National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Results EURL-*Salmonella* PT Typing 2022

serotyping & cluster analysis

Wilma Jacobs-Reitsma
EURL-*Salmonella*
With back-up from our
RIVM typing experts

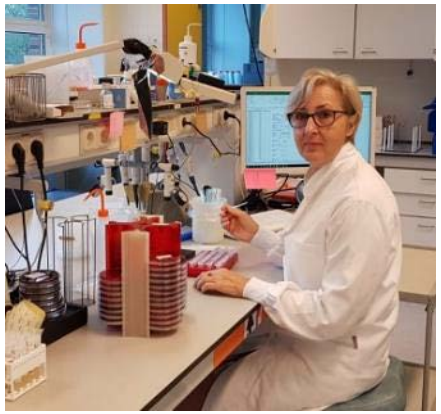
EURL Salmonella

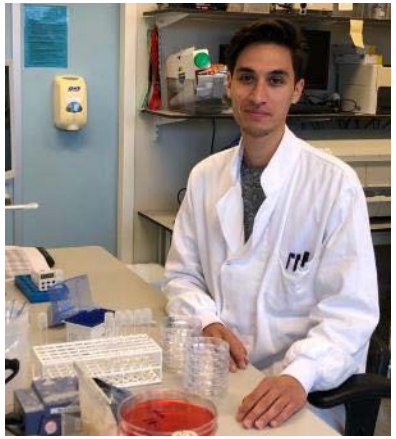
Co-funded by
the European Union

RIVM typing experts

- Serotyping:
  - Anjo Verbruggen
- MLVA:
  - Kim van der Zwaluw
- Cluster Analysis:
  - Maaike van den Beld
  - Angela van Hoek
  - Robin Diddens
  - The IDS-bioinformatics team
- Sample preparations:
  - Wendy van Overbeek

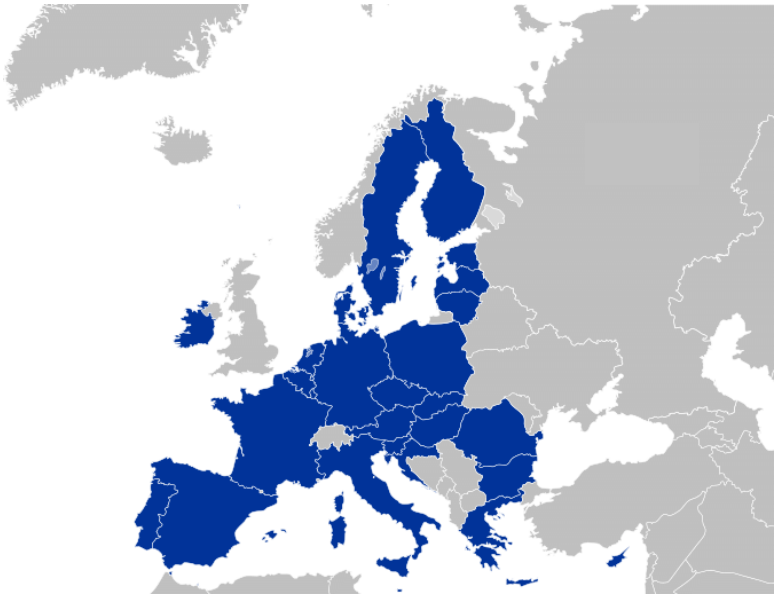
- EURL-*Salmonella* contact typing studies:
  - Wilma Jacobs





Participants PT Typing 2022

- National Reference Laboratories for *Salmonella* from all **27 EU** member states
- Kosovo, Moldova, Türkiye (EU candidate countries)
- Iceland, Norway, Switzerland (EFTA countries)
- United Kingdom (third country)



- Serotyping
  - **34** participants
- Optional Cluster Analysis
  - **20** participants

Part on Serotyping (27th PT)

- 20 different serovars of *Salmonella enterica* subsp. *enterica*
- 1 additional strain of an uncommon type (optional)
- Serotyping and reporting in accordance with the White-Kauffmann-le Minor scheme (2007) and the published supplements
  - Supplement 2003–2007 (no. 47) Guibourdenche et al., 2010
  - Supplement 2008-2010 (no. 48) Issenhuth-Jeanjean et al., 2014

- Laboratories have to report only those results, on which the identification of serovar names is based.
  - Examples of preferred reporting:

O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar name
9,12	g,m	-	Enteritidis
4,12	i	2	Typhimurium
4,5,12	i	-	4,5,12:i:-
6,7	-	1,5	6,7:-:1,5
42	g,t	-	42:g,t:-

Salmonella strains (1)

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
S4	1,4,[5],12	f,g,s	[1,2]	Agona
S13*	1,4,[5],12	g,m,s	[1,2]	Hato
S2	**1,4,[5],12**	**i**	**-**	**1,4,[5],12:i:-**
S18	**1,4,[5],12**	**i**	**1,2**	**Typhimurium**
S8*	4,[5],12	i	e,n,x	Farsta
S3*	4,12	z_{10}	1,6	Tudu
S1*	6,7	k	e,n,x	Singapore
S6*	6,7	l,z_{13}	e,n,x	Kenya
S12	**6,7,14**	**r**	**1,2**	**Virchow**
S20	**6,7,14**	**r**	**1,5**	**Infantis**

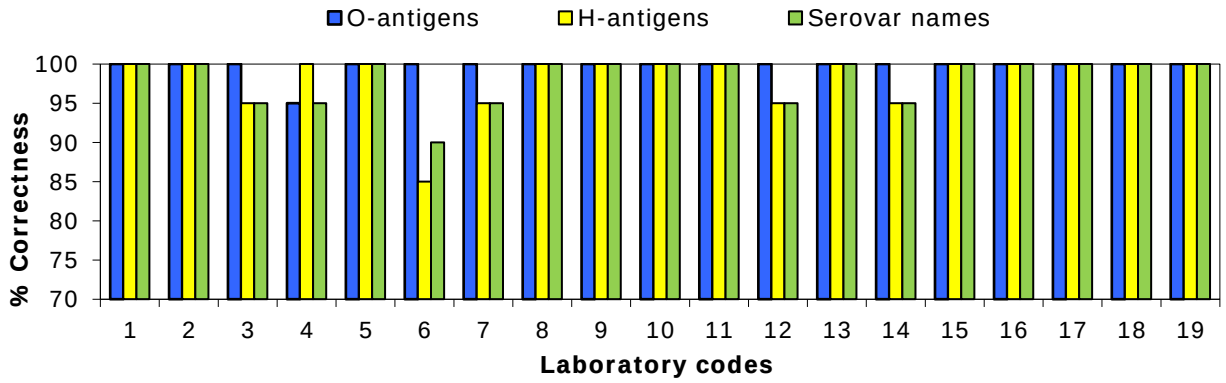
* Represented in an EURL-*Salmonella* PT Serotyping for the first time

