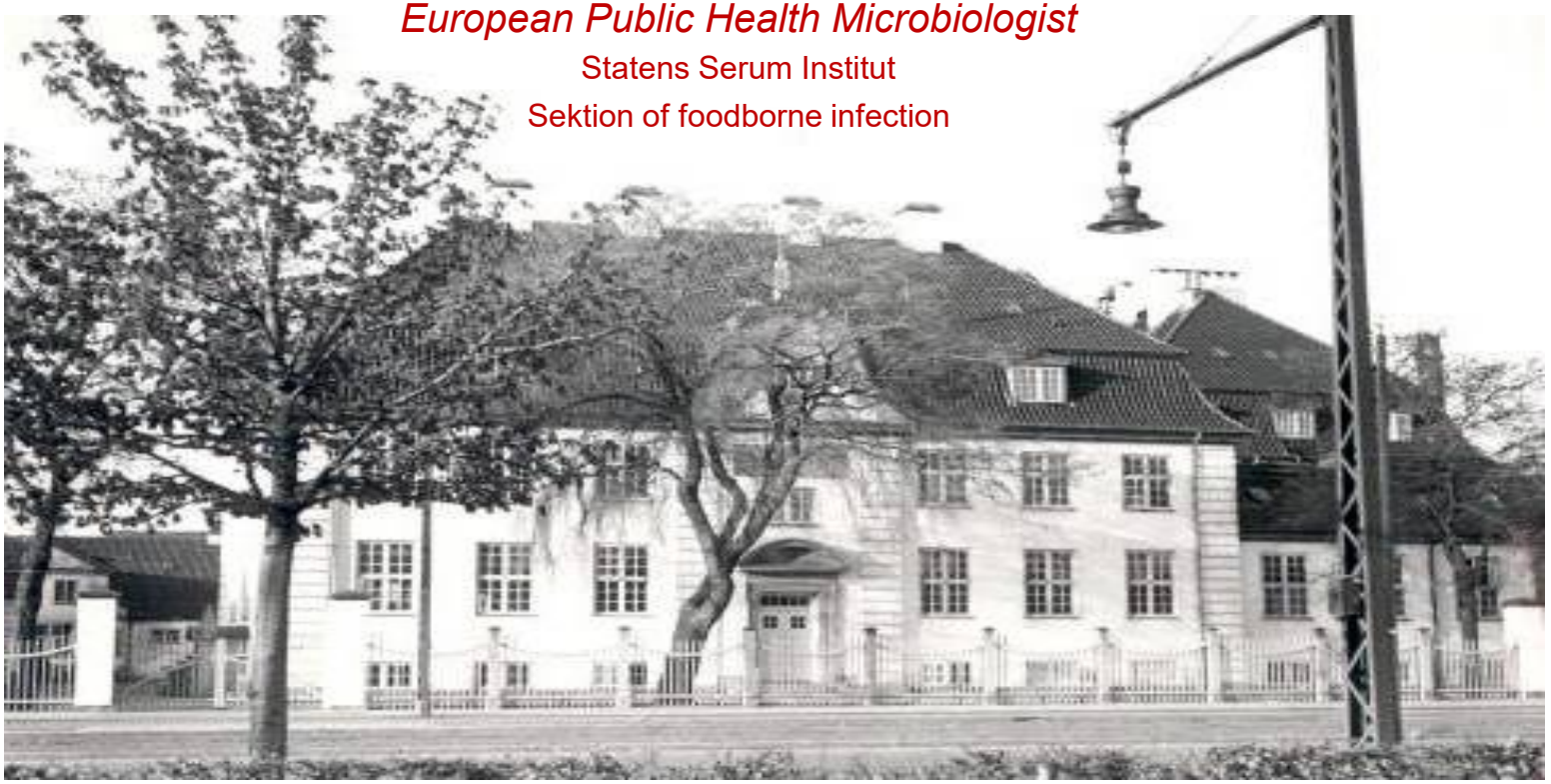
EXTERNAL QUALITY ASSESSMENT OF WGS-BASED CLUSTER ANALYSIS FOR NPHRLS

Susanne Schjørring, M.Sc, Ph.D
European Public Health Microbiologist
Statens Serum Institut
Sektion of foodborne infection



Molecular typing EQAs

STATENS SERUM INSTITUT

Funded:

By European Centre for Disease Prevention and Control (ECDC)

Organised:

Statens Serum Institut, Denmark, Section of Foodborne Infections

2 periods (2012-2016; 2017-2020)

Main objective of the EQAs:

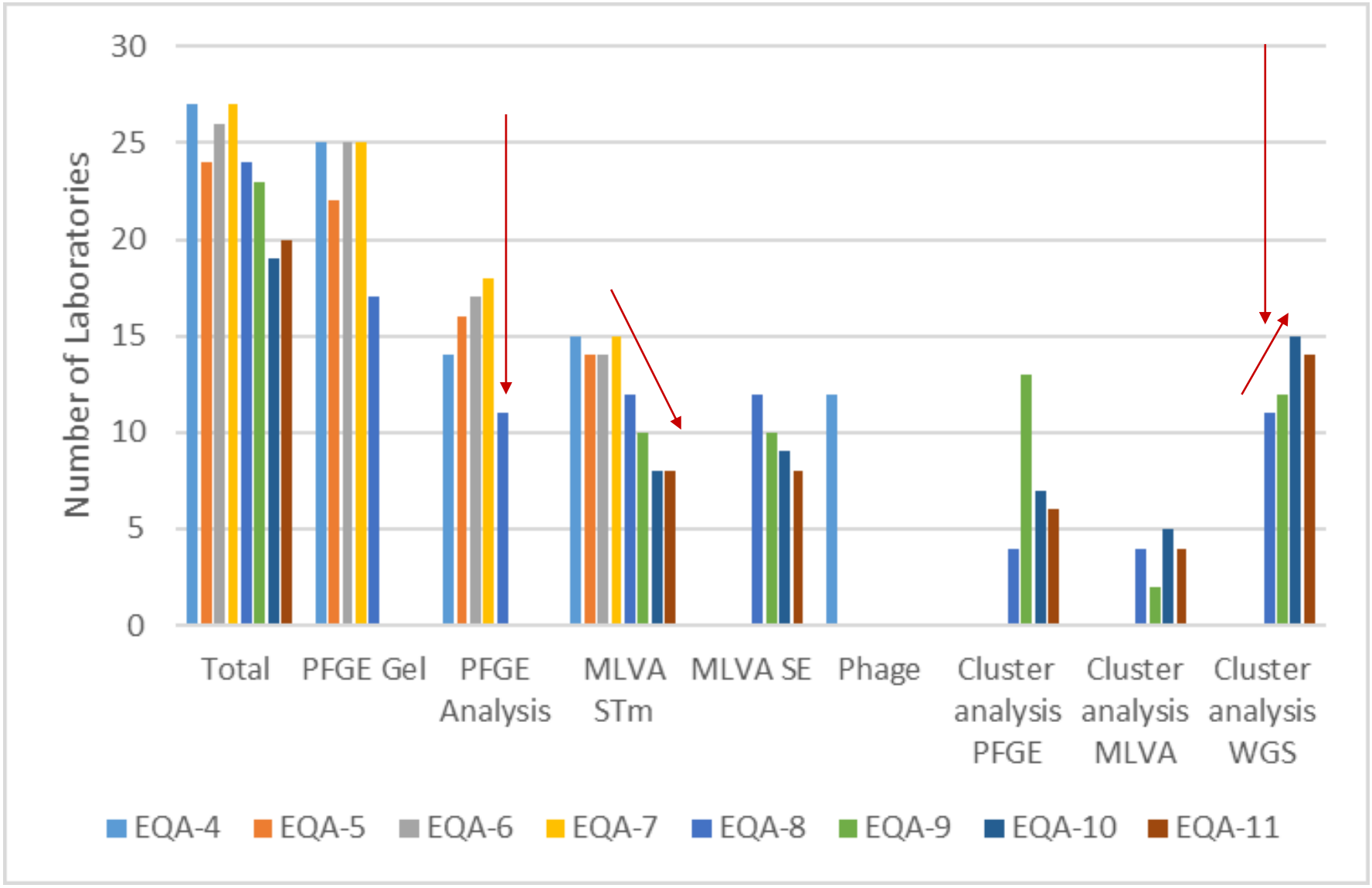
- assess the general standard of performance (‘state-of–the-art’)
- assess the effects of analytical procedures (method principle instruments, reagents, calibration)
- evaluate individual laboratory performance
- identify and justify problem areas
- provide continuing education
- identify needs for training activities

