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

25 years of Proficiency Tests on serotyping of *Salmonella*

Wilma Jacobs ***et al.***,

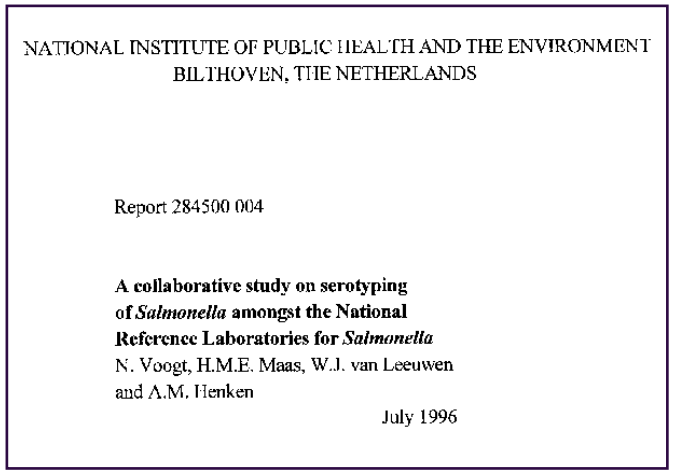
EURL-*Salmonella*, RIVM, Bilthoven

EURL *Salmonella*

Co-funded by the European Union

A bit of history

- RIVM in 1992 designated as the Community Reference Laboratory (CRL) for zoonotic *Salmonella* (92/117/EC)
- First collaborative study on serotyping amongst the NRLs for *Salmonella* took place in 1995
- Still an important task of the EURL [nowadays in (EU) 2017/625]


NATIONAL INSTITUTE OF PUBLIC HEALTH AND THE ENVIRONMENT
BILTHOVEN, THE NETHERLANDS

Report 284500 004

A collaborative study on serotyping of *Salmonella* amongst the National Reference Laboratories for *Salmonella*
N. Voogt, H.M.E. Maas, W.J. van Leeuwen and A.M. Henken

July 1996

Table 1 History of interlaboratory comparison studies on typing of *Salmonella* spp.

Study NRLs	Study ENLs	Year	Type and number of serotyping strains of *Salmonella* spp.	Number and type of phage typing strains	Antibiotic resistance testing	Reference
I		1995	18x spp. *enterica* 1x spp. *salamae* 1x spp. *houtenae*			Voogt et al., 1996 (RIVM report 284500004)
II		1996/ 1997	20x spp. *enterica*			Voogt et al., 1997 (RIVM report 284500008)
III		1998	20x spp. *enterica*	4 x SE 5x STM		Voogt et al., 1998 (RIVM report 284500010)
IV	I	1999	16x spp. *enterica*	10x SE 10x STM		Raes et al., 2000 (RIVM report 284500013)
V	II	2000	18x spp. *enterica* 1x spp. *salamae* 1x spp. *houtenae*	10x SE 10x STM	YES	Raes et al., 2001 (RIVM report 284500016)
VI	III	2001	19x spp. *enterica* 1x spp. *arizonae*	10x SE 10x STM	YES	Korver et al., 2002 (RIVM report 284500020)
VII	IV	2002	20x spp. *enterica*	10x SE 10x STM		Korver et al., 2002 (RIVM report 284500022)
VIII	V	2003	20x spp. *enterica*	10x SE 10x STM	YES	Korver et al., 2003 (RIVM report 330300002)
IX	VI	2004	20x spp. *enterica*	10x SE 10x STM	YES	Korver et al., 2005 (RIVM report 330300006)
X	VII	2005	20x spp. *enterica*	10x SE 10x STM	YES	Korver et al. 2006 (RIVM report 330300009
XI	VII	2006	20x spp. *enterica*	10x SE 10x STM		Berk et al. 2006 (RIVM report 330604001)
XII	VIII	2007	20x spp. *enterica*	10x SE 10x STM		This report → Berk et al. 2007 (RIVM report 330604005/2007

Study NRLs	Year	Type and number of strains in the study	Optional part on	Reference
XIII	2008	20x spp. *enterica*	Phage typing SE/STM	Berk, Maas, de Pinna, Mooijman (RIVM report 330604013/2010)
XIV	2009	20x spp. *enterica*	Phage typing SE/STM	Jacobs-Reitsma, Maas, de Pinna, Mooijman (RIVM report 330604021/2011)
XV	2010	20x spp. *enterica*	Phage typing SE/STM	Pol-Hofstad, Jacobs-Reitsma, Maas, de Pinna, Mooijman (RIVM report 330604024/2011)
XVI	2011	20x spp. *enterica* 1 optional uncommon	Phage typing SE/STM	Jacobs-Reitsma, Pol-Hofstad, Maas, de Pinna, Mooijman (RIVM report 330604027/2012)
XVII	2012	20x spp. *enterica* 1 optional uncommon	Phage typing SE/STM	Jacobs-Reitsma, Maas, de Pinna, Mooijman (RIVM report 330604032/2013)
XVIII	2013	20x spp. *enterica* 1 optional uncommon	Phage typing SE/STM PFGE	Jacobs-Reitsma, Maas, de Pinna, Mensink, Mooijman (RIVM report 2014-0009)
XIX	2014	20x spp. *enterica* 1 optional uncommon	Phage typing SE/STM PFGE	Jacobs-Reitsma, Maas, de Pinna, Mensink, Mooijman (RIVM report 2015-0081)
20	2015	20x spp. *enterica* 1 optional uncommon	PFGE	Jacobs-Reitsma, Maas, Bouw, Mooijman (RIVM report 2016-0043)
21	2016	20x spp. *enterica* 1 optional uncommon	PFGE	Jacobs-Reitsma, Verbruggen, Bouw, Mooijman (RIVM report 2017-0082)
22	2017	20x spp. *enterica* 1 optional uncommon	PFGE	Jacobs-Reitsma, Verbruggen, Bouw, Mooijman (RIVM report 2018-0022)
23	2018	20x spp. *enterica* 1 optional uncommon	PFGE	Jacobs-Reitsma, Verbruggen, Bouw, Mooijman (RIVM report 2019-0136)
24	2019	20x spp. *enterica* 1 optional uncommon	Cluster analysis (PFGE/MLVA/WGS)	Jacobs-Reitsma, Verbruggen, Diddens, van Hoek, Mooijman (RIVM report 2020-0084)
25	2020	20x spp. *enterica* 1 optional uncommon	Cluster analysis (PFGE/MLVA/WGS)	Jacobs-Reitsma, Verbruggen, Diddens, van Hoek, Mooijman (RIVM report 2021-0126)

Proficiency Test (PT)

- Collaborative studies, Interlaboratory comparison studies, External Quality Assurance studies (EQA), Proficiency Test (PT) (ISO 19043)

- Within EU Member States (and beyond)
- Of great importance to maintain high quality typing
- Uniformly typed, with comparable results
- Reliable results required in reporting systems for surveillance and for outbreak investigations

Salmonella serotyping in theory

- Gold Standard: “Kauffmann and White” scheme
  - Popoff MY & Le Minor L, 1992, 7th edition
  - Popoff MY, 2001, 8th edition
- Nowadays: “White-Kauffmann-Le Minor” scheme (WKLM)
  - Grimont PAD & Weill F-X, 2007, 9th edition
  - supplements no. 47 (2003-2007) and no. 48 (2008-2010)

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

Present number of serovars in each species and subspecies of *Salmonella*.

