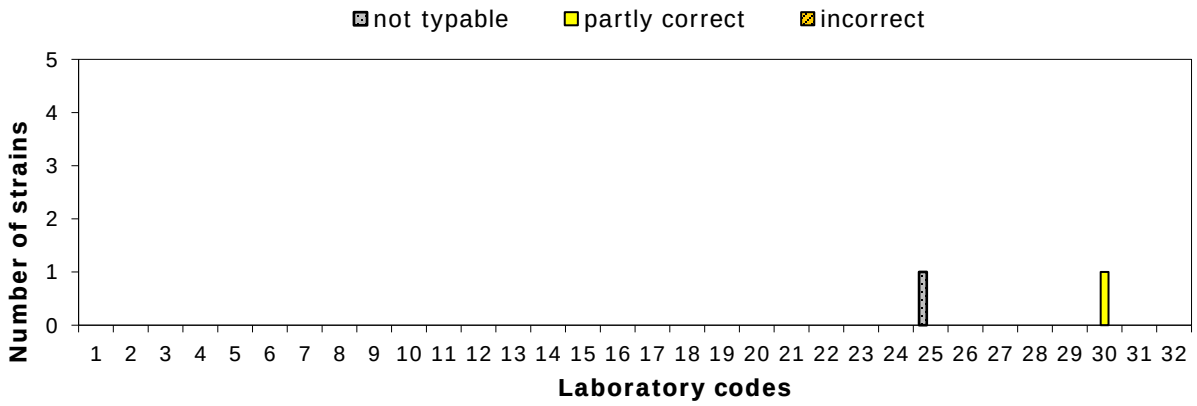

Figure 1. Evaluation of the type of errors for O-antigens, per participant

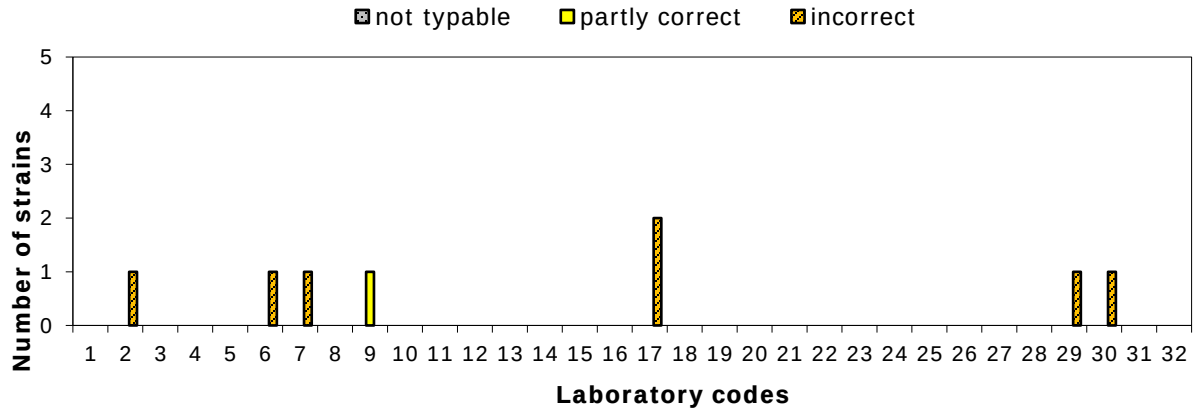

Figure 2. Evaluation of the type of errors for H-antigens, per participant

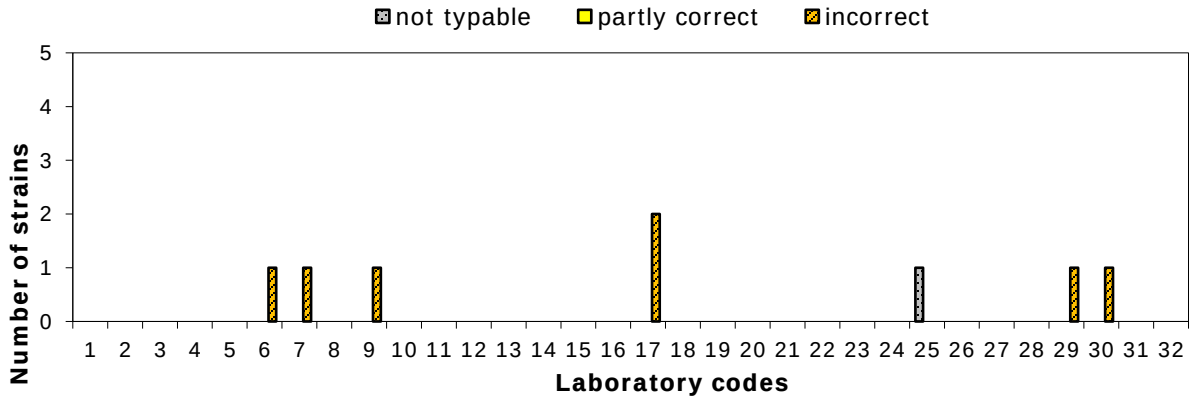

Figure 3. Evaluation of the type of errors in the identification of the serovar names, per participant

