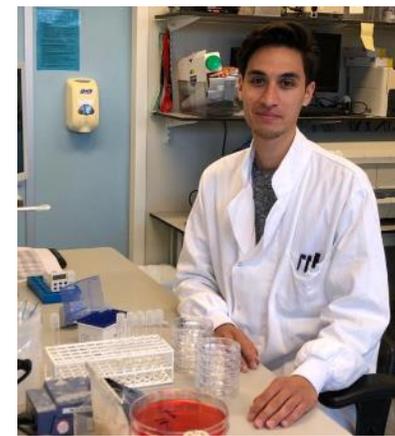






# RIVM typing experts

- › Serotyping:
  - Anjo Verbruggen
- › MLVA:
  - Kim van der Zwaluw
- › Cluster Analysis:
  - Maaïke van den Beld
  - Angela van Hoek
  - Robin Diddens
  - The IDS-bioinformatics team
- Sample preparations:
  - Wendy van Overbeek
- › EURL-*Salmonella* contact typing studies:
  - Wilma Jacobs



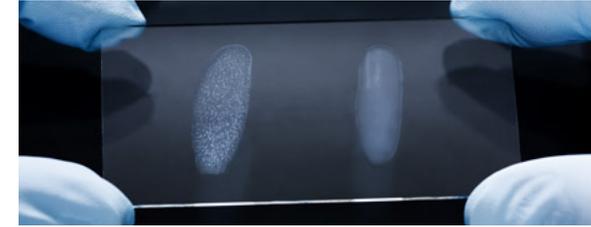


# Participants PT Typing 2021



- › National Reference Laboratories for *Salmonella* from all **27 EU** member states
- › Kosovo, Republic of North Macedonia, Serbia, Turkey (EU candidate countries)
- › Iceland, Norway, Switzerland (EFTA countries)
- › United Kingdom (third country)
  
- › Serotyping
  - **35** participants
- › Third PT Cluster Analysis
  - **19** participants





# Part on Serotyping (26<sup>th</sup> 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)
- › 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
S16	4, [5], 12	a	1, 7	Arechavaleta
S3*	<u>1</u> , 4, 12, 27	b	e, n, z15	Wagenia
S9*	<u>1</u> , 4, 12, [27]	b	l, w	Wien
<b>S19</b>	<b><u>1</u>, 4, [5], 12</b>	<b>i</b>	<b>1, 2</b>	<b>Typhimurium</b>
<b>S8</b>	<b><u>1</u>, 4, [5], 12</b>	<b>i</b>	-	<b>4, 5, 12:i:-</b>
S4	4, [5], 12	l, v	e, n, z15	Brandenburg
S6	6, 7, <u>14</u>	e, h	e, n, z15	Braenderup
S7	{6, 7, <u>14</u> } { <u>54</u> }	g, m, [p], s	[1, 2, 7]	Montevideo
<b>S10</b>	<b>6, 7, <u>14</u></b>	<b>r</b>	<b>1, 2</b>	<b>Virchow</b>
<b>S13</b>	<b>6, 7, <u>14</u></b>	<b>r</b>	<b>1, 5</b>	<b>Infantis</b>

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



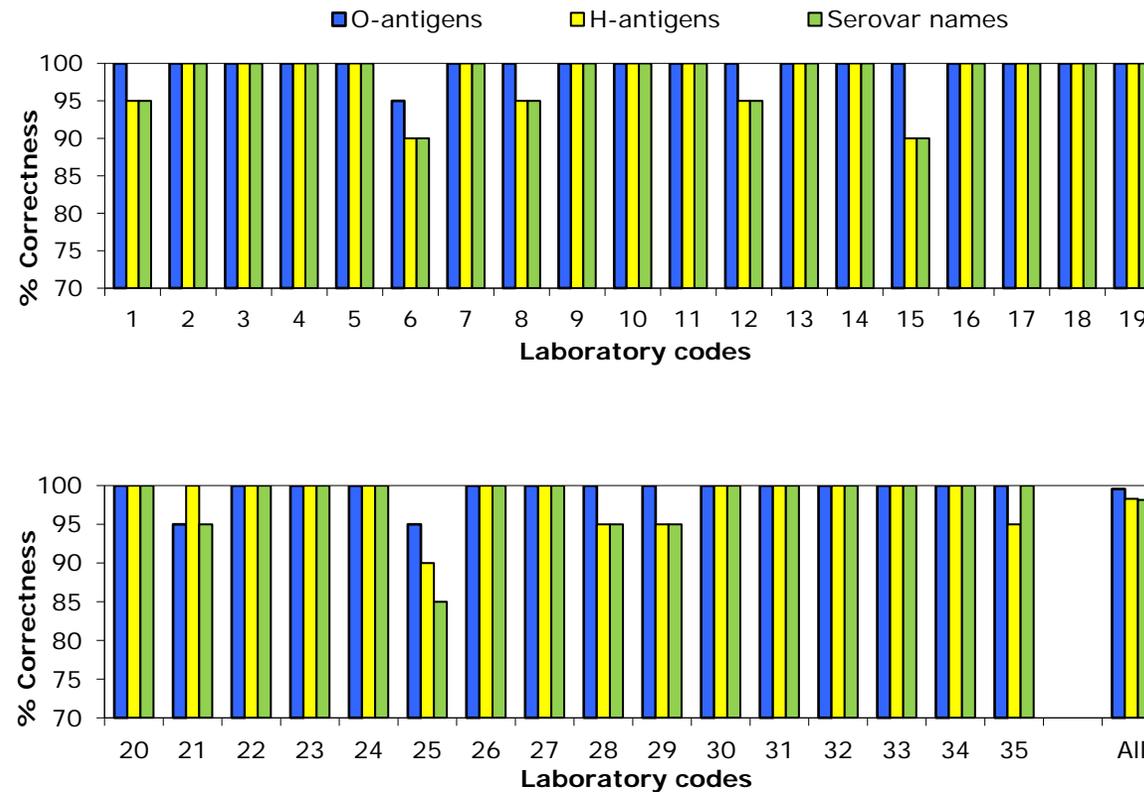
# Salmonella strains (2)

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar
S18	6,8, <u>20</u>	r,[i]	1,5	Bovismorbificans
<b>S15</b>	<b>6,8</b>	<b>z10</b>	<b>e,n,x</b>	<b>Hadar</b>
S12	8, <u>20</u>	z38	-	Apeyeme
<b>S1</b>	<b><u>1</u>,9,12</b>	<b>g,m</b>	-	<b>Enteritidis</b>
S11	<u>1</u> ,9,12	l,z13	e,n,x	Napoli
S17	3,{10}{ <u>15</u> }{ <u>15</u> ,34}	e,h	1,6	Anatum
S5	3,{10}{ <u>15</u> }{ <u>15</u> ,34}	y	1,5	Orion
S2*	11	e,h	1,2	Chingola
S20	<u>1</u> ,13,23	z29	-	Cubana
S14	28	z10	e,n,x	Umbilo
S21	50	r	1,5,(7)	50:r:1,5 (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 26/35 (74%) participants
- › 10 serovars completely correct identified by all participants:
  - Enteritidis (S1), Chingola (S2), Braenderup (S6), Montevideo (S7), Wien (S9), Virchow (S10), Infantis (S13), Hadar (S15), Anatum (S17), and Typhimurium (S19).