S. enterica	*n*
subsp. *enterica*	1586
subsp. *salamae*	522
subsp. *arizonae*	102
subsp. *diarizonae*	338
subsp. *houtenae*	76
subsp. *indica*	13
S. bongori	22
Total	2659

ELSEVIER

INSTITUT PASTEUR

Research in Microbiology 161 (2010) 26–29

www.elsevier.com/locate/resmic

Supplement 2003–2007 (No. 47) to the White-Kauffmann-Le Minor scheme

Martine Guibourdenche [a], Peter Roggentin [b], Matthew Mikoleit [c], Patricia I. Fields [c], Jochen Bockemühl [b], Patrick A.D. Grimont [a], François-Xavier Weill [a,*]

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Institut Pasteur

Research in Microbiology 165 (2014) 526–530

www.elsevier.com/locate/resmic

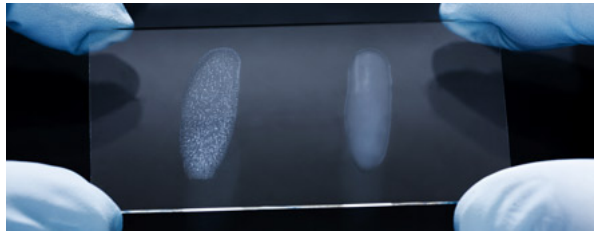
Supplement 2008–2010 (no. 48) to the White–Kauffmann–Le Minor scheme☆

Sylvie Issenhuth-Jeanjean [a], Peter Roggentin [b], Matthew Mikoleit [c], Martine Guibourdenche [a], Elizabeth de Pinna [d], Satheesh Nair [d], Patricia I. Fields [c], François-Xavier Weill [a,*]

Salmonella serotyping history

- Historically, names (often the geographical origin) were given to the different serovars
- Taxonomically more correct to name serovars by their antigenic formula
  - **O-antigens** (somatic) & H-antigens (flagellar), phase **H1** & **H2**
- Names were maintained only for subspecies *enterica* serovars
  - cover 99,5 % of isolated *Salmonella* strains
- **Correct indications for serovar 1,4,[5],12:i:1,2 according to WKLM (2007)**
  - *S. enterica* subsp. *enterica* serovar Typhimurium
  - *S. enterica* serovar Typhimurium
  - *Salmonella* ser. Typhimurium
    - Noticed in current practice most often: *S*. Typhimurium (e.g. **4,12:i:1,2**)

- II Bilthoven -> II 47:a:1,5 (*Salmonella enterica* subsp. *salamae*)



Salmonella serotyping in PT practice

- Naming/antigenic formula as detected
- O-antigens, H-antigens (two phases), [Vi- antigens]
- Sometimes additional biochemical tests needed
- Classical serology -> molecular techniques (->WGS)

Study I, 1995:

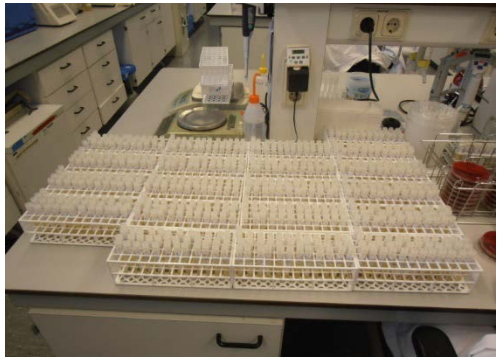
Table 3: *Different methods of agglutination used by the NRLs*

number of laboratories	object glass agglutination	agglutination in tubes	agglutination in microtitre trays
17	17	3	3

25th Study, 2020:

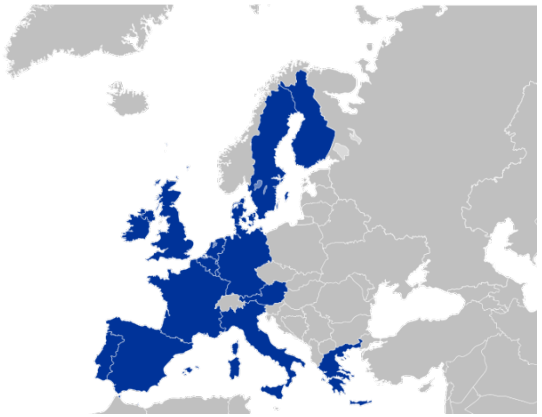
Classical serology was used by 33 participants, 6 of them mentioned the combined use of classical serology and Luminex assays (3) or multiplex/real time PCR (3). One participant used Whole Genome Sequencing (WGS); 3 WGS users were additional participants.

Protocol for a PT on *Salmonella* serotyping (1995 -2020)

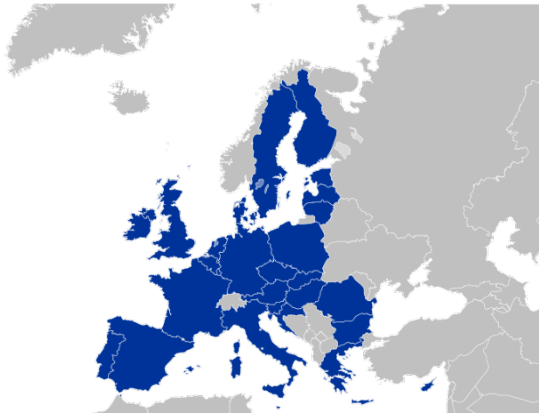


- 20 Strains (plus 1 bonus) to be tested by the participants
- Selection of strains in cooperation with Henny Maas and Anjo Verbruggen
  - (different) serovars of *Salmonella enterica* (subsp. *enterica*)
- Preparation of the parcels at Z&O
  - 1995: 20 x 16 = 320 tubes 2020: 21 x 37 = 740 tubes in 25 years: >13.500 tubes
- Transport of the parcels to participants under UN3373 conditions, PT Protocol
- Serotyping/reporting in accordance with the most recent “Kauffmann-White”
  - Paperwork, FAX, email, checks on re-entered results
  - Online result forms since 2012 (nowadays using formdesk)
- Evaluation of the results and communications
  - Correct reporting of detected O-antigens, detected H-antigens, and of serovar names
  - Since 2007: criteria for Good/Poor performance, based on Penalty Points
  - Individual performance reports, Interim summary report, Final RIVM report (www.eurlsalmonella.eu)