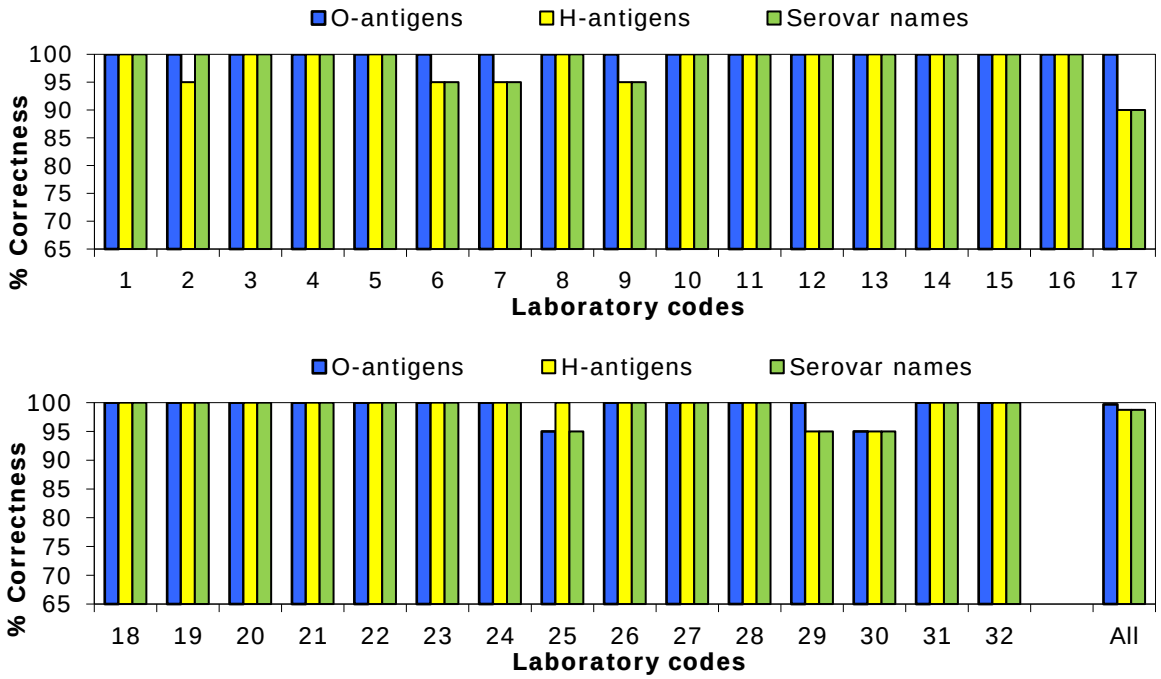

Figure 4. Percentages of correct serotyping results, per participant

Table 3. Evaluation of serotyping results per NRL

Lab code	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

Lab code	Penalty points	Good performance
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

Overall, the participants' performance in the PT Serotyping 2024 was very good. Table 4 shows the percentages of correctly typed strains, both for EU-MS participants only and for all participants. 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 and expertise to be able to perform properly (also see Annex A). Laboratory 33 will be contacted for further information exchange and a dedicated training may be organised before a potential follow-up study.

Table 4. 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*
N participants	27	32
N strains evaluated 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.

3.1.3. Serotyping results per strain

Annex A 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). Annex A also shows 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.

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

Annex C 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.