# Overview



Lab: REF	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	Lab: REF
	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4, 5, 12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	
1	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4, 5, 12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Kisangani	Anatum	Bovismorbificans	Typhimurium	Cubana	1
2	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	Typhimurium, monophasic (4,5,12:i:-)	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Archavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	2
3	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	3
4	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	Monophasic Salmonella typhimurium	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	4
5	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	5
6	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:-	Wien	Virchow	Nordrhein Albany	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	6
7	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	7
8	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	monophasic Typhimurium	Wien	Virchow	Lomalinda	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	8
9	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,[5],12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	9
10	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	10
11	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	11
12	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:e,n,x	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Archavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	12
13	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	13
14	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	14
15	Enteritidis	Chingola	Wagenia	Brandenburg	/	Braenderup	Montevideo	4,5,12:i:-	Wien	Virchow	Lomalinda	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	15
16	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	16
17	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	17
18	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	18
19	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	19
20	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	20
21	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	-:r:1,5	Typhimurium	Cubana	21
22	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	22
23	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	23
24	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	24
25	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:-	Wien	Virchow	Napoli	8,20:HME:-	Infantis	Djibuti	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	13,23:HME:-	25
26	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	Typhimurium monophasic Variant	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	26
27	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	Monophasic 4,5,12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	27
28	Enteritidis	Chingola	Abony	Brandenburg	Orion	Braenderup	Montevideo	1,4,5,12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	28
29	Enteritidis	Chingola	Wagenia	Kimuenza	Orion	Braenderup	Montevideo	4,5,12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	29
30	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	30
31	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	1,4,[5],12:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	31
32	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5:i:-	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	32
33	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4, 5, 12: i: -	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	33
34	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:- (mST)	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	34
35	Enteritidis	Chingola	Wagenia	Brandenburg	Orion	Braenderup	Montevideo	4,5,12:i:- (monophasic Typhimurium)	Wien	Virchow	Napoli	Apeyeme	Infantis	Umbilo	Hadar	Arechavaleta	Anatum	Bovismorbificans	Typhimurium	Cubana	35
X	0	0	1	1	0	0	0	1	0	0	3	1	0	1	0	1	0	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



# Details

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	Lab code
S-3	1,4,12,27	b	e,n,z15	Wagenia	REF
S-3	4,12,27	b	e,n,x	Abony	28
S-4	4,[5],12	l,v	e,n,z15	Brandenburg	REF
S-4	4	l,v	e,n,z15	Brandenbourg	21
S-4	4	l,v	e,nZ15	Brandenburg	23
S-4	4,12	l,v	e,n,x	Kimuenza	29
S-5	3,{10}{15}{15,34}	y	1,5	Orion	REF
S-5	3,10	y	/	/	15
S-6	6,7,14	e,h	e,n,z15	Braenderup	REF
S-6	6,7	e,h	e,nZ15	Braenderup	23
S-8	1,4,[5],12	i	-	4,5,12: i: -	REF
S-8	4,5,12	i	e,n,x	4,5,12: i: e,n,x	12
S-8	4,5,12	i	1,2	4,5,12: i: - (monophasic Typhimurium)	35
S-11	1,9,12	l,z13	e,n,x	Napoli	REF
S-11	9,46	l,z13,z28	e,n,z15	Nordrhein	6
S-11	9	a	e,n,x	Lomalinda	8
S-11	9	a	e,n,x	Lomalinda	15
S-12	8,20	z38	-	Apeyeme	REF
S-12	8,20	z4,z24	-	Albany	6
S-12	8,20	HME	-	8,20: HME: -	25
S-14	28	z10	e,n,x	Umbilo	REF
S-14	17	z10	e,n,x	Djibuti	25
S-15	6,8	z10	e,n,x	Hadar	REF
S-15	6,8	Z10	e,n,x	Hadar	11
S-16	4,[5],12	a	1,7	Archavaleta	REF
S-16	4,5,12	a	1,2	Kisangani	1
S-16	4,5,12	a	1,7	Archavaleta	2
S-16	4,5,12	a	1,7	Archavaleta	12
S-18	6,8,20	r,[i]	1,5	Bovismorbificans	REF
S-18	-	r	1,5	- : r: 1,5	21
S-18	6,8	r	s	Bovismorbificans	32
S-20	1,13,23	z29	-	Cubana	REF
S-20	13,23	HME	-	13,23: HME: -	25

	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

# Additional strain S21



> Examined by 34/37 labs in PT 2020; by 32/35 in PT 2021

Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	Lab code
S-21	50	r	1,5,(7)	IIIb 50:r:1,5	REF
S-21	11	r	1,5	senegal	22
S-21	50	-	1,5	50:-:1,5	6
S-21	50	r	1,5,7	50: r: 1,5,7	21
S-21	50	?	?	Subspec III**	31
S-21	IIIa 50	r	1,5,7	IIIa 50:r:1,5,7	96
S-21	61	r	1,5,7		10
S-21	61	r	5	IIIa arizonae	28
S-21	61	r	1,5,7	Diarizonae	30
S-21	-	-	-	-:-	16
S-21	?	r	5	OME + : r : 5	24
S-21	OME	r	1,5,7	OME : r : 1,5,7 (IIIb)	11

in variations:

S-21	50	r	1,5	Salmonella enterica subspecies diarizonae 50:r:1,5 (IIIb)	23x
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Strain code	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar	Lab code
S-21	50	r	1,5,(7)	50:r:1,5,(7) (IIIb)	REF
S-21	50	r	1,5,7	50:r:1,2,7	16
S-21	50	r		S. enterica ssp. diarizonae IIIb	11
S-21	50	l,v	z67	(VI)50:l,v:z67	29
S-21	50 or 61	r	1,5,7	IIIb 50 or 61:r:1,5,7	10
S-21	61	r	1,5,7	61:r:1,5,7	12
S-21	61	r	1,5,7	61:r:1,5,7 (IIIb)	18
S-21	61	r	5,7	61:r:5,7 (IIIb)	32
S-21	OME	r	1,5,7	OME : r : 1,5,7 (IIIb)	6
S-21	OME	-	-	OME: -:-	25
S-21	-	-	-	-	8

in variations:

S-21	50	r	1,5	Salmonella enterica subspecies diarizonae 50:r:1,5 (IIIb)	22 x
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# 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

Lab code	Penalty points	Good performance
1	1	yes
2	0	yes
3	0	yes
4	0	yes
5	0	yes
6	2	yes
7	0	yes
8	1	yes
9	0	yes
10	0	yes
11	0	yes
12	4	no
13	0	yes
14	0	yes
15	1	yes
16	0	yes
17	0	yes
18	0	yes
19	0	yes

Lab code	Penalty points	Good performance
20	0	yes
21	0	yes
22	0	yes
23	0	yes
24	0	yes
25	1	yes
26	0	yes
27	0	yes
28	1	yes
29	1	yes
30	0	yes
31	0	yes
32	0	yes
33	0	yes
34	0	yes
35	0	yes





# Follow-up study 1 participant

## Performance

### Follow-up on EURL-Salmonella PT Serotyping 2021



Number of penalty points: 0

Evaluation:  
Good Performance



Strain	Reference Results				Results labcode: 12			
	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar name	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar name
SF1	<u>1</u> ,9,12	g,m	-	Enteritidis	9, 12	g, m	-	S.Enteritidis
SF2	4,12	i	1,6	Agama	4, 12	i	1, 6	S.Agama
SF3	8, <u>20</u>	r,[i]	z6	Altona	8, 20	r	z <sub>6</sub>	S.Altona
SF4	<u>1</u> ,4,[5],12	f,g,s	[1,2]	Agona	4, 12	f, g, s	-	S.Agona
SF5	<u>1</u> ,4,[5],12	i	1,2	Typhimurium	4, 5, 12	i	1, 2	S.Typhimurium
SF6 <sup>a)</sup>	<u>1</u> ,4,[5],12	i	-	4,5,12:i:-	4, 12	i	-	<b>S.enterica subsp. enterica</b>
SF7	6,7, <u>14</u>	z10	l,w	Jerusalem	6, 7	Z <sub>10</sub>	l, w	S.Jerusalem
SF8	<u>1</u> ,4,[5],12	i	1,5	Lagos	4, 12	i	5	S.Lagos
SF9	3,{10}{ <u>15</u> }{15,34}	e,h	l,w	Meleagridis	3, 10	e, h	l, w	S.Meleagridis
SF10 <sup>b)</sup>	4,[5],12	i	e,n,x	Farsta	4, 5, 12	i	e, n, x	S.Farsta

<sup>a)</sup> monophasic variant of Typhimurium as determined by PCR.

<sup>b)</sup> Updated antigenic formula according to:

Issenhuth-Jeanjean et al., Supplement 2008-2010 (no. 48) to the White-Kauffmann-Le Minor scheme. Research in Microbiology, 2014, 165, pp. 526-530.

Lab 12: PCR analysis of the samples SF1, SF5 and SF6 was performed according to the method described in Maurischat et al 2015.

Results were as follows:

SF1- S. Enteritidis

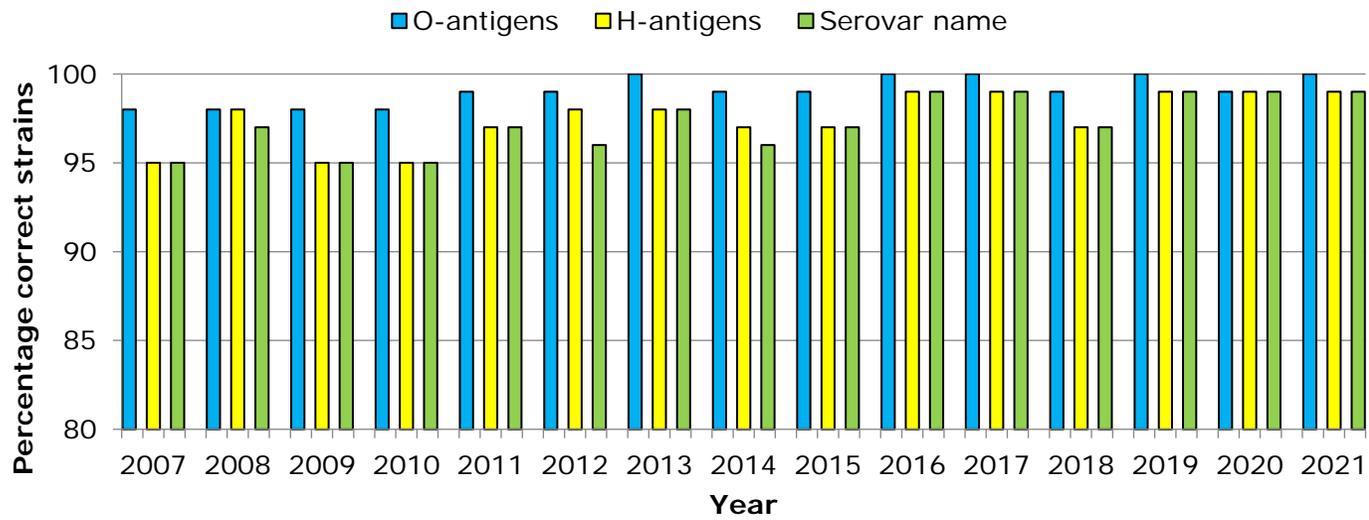
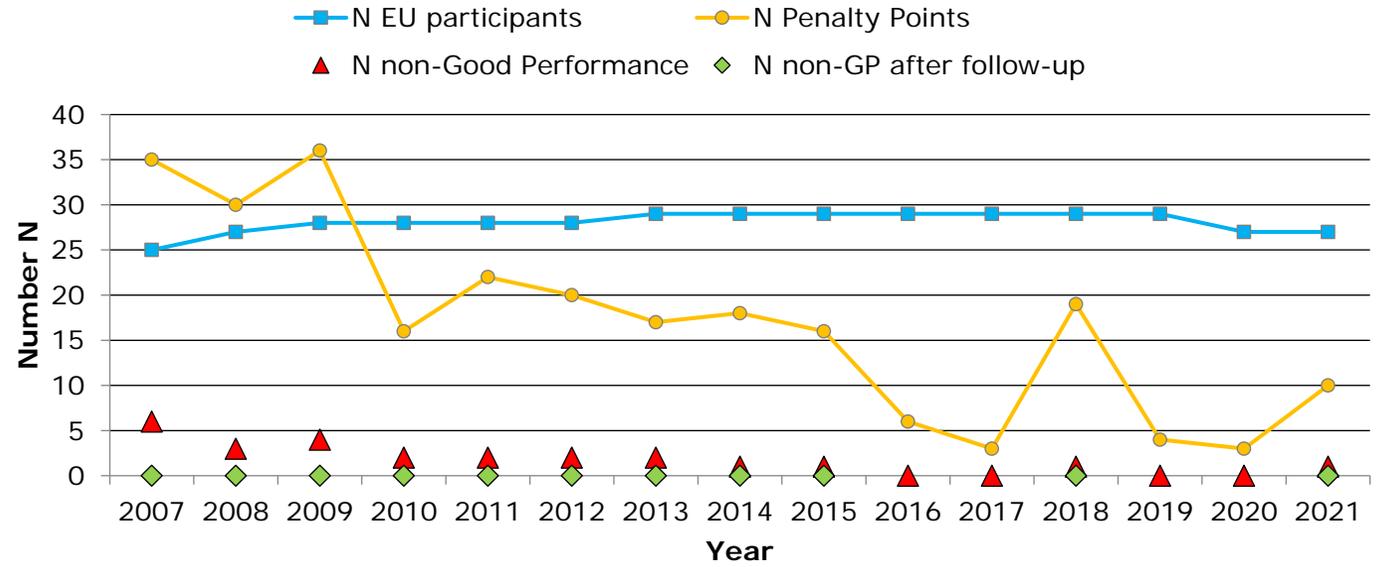
SF5- S. Typhimurium