EU-NRL Participants, plus… (1995 – 2020)



From 15 in 1995…



…to 28 in 2013

Albania	2
Austria	25
Belgium	25
Bulgaria	13
Croatia	12
Cyprus	17
Czech Republic	17
Denmark	25
Estonia	17
Finland	25
France	25
Germany	25
Greece	25
Hungary	17
Iceland	8
Ireland	25
Israel	2
Italy	25
Latvia	17
Lithuania	16
Luxembourg	25
Malta	13
Netherlands	25
North Macedonia, Republic of	10
Norway	21
Poland	17
Portugal	25
Romania	13
Serbia	7
Slovak Republic	16
Slovenia	17
Spain	25
Sweden	24
Switzerland	13
Turkey	5
UK England	25
UK Northern-Ireland	25

EU member state

(potential) Candidate EU member state

EFTA countries

Special request

Salmonella serovars (1995 – 2020)

- 491 x *S. enterica enterica* (names, except…)
- 5 x *S.* non-*enterica enterica* (no names)
- 164 different serovars
- 10 serovars in duplicate (or more) within 1 PT
- “Top 5” (EU legislation) since 2004,
  - Including the monophasic variant of serovar Typhimurium since 2010






Salmonella serovars (1995 – 2020)

No. of serovars	Used
1	33 x
1	32 x
2	21 x
1	20 x
1	11 x
2	9 x
3	8 x
2	7 x
2	6 x
8	5 x
11	4 x
22	3 x
27	2 x
81	1 x

Serovar	Used
Enteritidis	33 x
Typhimurium	32 x
Virchow	21 x
Infantis	21 x
Hadar	20 x
1,4,[5],12:1:-	11 x
Agona	9 x
Paratyphi B	9 x
Brandenburg	8 x
Derby	8 x
Dublin	8 x
Heidelberg	7 x
Poona	7 x
Kentucky	6 x
Stanley	6 x

Abaetetuba Abony Adelaide Adjame Agama Agbeni Agona Ahmadi Alachua Albany Altona Amsterdam Anatum Apeyeme Arechavaleta Augustenborg Baildon Banana Bardo Bareilly Benfica Berta Bispebjerg Blockley Bochum Bousso Bovismorbificans Bracknell Braenderup Brancaster Brandenburg Brazzaville Bredeney Canada Cannstatt Carno Cerro Chester Choleraesuis Coeln Colindale Corvallis Cotham Cubana Derby Dublin Duisburg Durban Eastbourne Eboko Emek Enteritidis Fluntern Fresno Galiema Give Glostrup Gloucester Goettingen Goldcoast Grumpensis Hadar Haifa Havana Heidelberg Herston Hessarek Indiana Infantis Irumu Isangi Jangwani Javiana Jerusalem Jukestown Kaapstad Kapemba Kedougou Kentucky Kiambu Kingston Kintambo Kivu Kottbus Krefeld Lagos Langenhorn Larochelle Lawndale Leeuwarden Lexington Lille Liverpool Livingstone Llandoff London Manchester Manhattan Matadi Mbandaka Meleagridis Menston Mikawasima Mississipi Molade Montevideo Muenchen Muenster Napoli Newport Nigeria Odozi Ohio Okatie Oranienburg Ordonez Orion Ouakam Panama Paratyphi B Java Plymouth Poona Potsdam Putten Reading Richmond Rissen Rubislaw Ruiru Saarbruecken Saintpaul Sandiego Saphra Schwarzengrund Senftenberg Southampton Stanley Stanleyville Stendal Stockholm Stourbridge Szentes Taksony Teddington Telelkebir Tennessee Thompson Typhimurium 1,4,[5],12:1:- Tyresoe Uganda Umbilo Urbana Vinorahdy Virchow Virginia Waycross Weltevreden Wernigerode Winslow Worthington Yaba Yoruba Zega

Collaborative study I, 1995

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July 1996

The conclusions of this study are:

- Seven of the 17 participants identified the 20 selected strains correctly; three laboratories typed one strain incorrectly and six laboratories typed 2-5 strains incorrectly. One laboratory reported six strains as not typable.

- Seven of the 18 strains of subspecies *enterica* were identified correctly by all participants; the strain belonging to the subspecies *salamae* was identified correctly by eight participants and the strain belonging to the subspecies *houtenae* by 12 participants.

- The 3 main reasons of incorrect identification were:

1. The O and/or H antigens were incorrectly detected;
2. The strains were identified on the grounds of an incomplete antigenic formula by means of the Kauffmann-White scheme;
3. The antigenic formula was interpreted incorrectly.

- It is better to identify strains by giving the antigenic formula as far as detected. Definite conclusions can be based only on agglutination with mono-specific antisera.

Collaborative study I, 1995

+ = correct
± = partly correct/incomplete
- = incorrect

Table 5: *Detection of O and H antigens of all 20 selected strains per laboratory*

labcode	O antigen detected +	O antigen detected ±	O antigen detected -	O antigen not typable	H antigen detected +	H antigen detected ±	H antigen detected -	H antigen not typable
1	20	-	-	-	20	-	-	-
2	16	1	-	3	12	2	-	6
3	20	-	-	-	17	1	2	-
4	20	-	-	-	18	2	-	-
5	20	-	-	-	20	-	-	-
6	18	2	-	-	14	5	1	-
7	20	-	-	-	20	-	-	-
8	20	-	-	-	19	1	-	-
9	14	3	-	3	17	-	-	3
10	20	-	-	-	20	-	-	-
11	15	3	1	1	14	3	2	1
12	20	-	-	-	20	-	-	-
13	19	-	-	1	18	-	-	2
14	18	2	-	-	14	6	-	-
15	19	1	-	-	18	1	1	-
16	20	-	-	-	20	-	-	-
17	18	-	2	-	16	4	-	-

Table 8: *Identification of all 20 selected strains per laboratory*

labcode	Identification +	Identification ±	Identification -	not typable
1	20	-	-	-
2	14	-	-	6
3	17	1	2	-
4	20	-	-	-
5	20	-	-	-
6	17	1	2	-
7	20	-	-	-
8	18	1	1	-
9	16	1	1	2
10	20	-	-	-
11	12	1	5	2
12	20	-	-	-
13	18	-	1	1
14	15	1	4	-
15	17	1	2	-
16	20	-	-	-
17	16	-	4	-