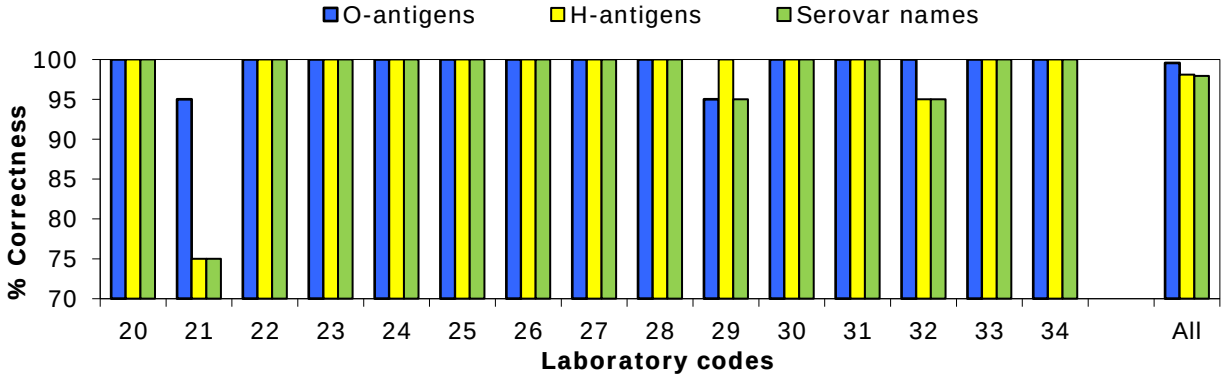
Salmonella strains (2)

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
S9	**6,8**	**z_{10}**	**e,n,x**	**Hadar**
S14	1,9,12	e,h	1,5	Eastbourne
S5	**1,9,12**	**g,m**	**-**	**Enteritidis**
S15*	3,10	z_{35}	z_6	Cairina
S11*	11	d	[e,n,x]	Chandans
S17*	1,13,23	d	1,5	Mishmarhaemek
S7	1,13,23	m,t	-	Kintambo
S16*	1,6,14,25	a	1,5	Garba
S10*	16	b	1,2	Hull
S19*	28	c	1,5	Hermannswerder
S21	47	k	z_{35}	47:k:z_{35} (IIIb)

* Represented in an EURL-*Salmonella* PT Serotyping for the first time

Percentages of correct serotyping results, per participant





Correct typing of strains

- 100% accurate serotype naming by 25/34 (74%) participants

- 10 serovars completely correct identified by all participants:
  - Singapore (S1), Agona (S4), Enteritidis (S5), Kenya (S6), Hadar (S7), Hull (S10), Virchow (S12), Hato (S13), Mishmarhaemak (S17), and Infantis (S20)

Overview

Lab: REF	S1 Singapore	S2 1,4,[5],12:i:-	S3 Tudu	S4 Agona	S5 Enteritidis	S6 Kenya	S7 Kintambo	S8 Farsta	S9 Hadar	S10 Hull	S11 Chandans	S12 Virchow	S13 Hato	S14 Eastbourne	S15 Cairina	S16 Garba	S17 Mishmarhaemek	S18 Typhimurium	S19 Hermannswerder	S20 Infantis	Lab: REF
1	Singapore	4:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandas	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	1
2	Singapore	I 4:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	2
3	Singapore	4,12:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	3,10:-:z6	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	3
4	Singapore	4,12:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Waedenswil	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	4
5	Singapore	1,4,5,12:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	5
6	Singapore	Typhimurium monofaza	Tudu	Agona	Enteritidis	Kenya	enterica II	Farsta	Hadar	Hull	Chandans	Virchov	Hato	Eastbourne	enterica II	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	6
7	Singapore	4,12:i:-	Tudu	Agona	Enteritidis	Kenya	Agbeni	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	7
8	Singapore	1,4,12:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	8
9	Singapore	4,12:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	9
10	Singapore	monophasic Typhimurium	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	10
11	Singapore	4,12:i:- (mST)	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	11
12	Singapore	4,5,12:i:-	Tudu	Agona	Enteritidis	Kenya	Agbeni	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	12
13	Singapore	monophasic Typhimurium	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	13
14	Singapore	Tumodi	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	14
15	Singapore	4,12:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	15
16	Singapore	4,12:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	16
17	Singapore	Monophasic Typhimurium 4:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	17
18	Singapore	4,5,12:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	18
19	Singapore	Sub I 4,12:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	19
20	Singapore	4,12:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	20
21	Singapore	Monophasic Salmonella Typhimurium	Lexington	Agona	Enteritidis	Kenya	Agbeni	Chester	Hadar	Hall	Findorff	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Vanier	Infantis	21
22	Singapore	4,5,12:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	22
23	Singapore	4,12:i: -	Tudu	Agona	Enteritidis	Kenya	Kintambo	Fasta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	23
24	Singapore	4:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	4,5:i:e,n,x	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	24
25	Singapore	4:i:- (monophasic Typhimurium)	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	25
26	Singapore	4,12:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	26
27	Singapore	4,12:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Vichow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	27
28	Singapore	4,12:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	28
29	Singapore	4,12:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Sanjuan	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	29
30	Singapore	Typhimurium, monophasic (4,12:i:-)	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	30
31	Singapore	4,12:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	31
32	Singapore	4,12:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	4,5,12:i:-	Hermannswerder	Infantis	32
33	Singapore	4,5:i:-	Tudu	Agona	Enteritidis	Kenya	Kintambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	33
34	Singapore	4,[5],12:i:- (monophasic Typhimurium)	Tudu	Agona	Enteritidis	Kenya	Kimtambo	Farsta	Hadar	Hull	Chandans	Virchow	Hato	Eastbourne	Cairina	Garba	Mishmarhaemek	Typhimurium	Hermannswerder	Infantis	34
X	0	1	1	0	0	0	4	1	0	0	1	0	0	1	1	1	0	1	1	0	**X**

remark (e.g. spelling errror)
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