Methods

STATENS SERUM INSTITUT

		Years (Rounds)							
		2012-2013	2013-2014	2014-2015	2015-2016	2017-2018	2018-2019	2019-2020	2020-2021
Listeria	Serotype	x	x	x	x	x	x	x	x
	PFGE assesment and analysis	x	x	x	x				
	Cluster analysis (PFGE/WGS)					x	x	x	x
STEC	Serotype	x	x	x	x	x	x	x	x
	Virulence profile	x	x	x	x	x	x	x	x
	Phenotypic test	x	x	x	x				
	PFGE assesment and analysis	x	x	x	x				
	Cluster analysis (PFGE/WGS)					x	x	x	x
Salmonella	Phage typing STm and SE	x							
	MLVA STm	x	x	x	x	x	x	x	x
	MLVA SE					x	x	x	x
	PFGE assesment and analysis	x	x	x	x	x			
	Cluster analysis (PFGE/MLVA/WGS)					x	x	x	x

Participation (Salmonella EQA-4 - EQA-11)



Cluster analysis (EQA-8 – EQA-11)

STATENS SERUM INSTITUT

Perform a cluster analyses by using PFGE-, MLVA- or WGS-derived data

- Report the isolates identified as being closely related (outbreak)
- Submit distance between one (cluster isolates) and the other test isolates
  - Band difference in PFGE (total bands /shared bands)
  - MLVA profiles
  - SNP distances/ allele differences (wgMLST/cgMLST) (WGS)
    - If using WGS, the submission should include the fastq- files

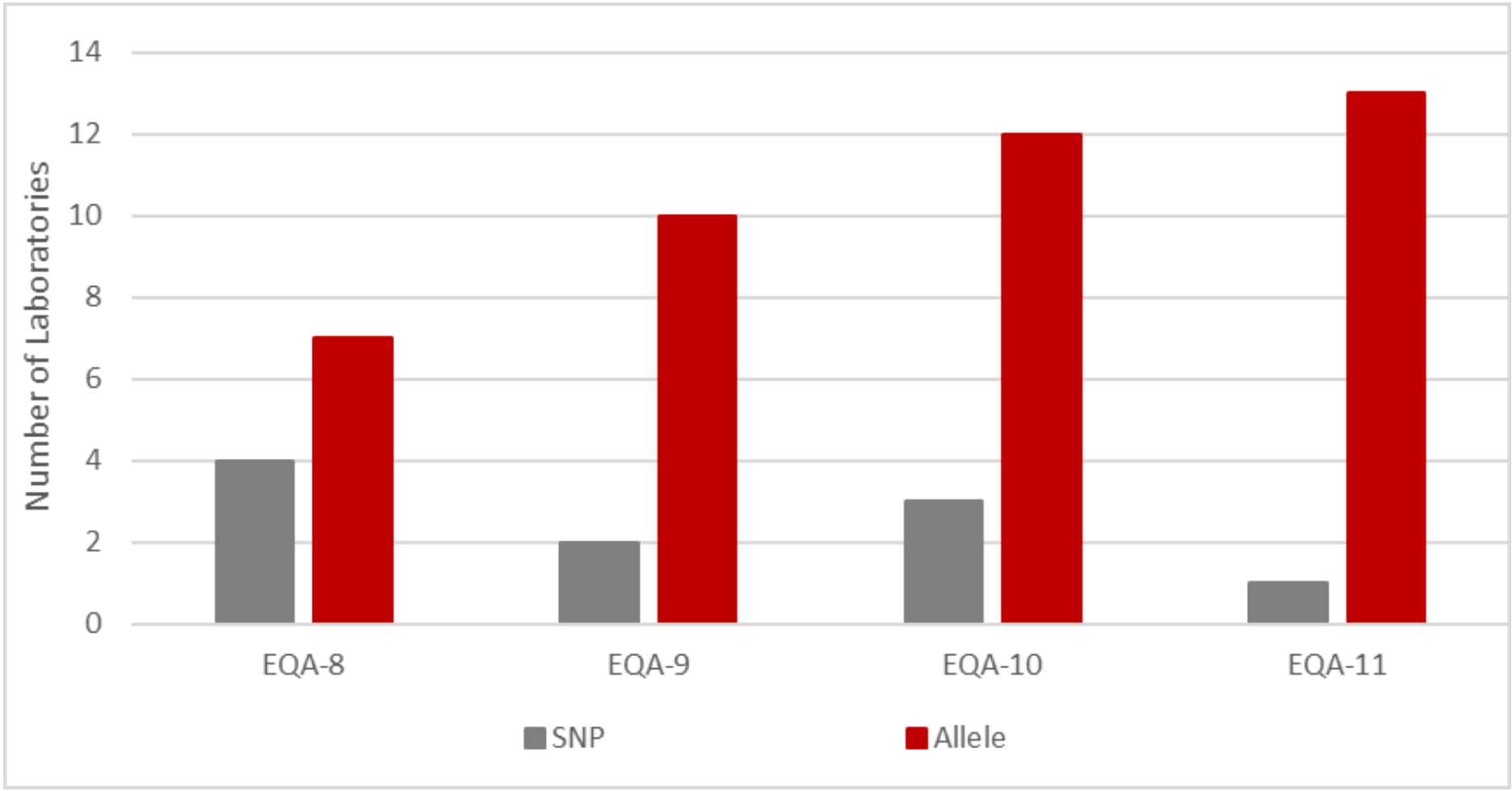
Evaluation:

- The ability to detect a cluster of closely related isolates bases on a pre-defined categorization by the organizer (WGS)

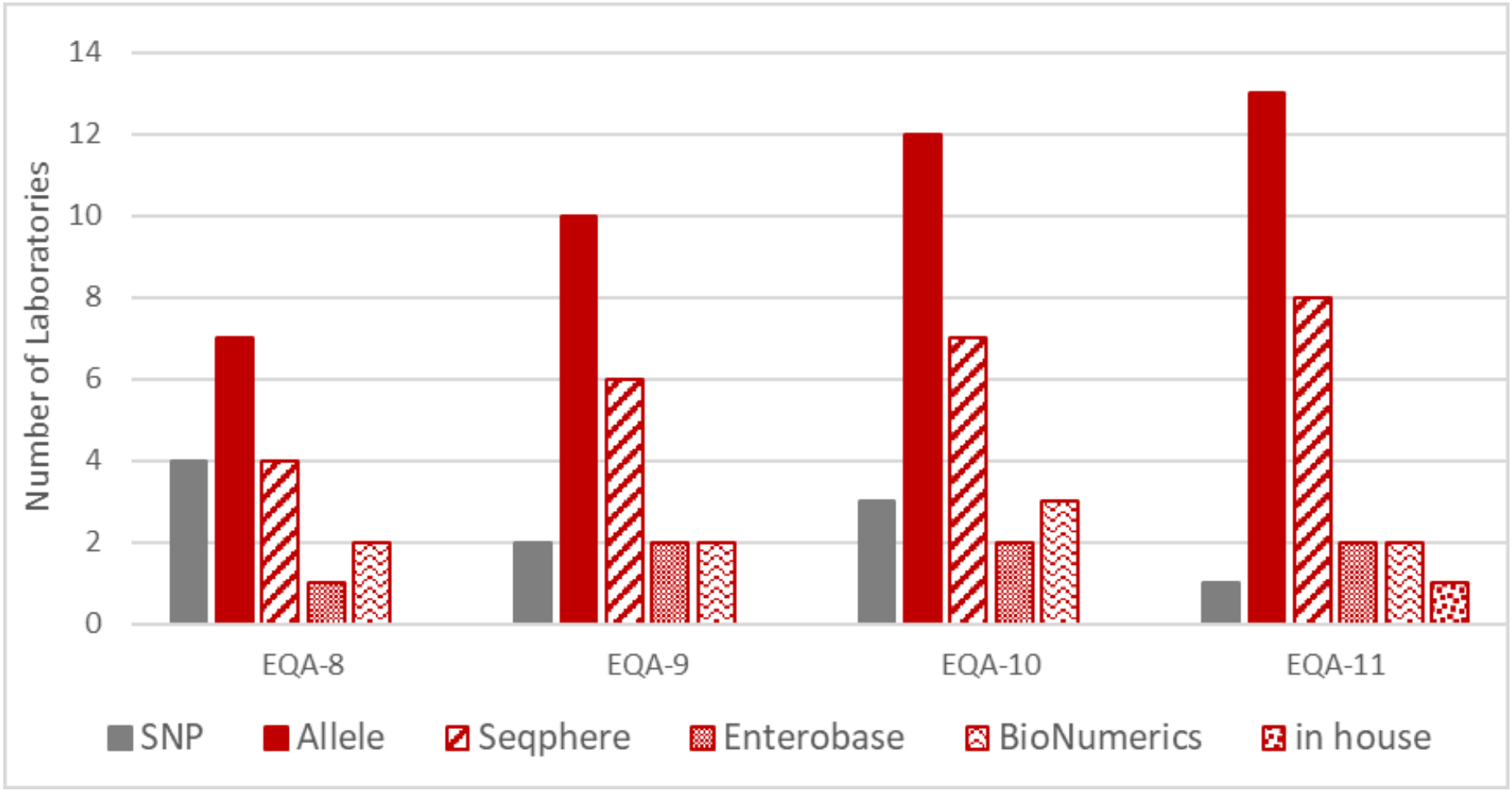
  (mimicking an outbreak situation)
- The submitted raw reads were “evaluated“ by the SSI in-house quality control pipeline

Submission: 1 main analysis + 1-2 additional analysis

Main analysis



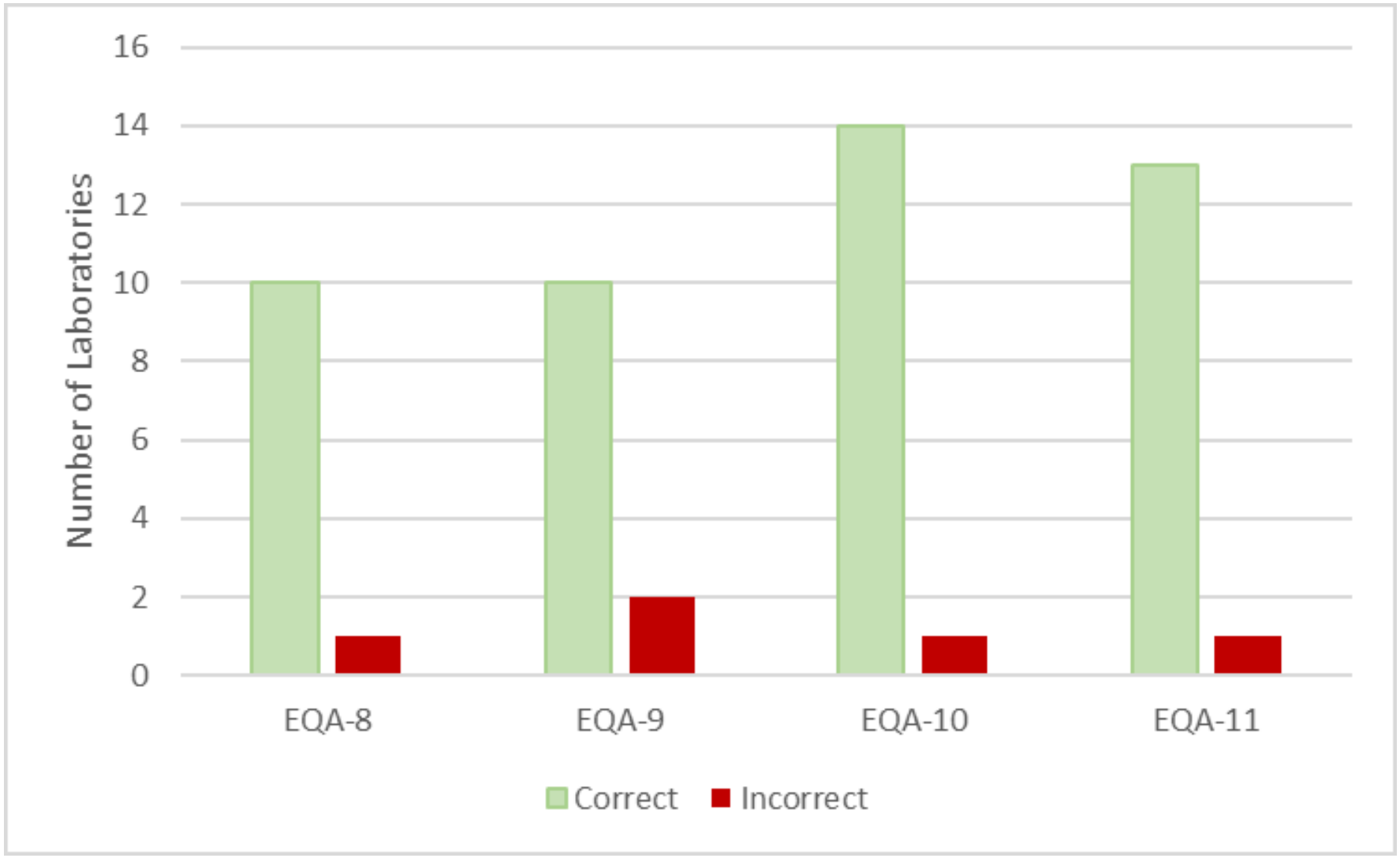
Main analysis (approach)