Table 9: *Identification of the 18 strains belonging to the subspecies enterica by the 17 participants*

strain no	serotype	Identification +	Identification -	not typable
1	S. Llandoff	15	1	1
2	S. Give	16	1	-
3	S. Virchow	17	-	-
4	S. Oranienburg	14	3	-
6	S. Enteritidis	16	1	-
7	S. Lille	11	3	3
8	S. Give	16	1	-
9	S. Berta	17	-	-
10	S. Typhimurium	17	-	-
11	S. Poona	17	-	-
12	S. Llandoff	13	2	2
14	S. Dublin	16	1	-
15	S. Infantis	17	-	-
16	S. Worthington	17	-	-
17	S. Fluntern	13	3	1
18	S. Kentucky	17	-	-
19	S. Enteritidis	16	1	-
20	S. Agona	16	1	-

e.g. the second study:

NATIONAL INSTITUTE OF PUBLIC HEALTH AND THE ENVIRONMENT
BILTHOVEN, THE NETHERLANDS

Report 284500 008
Test results of *Salmonella* serotyping
in the Member States of the European Union
A collaborative study amongst the National
Reference Laboratories for *Salmonella*
N. Voogt, H.M.E. Maas, W.J. van Leeuwen
and A.M. Henken

September 1997

4.2 Taxonomy and nomenclature of the typed strains

The first letter of serovars belonging to *S. enterica* subspecies *enterica* is a capital letter. Ten of the 15 participants wrote the identified serotype with a capital letter. Of the remaining NRLs three (labcode 2, 7 and 15) reported the name with a small letter and two participants (labcode 4 and 8) wrote the whole name in capital letters.

One laboratory (labcode 7) used S. Ardwick as well as S. Bissen to characterize strain no. 13. S. Ardwick is the name of the serotype withdrawn from the Kauffmann-White scheme, 1992 (3). Other laboratories (labcode 5 and 16) used the withdrawn name S. St. Paul instead of S. Saintpaul. In this study these withdrawn names were interpreted as incomplete (+). The identification S. Tenesse instead of S. Tennessee was interpreted as incomplete (±) as well, because incorrect writing of a name could give misleading in surveillance results.

Strains number 10 and 20 were S. Paratyphi B var. Java. This serotype is isolated mainly from animal sources, while the serotype S. Paratyphi B is a 'human type'. The difference between S. Paratyphi B var. Java and S. Paratyphi B depends on biotyping. If a strain is d-tartrate positive it is called variety Java. The strain is called S. Paratyphi B when the d-tartrate reaction is negative. Three laboratories (labcode 3, 5 and 10) reported strain 10 as S. Paratyphi B var. Java. Just one laboratory (labcode 10) identified strain 20 as S. Paratyphi B var. Java.
The withdrawn name from the Kauffmann-White scheme is S. Java. Three laboratories (labcode 13, 14 and 15) still used this name, probable to make clear that it was not a S. Paratyphi B. Therefore it was interpreted as correct (+). All identifications of strain 10 and 20 reported by the participants and the interpretation in this study are shown in Table 4.

Interlaboratory comparison study XII, 2007

rivm

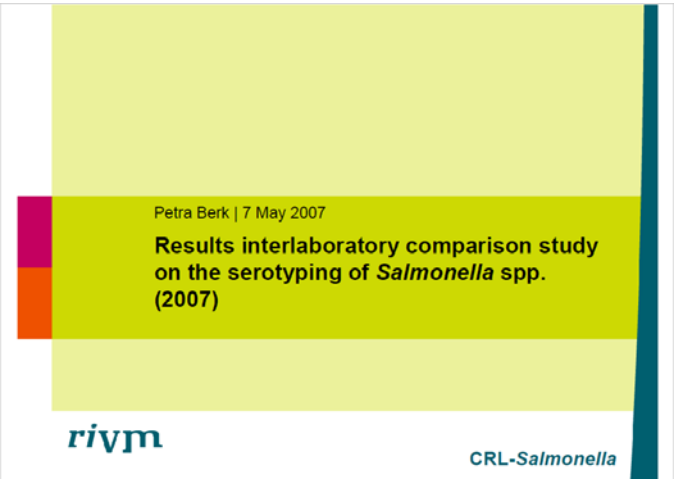
30 participants in 2007

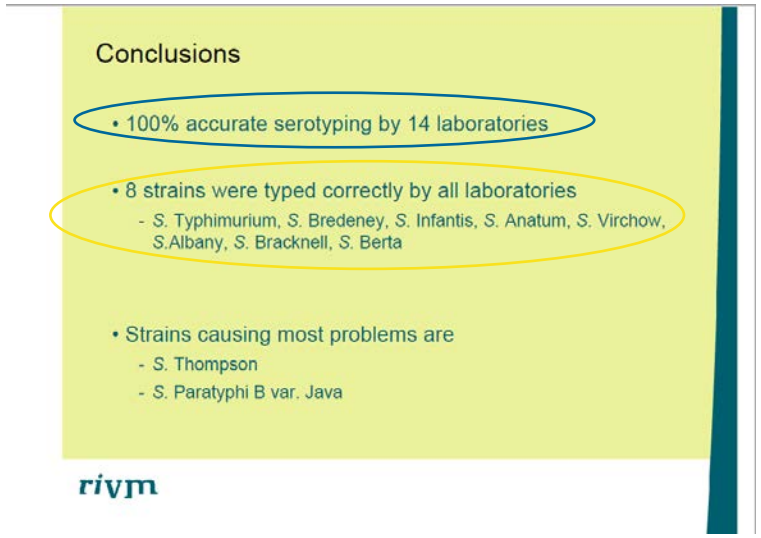
Presentation at the annual CRL-*Salmonella* Workshop

RIVM Report 330604005/2007

Twelfth CRL-*Salmonella* interlaboratory comparison study (2007) on typing of *Salmonella* spp.