Details

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	Lab code
S-2	**1,4,[5],12**	**i**	**-**	**1,4,[5],12:i:-**	**REF**
S-2	1,4,12	i	z6	Tumodi	14
S-3	**4,12**	**z10**	**1,6**	**Tudu**	**REF**
S-3	3	z10	1,5	Lexington	21
S-7	**1,13,23**	**m,t**	**-**	**Kintambo**	**REF**
S-7	1,13,23	g,m,t	1,5	enterica II	6
S-7	13,23	g,m,t	-	Agbeni	7
S-7	13,23	g,m	-	Agbeni	12
S-7	13,23	g,m,t	-	Agbeni	21
S-7	13,23	m,t	-	Kimtambo	34
S-8	**4,[5],12**	**i**	**e,n,x**	**Farsta**	**REF**
S-8	4,5,12	e,h	e,n,x	Chester	21
S-8	4,5,12	i	e,n,x	Fasta	23
S-8	4,5	i	e,n,x	4,5:i:e,n,x	24
S-10	**16**	**b**	**1,2**	**Hull**	**REF**
S-10	16	b	1,2	Hall	21
S-11	**11**	**d**	**[e,n,x]**	**Chandans**	**REF**
S-11	11	d	e,n,x	Chandas	1
S-11	11	d	z6	Findorff	21
S-12	**6,7,14**	**r**	**1,2**	**Virchow**	**REF**
S-12	6,7	r	1,2	Virchov	6
S-12	6,7	r	1,2	Vichow	27
S-14	**1,9,12**	**e,h**	**1,5**	**Eastbourne**	**REF**
S-14	9,46	e,h	1,5	Waedenswil	4
S-15	**3,10**	**z35**	**z6**	**Cairina**	**REF**
S-15	3,10	-	z6	3,10:-:z6	3
S-15	3,10	z35	e,n,x,z15	enterica II	6
S-15	3,1	z35	z6	Cairina	13
S-16	**1,6,14,25**	**a**	**1,5**	**Garba**	**REF**
S-16	6,7,14	a	1,5	Sanjuan	29
S-18	**1,4,[5],12**	**i**	**1,2**	**Typhimurium**	**REF**
S-18	4,5,12	i	-	4,5,12:i:-	32
S-19	**28**	**c**	**1,5**	**Hermannswerder**	**REF**
S-19	28	z	1,5	Vanier	21

- 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

Strain S8: 4,**5**,12:i:e,n,x

World Health Organization

Institut Pasteur

WHO Collaborating Centre for Reference and Research on *Salmonella*

ANTIGENIC FORMULAE OF THE *SALMONELLA* SEROVARS

2007

9th edition

Patrick A.D. Grimont & François-Xavier Weill

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Supplement 2008–2010 (no. 48) to the White–Kauffmann–Le Minor scheme☆

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WIKIPEDIA The Free Encyclopedia

Farsta is a borough (*stadsdelsområde*) of Söderort in the southern part of Stockholm Municipality, Sweden. [1] [2]

Farsta	4,12	i	e,n,x

Table 3
New variants of previously described *Salmonella* serovars recognized by the WHO Collaborating Centre for Reference and Research on *Salmonella*, 2008–2010.

IP strain number	Antigenic formula	Other character[a]	Source	Area of contamination or isolation	Year	Received from[b]	Serovar name	MLST type	Updated antigenic formula
S. enterica* subsp. *enterica									
9763/08	1,13,23:z_4,z_{23}:–		Human feces	France	2008	IP	Ajiobo	ST1065	1,13,23:z_4,z_{23}:–
9766/08	3,10:g,s,t:–:Rz_{27}		Poultry environment	La Réunion[c]	2008	IP	Westhampton	ST185	3,{10}{15}{15,34}:g,s,t:–:[Rz_{27}], [Rz_{37}]
9768/08	1,4,5,12:z:1,2		Human feces	France	2007	IP	Shubra	ST1394	1,4,[5],12:z:1,2
9815/08	6,14,25:k:e,n,x		Rosemary herb	Germany	2008	IHU	Trumersee	ST1701	1,6,14,25:k:e,n,x
9838/08	45:m,t:–:Rz_{45}		Poultry	Senegal	2008	IP	Apapa	ST1049	45:m,t:–:[Rz_{45}]
9957/09	1,3,19:d:l,w:Rz_{45}		Environment	France	2009	IP	Tilburg	ST1736	1,3,19:d:l,w:[Rz_{45}], [Rz_{49}]
9959/09	4,12:b:e,n,z_{15}		Human feces	France	2009	IP	Wagenia	ST1667	1,4,12,[27]:b:e,n,z_{15}
10035/09	4,5,12:i:e,n,x	$Dtar^-$, Gas^-	Poultry environment	France	2009	IP	Farsta	ST886	4,[5],12:i:e,n,x

S-8	4,[5],12	i	e,n,x	Farsta	REF
S-8	4,5,12	e,h	e,n,x	Chester	21
S-8	4,5,12	i	e,n,x	Fasta	23
S-8	4,5	i	e,n,x	4,5:i:e,n,x	24
S-8	4	i	e,n,x	Farsta	5x
S-8	4,12	i	e,n,x	Farsta	20x
S-8	4,5	i	e,n,x	Farsta	2x
S-8	4,5,12	i	e,n,x	Farsta	4x

Additional strain S21

- Examined by 29/34 participants

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	Lab code
S-21	**47**	**k**	**z_{35}**	**47:k:z_{35}**	**REF**
S-21	47	k	z_{35}	Lyon III b	21
S-21		k	z_{35}	S.enterica subsp. diarizonae IIIb	10
S-21	OME+	k	z_{35}	OME+:k:z35	24
S-21	47	k	-	enterica subsp diarizonae	7
S-21	47	k	-	47:k:-	3

S-21	47	k	z_{35}	47:k:z_{35}	7x

in variations:

S-21	47	k	z_{35}	*Salmonella enterica* subspecies *diarizonae* 47:k:z_{35} (IIIb)	17x

Evaluation of Performance

- **“Good Performance” (**Workshop Bilthoven, 2007)

- **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 serotypes to another strain.
- **1 penalty point:** Incorrect typing of all other *Salmonella* serotypes.

- For each NRL-*Salmonella* the total amount of penalty points is determined.
- “Good Performance” is: less than 4 penalty points.
- A follow-up is obligatory for EU-NRLs with 4 penalty points or more.

Evaluation of Performance

Laboratory code	Penalty points	Good performance
1	0	yes
2	0	yes
3	0	yes
4	1	yes
5	0	yes
6	2	yes
7	1	yes
8	0	yes
9	0	yes
10	0	yes
11	0	yes
12	1	yes
13	0	yes
14	4	no
15	0	yes
16	0	yes
17	0	yes

Laboratory code	Penalty points	Good performance
18	0	yes
19	0	yes
20	0	yes
21	5	no
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	0	yes
31	0	yes
32	1	yes
33	0	yes
34	0	yes