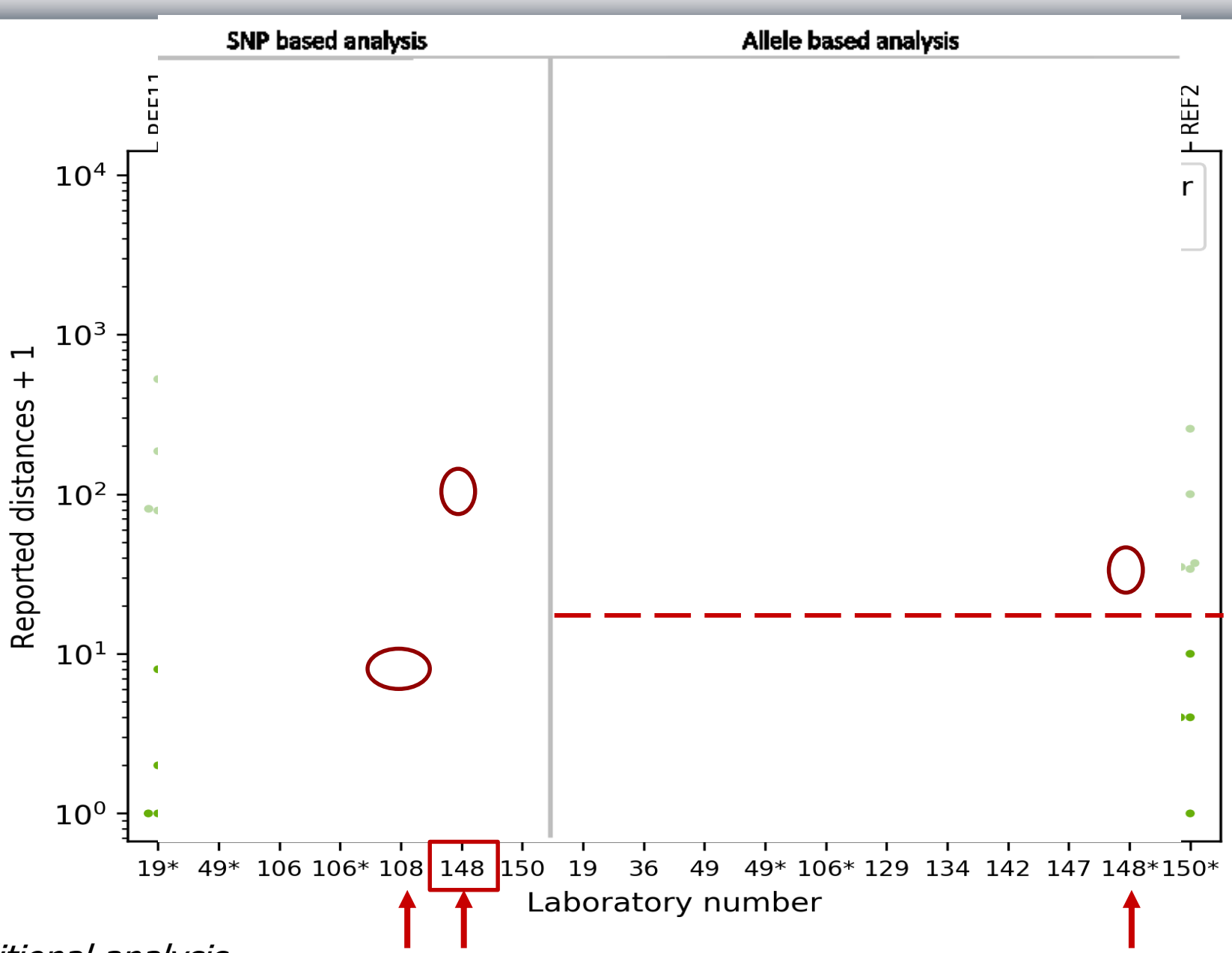
Annex 7. Reported sequencing details

Sequencing performed	Protocol (library preparation)	Commercial kit	Sequencing platform
In own laboratory	Commercial kits	NexteraXT	NextSeq
In own laboratory	Commercial kits	Nextera™ XT DNA Library Preparation Kit	NextSeq
In own laboratory	Commercial kits	Illumina Nextera DNA Prep	MiSeq
In own laboratory	Commercial kits	Illumina Nextera DNA Flex	MiSeq
In own laboratory	Commercial kits	Ion Xpress TM Plus Fragment Library Kit for AB Library Builder TM System	Ion Torrent S5XL
In own laboratory	Commercial kits	Nextera DNA Flex	NextSeq
Externally	Commercial kits	Nextera XT DNA preparation kit	MiSeq
In own laboratory	Commercial kits	Nextera XT	MiSeq
In own laboratory	Commercial kits	Nextera Flex Illumina	MiniSeq Illumina
In own laboratory	Commercial kits	Illumina DNA prep kit	NextSeq
In own laboratory	Commercial kits	Nextera (Illumina)	MiSeq
In own laboratory	Commercial kits	NexteraXT (Illumina)	NextSeq
In own laboratory	Commercial kits	Nextera XT DNA Library Kit, Illumina	MiSeq
In own laboratory	Commercial kits	Kapa HyperPlus (Roche)	NextSeq