P.A. Berk
H.M.E. Maas
E. de Pinna, Health Protection Agency, London
K.A. Mooijman





Study XII, 2007

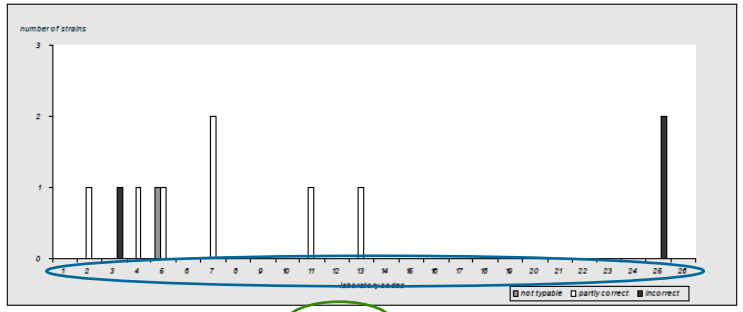


Figure 1 Evaluation of serotyping of O-antigens per NRL

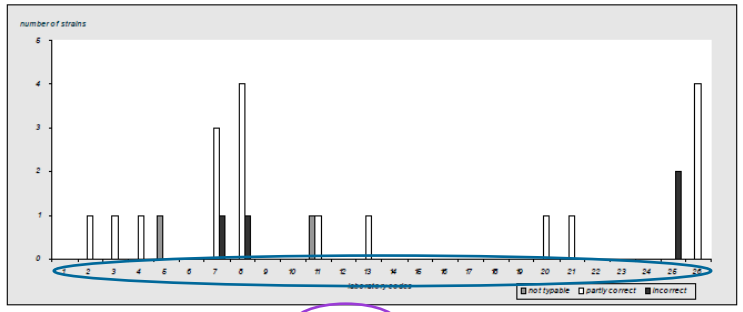


Figure 2 Evaluation of serotyping of H-antigens per NRL

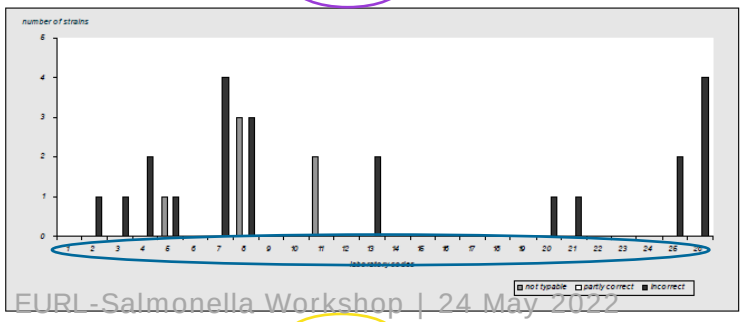


Figure 3 Evaluation of the correct serovar names per NRL

not typable | partly correct | incorrect

Labcode	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
CRL	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
1	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
2	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
3	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
4	Bredeney	Harburg	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
5	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Larochelle	not typable	Indiana	Typhimurium
6	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
7	Bredeney	Bareilly	Chincol	Infantis	Anatum	Hadar	Newport	Gloucester	Indiana	Typhimurium
8	Bredeney	not typable	untypable	Infantis	Anatum	Hadar	Newport	Hato	not typable	Typhimurium
9	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
10	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
11	Bredeney	not typable	Manchester	Infantis	Anatum	Hadar	Newport	not typable	Indiana	Typhimurium
12	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
13	Bredeney	Thompson	Manchester	Infantis	Anatum	Djugu	Newport	Stanley	Indiana	Typhimurium
14	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
15	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
16	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
17	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
18	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
19	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
20	Bredeney	Isangi	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
21	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
22	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
23	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
24	Bredeney	Thompson	Manchester	Infantis	Anatum	Hadar	Newport	Paratyphi B var Java	Indiana	Typhimurium
25	Bredeney	Thompson	Manchester	Infantis	Anatum	Indiana	Newport	Paratyphi B var Java	Hadar	Typhimurium
26	Bredeney	Isangi	Edmonton	Infantis	Anatum	Hadar	Newport	Abony	Indiana	Typhimurium

Labcode	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20
CRL	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
1	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
2	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Gateshead	Bracknell	Berta	Brandenburg
3	Virchow	Madiago	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
4	Virchow	Yoruba	Albany	Senftenberg	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
5	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
6	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
7	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Bredeney
8	Virchow	Shamba	Albany	Cannstatt	Elisabethville	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
9	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
10	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
11	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
12	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
13	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
14	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
15	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
16	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
17	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
18	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
19	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
20	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
21	Virchow	Yoruba	Albany	Kouka	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
22	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
23	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
24	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
25	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Bareilly	Enteritidis	Bracknell	Berta	Brandenburg
26	Virchow	Yoruba	Albany	Cannstatt	Weltevreden	Mikawasima	Enteritidis	Bracknell	Berta	Brandenburg

Collaborative study XII, 2007

Proposal for definition of good performance (1)

- A distinction between the 5 most important serovars (*S.* Enteritidis, *S.* Typhimurium, *S.* Hadar, *S.* Infantis, *S.* Virchow) and other strains
- The use of penalty points per wrong serovar name

rivm

Proposal for definition of good performance (2)

- 4 penalty points
  - Incorrect typing of *S.* Enteritidis, *S.* Typhimurium, *S.* Hadar, *S.* Infantis or *S.* Virchow
  - Assigning the serovar names of *S.* Enteritidis, *S.* Typhimurium, *S.* Hadar, *S.* Infantis or *S.* Virchow to another strain
- 1 penalty point
  - Incorrect typing of other strains
- Determining total amount of penalty points per NRL
- Good performance: <4 penalty points

rivm

Proposal Follow up

- Laboratories which have not reach the level of good performance (<4 penalty points) will receive a new set of (10) strains for serotyping.
- Laboratories with labcodes: 2, 7, 8, 13, 25 and 26

rivm

Table 11 Evaluation of serotyping results per NRL

Labcode	Penalty points	Good performance?	Labcode	Penalty points	Good performance?
1	0	Yes	14	0	Yes
2	4	No	15	0	Yes
3	1	Yes	16	0	Yes
4	2	Yes	17	0	Yes
5	1	Yes	18	0	Yes
6	0	Yes	19	0	Yes
7	4	No	20	1	Yes
8	6	No	21	1	Yes
9	0	Yes	22	0	Yes
10	0	Yes	23	0	Yes
11	2	Yes	24	0	Yes
12	0	Yes	25	8	No
13	5	No	26	4	No

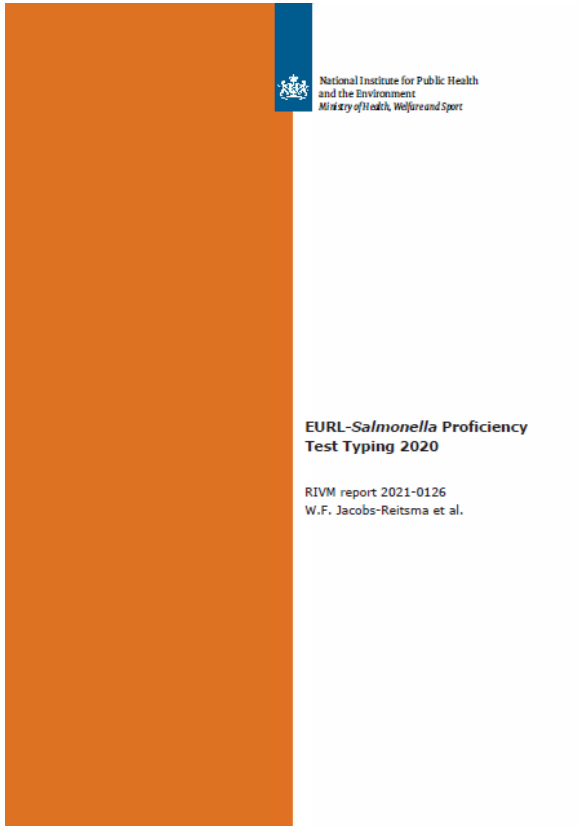
Table 15 Evaluation of serotyping results per NRL for the follow up

Labcode	Penalty points	Good performance?
2	0	Yes
7	0	Yes
8	0	Yes
13	0	Yes
25	0	Yes
26	2	Yes

Not achieving "Good Performance" ?