SF6- monophasic S. Typhimurium

**NOTE** that the preferred reporting for the serovar name of strain SF6 would have been: 4,12:i:-



# Results in time (EU NRLs only)





## Conclusions PT serotyping 2021 (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 26/35 (74%) participants
  - 6 participants with 1 penalty point
  - 1 participant with 2 penalty points
  - 1 participant with 4 penalty points
  
- › 34 participants “Good Performance”
- › 1 participant “Good Performance” after Follow-up





## Part on Cluster Analysis (third 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



# Evaluation cluster analysis per methodology

- › Participants were asked to report per strain if a clustering match was found with the reference outbreak strain (**21SCA-REF**)
  - Comparing the participants' results to the expected results, as pre-defined by the EURL-*Salmonella* (Protocol PT Typing 2021)

Like in 2020, the cluster analysis 2021 is mimicking an outbreak situation, with a *Salmonella* Enteritidis ST11, MLVA type 03-10-04-04-01 as the reference strain. WGS data on this strain (fastq-files) will be made available through a secure ftp server.

For this particular PT 2021 situation, the cluster definition is set at maximum 7 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 **21SCA06** and **21SCA09** to be (part of) one cluster
- › Deviations from the expected results are indicated in blue

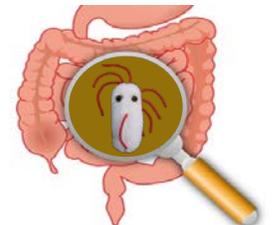


# Strains *Salmonella* Cluster Analysis PT 2021

- › 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.
- › 9 strains with stable and consistent MLVA and cgMLST results selected for inclusion in the PT
- › Technical duplicates: 21SCA06 and 21SCA09
  - Shipment tubes prepared from the same blood agar plate culture

Strain code	Serovar	ST	MLVA-profile	Origin
21SCA01	Enteritidis	11	2-9-9-4-2	Human
21SCA02	Enteritidis	183	2-11-9-3-1	Human
21SCA03	Enteritidis	183	2-11-9-3-1	Human
21SCA04	Enteritidis	11	3-10-4-4-1	Human
21SCA05	Enteritidis	1925	3-10-5-4-1	Human
<b>21SCA06</b> <sup>a)</sup>	Enteritidis	11	3-10-4-4-1	Human
21SCA07	Enteritidis	3406	2-14-NA-7-NA	Human
21SCA08	Enteritidis	11	3-10-4-4-1	Human
<b>21SCA09</b> <sup>a)</sup>	Enteritidis	11	3-10-4-4-1	Human
21SCA10	Enteritidis	11	1-10-7-3-2	Human

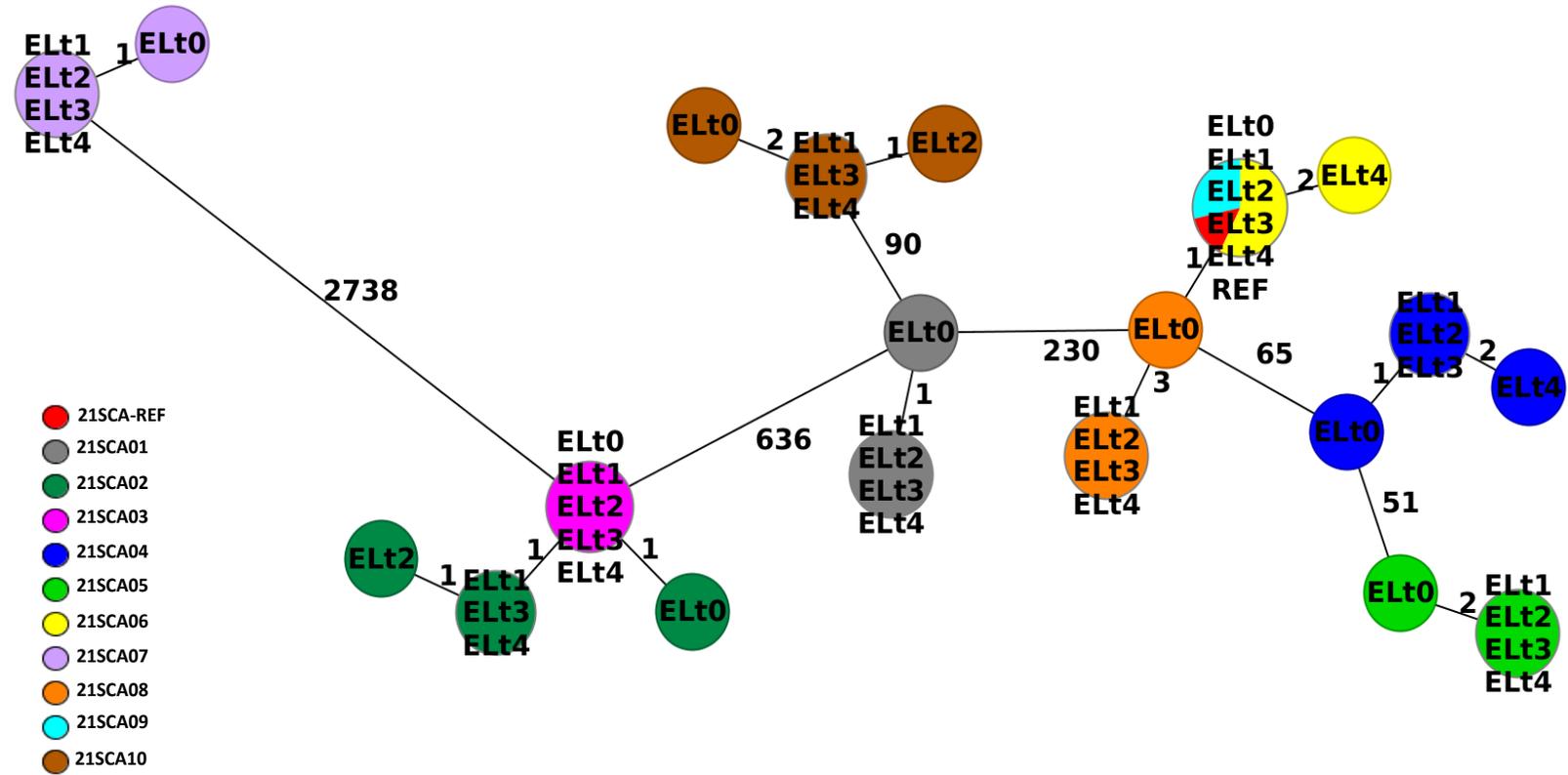
<sup>a)</sup> Technical duplicates (in bold)