Correct cluster identified



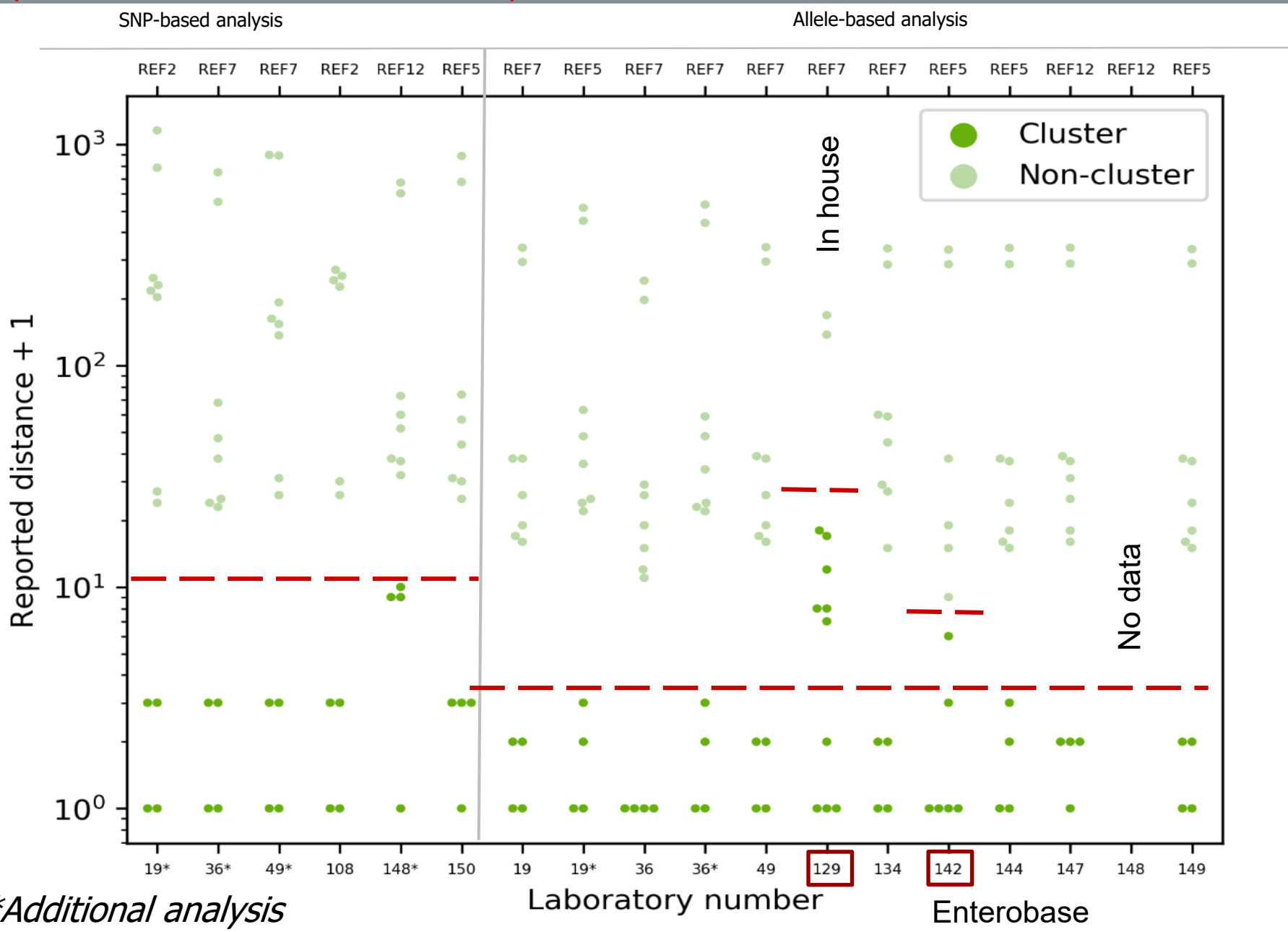
EQA-8 (*S.* Enteritidis, ST11x9, ST10, ST183, ST1925)



**Additional analysis*

EQA-9 *S.* monophasic Typhimurium, (ST34x10, ST19, ST2212)

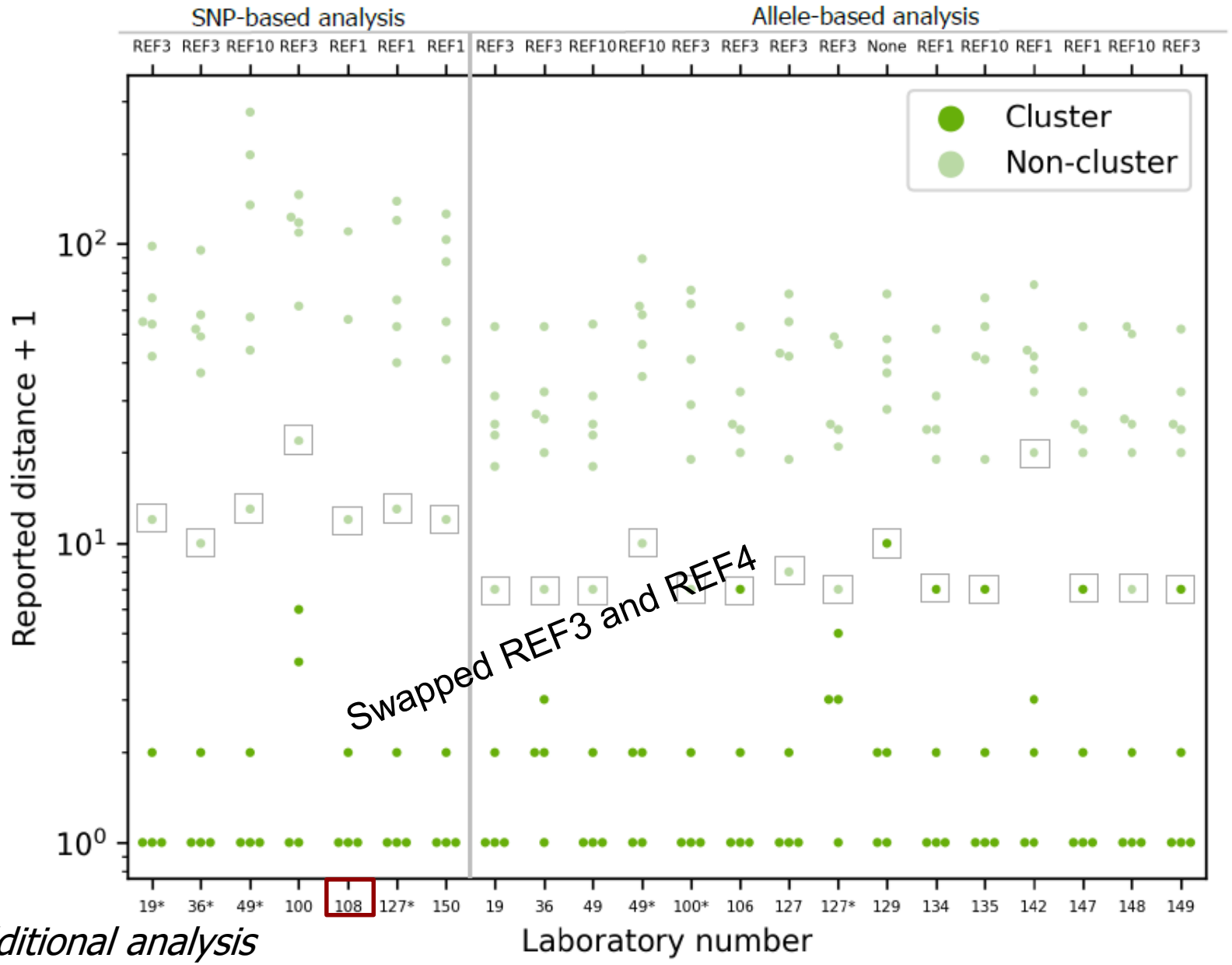
STATENS SERUM INSTITUT



**Additional analysis*

EQA-10 *S.* monophasic Typhimurium, (ST34x7, ST4430, ST4431, ST5296)