Follow-up study 2 EU participants

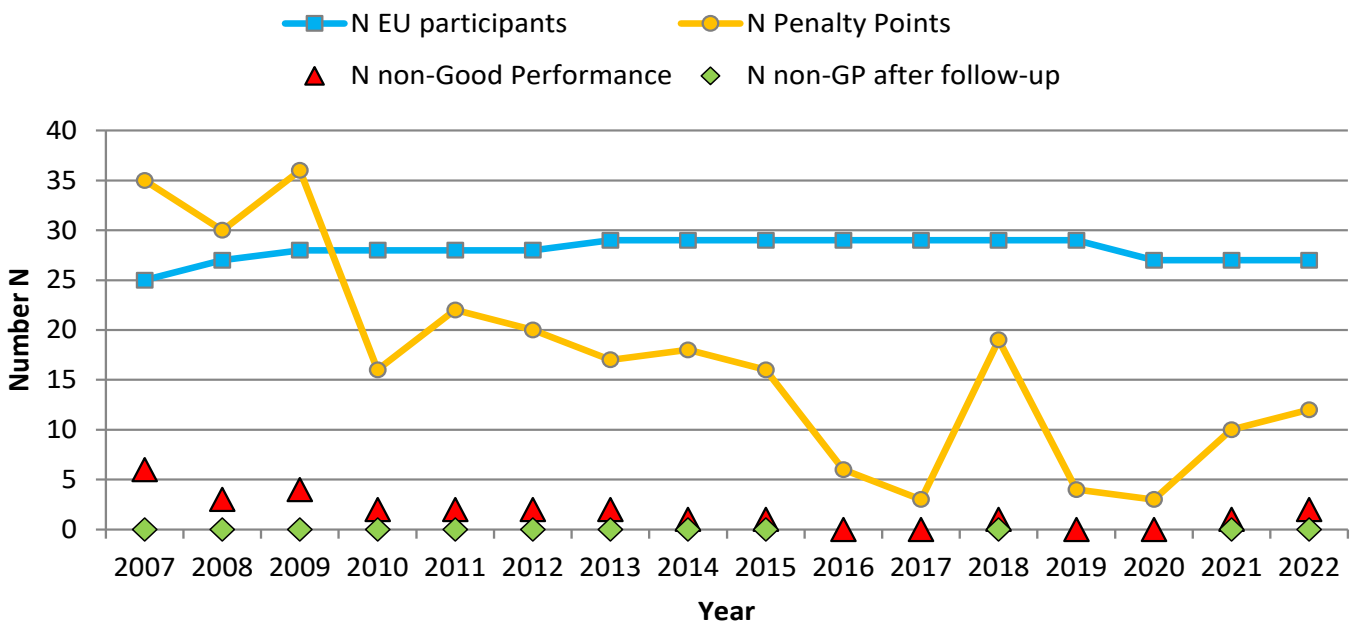
Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
SF1	28	c	1,5	Hermannswerder
SF2	3,{10}{15}{15,34}	e,h	1,6	Anatum
SF3[a)]	4,[5],12	i	e,n,x	Farsta
SF4[b)]	**1,4,[5],12**	**i**	**-**	**1,4,[5],12:i:-**
SF5	16	c	l,w	Yoruba
SF6	1,13,23	m,t	-	Kintambo
SF7	3,{10},{15}	r	z_6	Weltevreden
SF8	11	d	[e,n,x]	Chandans
SF9	4,12	z10	1,6	Tudu
SF10	{6,7,14}{54}	g,m,[p],s	[1,2,7]	Montevideo

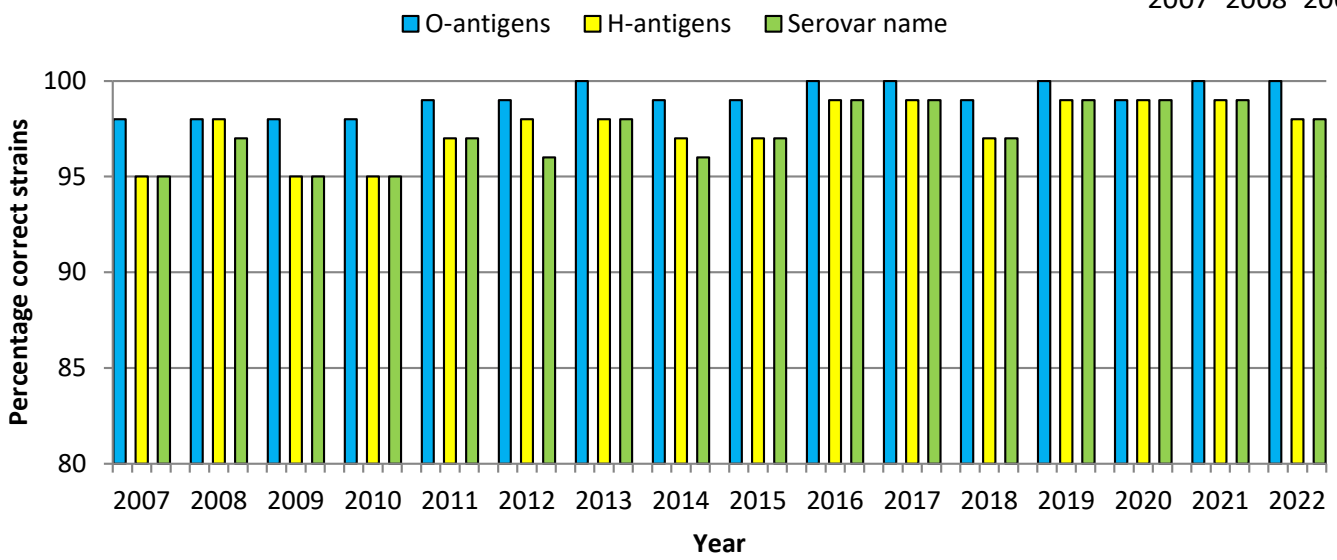
[a)] in accordance with Supplement 2008-2010 (no. 48) to the White-Kauffmann-Le Minor scheme.

[b)] monophasic variant of Typhimurium based on genomic sequences.

Laboratory code	Penalty points	Good performance
14	0	yes
21	0	yes

Results in time (EU NRLs only)





Conclusions PT serotyping 2022 (all participants)

- O-antigens: nearly 100% of strains typed correctly
- H-antigens: 98% of strains typed correctly
- Serovar names: 98% of strains typed correctly

- 100% accurate serotyping by 25/34 (74%) participants
  - 5 participants with 1 penalty point
  - 1 participant with 2 penalty points
  - 1 participant with 4 penalty points (mistake in ‘top-5’)
  - 1 participant with 5 penalty points

- 32 participants “Good Performance”
- 2 participants “Good Performance” after Follow-up

Part on Cluster Analysis (4th PT)

- Cluster analysis using MLVA and/or WGS
  - Participants' own routine method(s) of choice

- MLVA
  - Result form: allelic profile, **cluster analysis**
- WGS
  - Result form: wet-lab/dry-lab protocols, **cluster analysis**
  - Uploading: raw reads (fastq files)
  - Emailing: distance matrix

‘Wet’ strains *Salmonella* Cluster Analysis PT 2022

- More extensive pre-testing (MLVA and WGS)
  - DNA extraction, library preparation, sequencing performed in-house; WGS platform: Illumina NextSeq. Raw data processing: in-house developed Juno-assembly pipeline which includes SPAdes 3.15.3. Cluster analysis: Ridom SeqSphere+, using the cgMLST Enterobase v2.0 scheme.
- 6 strains with stable and consistent MLVA and cgMLST results selected for inclusion in the PT
- Technical duplicates: 22SCA01/22SCA05 and 22SCA04/22SCA06
  - Shipment tubes prepared from the same blood agar plate culture