# CA strains, (pre-)testing data MLVA/WGS

- ELt0: original WGS data from the human surveillance strains in 2019
- ELt1: WGS data initial pre-testing
- ELt2: WGS data after 10 times sub-culturing
- ELt3: WGS data at the start of the PT (November 2021)
- ELt4: WGS data at the end of the data submission period (February 2022)
- Identical MLVA data for all strains on t0, t1, t2, t3, t4





# 19 Participants in Cluster Analysis PT 2021

- › 5 x MLVA
- › 19 x WGS (23 submissions)
  - 2 participants: both cgMLST-based and reference-based SNP data
  - 1 participant: cgMLST-based as well as both assembly- and reference-based SNP data



	2019	2020	2021
PFGE	6	2	na
MLVA	8	6	5
WGS	14	21	19
Overall	18	21	19



# Individual results MLVA (for information only)

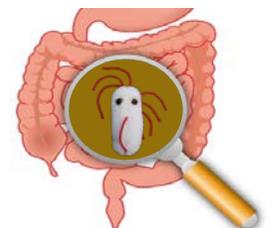
› Loci: SENTR7-SENTR5-SENTR6-SENTR4-SE-3

Labcode	21SCA01	21SCA02	21SCA03	21SCA04	21SCA05
<b>Expected</b>	<b>2-9-9-4-2</b>	<b>2-11-9-3-1</b>	<b>2-11-9-3-1</b>	<b>3-10-4-4-1</b>	<b>3-10-5-4-1</b>
6	2-9-9-4-2	2-11-9-3-1	2-11-9-3-1	3-10-4-4-1	3-10-5-4-1
7	2-9-9-4-2	2-11-9-3-1	2-11-9-3-1	3-10-4-4-1	3-10-5-4-1
11	2-9-9-4-2	2-11-9-3-1	2-11-9-3-1	3-10-4-4-1	3-10-5-4-1
23	2-9-9-4-2	2-11-9-3-1	2-11-9-3-1	3-10-4-4-1	3-10-5-4-1
35	2-9-9-4-2	2-11-9-3-1	2-11-9-3-1	3-10-4-4-1	3-10-5-4-1

Labcode	21SCA06 <sup>a)</sup>	21SCA07	21SCA08	21SCA09 <sup>a)</sup>	21SCA10
<b>Expected</b>	<b>3-10-4-4-1</b>	<b>2-14-NA-7-NA</b>	<b>3-10-4-4-1</b>	<b>3-10-4-4-1</b>	<b>1-10-7-3-2</b>
6	3-10-4-4-1	2-14-NA-7-NA	3-10-4-4-1	3-10-4-4-1	1-10-7-3-2
7	3-10-4-4-1	2-14-NA-7-NA	3-10-4-4-1	3-10-4-4-1	NA-10-7-3-2
11	3-10-4-4-1	2-14-NA-7-NA	3-10-4-4-1	3-10-4-4-1	1-10-7-3-2
23	3-10-4-4-1	2-14-0-7-0	3-10-4-4-1	3-10-4-4-1	1-10-7-3-2
35	3-10-4-4-1	2-14-NA-7-NA	3-10-4-4-1	3-10-4-4-1	1-10-7-3-2

<sup>a)</sup> Technical duplicates.

  Deviation from the expected result





# Evaluation MLVA-based cluster analysis (1)

- › 5 participants
- › Cluster definition from Protocol PT Typing 2021
  - no loci with a different number of repeats by MLVA
- › Outbreak strain (21SCA-REF): *Salmonella* Enteritidis, ST11, MLVA type 3-10-4-4-1
- › Participants' results compared to the expected results
  - Technical duplicates 21SCA06 and 21SCA09 within one cluster



# Evaluation MLVA-based cluster analysis (2)

- MLVA-cluster: 21SCA04, 21SCA06, 21SCA08 and 21SCA09
  - (second MLVA-cluster: 21SCA02, 21SCA03)

Strain code	Serovar	ST	MLVA-profile
21SCA01	Enteritidis	11	2-9-9-4-2
21SCA02	Enteritidis	183	2-11-9-3-1
21SCA03	Enteritidis	183	2-11-9-3-1
21SCA04	Enteritidis	11	3-10-4-4-1
21SCA05	Enteritidis	1925	3-10-5-4-1
<b>21SCA06<sup>a)</sup></b>	Enteritidis	11	3-10-4-4-1
21SCA07	Enteritidis	3406	2-14-NA-7-NA
21SCA08	Enteritidis	11	3-10-4-4-1
<b>21SCA09<sup>a)</sup></b>	Enteritidis	11	3-10-4-4-1
21SCA10	Enteritidis	11	1-10-7-3-2

<sup>a)</sup> Technical duplicates (in bold)

Labcode	21 SCA01	21 SCA02	21 SCA03	21 SCA04	21 SCA05	21 SCA06 <sup>a)</sup>	21 SCA07	21 SCA08	21 SCA09 <sup>a)</sup>	21 SCA10
<b>Expected</b>	<b>No</b>	<b>No</b>	<b>No</b>	<b>Yes</b>	<b>No</b>	<b>Yes</b>	<b>No</b>	<b>Yes</b>	<b>Yes</b>	<b>No</b>
6	No	No	No	Yes	No	Yes	No	Yes	Yes	No
7	No	No	No	Yes	No	Yes	No	Yes	Yes	No
11	No	No	No	Yes	No	Yes	No	Yes	Yes	No
23	No	No	No	Yes	No	Yes	No	Yes	Yes	No
35	No	No	No	Yes	No	Yes	No	Yes	Yes	No

<sup>a)</sup> Technical duplicates.