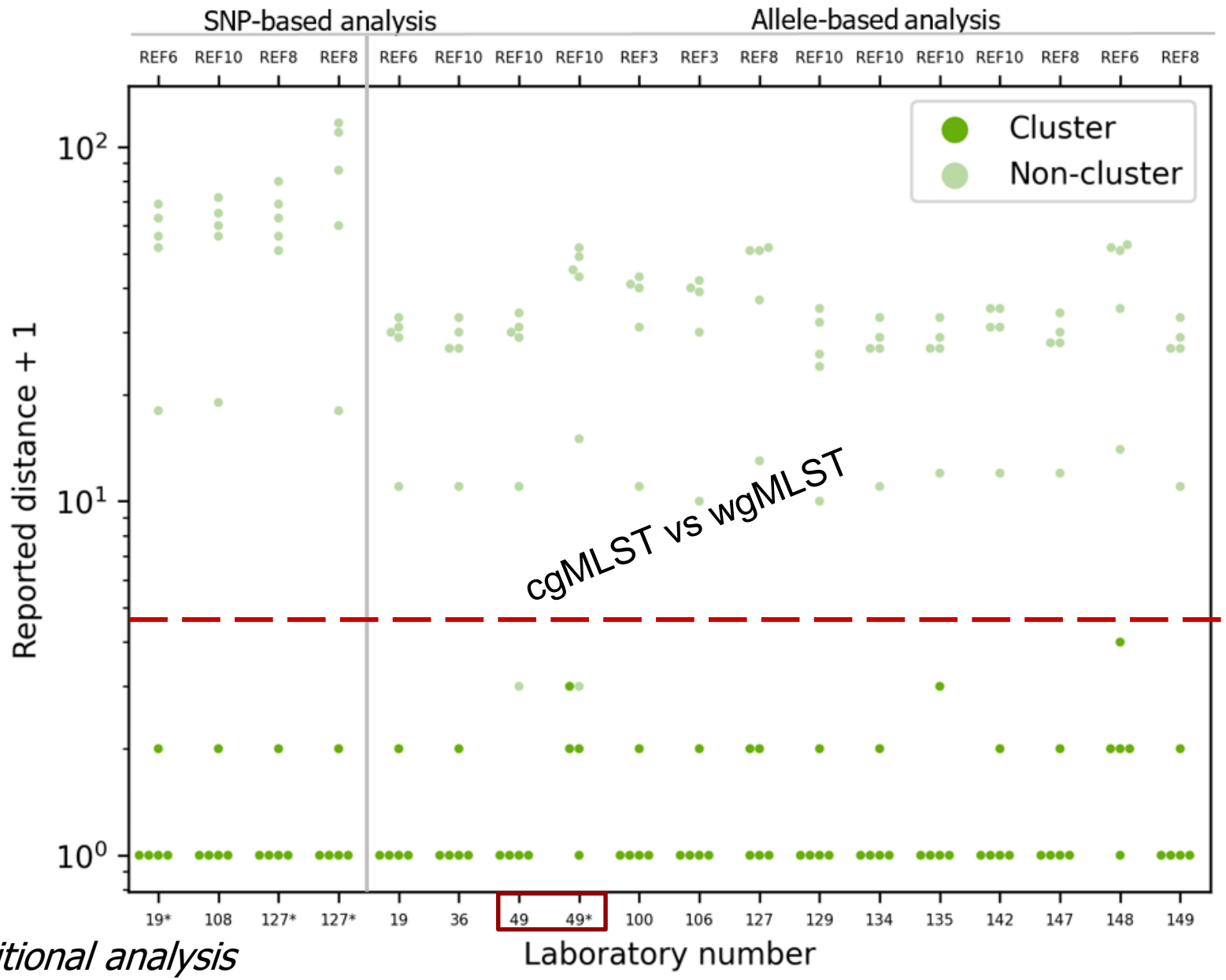
STATENS SERUM INSTITUT



**Additional analysis*

EQA-11 (*S.* Enteritidis, ST11x10)