Strain code	Serovar	ST	MLVA profile	Origin
22SCA01[a)]	Enteritidis	11	3-10-5-3-1	Human
22SCA02[c)]	Enteritidis	11	3-10-4-4-1	Human
22SCA03	Enteritidis	11	2-9-7-4-2	Human
22SCA04[b)] REF	Enteritidis	11	3-10-6-3-1	Human
22SCA05[a)]	Enteritidis	11	3-10-5-3-1	Human
22SCA06[b)]	Enteritidis	11	3-10-6-3-1	Human

a) Technical duplicates

b) Technical duplicates

c) Biological duplicate strain 2021SCA08

‘Dry’ strains *Salmonella* Cluster Analysis PT 2022

- More extensive pre-testing (2021/2022 WGS only)
  - DNA extraction, library preparation, sequencing performed in-house; WGS platform: Illumina NextSeq. Raw data processing: in-house developed Juno-assembly pipeline which includes SPAdes 3.15.3. Cluster analysis: Ridom SeqSphere+, using the cgMLST Enterobase v2.0 scheme.
- 6 strains/data selected for inclusion in the PT
- Contaminated strain included: 22SCA13

Strain code	Serovar	ST	MLVA profile	Origin
22SCA11[c)]	Enteritidis	11	3-10-4-4-1	Human
22SCA12[c)]	Enteritidis	11	3-10-4-4-1	Human
22SCA13*	Enteritidis	11	n.a.	Unknown
22SCA14	Enteritidis	11	2-10-8-5-1	Human
22SCA15[d)]	Enteritidis	11	3-10-5-3-1	Human
22SCA16	Enteritidis	11	2-8-5-15-NA	Human

c) Strain 2021SCA08, raw data PT 2021 from 2 different Laboratories

d) Biological duplicate strain 22SCA01

* *S.* Enteritidis strain contaminated with *E. coli*

Evaluation cluster analysis per methodology

- Participants were asked to report per strain if a clustering match [yes or no] was found with the reference outbreak strain (22SCA-REF)
  - Comparing the participants' results to the expected results, as pre-defined by the EURL-*Salmonella* (Protocol PT Typing 2022)

Like before, the cluster analysis 2022 is mimicking an outbreak situation, with a *Salmonella* Enteritidis ST11, MLVA type 3-10-6-3-1 as the reference strain. WGS data on this strain (fastq-files, md5checksums) will be made available through a secure ftp server.

For this particular PT 2022 situation, the cgMLST-based cluster definition is set at maximum 6 allelic differences from the reference sequence (WGS).
For MLVA, the cluster definition is set at no loci with a different number of repeats.

- No specific performance criteria were set for this PT on cluster analysis
- As a minimum, it was expected that participants would report the technical duplicate strains 22SCA01/22SCA05 and 22SCA04/22SCA06 to be (part of) one cluster
- Deviations from the expected results are indicated in blue

20 Participants in Cluster Analysis PT 2022

- 5 x MLVA
- 20 x WGS (26 submissions)
  - 4 participants: both cgMLST-based and SNP-based data
  - 1 participant: 2x cgMLST-based and 1x reference-based SNP data

	2019	2020	2021	2022
PFGE	6	2	na	na
MLVA	8	6	5	5
WGS	14	21	19	20
WGS: cgMLST	9	15	13	17
WGS: SNP	5	8	10	10
Partipants overall*	18	21	19	20

*excluding multiple data submissions per participant

Individual results MLVA (for information only)

- Loci:SENTR7-SENTR5-SENTR6-SENTR4-SE-3

	Strain code					
Lab code	**22SCA01**	**22SCA02**	**22SCA03**	**22SCA04**	**22SCA05**	**22SCA06**
Expected	**3-10-5-3-1**	**3-10-4-4-1**	**2-9-7-4-2**	**3-10-6-3-1**	**3-10-5-3-1**	**3-10-6-3-1**
1	3-10-5-3-1	3-10-4-4-1	2-9-7-4-2	3-10-6-3-1	3-10-5-3-1	3-10-6-3-1
17	3-10-5-3-1	3-10-4-4-1	2-9-7-4-2	3-10-6-3-1	3-10-5-3-1	3-10-6-3-1
19	5-2-3-7-6-6-11	5-2-3-7-6-6-11	5-2-3-7-6-6-11	5-2-3-7-6-6-11	5-2-3-7-6-6-11	5-2-3-7-6-6-11
28	3-10-5-3-1	3-10-4-4-1	2-9-7-4-2	3-10-6-3-1	3-10-5-3-1	3-10-6-3-1
33	3-10-5-3-1	3-10-4-4-1	2-9-7-4-2	3-10-6-3-1	3-10-5-3-1	3-10-6-3-1

In blue: Deviation from the expected result.

Evaluation MLVA-based cluster analysis (1)

- 5 participants
- Cluster definition from Protocol PT Typing 2022
  - no loci with a different number of repeats by MLVA
- Outbreak strain (22SCA-REF): *Salmonella* Enteritidis, ST11, MLVA type 3-10-6-3-1
- Participants' results compared to the expected results
  - Technical duplicates 22SCA01 and 22SCA05 within one cluster
  - Technical duplicates 22SCA04 (REF) and 22SCA06 within one cluster

Evaluation MLVA-based cluster analysis (2)

- MLVA-cluster: 22SCA04, 22SCA06
  - (second MLVA-cluster: 22SCA01, 22SCA05)

	Strain code					
Labcode	**22SCA01**	**22SCA02**	**22SCA03**	**22SCA04**	**22SCA05**	**22SCA06**
Expected	**No**	**No**	**No**	**Yes**	**No**	**Yes**
1	No	No	No	Yes	No	Yes
17	No	No	No	Yes	No	Yes
19*	Yes	Yes	Yes	Yes*	Yes	Yes*
28	No	No	No	Yes	No	Yes
33	No	No	No	Yes	No	Yes

In blue: Deviation from the expected result.

* The allelic profiles were not reported in the expected format, therefore the evaluation of the cluster analysis results cannot be done according to the PT Typing 2022 Protocol.