- 5/5 participants reported as expected
  - Technical duplicates 21SCA06 and 21SCA09 within one cluster



# WGS protocols used by the 19 participants

Labcode	Wet lab <sup>a)</sup>	WGS platform	Data analysis	Tool for analysis	Method for cluster analysis
1	In-Out-Out	Illumina NovaSeq	SNP-based - assembly-based	CSIPhylogeny 1.4	Maximum likelihood (ML)
2	In-In-In	Illumina MiSeq	SNP-based - assembly-based	In house pipeline	Maximum likelihood (ML)
6-cgMLST	In-Out-Out	Illumina MiSeq	cgMLST-based	PyMLST v1	hierarchical clustering
6-SNPa	In-Out-Out	Illumina MiSeq	SNP-based - assembly-based	Roary and Prank	Maximum likelihood (ML)
6-SNP <sub>r</sub>	In-Out-Out	Illumina MiSeq	SNP-based - reference-based	BWA, bcftools, RAxML	Maximum likelihood (ML)
7	In-In-In	Illumina MiSeq	cgMLST-based	Ridom SeqSphere	Minimum Spanning Tree (MST)
10-cgMLST	In-Out-Out	Illumina NovaSeq	cgMLST-based	Ridom SeqSphere	Minimum Spanning Tree (MST)
10-SNP <sub>r</sub>	In-Out-Out	Illumina NovaSeq	SNP-based - reference-based	in-house pipeline iVARCall2	Maximum likelihood (ML)
11	In-In-Out	Illumina NextSeq	SNP-based - reference-based	MINTyper 1.0	Minimum Spanning Tree (MST)
12	In-In-In	Illumina MiSeq	cgMLST-based	Linux cgmlst finder	Minimum Spanning Tree (MST)
14	In-In-In	Illumina MiniSeq	cgMLST-based	Ridom SeqSphere	Minimum Spanning Tree (MST)
16	In-In-In	Illumina MiSeq	cgMLST-based	Ridom SeqSphere	Minimum Spanning Tree (MST)
19-cgMLST	In-In-In	Illumina NextSeq	cgMLST-based	inhouse automated ChewieSnake Pipeline	single linkage hierarchical clustering
19-SNP <sub>r</sub>	In-In-In	Illumina NextSeq	SNP-based - reference-based	inhouse automated SnippySnake Pipeline	single linkage hierarchical clustering
21	In-In-In	Illumina MiSeq	SNP-based - reference-based	In-house pipeline	Minimum Spanning Tree (MST)
22	In-In-In	Illumina NextSeq	cgMLST-based	Ridom SeqSphere	Minimum Spanning Tree (MST)
23	In-In-In	Illumina MiSeq	cgMLST-based	ChewBBaCa	Minimum Spanning Tree (MST)
24	In-In-In	Illumina MiSeq	cgMLST-based	BioNumerics	Minimum Spanning Tree (MST)
26	In-Out-Out	Illumina NovaSeq	cgMLST-based	chewbbaca	Neighbor joining (NJ)
27	In-In-In	Illumina NextSeq	SNP-based - reference-based	Snippy, Gubbins	Maximum likelihood (ML)
30	In-In-In	Illumina MiSeq	SNP-based - assembly-based	CGE CSIPhylogeny 1.4	ML included in CSYPhylogeny
34	In-In-In	Illumina NextSeq	cgMLST-based	EnterobaseChewBBACA	MSTreeV2
35	In-In-In	Illumina MiSeq	cgMLST-based	in-house Galaxy pipeline	Neighbor joining (NJ)
EURL-Salm	In-In-In	Illumina NextSeq	cgMLST-based	Ridom SeqSphere	Minimum Spanning Tree (MST)



# Data quality criteria used by the participants

- › Comparable to the PT Typing 2019 and 2020
- › Variety in naming, as well as in thresholds
- › Complete overview will be included in the Full Report PT Typing 2021

	md5 checksum	
Labcode	21SCA_REF_R1.fq.gz	21SCA_REF_R2.fq.gz
<b>Expected</b>	<b>206064240c9fd1f9b89bbd74db4c31d6</b>	<b>4eb99f380cd3d3ca72642079528d4506</b>
1	206064240C9FD1F9B89BBD74DB4C31D6	4EB99F380CD3D3CA72642079528D4506
2		
6	206064240c9fd1f9b89bbd74db4c31d6	4eb99f380cd3d3ca72642079528d4506
7	206064240c9fd1f9b89bbd74db4c31d6	4eb99f380cd3d3ca72642079528d4506
10	<b>e8e2aaff2830d4c2833d7ca203ecba01</b>	<b>4078d467927d6ae2573984a2a8e02a0c</b>
11	206064240c9fd1f9b89bbd74db4c31d6	4eb99f380cd3d3ca72642079528d4506
12	206064240c9fd1f9b89bbd74db4c31d6	4eb99f380cd3d3ca72642079528d4506
14		
16	206064240c9fd1f9b89bbd74db4c31d6	4eb99f380cd3d3ca72642079528d4506
19	206064240c9fd1f9b89bbd74db4c31d6	4eb99f380cd3d3ca72642079528d4506
21	206064240c9fd1f9b89bbd74db4c31d6	4eb99f380cd3d3ca72642079528d4506
22	206064240c9fd1f9b89bbd74db4c31d6	4eb99f380cd3d3ca72642079528d4506
23		
24	NA	NA
26	206064240c9fd1f9b89bbd74db4c31d6	4eb99f380cd3d3ca72642079528d4506
27	206064240c9fd1f9b89bbd74db4c31d6	4eb99f380cd3d3ca72642079528d4506
30	206064240c9fd1f9b89bbd74db4c31d6	4eb99f380cd3d3ca72642079528d4506
34	206064240c9fd1f9b89bbd74db4c31d6	4eb99f380cd3d3ca72642079528d4506
35		
EURL-Salm	206064240c9fd1f9b89bbd74db4c31d6	4eb99f380cd3d3ca72642079528d4506

md5 checksum	
21SCA_REF_R1.fq	21SCA_REF_R2.fq
<b>e8e2aaff2830d4c2833d7ca203ecba01</b>	<b>4078d467927d6ae2573984a2a8e02a0c</b>