- The EURL contacts the NRL and asks for possible technical problems;
- The EURL organises a follow-up study with number and type of samples dependant on the problems of the NRL;
- In case of repeated poor performance:
  - Contact NRL for possible technical problems;
  - Offer training either at EURL or at NRL;
  - Inform European Commission → EC may contact Competent Authority relevant country. CA can decide to withdraw the NRL task of the relevant institute.
- In past years, several individual trainings given on serotyping of *Salmonella*

(25th) Proficiency Test Serotyping 2020



Conclusions 25th serotyping study (all participants)

- O-antigens: 99% of strains typed correctly
- H-antigens: 98% of strains typed correctly
- Serovar names: 97% of strains typed correctly

- 100% accurate serotyping by 29/37 (78%) participants
  - 8 participants with only 1 or 2 mistakes in the naming

- 9 serovars completely correct identified by all participants:

Bousso (S2), Hadar (S3), Zega (S6), Typhimurium (S13), Larochelle (S14), Virchow (S15), Enteritidis (S16), Benfica (S17), and Infantis (S18)

- All 37 laboratories (= all EU-MS) "Good Performance"
  - *No Follow-up needed!*

18 Results Salmonella PT Typing 2020 | 28 May 2021

(25th) Proficiency Test Serotyping 2020

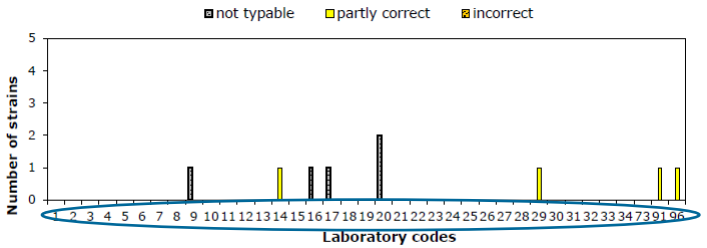


Figure 3 Evaluation of type of errors for O-antigens, per participant

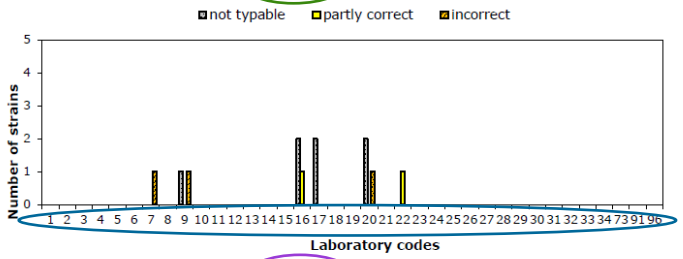


Figure 4 Evaluation of type of errors for H-antigens, per participant

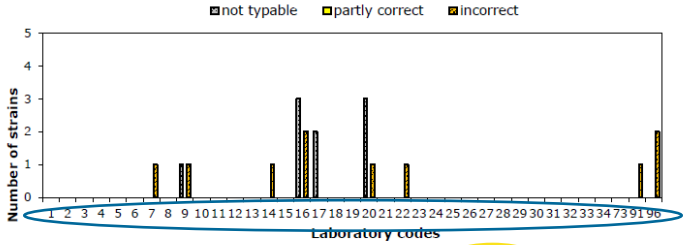


Figure 5 Evaluation of the type of errors in the identification of the serovar names, per participant

Lab code	Penalty points	Good performance
1	0	yes
2	0	yes
3	0	yes
4	0	yes
5	0	yes
6	0	yes
7	1	yes
8	0	yes
9	1	yes
10	0	yes
11	0	yes
12	0	yes
13	0	yes
14	1	yes
15	0	yes
16	2	yes
17	0	yes
18	0	yes
19	0	yes

Lab code	Penalty points	Good performance
20	1	yes
21	0	yes
22	1	yes
23	0	yes
24	0	yes
25	0	yes
26	0	yes
27	0	yes
28	0	yes
29	0	yes
30	0	yes
31	0	yes
32	0	yes
33	0	yes
34	0	yes
73	0	yes
91	1	yes
96	2	yes