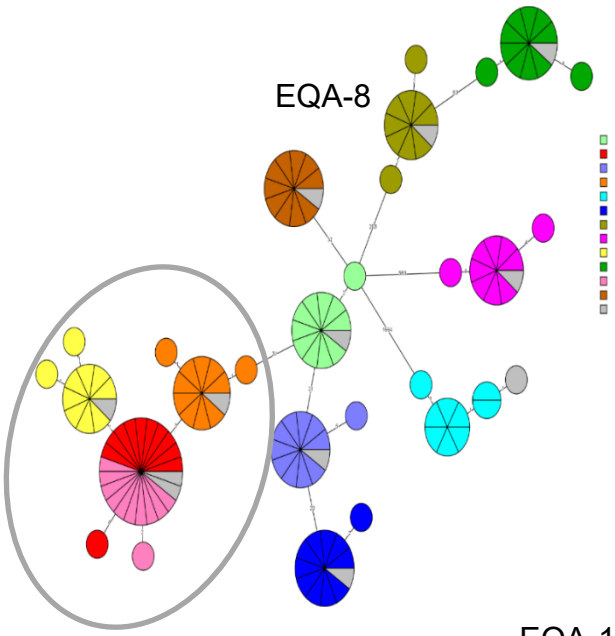
STATENS SERUM INSTITUT

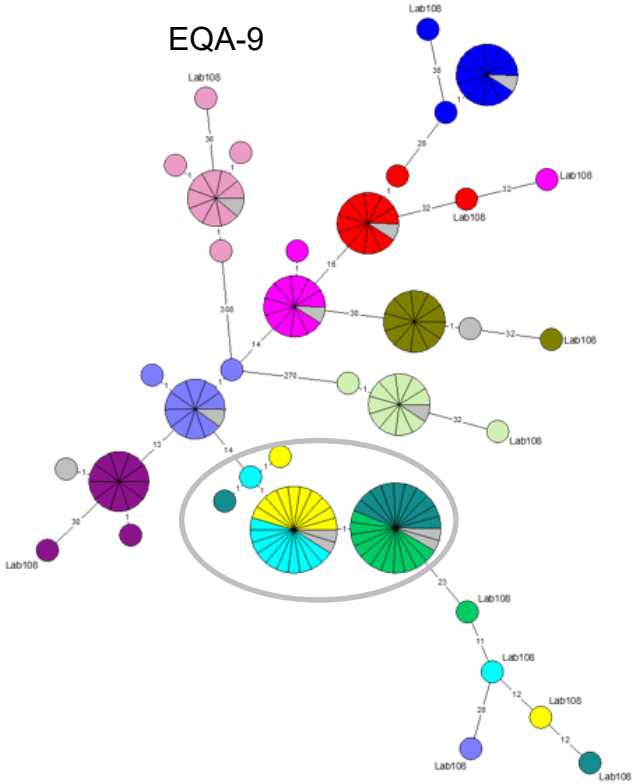


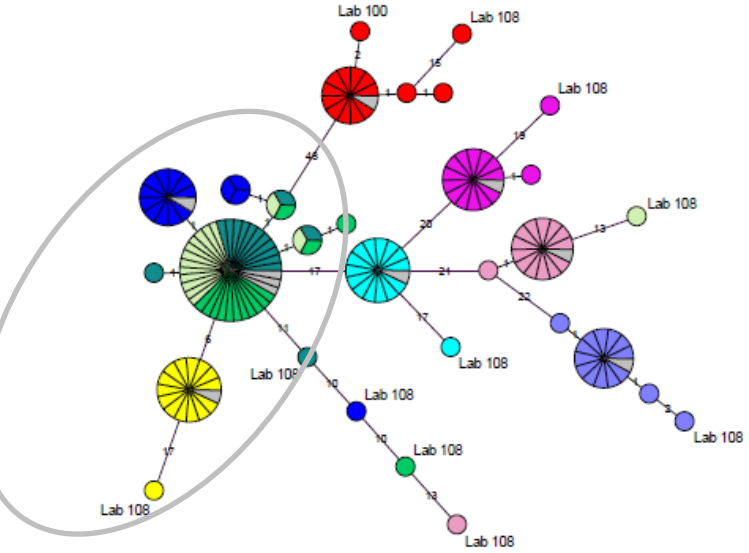
**Additional analysis*

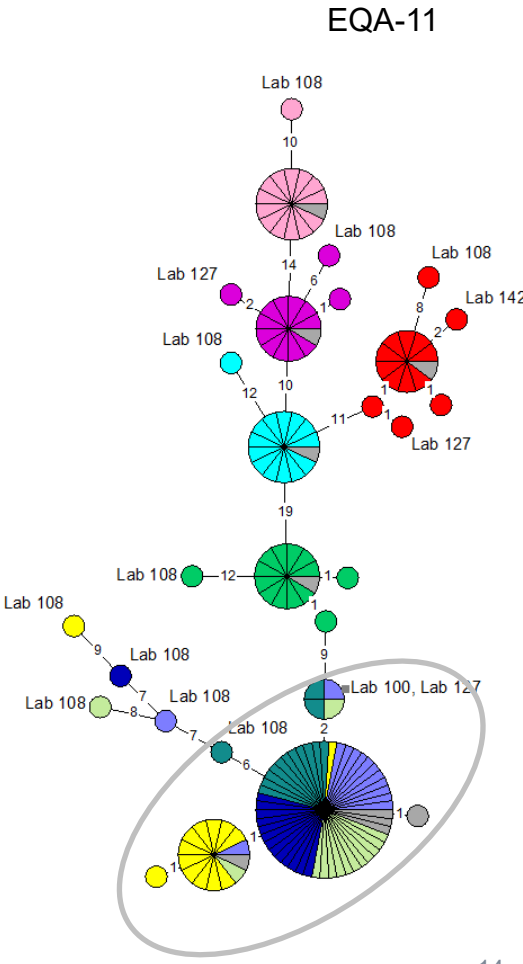
Fastq files analysed by SSI minimum spanning tree cgMLST

STATENS SERUM INSTITUT

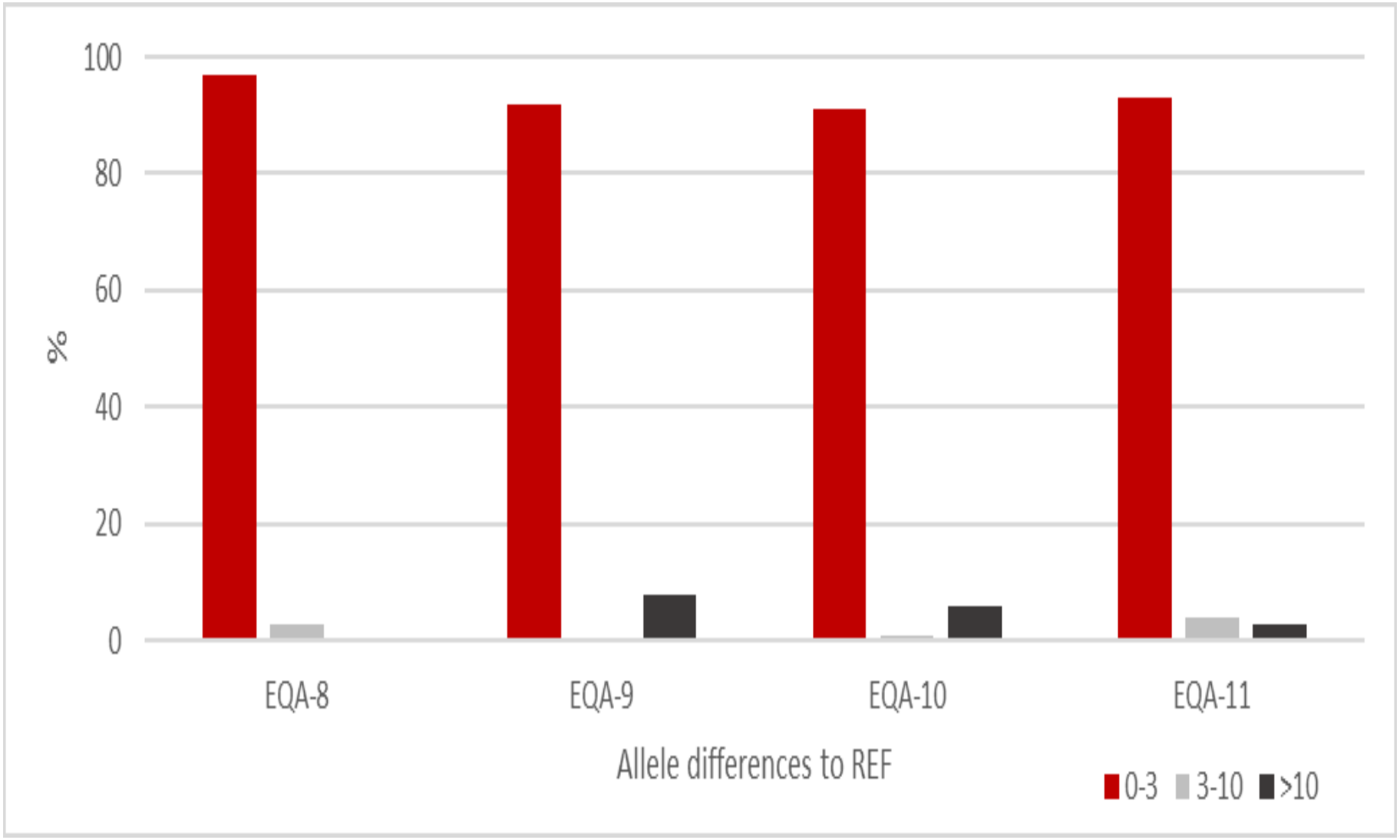








Allele difference from SSI sequences



Additional genomes

STATENS SERUM INSTITUT

- **EQA-10 and EQA-11**
  - 5-6 additional genomes

- Part of the already identified cluster Yes/No?
- Explain what you observe ….