Md5 checksum PT 2020: 11/21  
PT 2021: 14/19



# Evaluation WGS-based cluster analysis (1)

- › 19 participants (23 submissions)
- › Cluster definition from Protocol PT Typing 2021
  - set at maximum 7 allelic differences from the reference
- › 21SCA-REF: *Salmonella* Enteritidis, ST11, MLVA type 3-10-4-4-1
  - 21SCA\_REF\_R1.fq.gz & 21SCA\_REF\_R2.fq.gz
- › Participants' results compared to the expected results
  - Technical duplicates 21SCA06 and 21SCA09 within one cluster





<u>Labcode-method</u>	21 SCA01	21 SCA02	21 SCA03	21 SCA04	21 SCA05	21 SCA06 <sup>a)</sup>	21 SCA07	21 SCA08	21 SCA09 <sup>a)</sup>	21 SCA10
<b>Expected</b>	No	No	No	No	No	Yes	No	Yes	Yes	No
1-SNP <sub>a</sub>	No	No	No	No	No	Yes	No	No	Yes	No
2-SNP <sub>a</sub>	No	No	No	No	No	Yes	No	Yes	Yes	No
6-cgMLST	No	No	No	No	No	Yes	No	Yes	Yes	No
6-SNP <sub>a</sub>	No	No	No	No	No	Yes	No	Yes	Yes	No
6-SNP <sub>r</sub>	No	No	No	No	No	Yes	No	Yes	Yes	No
7-cgMLST	No	No	No	No	No	Yes	No	Yes	Yes	No
10-cgMLST	No	No	No	No	No	Yes	No	Yes	Yes	No
10-SNP <sub>r</sub>	No	No	No	No	No	Yes	No	Yes	Yes	No
11-SNP <sub>r</sub>	No	No	No	No	No	Yes	No	Yes	Yes	No
12-cgMLST	No	No	No	No	No	Yes	No	Yes	Yes	No
14-cgMLST	No	No	No	No	No	Yes	No	Yes	Yes	No
16-cgMLST	No	No	No	No	No	Yes	No	Yes	Yes	No
19-cgMLST	No	No	No	No	No	Yes	No	No	Yes	No
19-SNP <sub>r</sub>	No	No	No	No	No	Yes	No	No	Yes	No
21-SNP <sub>r</sub>	No	No	No	No	No	Yes	No	No	Yes	No
22-cgMLST	No	No	No	No	No	Yes	No	Yes	Yes	No
23-cgMLST	No	No	No	No	No	Yes	No	Yes	Yes	No
24-cgMLST	No	No	No	No	No	Yes	No	Yes	Yes	No
26-cgMLST	No	No	No	No	No	Yes	No	No	Yes	No
27-SNP <sub>r</sub>	No	No	No	No	No	Yes	No	No	Yes	No
30-SNP <sub>a</sub>	No	No	No	No	No	Yes	No	No	Yes	No
34-cgMLST	No	No	No	No	No	Yes	No	No	Yes	No
35-cgMLST	No	No	No	No	No	Yes	No	No	Yes	No

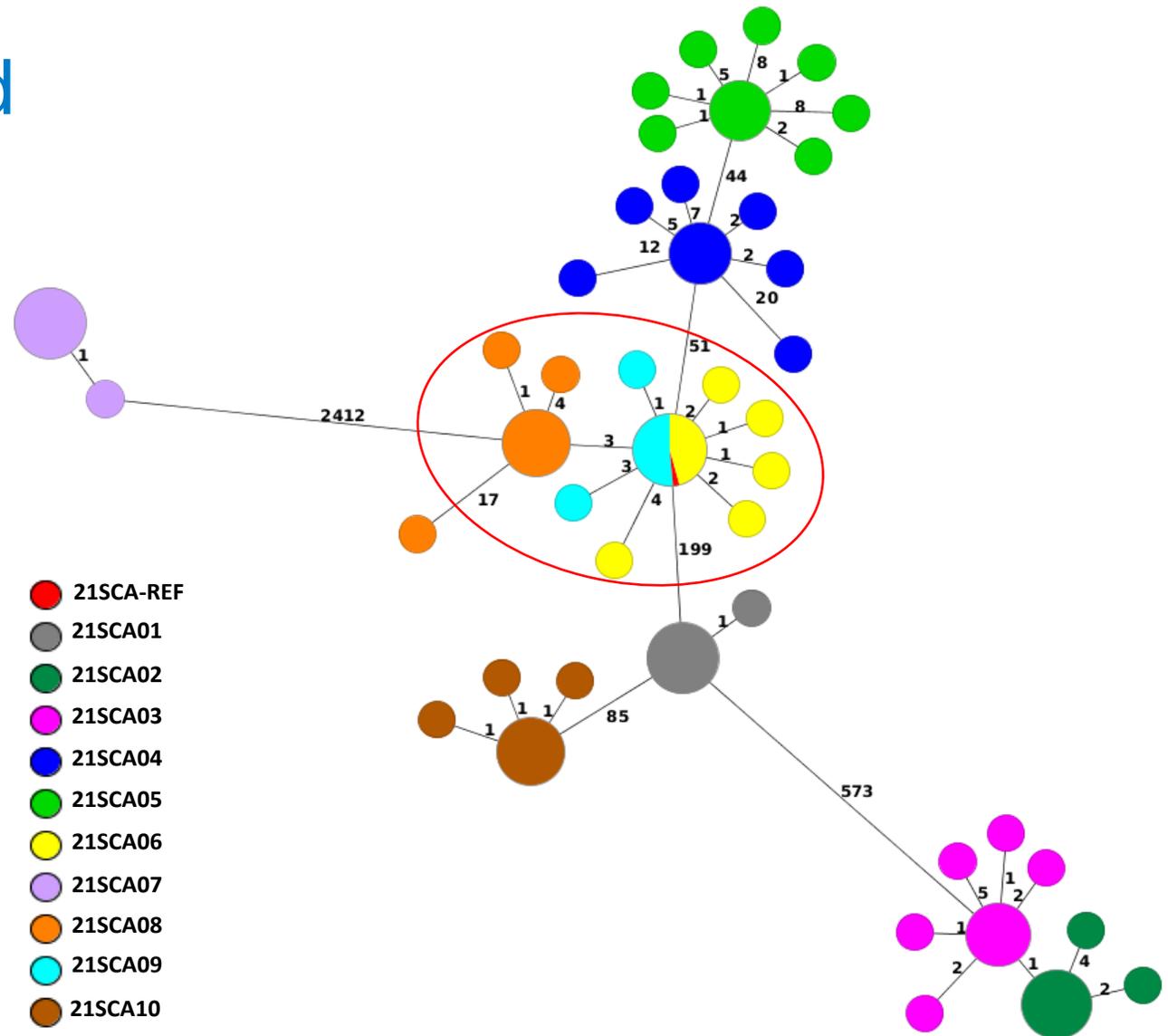
- › 14/23 submissions reported as expected
  - Technical duplicates 21SCA06 and 21SCA09 within one cluster in all submissions