not typable partly correct incorrect

(25th) Proficiency Test Serotyping 2020

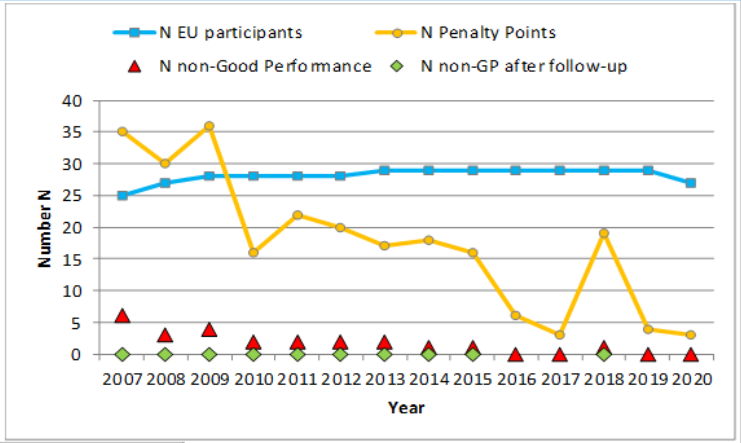
Lab:	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	Lab:
REF	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	1,4,[5],12:i:-	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	REF
1	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4,12:i:-	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	1
2	Jukestown	Bousso	Istanbul	Brancaster	Muenchen	Zega	Agbeni	1,4,12:i:-	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	2
3	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4,12:i:-	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	3
4	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	1,4,12;i;-	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	4
5	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4,12:i:-	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	5
6	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4,12:i:-	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	6
7	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4,12:i:-	Odozi	Tyresoe	Stendal	Lagos	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	7
8	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4,5,12:i:-	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	8
9	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	monophasic Typhimurium	Odozi	Tyresoe	Stendal	-	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Uppsala	Apeyeme	9
10	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	Typhimurium Monophasic	Odozi	Tyresoe	Stendal	Fulica / Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	10
11	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	monophasic Typhimurium	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	11
12	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4,12:i:-	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	12
13	Jukestown	Bousso	Hadar	Brancaster	Muenchen	Zega	Agbeni	1,4,5,12:i:-	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	13
14	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4,5,12:i:-	Odozi	Tyresoe	Stendal	Paratyphi A	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	14
15	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4,12:i:-	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	15
16	Jukestown	Bousso	Hadar	4,12:HME:-	Virginia	Zega	Agbeni	Typhimurium	OMC:k:e,n,z15	Azteca	Stendal	4,12:a:1,5	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	8,20:HME:-	16
17	I:13,23:i:-	Bousso	Hadar	Brancaster	Virginia	Zega	-:gm:-	I:4:i:- (monophasic TM)	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	17
18	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4,12:i:-	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	18
19	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	Typhimurium monophasic variant	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	19
20	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4,5,12:i:-	?	Tyresoe	Stendal	?	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Uppsala	?	20
21	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	1,4,12; i; -	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	21
22	jukestown	bousso	hadar	brancaster	virginia	zega	agbeni	Monophasic Salmonella typhimurium	odozi	tyresoe	tours	hessarek	typhimurium	larochelle	virchow	enteritidis	benfica	infantis	canada	apeyeme	22
23	Jukestown	Bousso	Hadar	Brancaster	Muenchen	Zega	Agbeni	4,12:i:-	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	23
24	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4 : i : -	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	24
25	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	1,4,12:i:- (mST)	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benefica	Infantis	Canada	Apeyeme	25
26	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4,12:i:-	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	26
27	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4, 5, 12: i: -	Odozi	Tyresoe	Stendal	Fulica	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	27
28	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4,5,12:i:-	Odozi	Tyresoe	Stendal	Fulica, Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apayeme	28
29	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4,(5),12:i:-	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	29
30	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	Monophasic Typhimurium	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	30
31	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4:i:-	Obdozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	31
32	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	Typhimurium, monophasic 4,12 : i -	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	32
33	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4:i:-	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	33
34	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4,12:i:-	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	34
73	Jukestown	Bousso	Hadar	Brancaster	Virginia	Zega	Agbeni	4,[5],12:i:-	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	73
91	Jukestown	Bousso	Hadar	Brancaster	Muenchen	Zega	Agbeni	Typhimurium - monophasic	Odozi	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	91
96	Juketown	Bousso	Hadar	Brancaster	Muenchen	Zega	Agbeni	I 4,[5],12:i:-	Angoda	Tyresoe	Stendal	Hessarek	Typhimurium	Larochelle	Virchow	Enteritidis	Benfica	Infantis	Canada	Apeyeme	96
X	0	0	0	0	2	0	0	1	1	1	1	2	0	0	0	0	0	0	2	0	X

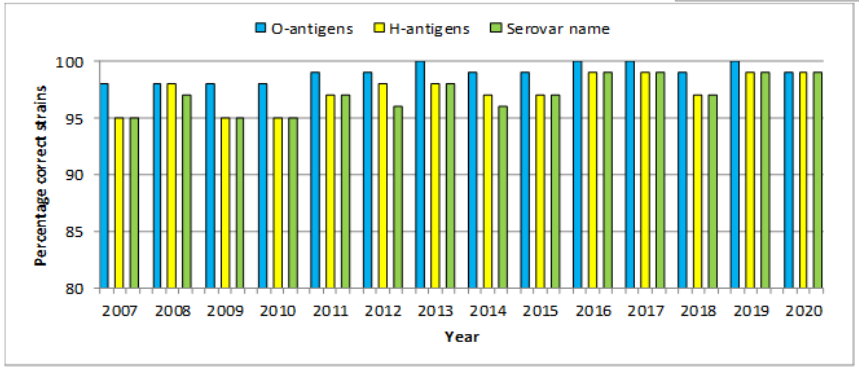
- remark (e.g. spelling errror)
- not typable (e.g. antisera not available, rough strain)
- partly correct; in the naming: no penalty points
- incorrect; in the naming: 1 penalty point
- incorrect; in the naming: 4 penalty points

X = number of deviating laboratories (by penalty points) per strain

(25th) Proficiency Test Serotyping 2020

Results in time (EU NRLs only)