Modified the genomes (mimicking a true outbreak situation)

- Contamination (by a different species or same species)
- Low coverage
- Good quality Fastq files
- Fasta files

Examples

STATENS SERUM INSTITUT

- EQA-10
  - A NonCluster isolate mixed with a *Klebsiella pneumonia* (approx. 10%)
    - 60% identified the contamination
    - 7% concluded is was a cluster isolate (1 lab – not identified the contamination)
    - 93% concluded is was NOT a cluster isolate

- EQA-11
  - A cluster isolate mixed with a *Citrobacter* (approx. 10%)
    - 72% identified the contamination
    - 78% concluded is was a cluster isolate, (22% ND)

  - A cluster isolate mixed with a *Salmonella* ST34 (approx. 20%), same species contamination
    - 100% identified the contamination
    - 7% concluded is was a cluster isolate (1 lab, but would re-run the sample)
    - 29% concluded is was NOT a cluster isolate, (64% ND)

Summery

STATENS SERUM INSTITUT

- Up to 15 laboratories in EU/EEA participated using WGS
- 64-93% use allele based approach, primarily SeqSphere, Enterobase (3002)
- In general the performance were high ~ 83-93% identified the correct cluster
- Similar results (allelic difference /SNP distances within the cluster)

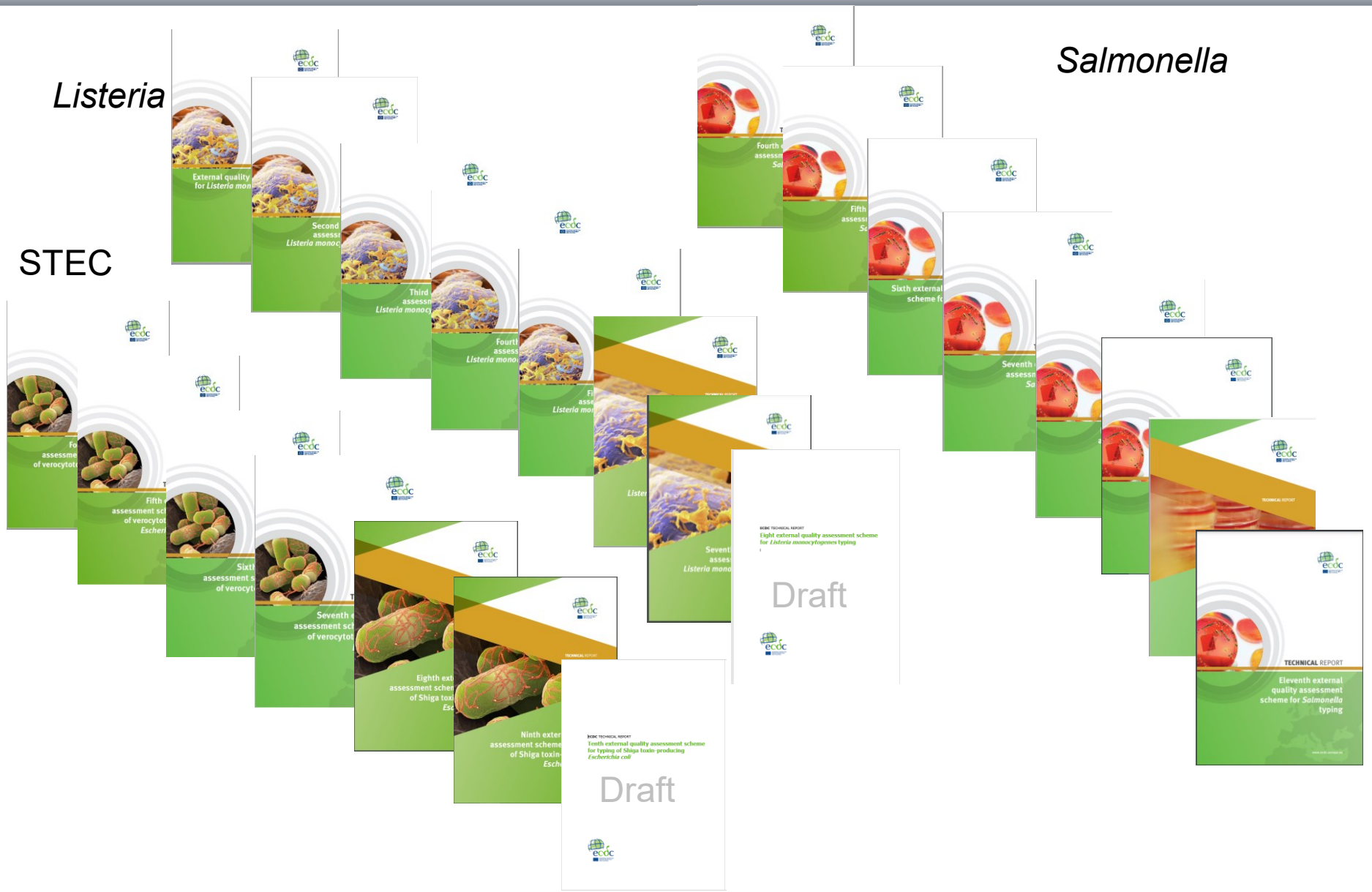
- Both SNP and allele based methods is useful for interlaboratory comparability
  - cgMLST results were at a comparable level
  - The reported SNP results showed more variability (Using a non-standardised)

- The reported results give no clear indication on the influence of the used analysis tools (assembler, allele calling method/software)

- Data with contamination of the same species is the most difficult to use

Published EQA reports since 2012

STATENS SERUM INSTITUT



Link to ECDC reports: https://www.ecdc.europa.eu/en/publications-data

ACKNOWLEDGEMENTS

All of you!
All the Public Health Reference Laboratories for *Salmonella*

Taina Niskanen (ECDC)

EQA-team at SSI: Gitte Sørensen, Louise Dahl, Kristoffer Kiil and Eva Møller Nielsen
Section of Foodborne Infections

THANK YOU FOR YOUR ATTENTION

FWD AMR· RefLabCap

SUSANNE SCHJØRRING (SSC@SSI.DK)