Strain code	Serovar	ST	MLVA profile
22SCA01[a)]	Enteritidis	11	3-10-5-3-1
22SCA02[c)]	Enteritidis	11	3-10-4-4-1
22SCA03	Enteritidis	11	2-9-7-4-2
22SCA04[b)] REF	Enteritidis	11	3-10-6-3-1
22SCA05[a)]	Enteritidis	11	3-10-5-3-1
22SCA06[b)]	Enteritidis	11	3-10-6-3-1

a) Technical duplicates
b) Technical duplicates
c) Biological duplicate strain 2021SCA08

- 4/5 participants reported as expected
  - Technical duplicates 22SCA04/22SCA06 and 22SCA01/22SCA05, each within one cluster

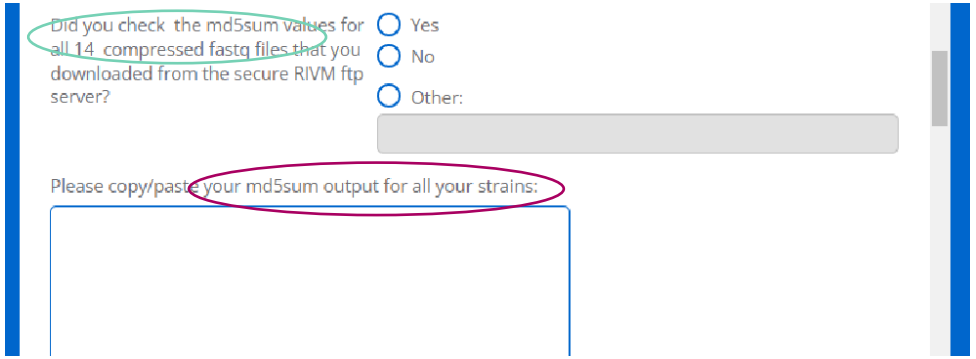
WGS protocols used by the 20 participants

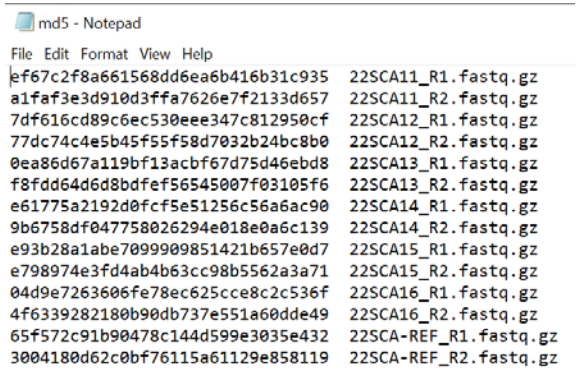
Labcode	Wet lab[a)]	WGS platform used	Data analysis	Tool for analysis	Method for cluster analysis
32	In-In-In	MiniSeq	cgMLST-based	Ridom SeqSphere	Distance matrix only
14-cgMLST	In-In-In	Illumina MiSeq	cgMLST-based	galaxy.sciensano	Maximum likelihood (ML)
1	In-In-In	Illumina MiSeq	cgMLST-based	Ridom SeqSphere	Minimum Spanning Tree (MST)
2	In-In-In	Illumina NextSeq	cgMLST-based	Ridom SeqSphere	Minimum Spanning Tree (MST)
3	In-In-In	Illumina MiSeq	cgMLST-based	BioNumerics	Minimum Spanning Tree (MST)
8-cgMLST	In-Out-Out	Illumina NovaSeq	cgMLST-based	Ridom SeqSphere	Minimum Spanning Tree (MST)
19	In-In-In	Illumina Miniseq	cgMLST-based	Ridom SeqSphere	Minimum Spanning Tree (MST)
23	In-In-In	Illumina NextSeq 2000	cgMLST-based	Ridom SeqSphere	Minimum Spanning Tree (MST)
24	In-In-In	Illumina MiSeq	cgMLST-based	Ridom SeqSphere	Minimum Spanning Tree (MST)
28-cgMLST	In-In-In	Illumina MiSeq	cgMLST-based	PyMLST v1	Minimum Spanning Tree (MST)
33	In-In-In	Illumina MiSeq	cgMLST-based	ChewBBaCa	Minimum Spanning Tree (MST)
EURL-Salm	In-In-In	Illumina NextSeq	cgMLST-based	Ridom SeqSphere	Minimum Spanning Tree (MST)
17	In-In-In	Illumina MiSeq	cgMLST-based	in-house galaxy	MSTreeV2
7	In-In-In	Illumina MiSeq	cgMLST-based	linux command line	Neighbor joining (NJ)
27-cgMLST1	In-In-In	Illumina NextSeq	cgMLST-based	inhouse chewieSnake pipeline (Enterobase scheme)	single linkage hierarchical clustering
27-cgMLST2	In-In-In	Illumina NextSeq	cgMLST-based	Ridom SeqSphere+Enterobase scheme	single linkage hierarchical clustering
30-cgMLST	In-In-In	Illumina MiSeq	cgMLST-based	chewBBACA using the scheme from Enterobase	Allele differences based on chewBBACA output
27-SNPr	In-In-In	Illumina NextSeq	SNP-based - reference-based	SNP-analysis using SnippySnake pipeline	single linkage hierarchical clustering
26	In-In-In	Illumina MiSeq	SNP-based - assembly-based	CSIPhylogeny 1.4	Maximum likelihood (ML)
30-SNPa	In-In-In	Illumina MiSeq	SNP-based - assembly-based	In house pipeline based on parSNP, Gubbins	Maximum likelihood (ML)
8-SNPr	In-Out-Out	Illumina NovaSeq	SNP-based - reference-based	SNIPPY	Maximum likelihood (ML)
10	In-In-Out	Illumina NextSeq	SNP-based - reference-based	https://cge.food.dtu.dk/services/CSIPhylogeny/	Maximum likelihood (ML)
14-SNPr	In-In-In	Illumina MiSeq	SNP-based - reference-based	Galaxy Sciensano	Maximum likelihood (ML)
16	In-In-In	Illumina MiSeq	SNP-based - reference-based	CSIPhylogeny (https://cge.food.dtu.dk/services/CSIPhylogeny/)	Maximum likelihood (ML)
28-SNPr	In-In-In	Illumina MiSeq	SNP-based - reference-based	BWA, bcftools, RAxML	Maximum likelihood (ML)
9	In-In-In	Illumina MiSeq	SNP-based - reference-based	Python script	Minimum Spanning Tree (MST)
29	In-In-In	Illumina MiSeq	SNP-based - reference-based	Snippy, Snapper DB, Gubbins, RAxML, iToL	ML and SNP address analysis

[a)] Wet lab preparations: DNA extraction, Library preparation, sequencing. IN: In-house, Out: Outsourced.

Data quality criteria used by the participants

- Comparable to the PT Typing 2019, 2020, 2021
- Complete overview will be included in the Final Report PT Typing 2022
- MD5: data evaluation will be included in Interim summary report or Final report
  - MD5 can be used as a checksum to verify data integrity against unintentional corruption, e.g. as a result of a faulty file transfer. The MD5 hashes (*message digests*) are typically represented as a sequence of 32 hexadecimal digits.





MD5 checksum PT 2020: 11/21
PT 2021: 14/19
PT 2022: 16/20

Evaluation WGS-based cluster analysis (1)

- 20 participants (26 submissions)
- Participants were asked to report per strain:
  - Whether the data passed the internal Quality Control (QC) criteria or not
  - Whether a clustering match was found with the reference outbreak strain (22SCA-REF)
- Protocol Typing 2022: Exclude strains from the cluster analysis if the data did not pass your QC.