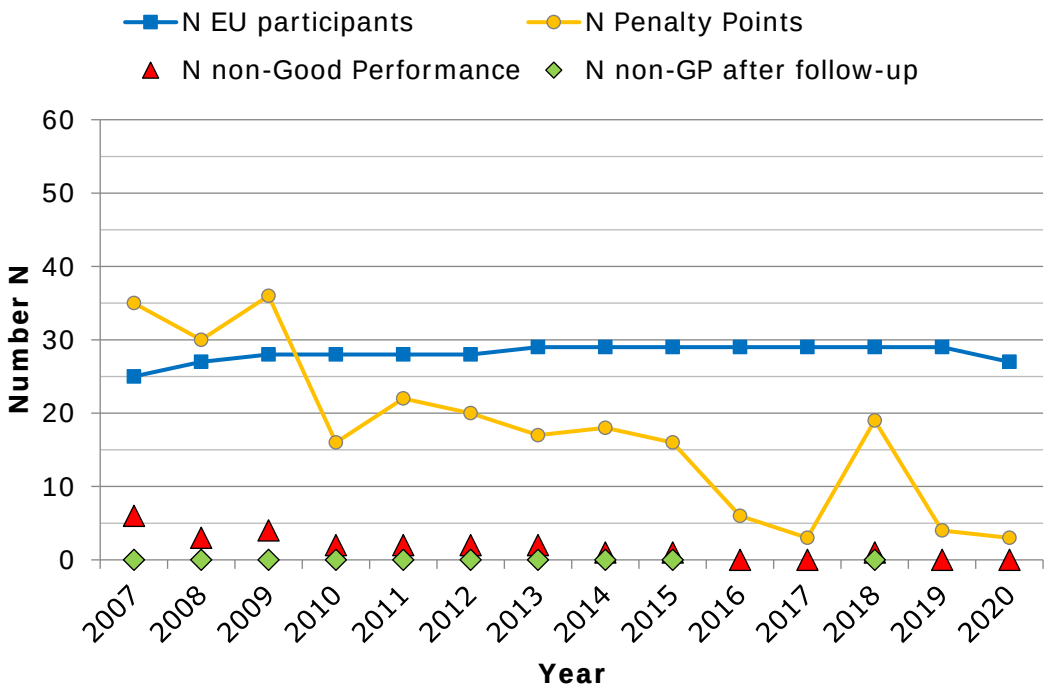


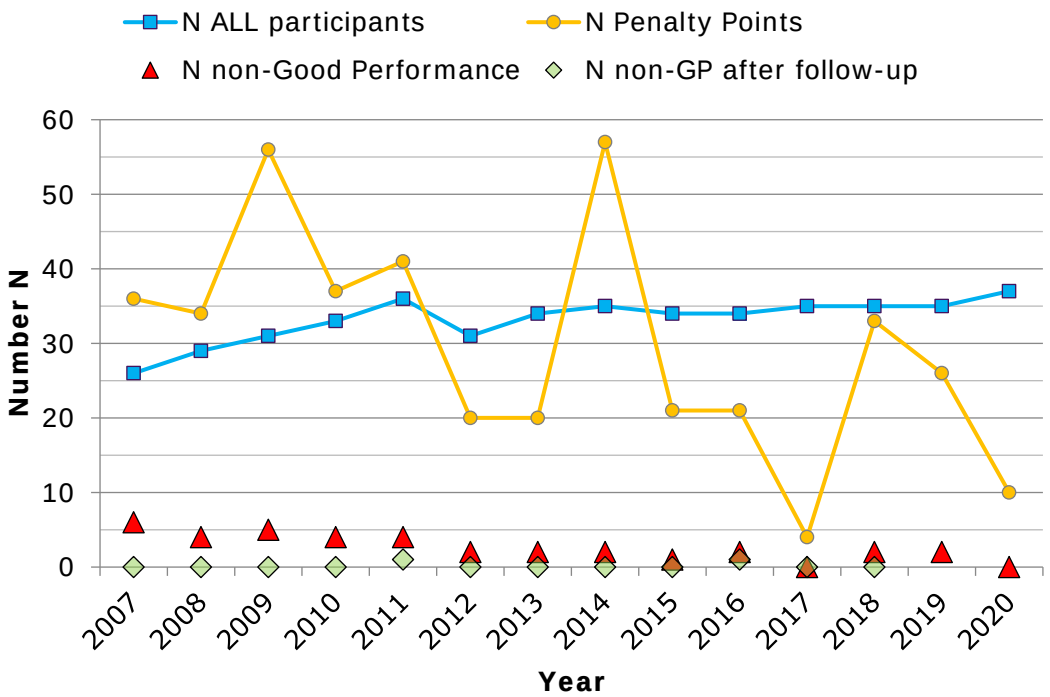


17

Results Salmonella PT Typing 2020 | 28 May 2021

EU Member states – ALL participants 2007 - 2020

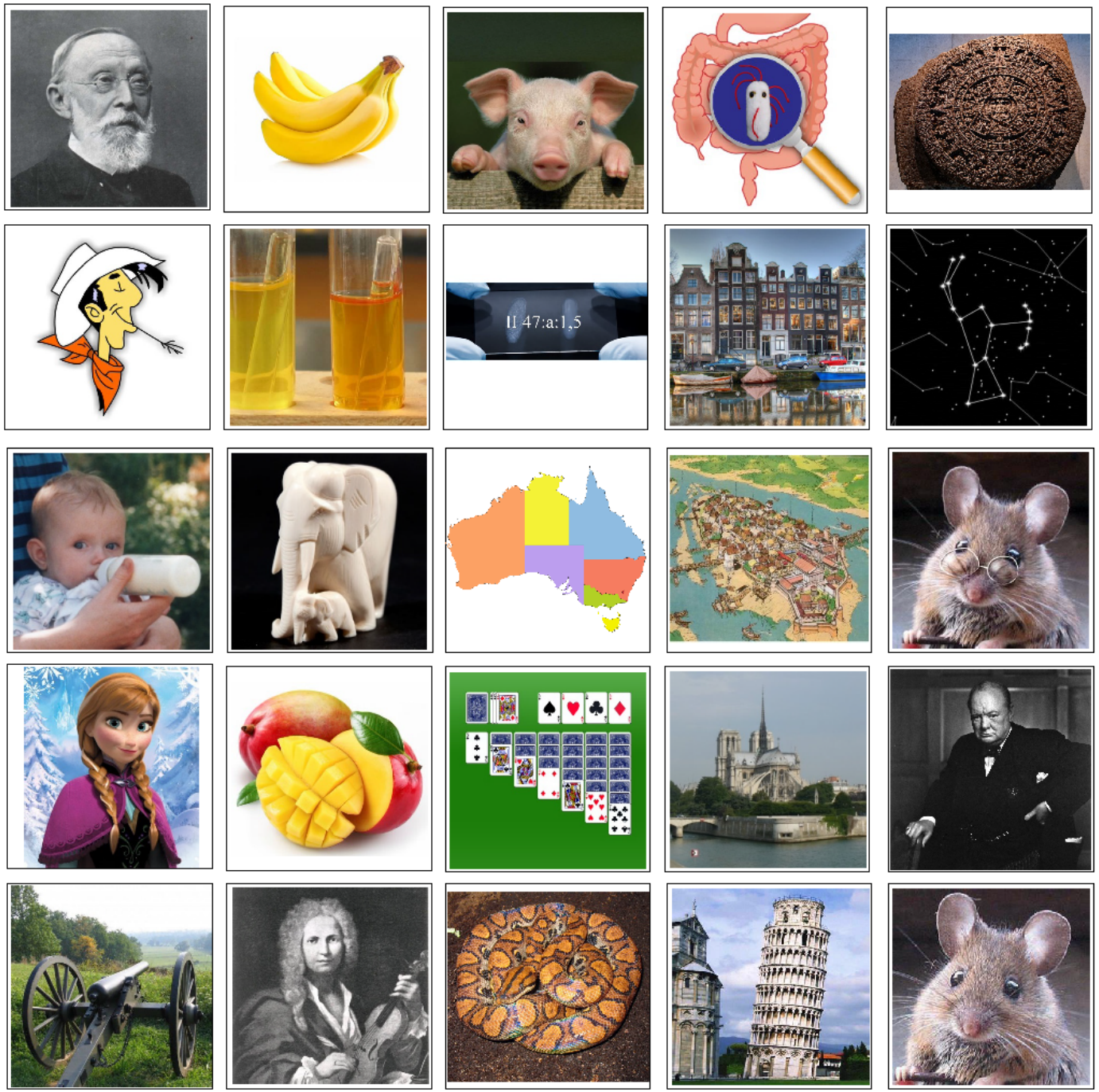




Conclusions

- Valuable information from the past
- Still to be used in the future
- (sero)typing of *Salmonella* is (rapidly) evolving
- PTs on (sero)typing of *Salmonella* remain of importance
- Continuous requirement of comparable typing results within the EU (and beyond) in both surveillance and outbreak investigations

- We may continue to organize PTs for the next 25 years (?!), but at least we start with the upcoming 27th PT in November 2022.


II 47:a:1,5