List of abbreviations

EFTA	European Free Trade Association
EU	European Union
EURL-*Salmonella*	European Union Reference Laboratory for *Salmonella*
NRLs-*Salmonella*	National Reference Laboratories for *Salmonella*
REF	Reference
RIVM	National Institute for Public Health and the Environment

References

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Contacts

Wilma Jacobs-Reitsma: wilma.jacobs@rivm.nl
Anjo Verbruggen: anjo.verbruggen@rivm.nl
Kirsten Mooijman: kirsten.mooijman@rivm.nl

EURL-*Salmonella* website: www.eurlsalmonella.eu

National Institute for Public Health and the Environment (RIVM)
Centre for Zoonosis and Environmental microbiology (Z&O/ internal mailbox 63)
Antonie van Leeuwenhoeklaan 9
P.O. Box 1, 3720 BA Bilthoven, The Netherlands

Annex A. Serotyping results per strain (S1 – S20) and laboratory (1 - 33)

Lab: REF	S1 Enteritidis	S2 Menston	S3 Infantis	S4 Okatie	S5 Moroto	S6 Molade	S7 Bolton	S8 Typhimurium	S9 Carmel	S10 Lagos
1	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
2	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
3	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
4	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
5	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
6	Enteritidis	Menston	Infantis	Okatie	Moroto	Bazenheid	Bolton	Typhimurium	Carmel	Lagos
7	enteritidis	6,7:g,s,t:-	infantis	13,23:g,s,t,:-	moroto	molade	bolton	typhimurium	carmel	lagos
8	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
9	Enteritidis	Menston	Infantis	Agbeni	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
10	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
11	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
12	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
13	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
14	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
15	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
16	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
17	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Lamberhurst	Typhimurium	Carmel	Lagos
18	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
19	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
20	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
21	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
22	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
23	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
24	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
25	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
26	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
27	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
28	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
29	Enteritidis	Menston	Infantis	Okatie	Moroto	Remiremont	Bolton	Typhimurium	Carmel	Lagos
30	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
31	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
32	Enteritidis	Menston	Infantis	Okatie	Moroto	Molade	Bolton	Typhimurium	Carmel	Lagos
33	Enteritidis	C1	Virchow	C2	C2	B	E	Typhimurium	-	C2
X	0	0	0	1	0	2	1	0	0	0

S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	Lab:
4,5,12:i:-	**Ridge**	**Oslo**	**Kisarawe**	**Virchow**	**Give**	**Charity**	**Southbank**	**Hadar**	**Indiana**	**REF**
Typhimurium, monophasic	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	1
4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	2
I 4:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	3
Monophasic Typhimurium	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	4
4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	5
4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	6
4,5,12:i:-	ridge	oslo	kisarawe	virchow	give	fischerkietz	3,10:m,t:-	hadar	indiana	7
4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	8
Typhimurium monofaza	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	9
4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	10
4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	11
1,4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	12
Typhimurium, monophasic	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	13
Monophasic Variant S. Typhimurium	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	14
4,[5],12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	15
monophasic Typhimurium	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	16
4,5,12:i:-	Ridge	Oslo	Mannheim	Virchow	Give	Charity	Southbank	Hadar	Indiana	17
4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	18
4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	19
Monophasic variant (4,5,12:i:-)	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	20
4,[5],12:i:- (monophasic Typhimurium)	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	21
4,5,12:i:- (mST)	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	22
4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	23
4,5,12: i: -	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	24
4:i:-	Ridge	Olso	Kisarawe	Virchow	Give	Lindern/Charity	Southbank	Hadar	Indiana	25
monophasic Typhimurium	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	26
4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	27
4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	28
4,5:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	29
Monophasic Salmonella Typhimurium	Ridge	Oslo	Kisarawe	Virchow	Give	Manhattan	Southbank	Hadar	Indiana	30
4,5,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	31
4,12:i:-	Ridge	Oslo	Kisarawe	Virchow	Give	Charity	Southbank	Hadar	Indiana	32
C2	D	C1	B	Typhimurium	C1	Hadar	Infantis	B	Typhimurium	33
0	0	0	1	0	0	2	0	0	0	**X**

remark (e.g. spelling error)
not typable (e.g. antisera not available, rough strain)
partly correct, in the naming: no penalty points
incorrect; in the naming: 1 penalty point
incorrect; in the naming: 4 penalty points

X = number of deviating laboratories (by penalty points) per strain, *Laboratory 33 excluded*.

Results for strain S21 are given in Annex C.

Annex B. Details per strain that caused problems in serotyping

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

NOTE: Laboratory 33 excluded.

*Remark Lab 25 regarding S17: we did not have O25 and O24,25 or control strains in house. Therefore we could not distinguish between Lindern and Charity.

Reference strain
remark (e.g. spelling error)
not typable (e.g. antisera not available, rough strain)
partly correct; in the naming: no penalty points
incorrect; in the naming: 1 penalty point
incorrect; in the naming: 4 penalty points

Annex C. Details on serotyping results strain S21

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

Reference strain
remark, deviations in the results on optional strain S21
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’.