# Evaluation WGS-based cluster analysis (3)

## > MST of the strains from the participants' processed raw data

- Juno Assembly\_pipeline
- Ridom SeqSphere+, cgMLST (3002), pairwise ignoring missing values
- NB: Allelic differences not on scale





# Several remarks from participants...

- › Lab 2: Based on *the criteria with a maximum number of 7 allelic differences* I interpret that this is based on *cgMLST allelic differences*. 21SCA08 has in *our SNP-analysis 10 SNP differences* compared to 21SCA-REF. Assuming that SNP analysis has a higher resolution than cgMLST we have included this strain as part of the cluster.
- › Lab 6: For the cluster definition for WGS SNP-based - *assembly-based* we use at maximum *7 SNPs differences* from the reference sequence.
- › Lab 6: We use at maximum *10 SNPs differences* from the reference sequence for the cluster definition for WGS SNP-based - *reference-based*.
- › Lab 10: Considering a *threshold of 21SNP* (Pightling et al., 2018), strains 21SCA06,08 and 09 are in the same cluster as the reference.
- › Lab 19: *21SCA06 and 21SCA09 showed 0 SNPs* to the reference strain. *SCA08 showed 9 SNPs*. We would report SCA08 as a potential genetic match to be confirmed by another analysis strategy.
- › Lab 19: *21SCA08* showed a genetic distance of *8 allelic differences* to the Reference Sequence. *In real life*, we would inform the involved partners/outbreak team to confirm this result whether these are 7 AD with a second NGS- analysis workflow (e.g. Ridom SeqSphere).

Per submission:

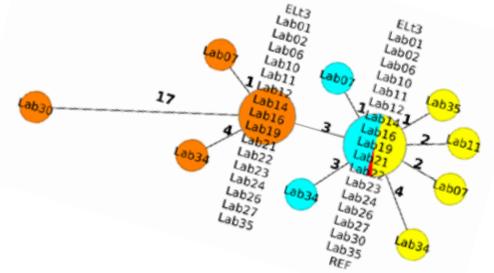


*Distance matrix data*  
(REF versus strains)

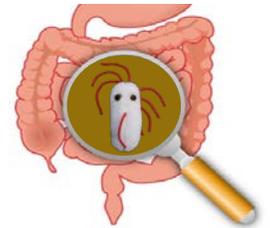
Labcode-method	21SCA-REF	21SCA01	21SCA02	21SCA03	21SCA04	21SCA05	21SCA06	21SCA07	21SCA08	21SCA09	21SCA10		21SCA08
1-SNPa	0	427	1444	1444	136	152	5	29352	11	4	444		No
2-SNPa	0	427	1461	1461	133	149	1	40860	10	1	450		Yes
6-SNPa	0	288	974	977	97	106	0	29800	3	0	302		Yes
30-SNPa	0	420	1435	1436	142	166	0	29291	44	2	441		No
6-SNPPr	0	453	1563	1561	140	157	0	40941	9	0	481		Yes
10-SNPPr	0	455	1571	1567	136	155	2	45908	10	1	476		Yes
11-SNPPr	0	383	1361	1360	129	147	0	29299	9	0	414		Yes
19-SNPPr	0	431	1464	1461	133	146	0	44867	9	0	449		No
21-SNPPr	0	376	1265	1262	105	126	0	37250	8	0	390		No
27-SNPPr	0	401	1378	1378	126	141	1	39910	11	1	424		No
6-cgMLST	0	233	682	680	69	83	0	2745	6	0	234		Yes
7-cgMLST	0	232	681	681	67	82	0	2749	4	0	232		Yes
10-cgMLST	0	232	680	680	67	81	0	2746	4	0	232		Yes
12-cgMLST	0	243	707	708	76	89	2	2767	9	3	248		Yes
14-cgMLST	0	232	680	680	67	81	0	2745	4	0	232		Yes
16-cgMLST	0	232	681	680	67	81	0	2750	4	0	232		Yes
19-cgMLST	0	233	691	692	70	85	1	2716	9	1	238		No
22-cgMLST	0	232	680	681	67	82	0	2750	4	0	232		Yes
23-cgMLST	0	228	691	691	69	85	0	2703	6	0	232		Yes
24-cgMLST	0	200	200	200	69	86	0	200	5	0	200		Yes
26-cgMLST	0	314	928	926	109	127	4	3467	8	3	318		No
34-cgMLST	0	236	697	700	72	86	1	2737	10	2	241		No
35-cgMLST	0,0000	0,0825	0,2425	0,2415	0,0257	0,0307	0,0000	0,9289	0,0039	0,0000	0,0825		No
ELt3-cgMLST	0	232	681	681	67	81	0	2750	4	0	232		Yes



# Conclusions PT Cluster Analysis 2021



- › 19 participants overall; 5 MLVA and 19 (23) WGS
- › And again: a lot of information and interesting data obtained!
- › Extended pre-testing for strain selection
- › Pre-defined “Cluster definitions”, (REF strain, expected results)
- › No performance criteria were set for this PT
  - Participants’ result compared to expected results: MLVA: 5/5, WGS: 14/23
  - Technical duplicates 21SCA06/21SCA09 assigned within 1 cluster: MLVA: 5/5, WGS: 23/23
- › **4<sup>th</sup> PT on (optional) Cluster Analysis in 2022**
  - using MLVA (?) and/or WGS
  - Defined cluster analysis (including SNP-based analysis) on 10 *Salmonella* (SE ?) strains
    - Simulation of an outbreak-related request from the EURL-*Salmonella* (EFSA/ECDC) to the NRL-network
    - A WGS reference sequence will be provided
    - Proposal (?): 5 strains ‘wet’ analysis plus 5 strains ‘dry’ analysis





# Provisional Planning of 27<sup>th</sup> Typing Study (2022)

Week	Date	Subject
39	Week of 26 September	Emailing of the link to the registration form for the typing study. Please <b>register by 14 October 2022</b> at the latest.
43	Week of 24 October	Emailing of the protocol 2022.
45	Monday 7 November	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
45	Week of 7 November	<i>Upon receipt:</i> 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 MLVA and/or WGS Cluster Analysis to the participants in a separate email.
50	16 December 2022 at the latest	Deadline for completing the electronic submission of <b>Serotyping</b> results: <b>16 December 2022</b> . After this deadline, the result form for serotyping will be closed.
	27 January 2023 at the latest	Deadline for completing the electronic submission of <b>MLVA/WGS Cluster Analysis</b> results: <b>27 January 2023</b> .



*Thank you for your attention and your participation !*



*Hartelijk dank !*