- Cluster definition from Protocol PT Typing 2022
  - set at maximum 6 allelic differences from the reference
- 22SCA-REF: *Salmonella* Enteritidis, ST11, MLVA type 3-10-6-3-1
  - 22SCA_REF_R1.fq.gz & 22SCA_REF_R2.fq.gz
- Participants' results compared to the expected results
  - Technical duplicates 22SCA01/22SCA05 and 22SCA04/22SCA06 within one cluster
  - Contaminated strain 22SCA13 excluded from the cluster analysis (+ distance matrix)

Evaluation WGS-based cluster analysis (2)

Labcode-method	Strain code											
	22 SCA01	22 SCA02	22 SCA03	22 SCA04	22 SCA05	22 SCA06	22 SCA11	22 SCA012	22 SCA13	22 SCA14	22 SCA15	22 SCA16
Expected	**Yes**	**No**	**No**	**Yes**	**Yes**	**Yes**	**No**	**No**	**n.a.**	**No**	**Yes**	**No**
1-cgMLST	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
2-cgMLST	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
3-cgMLST	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
7-cgMLST	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
8-SNPr	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
8-cgMLST	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
9-SNPr	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
10-SNPr	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
14-cgMLST	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14-SNPr	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16-SNPr	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
17-cgMLST	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
19-cgMLST	Yes	No	Yes	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
23-cgMLST	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
24-cgMLST	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
26-SNPa	Yes	Yes	No	No	Yes	Yes	No	No	n.a.	No	Yes	No
27-cgMLST1	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
27-cgMLST2	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
27-SNPr	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
28-cgMLST	Yes	No	No	Yes	Yes	Yes	No	No	No	No	Yes	No
28-SNPr	Yes	No	No	Yes	Yes	Yes	No	No	No	No	Yes	No
29-SNPr	Yes	No	No	Yes	Yes	Yes	No	No	No	No	Yes	No
30-SNPa	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
30-cgMLST	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
32-cgMLST	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No
33-cgMLST	Yes	No	No	Yes	Yes	Yes	No	No	n.a.	No	Yes	No

n.a.: Not applicable (QC not passed)

- 19/26 submissions reported as expected
  - Misunderstanding in reporting results by Lab 14?
  - Technical duplicates 22SCA01 and 22SCA05 within one cluster in 24/24 submissions
  - Technical duplicates 22SCA04 and 22SCA06 within one cluster in 23/24 submissions
  - Strain 22SCA13 excluded from cluster analysis in 21/26 submissions
    - Still in distance matrix Lab 16
    - Note on detected contamination by Lab 28

Excluding contaminated strain 22SCA13

Labcode	Strain 22SCA13 excluded from cluster analysis	Reason(s) not passing QC
1	Yes	Contamination Check Result: Potential contamination by second species above 10% detected: Escherichia coli; genome size too big (12.7 MB); strain excluded for MST and matrix
2	Yes	total length too high, # contigs too high, GC% too low, contamination too high: mostly E. coli reads
3	Yes	Failed on assembly size, contig number and N50 values. Contamination confirmed using Kmer Finder.
7	Yes	Contamination with E.coli, number of contigs over 3400, total lenght is oversize
8	Yes	Contamination by Escherichia 42.26%: coli(39.96%) - Salmonella 37.83%: enterica(37.57%)
9	Yes	Contamination and failed assembly
10	Yes	purity of culture; CG%; No. contigs; genome size
16	Yes	Total length 10,7Mbp, GC% 50,9, 3490 contigs, contamination confirmed by KmerFinder
17	Yes	contaminated with E. coli, low N50, total length 2x, number of contigs too high, <90% MLST loci detected
19	Yes	final assembly lenght too large
23	Yes	Contaminated with E. coli, assembly size too big for Salmonella
24	Yes	Contamination with E. coli
26	Yes	N. contigs >500; Total length higher than expected; N50<15000
27	Yes	Fail: Total length 12,718,480 bp; Read Fraction Majority Genus 0.488; Contam SNVs 2508 (inter and intra contamination); Warning: # Contigs 5,602; N50 11,563; Single copy orthologs 0.500; Duplication Rate 1.415; GC 51.07
30	Yes	Contamination with other species (E. coli)
32	Yes	Potential contamination by second species above 10% detected: Escherichia coli
33	Yes	contamination status = True
14	No	
28	No*	Sample 22SCA13 was contaminated (only about 35% of the reads were classified as belonging to the Salmonella taxon). We select those reads removing that way the contamination. Thus, we continue the analysis just with the reads classified as Salmonella.
29	No	

Per submission: *Distance matrix* data

	Strain code												
Labcode-method	22 SCA-REF	22 SCA01	22 SCA02	22 SCA03	22 SCA04	22 SCA05	22 SCA06	22 SCA11	22 SCA12	22 SCA14	22 SCA15	22 SCA16	22 SCA13
1-cgMLST	0	2	50	218	0	2	0	50	50	249	2	544	
2-cgMLST	0	2	50	220	0	2	0	50	50	251	3	546	
3-cgMLST	0	1	51	225	0	1	0	51	51	259	1	561	
7-cgMLST	0	4	54	223	1	4	1	54	54	251	4	552	
8-cgMLST	0	2	50	218	0	2	0	50	50	250	2	544	
14-cgMLST	0	6	103	411	0	6	0	103	103	472	6	1220	414
17-cgMLST	0	6	58	229	1	6	1	58	58	262	5	567	
19-cgMLST	0	3	51	219	1	3	1	51	52	250	3	545	
23-cgMLST	0	2	50	220	0	2	0	50	51	251	2	546	
24-cgMLST	0	2	49	218	0	2	0	50	50	249	2	543	
27-cgMLST1	0	4	53	220	1	4	1	53	54	250	4	544	213[a)]
27-cgMLST2	0	2	51	220	0	3	0	51	51	251	2	546	
28-cgMLST	0	3	53	212	2	5	1	48	49	246	3	537	232[b)]
30-cgMLST		4			1	4	1		54		5		
32-cgMLST	0	2	50	219	0	2	0	50	50	248	2	545	
33-cgMLST	0	4	64	246	0	4	0	64	65	270	4	606	
EURL-Salm-cgMLST	0	2	50	220	0	2	0	50	50	251	3	546	
26-SNPa	0	7	1	126	455	9	3	108	110	521	9	1413	
30-SNPa	0	10	111	453	4	10	4	111	109	516	11	1220	
8-SNPr	0	6	123	501	0	6	0	117	118	596	6	1321	
9-SNPr	0	6	98	447	0	6	0	98	98	500	6	1223	
10-SNPr	0	6	101	443	0	6	0	101	101	506	6	1447	
14-SNPr	0	6	109	449	0	6	0	109	109	504	6	1327	468
16-SNPr	0	9	111	451	3	9	2	109	112	520	8	1420	403[c)]
27-SNPr	0	6	108	479	0	6	0	108	108	531	6	1683	
28-SNPr	0	7	112	495	2	7	1	114	112	636	6	1765	516[b)]
29-SNPr	0	6	102	418	1	6	1	102	102	461	6	1127	418

[a)] 22SCA13 QC failed and will not be included for reporting. However we checked the allelic differences for own interests.

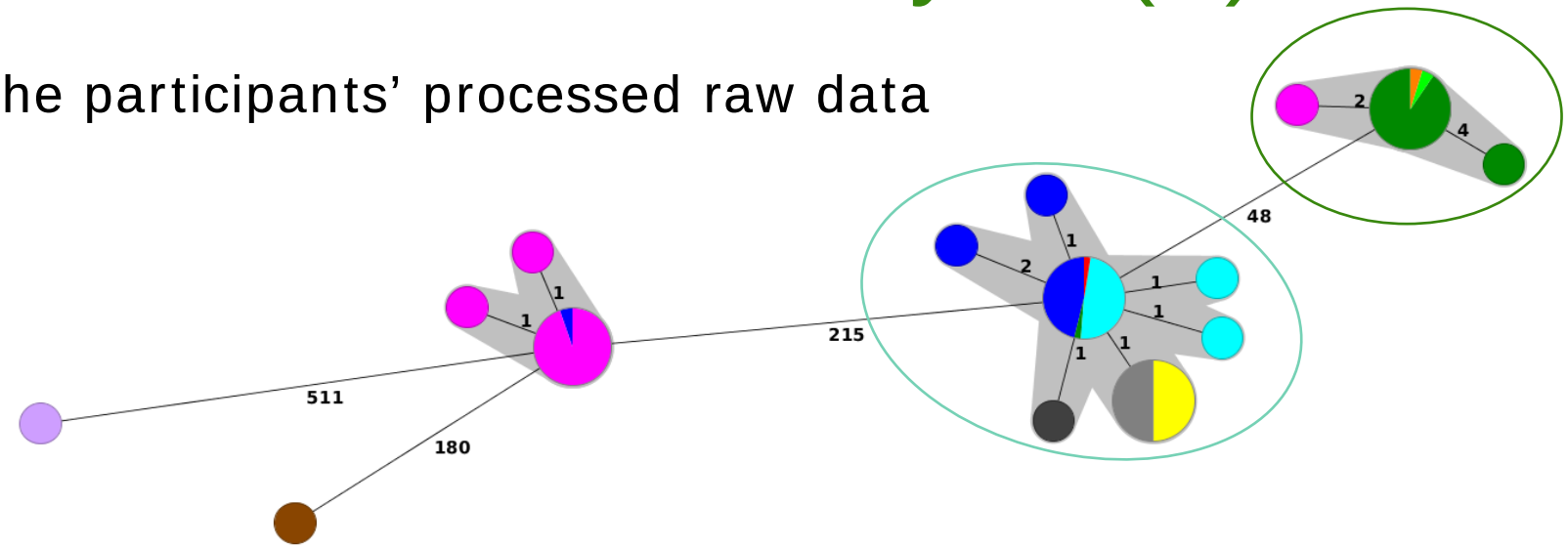
[b)] Sample 22SCA13 was contaminated (only about 35% of the reads were classified as belonging to the Salmonella taxon). We select those reads removing that way the contamination. Thus, we continue the analysis just with the reads classified as Salmonella.

[c)] Reported to be excluded from the cluster analysis

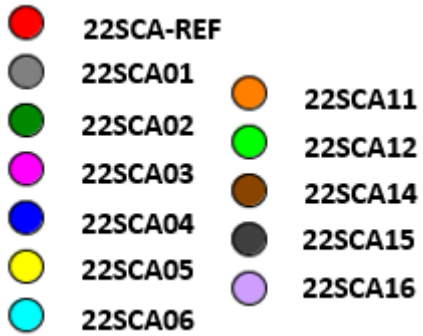
- 19/26 submissions reported as expected
  - Misunderstanding in reporting results by Lab 14?
  - Technical duplicates 22SCA01 and 22SCA05 within one cluster in 24/24 submissions
  - Technical duplicates 22SCA04 and 22SCA06 within one cluster in 23/24 submissions
  - Strain 22SCA13 excluded from cluster analysis in 21/26 submissions
    - Still in distance matrix Lab 16
    - Note ondetected contamination by Lab 28

Evaluation WGS-based cluster analysis (3)

- MST of the strains from the participants' processed raw data and the 'dry' strains
  - Juno Assembly_pipeline
  - Ridom SeqSphere+, cgMLST (3002)
  - Note: Allelic differences not on scale



- Reference cluster:
  - 22SCA-REF, 22SCA04, 22SCA06, 22SCA01, 22SCA05
- Optional: any further clusters reported
  - Second cluster: 22SCA02, 22SCA11, 22SCA12
  - Any further clusters reported: 18/26
  - Second cluster identified as expected: 15/18



Conclusions PT Cluster Analysis 2022

- **20** participants overall; **5 MLVA** and **20 WGS** (with 26 submissions)
- Extended pre-testing for strain selection
- 6 ‘wet’ strains (MLVA and WGS) and 6 ‘dry’ strains (WGS only)
- Pre-defined “Cluster definitions”, (REF strain, expected results)
- Contaminated strain 22SCA13 (failing QC) had to be excluded from cluster analysis (WGS)
- No performance criteria were set for this PT
  - Participants’ results compared to expected results: MLVA: 4/5, WGS: 15/20 (19/26)
  - Contaminated strain 22SCA13 excluded from WGS cluster analysis: 17/20 (21/26)

- **5th PT on (optional) Cluster Analysis, WGS only, in 2023**
  - Defined cluster analysis on 10-12 *Salmonella* strains
    - Simulation of an outbreak-related request from the EURL-*Salmonella* (EFSA/ECDC) to the NRL-network
    - A WGS reference sequence will be provided (plus md5sum values)
    - Proposal: 5-6 strains ‘wet’ analysis plus 5-6 strains ‘dry’ analysis

Provisional Planning of 28th Typing Study (2023)

Week	Date	Subject
39	Week of 25 September	Emailing of the link to the registration form for the typing study. Please **register by 20 October 2023** at the latest.
43	Week of 23 October	Emailing of the protocol 2023.
45	Monday 6 November	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
45	Week of 6 November	*Upon receipt*: Starting the identification of the strains, according to the usual practice of the laboratory. Sending the link for the result form on Serotyping to the participants. Sending the link for the result form on WGS Cluster Analysis to the participants in a separate email.
50	15 December 2023 at the latest	Deadline for completing the electronic submission of **Serotyping** results: **15 December 2023.** After this deadline, the result form for serotyping will be closed.
5	31 January 2024 at the latest	Deadline for completing the electronic submission of **WGS Cluster Analysis** results: **31 January 2024.**

Still option for MLVA evaluation?

-Within the 20 strains for Serotyping:

1x Enteritidis
1x Typhimurium
1x monophasic variant Typhimurium

-Submit MLVA results on 3 strains
-Compare those results among the MLVA participants (minimum of 3)
-Will be asked for in registration form

Thank you for your attention and your participation !

Hartelijk dank !